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CASE REPORT

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Ozkan G, Ulusoy S, Guvercin B, Menteşe A, Karahan SC, Yavuz A. A new player in chronic kidney disease mineral and bone disorder: tenascin-C. Int J Artif Organs. 2015;38:481-7.

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West JB: Respiratory Physiology (2nd ed). Baltimore: Williams and Wilkins, 1974; 72-5.

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For the citations from the thesis:

Kulu A. Evaluation of Quality of Life After Surgical Interventions Applied to Patients with Bladder Tumors, Trakya University, Institute of Health Sciences, Department of Nursing. Master Thesis. 2010; Edirne.

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Felek S, Kılıç SS, Akbulut A, Yıldız M. A case of phylgellosis with visual hallucinations. XXVI. Turkish Microbiology Congress Abstract Book, 22-27 September 2000, Antalya, Mars Printing House, 1994, p.53-6.

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INSTRUCTIONS TO AUTHORS

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- After revisions, I would like to see it again (The work is taken into a second-round evaluation after it is submitted by its authors)
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CONTENTS

ORIGINAL ARTICLE

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Dosimetric Comparison of Intensity Modulated Radiotherapy and Simultaneous Integrated Boost Techniques in the Treatment of Glioblastoma Multiforme

Glioblastoma Multiform Tedavisinde Yoğunluk Ayarlı Radyoterapi ve Simultane Entegre Boost Tekniklerinin Dozimetrik Karşılaştırılması

Hamit BAŞARAN, Gökçen İNAN, Osman Vefa GÜL

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ABSTRACT

Aim: The aim of this study was to compare dosimetric advantages of using intensity-modulated radiation therapy (IMRT) and simultaneous-boost (SIB-IMRT) techniques for glioblastoma multiform (GBM).

Materials and Methods: Ten patients with GBM were retrospectively selected between the years of 2020 and 2021. For all patients, two treatment plans were created. The plans were calculated using anisotropic analytical algorithm with 6 MV photon energy. Treatment doses were 50 Gy for planned target volume (PTV) (50 Gy), 10 Gy for PTV (60 Gy) and 60 Gy for PTV (60 Gy), which is planned as 2 Gy per daily fraction in IMRT technique. In the SIB-IMRT technique, which provides different dose levels in target volumes simultaneously in 25-day fractions, was used. All plans were compared with respect to the doses received by PTV and the organ at risk including brain system, optic chiasma, optic nerves, eyes, the dose homogeneity index (HI), conformity indexes (CI) and total monitor unit counts required for the treatment.

Results: The average doses for PTV were 60.62±0.33 Gy for the IMRT technique and 60.58±0.32 Gy for the SIB-IMRT technique. The average doses for PTV, for both techniques were found to be similar. The average HI value for PTV (60 Gy) was 0.05±0.009 in IMRT, 0.13±0.197 in SIB-IMRT, 0.97±0.02 in IMRT, and 0.35±0.06 in SIB-IMRT, respectively. As a result of the statistical comparison, a significant difference was observed in HI and CI values between IMRT and SIB-IMRT in the analysis of the values of PTV (p=0.004, p=0.001). When the SIB-IMRT plans were compared with the IMRT plans, it was observed that the mean doses received by critical organs such as optic chiasma, optic nerve, and eye were significantly decreased in the SIB-IMRT technique (p=0.000).

Conclusion: When the IMRT technique for GBM treatment was compared with the SIB-IMRT technique, SIB-IMRT provided better protection for organ at risk. SIB-YART plans may be clinically acceptable treatment modalities for GBM cancers.

Keywords: Glioblastoma multiform, intensity-modulated radiation therapy, simultaneous integrated boost method

ÖZ

Amaç: Bu çalışmanın amacı glioblastoma multiform (GBM) tedavisinde yoğunluk ayarlı radyoterapi (YART) ve simultane entegre boost (SIB-YART) tekniklerini dozimetrik olarak karşılaştırmaktır.

Gereç ve Yöntem: Bölümümüzde 2020-2021 yılları arasında RT tedavisi alan 10 GBM hastası çalışmaya dahil edildi. Her hasta için aynı tümör ve kritik yapılar kullanılarak YART ve SIB-YART tekniklerinde planlar yapıldı. Tedavi dozları YART tekniğinde günlük fraksiyon başına 2 Gy olacak şekilde planlanan hedef hacime (planned target volume-PTV) (50 Gy) 50 Gy ve PTV'ye (60 Gy) 10 Gy ve toplamda PTV'de (60 Gy) 60 Gy'yi tamamlayacak şekilde planlandı. SIB-YART tekniğinde ise 25 günlük fraksiyonda eş zamanlı olarak hedef hacimlerde farklı doz seviyelerinin sağlandığı SIB tekniği kullanıldı. Planlar 6 MV foton enerjisi kullanılarak, anisotropik analitik algoritması ile Eclipse tedavi planlama sisteminde hesaplatıldı. PTV, beyin sapı, optik kiazma, optik sinir ve göz gibi risk altındaki organlar (RAO), doz homojenite indeksi (HI), konformite indeksi (CI), monitör üniteleri açısından YART planları SIB-YART planları ile karşılaştırıldı.

Address for Correspondence: Hamit BAŞARAN MD, Selçuk University Faculty of Medicine, Department of Radiation Oncology, Konya, Turkey
Phone: +90 543 607 61 65 **E-mail:** drhbasaran@gmail.com **ORCID ID:** orcid.org/0000-0002-2122-8720
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Bulgular: PTV için ortalama dozlar YART tekniği için 60,62±0,33 Gy iken, SIB-YART tekniği için 60,58±0,32 Gy'dir. Her iki teknik için PTV'nin aldığı ortalama dozlar benzerdir. PTV (60 Gy) için HI ortalama değeri sırasıyla YART'de 0,05±0,009 iken, SIB-YART'de 0,13±0,197, CI ise YART'de 0,97±0,02, SIB-YART için 0,35±0,06 olarak bulundu. Yapılan istatistiksel karşılaştırma sonucunda PTV'ye ait değerlerin analizinde YART ve SIB-YART arasında HI ve CI değerlerinde anlamlı bir fark görüldü (p=0,004, p=0,001). SIB-YART planları YART planları ile karşılaştırıldığında optik kiazma, optik sinir, göz gibi kritik organların aldığı ortalama dozların SIB-YART tekniğinde anlamlı olarak azaldığı görüldü (p=0,000).

Sonuç: GBM tedavisine yönelik YART tekniği SIB-YART tekniği ile karşılaştırıldığında, SIB-YART tekniğinin kritik organları daha iyi koruduğu görüldü. SIB-YART planları GBM kanserlerinde klinik olarak kabul edilebilir tedavi yöntemi olabilir.

Anahtar Kelimeler: Glioblastoma multiform, yoğunluk ayarlı radyoterapi, simultane entegre boost tekniği

INTRODUCTION

Glial tumors are the most common primary malignant brain tumors in adults[1]. Malignant gliomas (World Health Organization grade 3-4) constitute more than half of primary brain tumors, and approximately 75% of them are glioblastoma multiform (GBM) with grade IV[2,3]. It is known that malignant brain tumors, especially GBM, have lower survival rates and the worst prognosis due to their high progression potential[4]. The primary standard treatment for GBM treatment is surgery[5,6]. However, due to its high infiltrative character, GBM has high local recurrence rates even with the best surgical approach, which necessitates additional local treatments such as radiotherapy (RT). According to the results of phase III randomized studies, the standard adjuvant treatment of GBM is 60 Gy local RT ± alkylating agent-based chemotherapy[7]. In GBM RT, tumors can usually be located in or very close to critical radiation-sensitive structures such as the brain stem, optic chiasm, right optic nerve, left optic nerve, right orbit, and left orbit. The tolerance doses of these critical structures are lower than the targeted treatment doses, and this may cause damage to critical structures.

The aim in RT is to protect the critical structures around it in the best possible way, while giving the desired dose to the determined target volume[8]. Today, there are many RT options used in treatment. One of the most commonly used treatments in RT is intensity modulated RT (IMRT). In IMRT treatment techniques, the aim of treatment is determined in advance with the inverse planning system. In the optimization processes, it is tried to obtain a homogeneous and desired dose distribution in order to achieve these goals. In IMRT techniques, different fraction schemes can be applied to different target volumes simultaneously with the simultaneous integrated boost (SIB) method. In IMRT treatments for this purpose, organs at risk (OAR) and target volumes are displayed in three dimensions, and the most appropriate gantry angles and number of fields are determined, and treatment planning is made.

In this study, it was aimed to compare the current treatment plan of our patients with malignant glial tumors, who were treated with the IMRT technique, with the virtually created SIB-IMRT technique, dosimetrically.

MATERIALS AND METHODS

Patient Selection

For the study, 10 patients with malignant glial tumors who were treated with 60 Gy RT and CRT in the Department of Radiation Oncology, Faculty of Medicine Selçuk University between 2020 and 2021 were selected. Permission for this study was obtained from the Ethics Committee of Selçuk University Faculty of Medicine, with the decision dated 07 April 2021 and numbered 2021/198. The clinical and dosimetric characteristics of the patients selected for the study are given in Table 1.

Target Volume and Critical Organs

All patients were immobilized with a head and neck thermoplastic mask in the supine position. The images obtained by scanning 3 mm slice thickness over the area of interest in the computed tomography (CT) unit were transferred to the treatment planning system (Eclipse, version 15.1; Varian). Preoperative and postoperative axial T1 contrast and axial T2-FLAIR magnetic resonance (MR) images were fused to the planning CT image set for contouring. For gross tumor volume (GTV) determination, T1 contrast-enhanced and axial T2-FLAIR from preoperative MR images or the cavity and surrounding area of contrast on postoperative MR were defined as GTV_{50}.

The clinical target volume (CTV) CTV_{50} was created by adding an isometric 2-2.5 cm margin to the GTV_{50} to achieve the CTV, and the PTV_{50} was created by adding a 0.5 cm margin around the CTV_{50} for the planned target volume (PTV) definition. For the boost area, the GTV_{60} was contoured using preoperative MR axial T1 contrast-enhanced images. The CTV_{60} was created by adding an isometric 2-2.5 cm margin to the GTV_{60} and PTV_{60} was created by adding 0.5 cm margin around the CTV_{60}[9,10]. Brain stem, optic chiasm, right optic nerve, left optic nerve, right orbit and left orbita were contoured as critical organs. Target structures were removed with a 1 mm margin from each other in order to ensure sharp dose changes easily. By removing the parts of critical organs that intersected with the tumor with a margin of 2 mm, the mean dose values were reduced. The IMRT treatment technique was planned as PTV_{50}, 50 Gy from 2 Gy/25 fractions in phase 1, and then 60 Gy in total from PTV_{60} 2 Gy/5

fractions in phase 2. In the SIB-IMRT treatment technique, PTV_{60} was planned to be 2.4 Gy in 25 fractions.

Treatment Planning

In this study, the Varian Millennium 80-leaf collimators (Varian) treatment device available in our clinic was used. Dynamic IMRT and SIB-IMART treatment plans with 5 coplanar fields were created for GBM patients with IMRT and SIB-IMART techniques. IMRT plans were prepared with the inverse planning method using 6 MV X-rays. After the treatment plans were created, the optimization process was started. During the optimization process, minimum and maximum dose limitations were made to the target volumes, and it was aimed that 95% of the PTVs would receive 100% of the defined dose. Necessary dose limitations were made in order to give the lowest dose among the determined criteria to the organs at risk. Anisotropic Analytical Algorithm (v.15.1) was used for dose optimization and calculations of IMRT plans.

Plan Evaluation

Dose volume histograms (DVH) were used to compare the target volume and critical organ doses of the treatment plans. PTV and the doses taken by the OAR were evaluated by comparing DVHs from IMRT and SIB-IMRT plans. Homogeneity index (HI) and conformity index (CI) parameters are used to evaluate plans in different treatment options. The dose HI formula was defined according to the International Commission on Radiation Units report no: 83[11].

$$HI=\frac{(D_{2\%}-D_{98\%})}{D_{50\%}}$$

It is defined in the formula as "D_2 is the dose received by 2% of the target, D_{98} is the dose received by 98% of the target, and D_{50} is the dose received by 50% of the target". In cases where CI is equal to 1, we can talk about the ideal dose distribution. If CI is greater than 1, the irradiated volume is greater than the target volume, and if CI is less than 1, the target volume is partially irradiated. The CI index is used to estimate the degree of suitability of the plan[12]. It is calculated as the ratio of the volume of PTV receiving 98% of the dose to the total volume of PTV. This value was calculated automatically with the planning option. By using DVHs, D_{max}(Gy), D_{mean}(Gy) (maximum and mean doses at target volume), D_{98}, D_{95}, D_{50}, and D_2 data of PTV were compared. D_{max}(Gy) and D_{mean}(Gy) values were compared for optic nerves, brain stem, optic chiasm and orbits in critical organs. In addition, the monitor unit (MU) values of the plans were compared.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 25.1. The Paired samples t-test was used for statistical analysis of the difference between the two groups. A p value of <0.05 was considered statistically significant.

RESULTS

In Table 2, the mean of dose values of PTV_{60} and PTV_{50} obtained from IMRT and SIB-IMRT treatment plans, the mean of HI and CI values, and the mean numerical values for MU values, and the results of binary statistical analysis between techniques for 10 GBM patients are given. IMRT plans are more advantageous than SIB-IMRT plans in terms of covering the PTV_{60} target volume with the defined dose. The comparison of the plans showing the dose covering 95% of the targeted volume in IMRT and SIB-IMRT techniques is shown in Figure 1. It was observed that similar results were obtained in IMRT and SIB-

Table 1. Patients' clinical and dosimetric characteristics

Patient	Age	Gender	Pathology	Grade	IDH	Anatomical localization	Size (mm)	Excision	Dose/W	Treatment protocol
1. A. Ö.	70	E	GBM	IV	Mutant	Temporal	55	Total	60 Gy/5W	Adjuvant RT+TMZ
2. N. M.	65	E	GBM	IV	Mutant	Temporal	40	Total	60 Gy/5W	Adjuvant RT+TMZ
3. A. Ş.	67	K	GBM	IV	Mutant	Basal ganglion +Temporal	85	Subtotal	60 Gy/5W	Adjuvant RT+TMZ
4. M. D.	80	K	GBM	IV	Mutant	Frontotemporal	55	Subtotal	60 Gy/5W	Adjuvant RT+TMZ
5. H. Y.	55	K	GBM	IV	Mutant	Frontal	36	Total	60 Gy/5W	Adjuvant RT+TMZ
6. Z. Y.	55	E	Geliosarcoma	IV	Mutant	Temporal	35	Total	60 Gy/5W	Adjuvant RT
7. A. B.	70	E	GBM	IV	Mutant	Temporal	70	Subtotal	60 Gy/5W	Adjuvant RT+TMZ
8. A. Ç.	62	E	GBM	IV	Mutant	Temporal	27	Total	60 Gy/5W	Adjuvant RT+TMZ
9. H. U.	76	K	GBM	IV	Non-mutant	Temporoparietal	30	Total	60 Gy/5W	Adjuvant RT+TMZ
10. İ. D.	52	E	GBM	IV	Non-mutant	Temporal	35	Total	60 Gy/5W	Adjuvant RT+TMZ
11. İ. G.	33	E	Anaplastic oligoastrocytoma	III DSO 2007	Non-mutant	Parietal	53	Total	60 Gy/5W	Adjuvant RT+TMZ

F: Female, M: Male, IDH: Isocitrate dehydrogenase, GBM: Glioblastoma multiforme, RT: Radiotherapy, TMZ: Temozolomide, PCV: Procarbazine, CCNU, and vincristine

IMRT plans in terms of covering the PTV_{50} target volume with the defined dose. Since the ideal value of HI was "0", the plans with the most homogeneous dose distribution were found in the IMRT technique (p=0.004). Since the ideal value of CI was "1", the most conformal technique was also found in the IMRT technique (p=0.001). The comparison of dosimetric values between techniques for critical organs is given in Table 3. When SIB-IMRT plans were compared with IMRT plans, the mean doses received by the brain stem, optic chiasm, optic nerves, and eyes were found to be significantly lower in the SIB-IMRT technique (p values: 0.006, 0.000, 0.000 and 0.000, respectively). In addition, the maximum doses received by the brain stem, optic chiasm, optic nerves and orbits were significantly reduced by the SIB-IMRT technique (p values: 0.000, 0.002, 0.000 and 0.000, respectively). The DVH of a patient whose treatment plan was prepared with IMRT and SIB-IMART is shown in Figure 2. The mean MU counts for the IMRT and SIB-IMRT techniques were 501±31 and 860±111, respectively. The MU value required for the IMRT technique was found to be significantly lower than for the SIB-IMRT technique (p=0.000).

DISCUSSION

Currently, the standard treatment for GBM tumors is surgery, chemoradiotherapy and adjuvant chemotherapy[13]. In high-grade astrocytomas, no matter how extensively the tumor

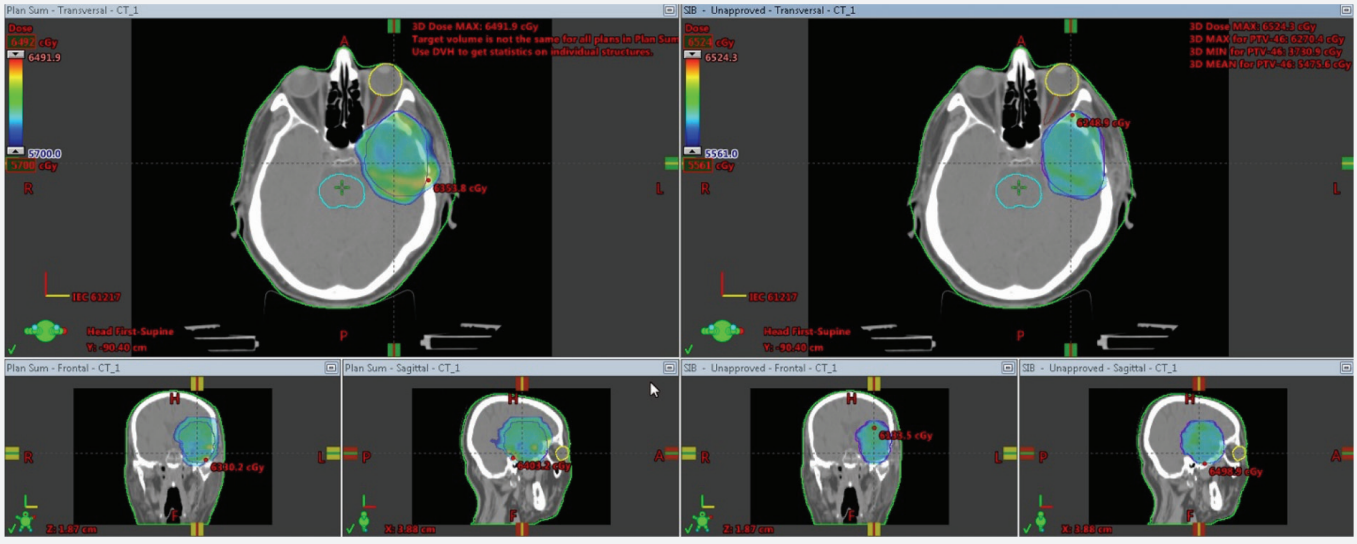

Figure 1. Plan comparison of IMRT and SIB-IMRT techniques

Table 2. Dosimetric values at planned target volume

OAR	IMRT (Mean±SD) (Gy)	SIB-IMRT (Mean±SD) (Gy)	Δ Mean±SD (IMRT SIB-IMRT)	p
PTV60				
$D_{98\%}$ (Gy)	59.87±0.30	57.80±0.20	2.06±0.38	**0.000**
$D_{95\%}$ (Gy)	60.44±0.39	58.42±0.15	2.02±0.40	**0.000**
$D_{50\%}$ (Gy)	60.77±0.54	60.42±0.15	0.34±0.46	0.489
$D_{2\%}$ (Gy)	62.80±0.37	62.32±0.21	0.47±0.41	0.547
D_{max} (Gy)	63.61±0.42	63.60±0.44	0.01±0.06	0.589
D_{mean} (Gy)	60.62±0.33	60.58±0.32	0.04±0.04	0.485
CI	0.97±0.02	0.35±0.06	0.62±0.08	**0.001**
HI	0.05±0.009	0.13±0.197	-0.08±0.19	**0.004**
MU	501±31	860±111	-359±101	**0.000**
PTV50				
$D_{98\%}$ (Gy)	48.97±0.32	48.38±0.41	0.59±0.40	0.001
$D_{95\%}$ (Gy)	49.68±0.18	49.41±0.31	0.26±0.37	0.050

*p<0.005.

SD: Standard deviation, OAR: Organ at risk, IMRT: Intensity-modulated radiation therapy, SIB: Simultaneous integrated boost, HI: Homogeneity index, CI: Conformity indexes, MU: Monitor unit, PTV: Planned target volume

tissue is surgically removed, the neoplastic cells at the microscopic level reproduce in the normal brain tissue due to their infiltrative structure. Therefore, RT is recommended to prevent the increase of residual cells or to eliminate the macroscopic tumor remaining after subtotal resection[14]. RT is an important treatment option in the treatment of malignant glial tumors. In this study, IMRT and SIB-IMRT plans were made for 10 cases diagnosed with malignant glial tumors, and they were compared dosimetrically in terms of target volume coverage, risky organ doses and MU.

Fogliata et al.[15] evaluated the potential benefits of IMRT and SIB-IMRT plans in head and neck patients in terms of planning and at a dosimetric level. Dose distributions were obtained with inverse planning IMRT for all plans and sliding window technique was used after IMRT optimization. They stated that the physical dose distribution and homogeneity were better for the plans obtained with the IMRT technique. They found that the V_{95} parameter was lower in SIB plans (p=0.002). They stated that the doses received by organs at risk, such as the spinal cord and parotid, were lower in the SIB-IMRT technique. Similarly, in our study, it was found that the plans obtained with the IMRT technique were more advantageous in terms of dose coverage, but the doses received by the OAR were lower in the SIB-IMRT technique.

Li et al.[16] compared IMRT and SIB-IMRT plans to deliver high doses to the prostate and lower doses to the pelvic region. They noted that the SIB-YART technique had potential advantages, including better preservation of critical structures, more efficient administration, shorter treatment time, and better biological efficacy. In parallel with this study, in this study conducted on 10 cases with a diagnosis of malignant glial tumor, it was observed that the mean doses received by the brain stem, optic chiasm, optic nerves and orbits were significantly lower in the SIB-IMRT technique when SIB-IMRT plans were compared with IMRT plans.

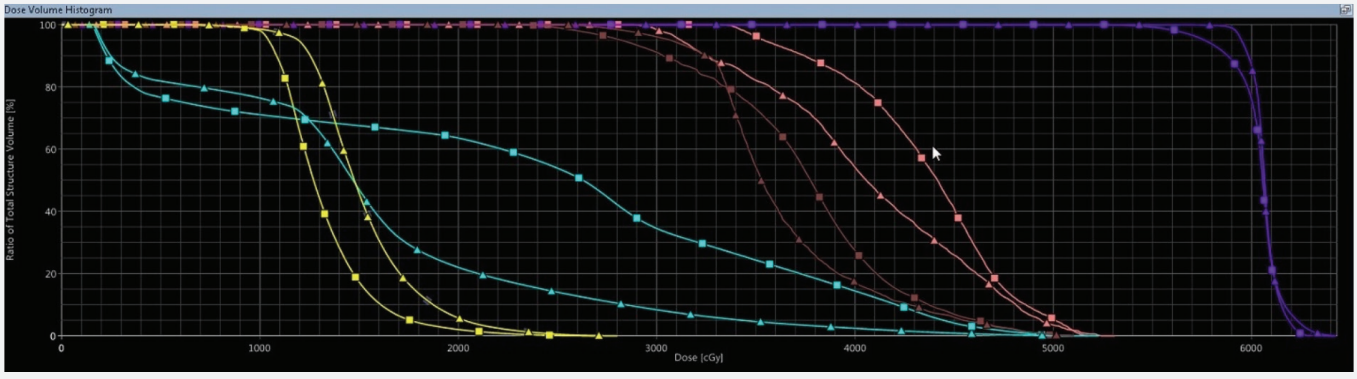

Figure 2. Dose volume histogram of a patient whose treatment plan was prepared with IMRT and SIB-IMRT (purple: PTV, blue: brainstem, pink: optic chiasm, brown: optic nerve, yellow: eye)

Table 3. Dosimetric values in organs at risk

OAR		IMRT (Mean±SD) (Gy)	SIB-IMRT (Mean±SD) (Gy)	Δ Mean±SD (IMRT SIB-IMRT)	p
Brainstem	D_{max}	52.88±1.58	51.43±1.04	1.44±0.70	**0.000**
	D_{mean}	19.87±5.61	19.18±5.86	0.68±0.60	**0.006**
Optic chiasm	D_{max}	44.67±6.23	42.41±7.23	2.26±1.68	**0.002**
	D_{mean}	28.35±7.04	26.11±7.13	2.23±1.10	**0.000**
Left optic nerve	D_{max}	44.84±8.70	42.59±8.62	2.24±0.96	**0.000**
	D_{mean}	31.25±5.76	28.45±5.43	2.80±1.07	**0.000**
Left eye	D_{max}	38.15±9.25	36.36±9.11	1.79±0.83	**0.000**
	D_{mean}	22.26±7.93	21.16±8.13	1.10±0.58	**0.000**
Right optic nerve	D_{max}	16.68±4.73	15.73±4.77	0.94±0.48	**0.000**
	D_{mean}	12.38±4.69	10.83±3.32	1.54±1.62	**0.000**
Right eye	D_{max}	15.17±4.15	14.37±3.66	0.80±0.82	**0.013**
	D_{mean}	6.22±2.85	5.32±2.56	0.89±0.44	**0.000**

*p<0.005.

SD: Standard deviation, OAR: Organ at risk, IMRT: Intensity-modulated radiation therapy, SIB: Simultaneous integrated boost

Onal et al.[17] compared sequential boost (SEB) technique and SIB techniques dosimetrically in volumetric modulated arc therapy (VMAT) and helical tomotherapy (HT). In their study, they stated that the SIB technique protects the heart better than the SEB technique in HT plans. In our study, it was observed that critical organs were better protected with the SIB technique.

Farzin et al.[18] compared the SIB and SEB method for VMAT in 20 patients with high-grade gliomas. In their study, in the SIB method, PTV received 54 Gy in 30 fractions with a dose of 1.8 Gy per fraction, while the tumor bed received 60 Gy from 2 Gy per fraction. According to their results, they found that both techniques were similar in terms of target coverage, but the SIB technique was significantly superior in protecting critical organs. The results obtained from this study were found to be similar to our study.

Nageeti et al.[19] compared the dosimetric coverage of PTV and OAR with SIB and SEB method in VMAT technique for 7 patients with a diagnosis of high-grade glioma. They stated in their study that although the protection of critical organs was similar for all plans, the use of SIB with fewer fractions of the total dose might be the best option for the treatment of patients with short survival without increasing toxicity. Contrary to this study, in our study, it was shown that the SIB-IMRT technique was more advantageous than IMRT plans because it protects critical structures at risk, and it provides a dosimetric advantage over IMRT plans because it protects healthy tissues.

In their study, Çelen and Kızılkaya[20] aimed to dosimetrically compare PTV and OAR with sequential IMRT and SIB-IMRT techniques to the entire breast and boost area in patients who underwent breast-conserving surgery. In their study, they gave 50 Gy/25 fractions to the whole breast and 10 Gy/5 fractions to the boost area to the patients who underwent sequential IMRT, and they gave a total of 50.4 Gy/28 fractions to the whole breast for patients who were applied SIB IMRT while, at the same time, they gave an additional dose of 60 Gy/28 fractions to the boost volume. In their study, in the administration of the SIB-IMRT technique and the sequential IMRT technique to the same side lung; the comparison of the mean doses of V5 value for 10 patients revealed no statistically significant results, while the comparison of the mean dose values for V_{20} value in 10 patients revealed a statistical significance. They demonstrated that with the SIB-IMRT technique, treatment could be performed with a lower dose at V20 in the ipsilateral lung. They stated that the SIB-IMRT technique might be suitable for standard use in breast-conserving RT to reduce irradiated excess normal tissue volumes and to reduce the dose in organs at risk. Similar results were obtained in our study, and it has been shown that the SIB technique can be used to reduce the dose of organs at risk.

Study Limitations

There are several limitations in our study. This is a dosimetric study and does not include vital aspects necessary for clinical use. The number of patients used for comparison was limited to 10, which can be expanded in the next study to obtain a better sample.

CONCLUSION

It is known that IMRT therapy has many advantages over conventional RT. IMRT therapy is capable of delivering a highly compatible dose of irradiation to the target while preserving surrounding tissues. In the SIB-IMRT technique, on the other hand, all target volumes can become conformal by using different fraction sizes simultaneously. The SIB-IMRT technique can also be an easier, more effective and error-free IMRT planning and implementation method, because the same plan is used throughout the entire treatment. Studies have shown that the use of SIB-IMRT provides dosimetric advantages due to shorter treatment time, potential radiobiological gains, and preservation of normal tissues.

Ethics

Ethics Committee Approval: Permission for this study was obtained from the Ethics Committee of Selçuk University Faculty of Medicine, with the decision dated 07 April 2021 and numbered 2021/198.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.B., G.İ., O.V.G., Design: H.B., G.İ., O.V.G., Data Collection or Processing: H.B., G.İ., O.V.G., Analysis or Interpretation: H.B., G.İ., O.V.G., Literature Search: H.B., G.İ., O.V.G., Writing: H.B., G.İ., O.V.G.

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

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ORIGINAL ARTICLE

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Evaluation of Anxiety and Caregiver Burden in the Mothers of 0-2-Year-Old Children with Food Allergy

Besin Alerjisi Olan 0-2 Yaş Çocukların Annelerinde Anksiyete ve Bakım Veren Külfetinin Değerlendirilmesi

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ABSTRACT

Aim: Patients with food allergy and their families have poor quality of life, anxiety, depression, and stress compared to healthy individuals, and the Caregiver Burden is high in the parents. In our study, it was aimed to evaluate the anxiety disorder that may accompany the mothers of food allergic patients aged 0-2 years, and to examine the Caregiver Burden in the mothers of food allergic patients.

Materials and Methods: A questionnaire about sociodemographic data, Hospital Anxiety Depression Scale (HADS), Psychological Symptom Checklist (SCL 90-R), and Zarit Caregiver Burden Scale were administered to the mothers of food-allergic children aged 0-2 years and the mothers of healthy children (MHC) as the control group.

Results: Sixty seven mothers of children with food allergy and 74 MHC were enrolled in the study. Zarit Caregiver Burden Scale was significantly higher in the mothers of children with food allergy than in the MHC (p=0.018). Mothers of food allergic children had a significantly higher overall score on the general SCL 90-R scale (p=0.045). While the hospital anxiety scale score was significantly higher in the mothers of children with a food allergy, there was no difference in the HADS (p=0.045, p=0.825, respectively).

Conclusion: Evaluation of mothers' emotional status such as burden, anxiety, and depression and coping with food allergy strategies can be neglected. Therefore, the requirement of psychosocial support for the mothers of children with food allergy, especially in the young age group, should be evaluated and provided when necessary.

Keywords: Anxiety, burden, food allergy, quality of life, Zarit Caregiver Burden Scale

ÖZ

Amaç: Besin alerjisi olan hastalar ve aileleri sağlıklı bireylere göre düşük yaşam kalitesi, anksiyete, depresyon ve strese sahip olup, ebeveynlerde bakım veren külfeti yüksektir. Çalışmamızda 0-2 yaş arası gıda alerjisi hastalarının annelerine eşlik edebilecek anksiyete bozukluğunun değerlendirilmesi ve gıda alerjisi olan hastaların annelerinde külfetin incelenmesi amaçlandı.

Gereç ve Yöntem: Gıda alerjisi olan 0-2 yaş arası çocukların annelerine ve kontrol grubu olarak sağlıklı çocukların annelerine sosyodemografik veriler, Hastane Anksiyete ve Depresyon Ölçeği (HADÖ), Psikolojik Belirti Tarama Listesi (SCL 90-R) ve Zarit Bakıcı Yükü Ölçeği ile ilgili anket uygulandı.

Bulgular: Çalışmaya gıda alerjisi olan 67 çocuk annesi ve sağlıklı çocuğu olan 74 anne alındı. Zarit Bakım Veren Külfet Ölçeği puanı, gıda alerjisi olan çocukların annelerinde sağlıklı çocukların annelerine göre anlamlı olarak daha yüksekti (p=0,018). Besin alerjisi olan çocukların anneleri, genel SCL 90-R ölçeğinde anlamlı olarak daha yüksek bir genel puana sahipti (p=0,045). Besin alerjisi olan çocukların annelerinde HADÖ puanı anlamlı olarak yüksekken, Hastane Depresyon Ölçeği puanında fark yoktu (sırasıyla p=0,045, p=0,825).

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Sonuç: Annelerin külfet, anksiyete, depresyon gibi duygusal durumlarının değerlendirilmesi ve besin alerjisi ile baş etmeye çalışma stratejileri ihmal edilebilir. Bu nedenle besin alerjisi olan özellikle küçük yaştaki çocukların annelerinin psikososyal destek gereksinimleri değerlendirilmeli ve gerekli yönlendirme sağlanmalıdır.

Anahtar Kelimeler: Anksiyete, besin alerjisi, külfet, hayat kalitesi, Zarit Bakım Veren Külfet Ölçeği

INTRODUCTION

The prevalence of food allergy varies between 5% and 10%, and it has increased over the last few decades[1]. The main principles of the treatment are the elimination of the culprit food, prevention from accidental ingestions, and treating acute reactions[2]. Studies have reported that patients with food allergies and their families have decreased quality of life, increased psychological disorders such as anxiety, depression, stress, and social isolation compared to healthy people[3]. Generally, studies have evaluated patients in the older age group, adolescents, and their families. There are a limited number of studies evaluating the mothers of patients with a diagnosis of food allergy aged 0-2 years[4,5].

Nutrition in the first two years is very important for the physical and neurological development of the baby. Elimination of food due to food allergy and restrictions in daily and social activities cause a decrease in the quality of life for both the patient and their family, and especially for caregiving mothers[3,6,7].

When the child and in some cases the nursing mother are recommended an elimination diet, a new era begins. During this period, the mother often seeks an answer to "What should I eat, what should my baby eat?" questions. The idea of living with the food allergy, elimination of the culprit food from diet, reading of the labels and preparation of allergen-free meals, the risk of sudden and life-threatening reactions, and the possibility of persistence can affect this anxiety and stress situation[3,8].

The caregiver burden scale was evaluated in mothers of children with chronic diseases such as cystic fibrosis and cerebral palsy, and the burden was found to be high[9,10]. Food allergy, like other chronic diseases, can last for a long time and the development of tolerance may not occur immediately. Close attention is required during the care of the food allergic patient. The only difference from chronic diseases is that the patient's health condition is generally good in periods when there is no reaction. However, these reactions can be sudden and life-threatening at unexpected times, and the dependence of the baby on the mother who provides primary care may cause a burden especially on the mother.

Although stress and anxiety disorders have been shown in the mothers of children with food allergies in the literature, they were not evaluated with the caregiver burden scale[3].

In our study, we aimed to evaluate the anxiety disorder that may accompany the mothers of patients with a diagnosis of food allergy aged 0-2 years. We aimed to evaluate the burden caused by the existing food allergy in the caregiving mother.

MATERIALS AND METHODS

Patients and Control Group

The mothers of patients who were diagnosed with food allergy between the ages of 0 and 2 years, admitted to our hospital's Pediatric Allergy and Immunology outpatient clinics, were included in the study. Mothers of healthy 0-2-year-old children who were admitted to our hospital's well child outpatient clinic were enrolled as the control group. Mothers of children with other chronic diseases were not enrolled to the study.

Sample Size Calculation

The number of participants participating in the study was calculated in the Minitab program using anxiety scores obtained in a similar study[11]. When Type I error was taken by 0.05 and the strength of the study was 80%, the number of people to be taken in each group was calculated as at least 70 (if assumed standard deviation=4.15 and differences=1.99).

Diagnosis of Food Allergy

Food allergy was diagnosed as a compatible clinical history with a wheal diameter >3 mm in the skin prick test and/or specific IgE ≥0.35 kU/L, and/or positive oral provocation test for the culprit food[1,2,12].

Exclusion Criteria

Mothers of patients older than 2 years, without a diagnosis of food allergy, and mothers who did not want to participate were excluded. Besides, mothers who declared that they had a psychiatric illness or underlying medical conditions during the evaluation and illiterate mothers were not included in the study.

Socio-Demographic Characteristics

All mothers in the patient and control groups were filled out a questionnaire containing questions regarding their educational status, family structure, number of people living at home, education, occupation, and monthly income. As a pilot study, the questionnaire was administered to ten mothers selected randomly and tested for clarity at the beginning of the study.

Psychosocial Assessment

To evaluate the stress and anxiety disorders of the mothers, all mothers were asked to complete the Psychological Symptom Checklist (SCL 90-R) and the Hospital Anxiety and Depression Scale (HADS). These scales were filled under the supervision of the investigators.

Hospital Anxiety and Depression Scale

The HADS is a self-report test that includes 14 items and is used to evaluate anxiety and depression symptoms. Each item is scored according to the Likert scale, and the total score is between 0 and 21 for both anxiety and depression subscales. The Turkish validity and reliability study was performed for Turkish society, the threshold values were found to be 7 for depression and 10 for anxiety[13].

Psychological Symptom Checklist

The SCL 90-R psychological symptom screening test is a self-assessment test consisting of 90 items. Each item scored with a Likert-type scale is used to screen the psychological symptoms. The primary symptom dimensions that are assessed are somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism, and a category of "additional items", which helps clinicians assess other aspects of the client's symptoms. It has been translated and validated into Turkish[14].

Zarit Caregiver Burden Scale

The Zarit Caregiver Burden Scale was used to assess the caregiver's burden. This scale has been translated and validated into Turkish[15]. It consists of 22 items rated on a 5-point Likert scale that ranges from 0 (never) to 4 (nearly always) with the sum of scores between 0 and 88. Higher scores indicate a greater burden. 0-20 points indicate low burden. In our study, it was accepted that mothers who scored 20 or less did not have a burden, and those who scored above had a burden[16].

The scoring and evaluation of the scales were made by the pediatric psychiatry clinic of our hospital, and the mothers deemed necessary were referred for additional psychiatric evaluation.

Ethics

This study has been conducted in accordance with the principles of Helsinki Declaration. Written informed consent was taken from all participants. This study was approved by the Ethics Committee of University of Health Sciences Turkey, Okmeydanı Training and Research Hospital (protocol no: 740, date: 24.10.2017).

Statistical Analysis

Statistical Package for the Social Sciences 22.1 was used for data analysis. The distribution of the data was analyzed using the Shapiro-Wilk's normality test. Since data did not conform to the normal distribution, median (minimum-maximum) and quantitative data were presented as numbers and percentages (%). The Mann-Whitney U test was used for paired groups since the data did not show normal distribution when compared to qualitative data. Quantitative data were compared using the chi-square test. Linear correlations between the Zarit Caregiver Burden Scale and others were analyzed by the Spearman correlation test. Statistical significance was evaluated as $p<0.05$.

RESULTS

The study included the mothers of 67 patients and the mothers of 74 healthy children as the age and sex-matched control group. The mean age of the mothers was also similar in both groups. The socio-demographic characteristics of the patient and control groups are given in Table 1.

The Zarit Caregiver Burden Scale scores of the mothers of the patients diagnosed with food allergy were significantly higher than those of the mothers of healthy children (p=0.018). The general score of the psychological symptom screening list (SCL 90-R) was found to be significantly higher in the mothers of children with food allergy (p=0.045). When the subtitles of the SCL 90-R scale were evaluated separately, no significant difference was found between the groups. While the HADS was significantly higher in the mothers of children with a food allergy, there was no difference in the hospital depression scale score (p=0.045, p=0.825, respectively). The results of all scales applied in the mothers of food-allergic children (MFAC) and controls are given in Table 2. There was no significant difference in all scores when compared according to the onset of symptoms. There was a statistically significant positive correlation coefficient (r) value between 0.46 and 0.66 with Zarit Caregiver Burden and all other scales (p=0.001).

Zarit Caregiver Burden Scale was found to be positive as having a burden in 42 (62.6%) MFAC and 35 (47.3%) mothers with healthy children (MHC). Among the mothers with positive Zarit Caregiver Burden Scale, the unemployment was higher and the monthly income of the family was significantly lower in MFAC (p=0.040 and p=0.024, respectively). The comparison of the socio-demographic characteristics of MFAC and MHC with positive Zarit Caregiver Burden Scale is given in Table 3.

DISCUSSION

Food allergy is a global health problem that affects many children and their families. In addition to its financial burden, the decrease in quality of life causes a burden to both the

patients and family members[17,18]. In our study, the burden was found to be higher in the MFAC aged 0-2 years compared to the MHC in the same age group. Besides, the HADS and SCL 90-R general score were found to be significantly higher in these mothers.

Studies have shown that the families of children with food allergies experience social and emotional deterioration and that parents experience fear and anxiety. It was found that mothers also experience more stress and anxiety than fathers[19]. It has been shown that some parents also transfer their anxiety to the child and this situation was especially observed in the parents of children with previous anaphylaxis history[20].

Many parents report that they hesitate to try new foods for their children and healthy siblings and felt anxious after food

Table 1. The socio-demographic characteristics of the patient and control groups

		MFAC (n=67)	MHC (n=74)	p value
Age (month) (median, min-max)		9 (3.0-23.0)	8.0 (1.0-24.0)	0.076¥
Gender (n, %)	Boy	38 (56.0)	39 (50.0)	0.845#
	Girl	29 (44.0)	35 (53.0)	
Parents are not divorced (n, %)		67 (100)	72 (97.3)	0.950#
Number of siblings (median, min-max)		1.0 (0.0-4.0)	1.0 (0.0-4.0)	0.575¥
Household population (median, min-max)		4.0 (3.0-10.0)	4.0 (3.0-7.0)	0.242¥
Age of the mother (year) (median, min-max)		28.0 (18.0-49.0)	30.0 (19.0-39.0)	0.078¥
Education of the mothers (n, %)	Primary school	11 (16.4)	15 (57.7)	0.104#
	Junior high school	19 (28.3)	11 (36.7)	
	High school	16 (23.8)	14 (46.7)	
	University	17 (25.3)	23 (57.3)	
Mother's employment status (n, %)	Employer	13 (19.5)	32 (71.1)	**0.004#**
	Unemployed	54 (80.5)	42 (43.8)	
Father's employment status (n, %)	Employer	66 (98.5)	71 (95.9)	0.684#
	Unemployed	1 (1.5)	3 (0.04)	
Total family income (n, %)	0-1000 TL	16 (23.9)	16 (50.0)	0.058#
	1000-2000	33 (49.2)	26 (44.1)	
	>2000 TL	16 (23.9)	32 (66.7)	

#Chi-square test, TL: Turkish liras, ¥Mann-Whitney U test, min-max: Minimum-maximum, SD: Standard deviation, MFAC: Mothers of food-allergic children, MHC: Mothers of healthy children

Table 2. Comparison of the results of all scales applied in the mothers of food allergic children and controls

	MFAC (n=67)	MHC (n=74)	p value¥
Zarit Caregiver Burden Scale	22.0 (2.0-53.0)	18.0 (0.0-59.0)	**0.018**
SCL somatization	0.67 (0.0-3.33)	0.50 (0.0-3.42)	0.111
SCL obsession	0.80 (0.0-3.20)	0.50 (0.0-3.30)	0.113
SCL interpersonal sensitivity	0.56 (0.0-3.67)	0.33 (0.0-3.89)	0.131
SCL depression	0.54 (0.0-3.62)	0.54 (0.0-3.69)	0.846
SCL anxiety	0.35 (0.0-3.50)	0.20 (0.0-2.90)	0.104
SCL hostility	0.33 (0.0-3.83)	0.33 (0.0-3.17)	0.442
SCL phobia	0.14 (0.0-3.0)	0.00 (0.0-2.71)	0.155
SCL paranoid ideation	0.50 (0.0-3.83)	0.33 (0.0-3.17)	0.114
SCL psychoticism	0.10 (0.0-2.40)	0.10 (0.0-2.70)	0.465
SCL addition	0.71 (0.0-3.14)	0.43 (0.0-11.4)	0.065
SCL general	0.60 (0.03-3.10)	0.37 (0.0-2.84)	**0.045**
HAD anxiety	6.00 (0.0-19.0)	4.00 (0.0-16.0)	**0.045**
HAD depression	5.00 (0.0-20.0)	5.00 (0.0-15.0)	0.825

¥Mann-Whitney U test, HAD: Hospital Anxiety and Depression Scale, SCL: Psychological Symptom Screening List, MFAC: Mothers of food-allergic children, MHC: Mothers of healthy children

allergy was diagnosed[21,22]. Mothers stated that they were more anxious especially on the day of the oral food provocation test compared to the other days[23]. However, in cases where the diagnosis of food allergy is confirmed, parental anxiety has been shown to decrease as necessary avoidance measures are taken[24,25]. Prescription of an adrenaline autoinjector has been shown to reduce anxiety in some mothers of children with food allergy[26]. However, parental anxiety for whom an avoidance diet was recommended did not affect the increasing compliance with adrenaline auto-injector carrying and treatment[27]. Also, no anxiety can result in accidental encounters and risky behaviors during follow-up. Fedele et al.[28] evaluated food allergy coping behaviors with four different adaptation models. They found that anxiety and psychosocial influence was less in families that gave a balanced response to this process. It was observed that compliance with the treatment was less and psychosocial involvement was higher in anxious high responder families.

Most studies involve older children and adolescents with food allergy. There is a limited number of studies evaluating anxiety in the parents of the food allergic patients aged 0-2 years. Cortes et al.[29] evaluated the mothers of the children with an average age of 26 months with the HADS and they found the anxiety scores high in 42.6% of the mothers. In our study, 14.3% of mothers of children with food allergies had higher anxiety scores and 40.6% had higher depression scores.

In the studies, parental anxiety related to food allergy was evaluated with the quality of life questionnaire and psychosocial symptom screening questionnaires. The variability of the results was attributed to the child's age group, the timeline of the food allergy diagnosis, and the disease course, and it was thought that it should also be evaluated during the follow-up period[3].

Parental anxiety was generally evaluated in both mothers and fathers in the studies. Zarit Burden Scale was found to be higher in the mothers of patients with chronic lung disease than in their fathers[10]. In our study, only mothers were evaluated because the caregivers in the first two years of age were mainly the mothers of the children.

Approximately 60% of the parents of children with chronic illness reported that they were in good emotional and physical conditions[9]. In the studies evaluating the burden in the families of food-allergic children, Food Allergy Quality of Life -Parental Burden questionnaire was generally used[30,31]. This questionnaire has not been translated and validated into Turkish. Therefore, in our study, the presence of burden in mothers was evaluated with the Zarit Caregiver Burden Scale, which was validated for Turkish[15]. The Zarit Caregiver Burden Scale was applied to family members caring for children with chronic diseases such as cystic fibrosis and cerebral palsy, and the burden was found to be high in these disease groups[9,10]. In our study, burden in the mothers of

Table 3. The comparison of socio-demographic characteristics of the mothers with food allergic and healthy children whose Zarit Caregiver Burden Scale was found to be positive

		MFAC (n=42)	MHC (n=35)	p value
Age (month) (median, min-max)		8.5 (3.0-23.0)	9.0 (1.0-24.0)	0.87¥
Gender (n, %)	Boy	24 (57.1)	21 (60.0)	0.983¥
	Girl	18 (42.9)	14 (40.0)	
Number of siblings (median, min-max)		1.0 (0.0-4.0)	0.0 (0.0-4.0)	0.160¥
Household population (median, min-max)		4.0 (3.0-10.0)	3.0 (3.0-7.0)	**0.048¥**
Age of the mother (year) (median, min-max)		28.0 (18.0-37.0)	30.0 (20.0-38.0)	0.084¥
Education of the mothers (n, %)	Primary school	7 (17.9)	4 (11.4)	0.43#
	Junior high school	11 (28.2)	6 (17.1)	
	High school	9 (23.1)	6 (17.1)	
	University	10 (25.6)	15 (42.9)	
Mother's employment status (n, %)	Employer	9 (23.1)	17 (48.6)	**0.040#**
	Unemployed	30 (76.9)	18 (51.4)	
Father's employment status (n, %)	Employer	38 (97.4)	33 (94.3)	0.924#
	Unemployed	1 (2.6)	2 (5.7)	
Family income (n, %)	0-1000 TL[a]	12 (31.6)	3 (8.6)	0.024#
	1000-2000[b]	14 (36.8)	12 (34.3)	[a-b]: 0.09
	>2000 TL[c]	12 (31.6)	20 (57.1)	[a-c]: 0.007
				[b-c]: 0.21

TL: Turkish liras, #chi-square test, ¥Mann-Whitney U test, min-max: Minimum-maximum, MFAC: Mothers of food-allergic children, MHC: Mothers of healthy children

children with food allergy was found to be significantly higher than in the MHC.

In our study, the rate of unemployment in the mothers of children with food allergy was found to be significantly higher than in the MHC. Also, the rate of unemployment was found to be higher in mothers with a high score of Zarit Caregiver Burden Scale. Therefore, the unemployment was thought to be a factor that would increase the caregiver burden of the mother.

Study Limitations

The course of food allergy, the culprit allergen, the age group, the type of food allergy can affect the anxiety and burden in the family. The limitation of our study is that the mothers were evaluated in terms of anxiety and burden after a cross-sectional diagnosis of food allergy and no comparison was made in terms of these sub-parameters. Its strength is that it is the first study in our country evaluating the burden in the MFAC aged in the first two years.

CONCLUSION

In conclusion, the management of food allergy usually includes strict avoidance, patient education and provision of emergency medication (adrenaline-autoinjectors). However, in our clinical practice, the evaluation of mothers' emotional status such as coping with a food allergy, burden, and anxiety-depression can be neglected. For this reason, the psychosocial support needs of mothers of children with a food allergy, especially in the young age group, should be evaluated and obtained when necessary because psychological, emotional, and social well-being of the caregivers increases the quality of care the child receives.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of University of Health Sciences Turkey, Okmeydanı Training and Research Hospital (protocol no: 740, date: 24.10.2017).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y., S.E., N.P.Y., D.Ö., Concept: E.Y., S.E., N.P.Y., Design: E.Y., N.P.Y., Data Collection or Processing: E.Y., D.Ö., S.E., N.P.Y., Ö.T., Analysis or Interpretation: Ö.T., E.Y., D.Ö., Literature Search: Ö.T., E.Y., D.Ö., S.E., Writing: D.Ö., Ö.T., E.Y., N.P.Y.

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The Expression Profiles of Angio-miRs in Glioblastomas Invasion Inhibited by Ruxolitinib

Ruksolitinib ile Engellenen Glioblastoma İnvazyonunda AnjiyomiR'lerin Ekspresyon Profili

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ABSTRACT

Aim: MicroRNAs (miR) have an essential role on the regulated gene expression in the human genome. In recent years, a specific miR group was called to angio-miRs due to their role in the angiogenesis, and recent study showed that they involved in the pathogenesis of gliomas. In this study, we investigated the changes in the expression profiles of angio-miRs in glioblastoma cells and identified relationship between these genes and invasion and tumor growth.

Materials and Methods: In this study, glioblastoma tumor spheroids were obtained using the human glioblastoma cell line U-87 MG. 50 nM, 100 nM and 200 nM ruxolitinib were applied to tumor spheroids for 48 hours by using Matrigell method. Tumor volume and invasion formation relative % tumor growth and relative % invasion area were measured in glioblastoma tumor spheroids after 48 hours of treatment. At the same time, quantitative real-time polymerase chain reaction (qRT-PZR) analysis was performed and miR expression profiles were determined. The most important (importance features) miRNAs selected along with the heatmap and volcano plot analyzes were used to display the pattern of the differentially expressed miRs using normalized miR expression profiles.

Results: When the effect of 50 nM, 100 nM and 200 nM ruxolitinib administration to tumor spheroids on tumor volume and invasion was evaluated, a significant difference was found at each dose applied. However, at the dose of 200 nM ruxolitinib, it was observed that the inhibitory effect of tumor invasion was the highest. When miR expression profiles obtained by qRT-PZR test with 200 nM ruxolitinib adminisration were evaluated, it was determined that the expression profiles of 10 miRs increased and the expression profiles of 4 miRs decreased.

Conclusion: In conclusion, angio-miR expression profiles are important because they enable us to better understand the prognostic process of gliomas. Because of their multiple silencing properties, they may contribute to the clinic with further studies in terms of their use as new therapeutic targets and prognostic biomarkers for glioblastoma.

Keywords: miR, glioblastoma, angiogenesis, invasion

ÖZ

Amaç: MikroRNA'lar (miR), insan genomunda gen ifadesinin düzenlenmesinde önemli rolü olan düzenleyicilerdir. Son yıllarda anjiyogenezde rol oynayan spesifik bir miR grubu tanımlanmış (anjiyo-miR) ve bazı anjiyo-miR'lerin gliomalarda etkin rol oynadıkları ortaya konmuştur. Bu çalışmada, glioblastoma hücrelerindeki anjiyo-miR'lerin ifade değerlerindeki değişiklikleri ve bu değişikliklerin invazyon ve tümör büyümesi ile ilişkisini araştırdık.

Gereç ve Yöntem: Bu çalışmada, insan glioblastoma hücre hattı U-87 MG kullanılarak glioblastoma tümör sferoidleri elde edildi. Matrigel yöntemi ile tümör sferoidlerine 48 saat süresince 50 nM, 100 nM and 200 nM ruksolitinib uygulandı. Kırk sekiz saat tedaviden sonra glioblastoma tümör sferoidlerinde tümör hacmi ve invazyon oluşumu relatif yüzde tümör gelişimi ve relatif yüzde invazyon alanı ölçüldü. Aynı zamanda, niceliksel gerçek zamanlı polimeraz zincir reaksiyonu (qRT-PZR) analizi yapıldı ve miR ifade değerleri belirlendi. Farklı şekillerde ifade edilen miRNA'ların modelini görüntülemek için normalize edilen miRNA ifade değerleri kullanılarak heatmap ve volcano plot analizleri ile seçilen en önemli (importance features) miRNA'lar gösterildi.

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Bulgular: Tümör sferoidlerine 50 nM, 100 nM ve 200 nM ruksolitinib uygulamasının tümör hacmi ve invazyon üzerine etkisi değerlendirildiğinde, uygulanan her dozda anlamlı fark saptandı. Ancak 200 nM ruksolitinib dozunda tümör yayılımını engelleyici etkisinin en yüksek olduğu gözlendi. 200 nM ruksolitinib uygulaması ile qRT-PZR testi ile elde edilen miR ifade değerleri incelendiğinde 10 miR'nin ifade değerlerinin arttığı, 4 tanesinin ifade değerlerinin ise azaldığı belirlendi.

Sonuç: Sonuç olarak anjiyo-miR ifade değerleri gliomaların prognostik sürecini daha iyi anlamamızı sağlayabilmeleri açısından önemlidirler. Çoklu susturma özellikleri sayesinde glioblastomalar için yeni terapötik hedefler ve prognostik biyobelirteçler olarak kullanılabilmesi açısından ileri çalışmalarla kliniğe katkı sağlayabilirler.

Anahtar Kelimeler: miR, glioblastoma, anjiyogenez, invazyon

INTRODUCTION

MicroRNAs (miR) are small, single-stranded RNA sequences of 21-25 nucleotides found in the human body. These non-protein-coding small RNA sequences play a role in physiological and pathological processes by inhibiting gene expression in the post-transcriptional modification step of protein synthesis[1]. These pathological processes include many types of cancer, including glioblastomas[2]. The close relationship of miRs with these pathologies has led to their being proposed as new therapeutic agents[3]. Recent studies reveal that these small RNAs can be used not only in the treatment phase, but also as a biomarker with varying expression values[4]. As a result, in recent years, miRs have become one of the most frequently studied subjects in laboratory and clinical studies, especially for incurable cancer types[1-4].

Glioblastomas are among the brain cancers originating from glial cells, growing fast and still fatal with its aggressive nature despite the developing technological opportunities. Although more than 100 subtypes have been defined with their heterogeneous molecular features, the common feature of all of them is high angiogenetic and invasion capacity[5,6]. Glioblastoma cells invade the surrounding healthy brain tissue and spread by sequential angiogenesis. This aggressive spread is thought to be the main difficulty in the treatment of glioblastomas[7]. From this point of view, targeting cytokines and associated signaling pathways involved in this high angiogenetic process seems to be a rational way to treat the disease. However, phase-2 and phase-3 studies of these treatment interventions have been conducted, and although some positive results have been obtained, a treatment protocol that has been included in routine treatment has not been determined yet[8]. For this, a better understanding of the complex molecular mechanism underlying the high angiogenic and invasive characteristics of gliomas is needed.

In recent years, many miRs, called angio-miRs, closely related to tumor angiogenesis have been identified in the field of cancer biology. Among them, angio-miRs such as miR_7, miR_296, miR_15b and miR_93 have been reported to play a role in glioblastomas[9]. In our laboratory, it was previously reported that ruxolitinib effectively inhibited the invasion of gliomas[10]. In another study, a close relationship of this effect with miR_17 and miR_20a was demonstrated[11]. In this study, the relationship between inhibited invasion characteristics and expression values of 34 angio-miRs in glioblastoma three-dimensional tumor clusters was investigated, again using ruxolitinib.

MATERIALS AND METHODS

Supply of Ruxolitinib and Preparation of Cell Line

Ruxolitinib (CAS 941678-49-5) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and human glioblastoma cell line U-87 MG (ATCC® HTB-14™) from American Type Culture Collection; (Manassas, VA, USA). During the study, cells were grown by using Eagle's Minimum Essential Medium (EMEM; catalog no. 30-2003) which was supplemented with 10% fetal bovine serum (Gibco Life Technologies, Grand Island, NY, USA), 1 mM glutamine, and 1% (final concentration) penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) and kept in a humidified incubator at 37 °C and 5% CO_2 throughout the entire study.

Construction of Glioblastoma Tumor Clusters and Matrigel Invasion Assay Protocol

Tumor clusters were created using the hanging drop method, with minor modifications[10,11]. Single-cell suspensions were obtained from trypsinized monolayer cell cultures and diluted to the desired cell density using complete EMEM medium with the addition of 0.5% methyl cellulose. 20 µL of cell suspension was pipetted to the inner surface of the top cover of non-adhesive, sterile polystyrene Petri dishes as 40 drops (final concentration of 750-1.000 cells per drop). The upper lids of the Petri dishes with the tumor droplets were inverted and 2 mL of phosphate-buffered saline was placed in the lower dish. After the cells were incubated for 72 hours, tumor cluster formation was observed using the ZEISS Axio Vert.A1 (Oberkochen, Germany) invert microscope. All tumor spheres were collected in 15 mL Falcon tubes and fresh tumor spheres of the same age were used in all experiments.

Twenty-four well plates were used for the Matrigel invasion assay. The plates were covered with an extracellular matrix and the excess of this matrix was removed to form a thin layer. Then, 40 µL of tumor clusters collected from the

previous section were taken and mixed with 100 µL of matrigel matrix (Corning, Corning, NY, USA) and 100 µL of collagen type I (Sigma Aldrich) in pre-chilled Eppendorf tubes. 40 µL (3-5 tumor clusters) of this mixture was put in each well. Afterwards, the plate was left in the incubator for 24 hours, then 1 mL of cell culture medium was added to each well. After 24 hours, tumor clusters were treated with vehicle or 50, 100, or 200 nM ruxolitinib for 48 hours. Five replicates were made for each process. After 48 hours of treatment, cell invasion was recorded for 48 hours using an inverted phase contrast light microscope at 20X magnification (ZEISS Axio Vert.A1) equipped with a digital camera. Tumor volume and tumor invasion were calculated according to our previous studies[10,11].

miR Isolation and Quantitative Real Time-polymerase Chain Reaction (RT-qPCR) Analyses

A group of miRNAs called angio-miRNAs (angio-miRs) is well defined in terms of target genes and expression profile and has been shown to play an important role in glioblastoma multiforme (GBM) pathogenesis. Whole genome-wide microarray studies have revealed that more than 50 miRs are involved in hypoxia-related pro- or anti-angiogenesis signaling[12-15]. In our study, 34 angio-miRs determined in this group were used. Isolation of miRs from tumor spheres 48 hours after the administration of ruxolitinib was performed using the mirVana™ miR Isolation Kit with phenol according to the kit protocol. cDNAs were synthesized using a TaqMan™ Advanced miR cDNA Synthesis Kit according to kit protocols. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed on a Quant studio 5 real time PCR (life technologies) using FastStart TaqMan® Probe Master (life technologies). The TaqMan probes and miR sequences used are given in Table 1. miR expression values were calculated by the $2^{-\Delta\Delta Ct}$ method, and RNU6B (Assay ID: 001093) was used as correction factor and endogenous control[16].

Statistical Analysis

The differences in the invasion rate and tumor volume between the control and experimental groups and the relative fold change in miR expression were compared with one-way analysis of variance and Tukey HSD test. Pearson correlation coefficient method was used for correlation analysis. Statistical analyses were performed using Statistical Package for the Social Sciences 20 software with a significance level of $p<0.05$. Heat map analysis, unsupervised hierarchical clustering analysis, volkano plot and feature importance analysis were performed using MetaboAnalyst 4.0 software to identify potential biomarkers/important miRs in three-dimensional tumor clusters with and without ruxolitinib.

RESULTS

In the study, 50, 100 and 200 nM doses of ruxolitinib were applied to glioblastoma tumor clusters and their effects were evaluated after 48 hours. Although a statistically significant effect was observed at each applied dose, it was observed that the effect reached the highest level at the 200 nM dose and this effect was consistent with our previous studies (Figure 1)[10,11]. Therefore, the relationship between miR expression values obtained by qRT-PCR test and this effect dose was analyzed and it was determined that the expressions of 10 miRs increased significantly, and 4 of them decreased significantly (Table 2). The heatmap showed that 200 nM ruxolitinib administration had the highest effect on miR expressions compared to the control group (Figure 2). In the next step, in order to determine the miRs directly related to ruxolitinib, first volcano plot and then OPLS-discriminant analysis were performed and the most important features were determined here. Nine miRs, which were found to be significantly correlated with ruxolitinib applications, which gave similar statistically significant results in both analyses, are summarized in Table 3. Correlation analyses of these identified miRs were then performed with

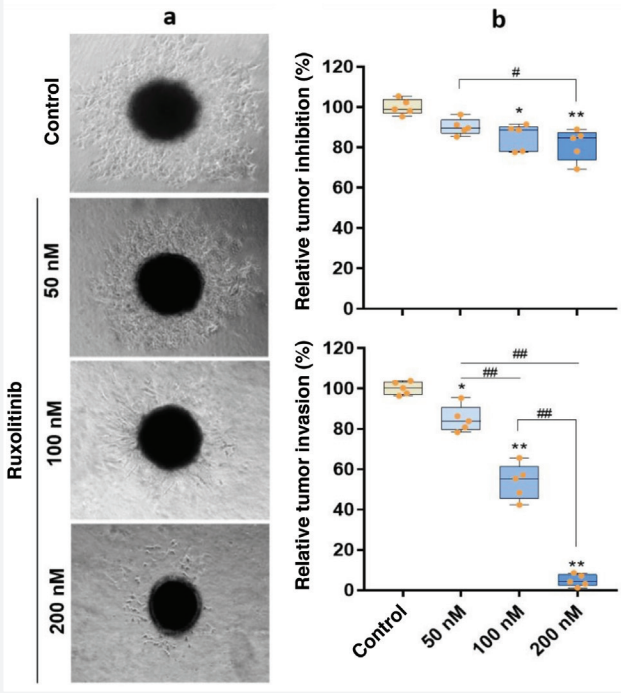

Figure 1. A) GBM tumor spheroids and invasion formation determined by Matrigel method, b) Relative % tumor growth and relative % invasion area in GBM tumor spheres treated with 50 nM, 100 nM and 200 nM ruxolitinib or vehicle for 48 hours. Bars mean±SE, n=5, * statistically different compared to control, #statistically different from other groups, one-way ANOVA, Tukey HSD test, $p\leq0.05$;**, ##$p\leq0.01$

GBM: Glioblastoma multiforme

tumor volumes and invasion rates, and as a result, 5 miRs [miR_15b(r:-0,659/-0,861]; miR_19a_3p(r:-0,713/-0,455); miR_31_3p(r:-0,461/-0,533); miR_155_3p(r:-0,572/-0,625); miR_200b_5p(r:-0,673/-0,957) among 9 miRs, which showed a statistically significant correlation with both tumor invasion and tumor growth, were detected (Figure 3).

DISCUSSION

In recent years, with the developments in the field of molecular biology, large volumes of data have begun to be examined, thus it has been demonstrated that miRs are closely related to tumor invasion, and various cancer-specific miRs have been defined[17-19]. It is suggested that inhibition of miRs specific to this cancer type is a new target that can be used in the treatment of cancer[1]. In the present study, miRs associated with U87 glioblastoma invasion, which we blocked using ruxolitinib that we previously used in our laboratory, were determined. In this context, it was determined that there was an increase in 10 miR expression values and a decrease in 4 miR expression values in GBM tumor spheres treated with ruxolitinib for 48 hours. It was determined that miR_15b, miR_18a_5p, miR_19a_3p, miR_21_5p, miR_27a_5p, miR_31_3p, miR_132_5p, miR_155_3p and miR_200b_5p had a statistically significant effect on the invasion and tumorigenesis of U87 GBM spheres.

miR_15b is a miR localized in the 3rd chromosome of the human genome and has been frequently studied in cancer studies

Table 1. Names of mikro-RNAs, Taq-Man probe ID and mature miRNA sequences

micro-RNA	Mature micro-RNA sequence	Taq-Man probe ID
hsa-miR-15b	UAGCAGCACAUCAUGGUUUACA	478313_mir
hsa-miR-18a-3p	ACUGCCCUAAGUGCUCCUUCUGG	477944_mir
hsa-miR-18a-5p	UAAGGUGCAUCUAGUGCAGAUAG	478551_mir
hsa-miR-19a-3p	UGUGCAAAUCUAUGCAAAACUGA	479228_mir
hsa-miR-19a-5p	AGUUUUGCAUAGUUGCACUACA	478750_mir
hsa-miR-21-3p	CAACACCAGUCGAUGGGCUGU	477973_mir
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	477975_mir
hsa-miR-23a-3p	AUCACAUUGCCAGGGAUUUCC	478532_mir
hsa-miR-31-3p	UGCUAUGCCAACAUAUUGCCAU	478012_mir
hsa-miR-31-5p	AGGCAAGAUGCUGGCAUAGCU	478015_mir
hsa-miR-92a-3p	UAUUGCACUUGUCCCGGCCUGU	477827_mir
hsa-miR-92a-5p	AGGUUGGGAUCGGUUGCAAUGCU	479205_mir
hsa-miR-101-3p	UACAGUACUGUGAUAACUGAA	477863_mir
hsa-miR-101-5p	CAGUUAUCACAGUGCUGAUGCU	478620_mir
hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG	477887_mir
hsa-miR-130a-3p	CAGUGCAAUGUUAAAAGGGCAU	477851_mir
hsa-miR-130a-5p	GCUCUUUUCACAUUGUGCUACU	483130_mir
hsa-miR-132-3p	UAACAGUCUACAGCCAUGGUCG	477900_mir
hsa-miR-132-5p	ACCGUGGCUUUCGAUUGUUACU	478705_mir
hsa-miR-155-3p	CUCCUACAUAUUAGCAUUAACA	477926_mir
hsa-miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGUU	483064_mir
hsa-miR-191-3p	GCUGCGCUUGGAUUUCGUCCCC	477951_mir
hsa-miR-191-5p	CAACGGAAUCCCAAAAGCAGCUG	477952_mir
hsa-miR-200b-3p	UAAUACUGCCUGGUAAUGAUGA	477963_mir
hsa-miR-200b-5p	CAUCUUACUGGGCAGCAUUGGA	478753_mir
hsa-miR-210-3p	CUGUGCGUGUGACAGCGGCUGA	477970_mir
hsa-miR-210-5p	AGCCCCUGCCCACCGCACACUG	478765_mir
hsa-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC	477981_mir
hsa-miR-221-5p	ACCUGGCAUACAAUGUAGAUUU	478778_mir
hsa-miR-296-3p	GAGGGUUGGGUGGAGGCUCUCC	478790_mir
hsa-miR-296-5p	AGGGCCCCCCCUCAAUCCUGU	477836_mir
hsa-miR-424-5p	CAGCAGCAAUUCAUGUUUUGAA	478092_mir

miRNA: MicroRNA

in recent years. Xia et al.[20] suggested that miR_15b played a role as a tumor inhibitory agent in cell cycle regulation in glioblastomas. In another study, increased miR_15b expression was reported in analyses performed in serum and cerebrospinal fluid of patients[21,22]. Finally, Chen et al.[23] reported that miR_15b showed its effect on gliomagenesis through sal-like protein 4. In our study, increased expression values of miR_15b were detected and it was thought to play an important role in the molecular process.

The miR_17-92 family is a polycistronic miR family that is involved in the coding of more than one protein and has

Table 2. Relative miR expression levels determined in GBM tumor spheres in the groups administered 200 nM ruxolitinib, compared to the control groups without treatment, and the results of the related statistical analysis

				95% CI	
miRNA	**Mean value**	**Standard deviation**	**p value**	**Decrease**	**Increase**
MiRs with significantly increased expression					
miR_15b	12.567	2.988	0.041	-22.321	-0.520
miR_18a_5p	154.640	18.073	0.000	-218.814	-87.006
miR_19a_3p	44.298	4.543	0.000	-59.818	-26.688
miR_21_3p	2.559	0.389	0.047	-2.900	-0.019
miR_27a_5p	6.543	1.927	0.120	-12.505	1.553
miR_31_3p	226.034	46.217	0.012	-391.855	-54.810
miR_130a_5p	4.144	0.802	0.046	-5.920	-0.064
miR_132_3p	1.824	0.135	0.002	-1.316	-0.325
miR_155_3p	2.657	0.418	0.038	-3.204	-0.101
miR_200b_5p	33.275	5.785	0.005	-52.840	-10.623
MiRs with significantly decreased expression					
miR_21_5p	0.370	0.093	0.002	0.308	1.259
miR_210_3p	0.546	0.103	0.019	0.097	0.974
miR_221_3p	0.619	0.060	0.003	0.159	0.673
miR_424_5p	0.537	0.129	0.036	0.053	1.393
miRs without significant change					
miR_18a_3p	1.508	0.351	0.984	-1.501	1.472
miR_19a_5p	3.132	0.934	0.232	-5.443	1.390
miR_23a_3p	0.627	0.125	0.074	-0.049	0.996
miR_27a_3p	0.667	0.114	0.109	-0.081	0.757
miR_31_5p	1.038	0.087	0.932	-0.346	0.376
miR_92a_5p	1.114	0.373	0.885	-1.458	1.266
miR_92a_3p	0.906	0.108	0.614	-0.297	0.491
miR_101_3p	2.072	1.446	0.683	-6.324	4.221
miR_101_5p	1.403	0.226	0.347	-1.215	0.445
miR_130a_3p	6.394	1.568	0.064	-11.106	0.331
miR_126_3p	0.881	0.081	0.335	-0.174	0.490
miR_132_5p	13.145	7.780	0.384	-40.506	16.229
miR_155_5p	3.413	1.562	0.663	-7.154	4.641
miR_191_3p	1.190	0.603	0.861	-2.389	2.012
miR_191_5p	0.873	0.074	0.229	-0.146	0.580
miR_200b_3p	2.555	0.799	0.295	-4.428	1.408
miR_210_5p	0.728	0.113	0.159	-0.128	0.735
miR_221_5p	2.629	1.243	0.823	-5.289	4.247
miR_296_3p	1.054	0.218	0.958	-0.802	0.845
miR_296_5p	1.843	0.281	0.278	-1.717	0.518

CI: Confidence interval, miRNA: MicroRNA

6 members[24]. In our study, we detected an increase in the expression values of miR_18a and miR_19a in this family (Table 2). It has been reported that the expression of mir_18a is increased in human prostate, breast and colorectal cancers, especially with its close relationship with apoptosis[19,25]. They reported a correlation between the increased tumor grade of

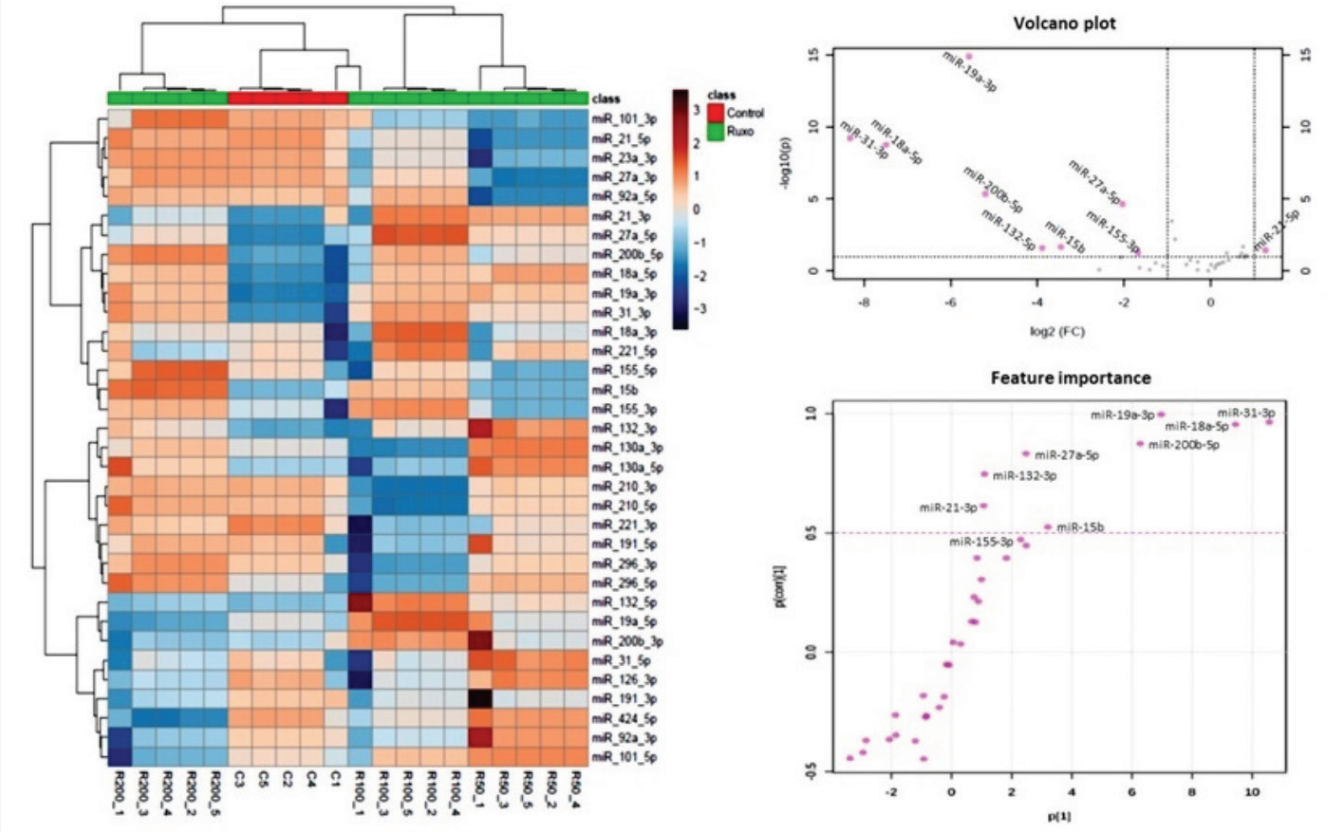

Figure 2. a) Heatmap analysis using normalized miR expression values (distance measurement parameter: euclidian, cluster algorithm: ward), b) The most important miRNAs selected with fold change threshold (x) 2 in Volcano plot analysis and t-test significance level threshold (y) 0.1. The red dashed line shows miRs significantly above the threshold (both fold increase and p values were undergone logarithmic transformation), c) S-graph combining covariance correlation [p (corr)] loading profile, showing variable significance obtained by OPLS-Discriminant analysis. All biomarker analyses were performed between 200 nM ruxolitinib-treated and untreated control groups

miRNA: MicroRNA

Table 3. The most important features of miRs determined by Volcano plot and Discriminant Analysis of Orthogonal-Orthogonal Projections to Latent Structures

	Volcano plot				Important feature	
miRNA	**FC**	**log2(FC)**	**p value**	**-log10(p)**	**p[1]**	**p(corr)[1]**
miR_15b	0.0910	-34.5750	0.0216	16.6640	320.8360	0.5251
miR_18a_5p	0.0056	-74.8870	1.74E-05	87.5930	944.6110	0.9546
miR_19a_3p	0.0209	-55.7790	1.31E-11	14.8820	69.7660	0.9965
miR_21_5p	23.9670	12.6110	0.0372	14.2940	106.7570	0.6144
miR_27a_5p	0.2433	-20.3910	2.27E-02	46.4320	247.7050	0.8329
miR_31_3p	0.0031	-83.1770	5.95E-06	92.2570	105.6860	0.9647
miR_132_5p	0.0674	-38.9060	0.0253	15.9630	109.7980	0.7473
miR_155_3p	0.3118	-16.8130	0.0528	1.2770	230.0530	0.4726
miR_200b_5p	0.0272	-51.9990	4.46E-03	53.5050	628.0550	0.8749

FC: Fold change, log: Logarithm, corr: Correlation, p: Significance level

glioblastomas and increased miR_18a expression values[26,27]. On the other hand, Jiang et al.[28] targeted miR_18a as responsible for the low expression values of the receptor-related orphan receptor A (RORA) protein, which they associated with a good prognosis, in high-grade glioblastomas. It has been suggested that the other member of the family, miR_19a, is expressed in many types of cancer and can be used as a biomarker of cancer progression[29]. Phosphatase and tensin homolog (PTEN) is a well-known tumor suppressor gene and is the target protein of miR_19a. It has been reported that with the inhibition of miR_19a, the activation of PTEN is increased and the invasion of glioma cells is prevented in this way[30]. In the study of Malzkorn et al.[31], they reported that there was an increase in miR_19a expression values in tissues obtained from people with a diagnosis of glioblastoma, and that miR_19a inhibition prevented cell proliferation of glioblastoma cells. In the present study, a statistically significant increase was detected in the expression values of miR_19a (Figure 3). These results suggest that the effect of ruxolitinib on tumor invasion may be related to miR_19a rather than miR_18a.

miR_31 is a miR known for its anti-cancer properties for many types of cancer, but also reported to inhibit metastasis in breast cancer[32,33]. Its oncogenic properties have been reported in lung and colon cancers[34,35]. Its decreased expression has been reported in glioblastomas compared to normal brain tissue[36]. The target protein of miR_31 is radixin[30]. Radixin is a member of the ezrin/radixin/moesin protein family, which is responsible for binding between the cell membrane and the cytoskeleton[37]. Wang et al.[38] reported that decreased miR_31a and high radixin expression were associated with poor Karnofsky performance and poor survival in patients with glioblastoma. In another study, it was reported that miR_31 inhibits nuclear factor-kappa B signaling, which is known to be highly effective in glioblastomas[39]. In our study, increased expression of miR_31a was detected with ruxolitinib administration (Table 2). This increased expression was also found to correlate with inhibition of invasion (Figure 3). Ruxolitinib is a janus kinase (JAK) inhibitor. It is thought that the target protein of miR_31, radixin, is also included in the structure of JAK[40]. These data suggest that there is a molecular link between JAK/STAT signaling and miR_31 in the invasion inhibitory effect of ruxolitinib, but this needs to be confirmed by further studies.

miR_155 is a well-known oncogenic miR with 147 target genes identified in the literature. The presence of many target genes is met with increasing interest in their clinical significance[41]. Elevated expression values have been reported in many types of cancer, including glioblastoma, lung cancer, breast cancer, Burkitt's lymphoma, and leukemia[41-43]. In the study by D'Urso et al.[44], they found an increase in the expression of miR_155 in both primary and secondary glioblastoma patients and suggested that it showed its oncogenic effect in glioblastomas through the target gene of miR_155, γ-*aminobutyric acid A receptor 1* (GABRA 1)[44]. GABRA 1 is the receptor for gamma acetyl amunobutyric acid (GABA), whose role in brain functions is well known. GABA is one of the main inhibitory neurotransmitters in the human brain. With its increased expression, it is known to increase tumor cell proliferation in glioblastomas[45]. In the present study, increased miR_155 expression was detected in U87

a

Variable		Tumour volume	Invasion rate
1. Tumour volume	Pearson's r	—	
	p-value	—	
2. Invasion rate	Pearson's r	0.707***	—
	p-value	< .001	—
3. miR_15b	Pearson's r	−0.659**	−0.861***
	p-value	0.002	< .001
4. miR_18a_5p	Pearson's r	−0.543*	−0.357
	p-value	0.013	0.123
5. miR_19a_3p	Pearson's r	−0.713***	−0.455*
	p-value	< .001	0.044
6. miR_21_5p	Pearson's r	0.138	−0.079
	p-value	0.561	0.741
7. miR_27a_5p	Pearson's r	−0.413	−0.136
	p-value	0.070	0.567
8. miR_31_3p	Pearson's r	−0.461*	−0.533*
	p-value	0.041	0.015
9. miR_132_5p	Pearson's r	−0.144	−0.058
	p-value	0.544	0.807
10. miR_155_3p	Pearson's r	−0.572**	−0.625**
	p-value	0.008	0.003
11. miR_200b_5p	Pearson's r	−0.673**	−0.957***
	p-value	0.001	< .001

* p < .05, ** p < .01, *** p < .001

b

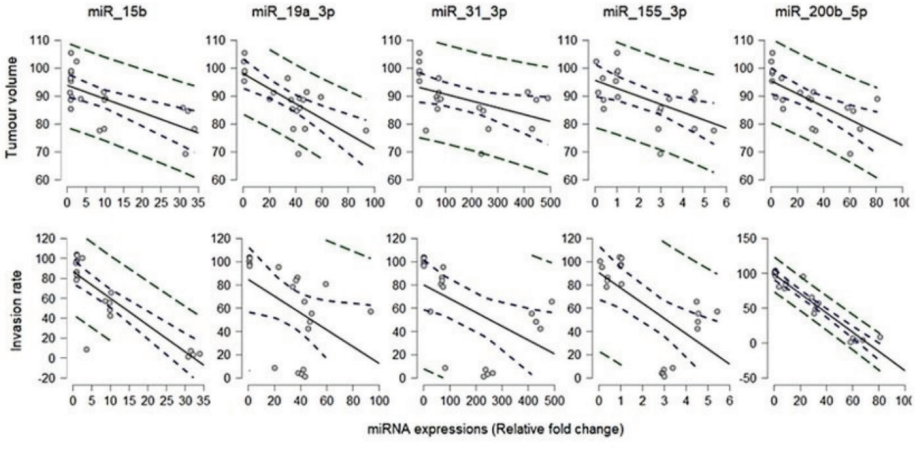

Figure 3. Tumor size, invasion rate, and the most significant angio-miR expression relationship a) Correlation matrix generated according to the Pearson correlation coefficient b) Scattered points, 95% confidence interval (blue line) and 95% predictive interval (green line)

invasion, which was strongly inhibited by ruxolitinib. This result suggests that ruxolitinib does not exert its inhibitory invasion effect on miR_155, which is known for its oncogenic effect.

miR_200b is a member of the miR_200 family and has been reported to be involved in many types of cancer, including glioblastomas[46]. It has been reported to inhibit tumor growth in malignant glioblastoma cell lines and human tissues with low expression values in cell lines[47]. It has been reported that it exerts its inhibitory effect on glioblastomas through its target gene, element-binding protein 1 (*CREB1*)[47]. Liu et al.[48] associated decreased expression of miR_200b with poor prognosis, and they suggested that this effect occurred on another target gene of miR_200b, *RAB* gene family[48]. Chang et al.[49] have reported that increased expression of RAB3C, a member of the RAN family, is associated with high grade and poor prognosis in colorectal cancers, and that the expression of this gene decreases with ruxolitinib administration and prevents cancer cell movement. In our study, a strong correlation was found between U87 cell growth and invasion, which was significantly inhibited by ruxolitinib, in miR_200b expression. Considering the close relationship of ruxolitinib with the RAB family, which is the target gene of this miR, we think that miR_200b may be closely related to the effect of ruxolitinib.

Study Limitations

Although the presented study reveals results that we think are important, it has some limitations. The most important limitation is that the study is an *in vitro* study. In addition, the validation of the obtained data through human tumor tissues can make the results even more reliable. Similarly, investigation of changes in protein expression along with changes in gene expression may contribute to the elucidation of the molecular mechanism more. In addition, we think that our data reflect some important potential clinical scenarios for ruxolitinib-angio-miR association in GBM patients.

CONCLUSION

We demonstrated that GBM growth and invasion modeled in tumor spheroids was significantly inhibited specifically by 200 nM ruxolitinib treatment. We also identified a strong interaction between ruxolitinib and angio-miRs in the ruxolitinib-treated groups. Our results revealed that miR_15b, miR_19a, miR_31_3p, miR_155_3p and miR_200b among 34 angio-miRs that we investigated were statistically significantly changed by ruxolitinib treatment, and all of them were associated with tumor growth and invasion. Our results suggest that ruxolitinib is an effective anti-tumor therapeutic in glioblastoma tumor spheroids, possibly by altering the expression profile of angio-miRs and thereby inhibiting angiogenesis-related signals. However, it is thought that more studies are needed to validate the data of our study and make it clinically usable.

Ethics

Ethics Committee Approval: Commercial cells were used in our study. Ethics committee approval is not required for such studies.

Informed Consent: There is no need.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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ORIGINAL ARTICLE

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Combining Histone ELISA and Quantitative PCR Assays to Extract and Quantify Histone Methylation-related Circulating DNA

Histon ELISA ve Nicel PCR Testlerinin Birleştirilerek Histon Metilasyonu ile İlişkili Dolaşımdaki DNA'nın İzolasyonu ve Ölçülmesi

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ABSTRACT

Aim: Liquid biopsy-based measurement of post-translational histone modifications (PTHMs) in bodily fluids of patients with cancer represents a novel area of biomarker research. Here, we tested the applicability of an approach combining enzyme-linked immunosorbent assays (ELISA)-like measurement of histone methylation and quantitative polymerase chain reaction to extract and quantify pericentric histone 4 lysine 20 trimethylation (H4K20me3)-related circulating DNA.

Materials and Methods: After adding plasma into ELISA wells and letting H4K20me3-related nucleosomes bind to the antibody, nucleosomal DNA was detached from immune complexes using a buffer including $NaHCO_3$, SDS, and proteinase K and purified. A single copy- and a multiple copy-sequence from pericentric regions of chromosomes 16 and 1 were amplified to quantify H4K20me3-related DNA from patients with colon cancer or colonic polyps.

Results: Relative plasma level of H4K20me3-related nucleosomal DNA was lower in the patients with colon cancer than in controls for both target sequences, with a statistically significant difference at the chr16-specific target (relative values were 0.037 vs. 0.073, respectively; p=0.008).

Conclusion: That H4K20me3-related nucleosomal DNA is present in decreased levels in colon cancer confirms our previous findings attained using other techniques. In conclusion, our findings indicate the applicability of direct extraction of protein-bound DNA from ELISA immune complexes and it could provide a surrogate means in the assessment of PTHMs or other protein-DNA interactions.

Keywords: Colon cancer, histone methylation, circulating nucleosomal DNA, ELISA

ÖZ

Amaç: Kanserli hastaların vücut sıvılarında post-translasyonel histon modifikasyonlarının (PTHM) likit biyopsi bazlı ölçümü, biyobelirteç araştırmalarının yeni bir alanını temsil etmektedir. Bu çalışmada, perisentrik histone 4 lizin 20 trimetilasyonu (H4K20me3) ile ilgili dolaşımdaki DNA'yı çıkarmak ve ölçmek için enzime bağlı immünosorbent testini (ELISA) ve nicel polimeraz zincir reaksiyonu birleştiren bir yaklaşımın uygulanabilirliğini test ettik.

Gereç ve Yöntem: ELISA kuyularına plazma eklendikten ve H4K20me3 ile ilgili nükleozomların antikora bağlanmasına izin verildikten sonra, $NaHCO_3$, SDS ve proteinaz K içeren bir tampon kullanılarak nükleozomal DNA bağışıklık komplekslerinden ayrıldı ve saflaştırıldı. Kolon kanseri ve kolon polipleri olan hastalardan H4K20me3 ile ilgili DNA'nın miktarını ölçmek için kromozom 16 ve 1'in perisentrik bölgelerinden tek kopya ve çoklu kopya dizisi çoğaltıldı.

Bulgular: H4K20me3 ile ilişkili nükleozomal DNA'nın göreceli plazma seviyesi, kolon kanserli hastalarda kontrollere göre daha düşük görüldü ve chr16'ya özgü hedefte istatistiksel olarak anlamlı bir fark saptandı (göreceli değerler sırasıyla 0,037'ye karşı 0,073 idi; p=0,008). H4K20me3 ile ilişkili nükleozomal DNA'nın kolon kanserinde düşük seviyelerde bulunması, diğer teknikler kullanılarak elde ettiğimiz önceki bulgularımızı doğrulamaktadır.

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Sonuç: Sonuç olarak, bulgularımız ELISA bağışıklık komplekslerinden proteine bağlı DNA'nın doğrudan çıkarılmasının uygulanabilirliğini göstermektedir ve PTHM'lerin veya diğer protein-DNA etkileşimlerinin değerlendirilmesinde farklı bir yöntem sağlayabilir.

Anahtar Kelimeler: Kolon kanseri, histon metilasyonu, dolaşımdaki nükleozomal DNA, ELISA

INTRODUCTION

In eukaryotic cells, nucleosomes are basic functional units of DNA packaging in chromatin. Each nucleosome is composed of an octamer of four core histones (H3, H4, H2A, and H2B), with wrapped DNA around them[1]. The genetic information stored in the context of chromatin is subjected to two main types of epigenetic modifications, i.e. DNA methylation and various histone modifications. Post-translational modifications of N-terminal tails of histone proteins protruding from the nucleosomes affect the chromatin structure, thus their dysregulation impacts gene expression and represents a common mechanism across human cancers contributing to oncogenesis through the induction of epigenetic, transcriptomic, and phenotypic alterations[2].

Histone methylation, which is catalyzed by histone methyltransferases on three levels (mono-, di-, or trimethylation), mainly takes place on lysine residues of histone H3 and H4 and is a dynamic process with key roles in development and differentiation. The outcomes of this modification are highly context-dependent and can be associated with transcriptional repression or activation. Methylation of H4K20 is associated with both transcriptional activation and repression depending on methylation states. H4K20me1 catalyzed by PR-Set7 is associated with transcriptional activation, whereas H4K20me2/3 catalyzed by SUV4-20H1/2 is associated with the repression of transcription by maintaining pericentric and telomeric heterochromatin[3]. Loss of H4K20me3 has been described as a hallmark of cancer[4].

Liquid biopsy-based detection of cancer-related alterations in PTHMs has been reported by our group and others[5-9]. In the present study, our goal was to demonstrate the practicability of the extraction of circulating nucleosomal DNA from antigen-antibody complexes in enzyme-linked immunosorbent assays (ELISA) wells. This resembles chromatin immunoprecipitation (ChIP) assays where, however, there are relevant differences between the two approaches. In ChIP assays, protein A/G agarose beads are usually employed to bind antibodies of interest, and this is often associated with significant non-specific binding of DNA or non-target proteins to beads[10-12]. In the approach used in the present study, we extracted H4K20me3-associated circulating DNA directly from H4K20me3-containing nucleosomes that bound to anti-H4K20me3 antibodies immobilized on assay strips. In this way, many background signals could be excluded. Following extraction, a single copy and a multiple copy sequence from pericentric regions of chromosomes 16 and 1 were amplified to quantify H4K20me3-related circulating DNA from patients with colon cancer or colonic polyps.

MATERIALS AND METHODS

Study Participants

The study cohort included individuals who underwent colonoscopy procedures at the Surgical Department of İstanbul University, İstanbul Faculty of Medicine Hospital. Individuals with pathologically confirmed polyps in the colon (control group, n=15) and those with pathologically confirmed colon cancer (cancer group, n=15) were enrolled in the study. The characteristics of the patients with colon cancer are shown in Table 1. The study was approved by the İstanbul University, İstanbul Faculty of Medicine Ethics Committee (approval no: 2018/1095) and informed consent was obtained from each participant. Written informed consent forms were read by each patient and signed approvals were obtained before their operation.

Quantification of Circulating Nucleosomes

Blood samples were taken into EDTA tubes before the colonoscopy procedure and immediately centrifuged (10 min at 717 × g) to obtain the plasma fraction. After re-centrifugation, plasma was stored in aliquots at -80 °C until assayed. Plasma levels of circulating nucleosomes were determined using the Cell-Death Detection ELISA kit (Roche Diagnostics, Mannheim, Germany), as previously reported[6]. We applied 20 µL of plasma twice and the mean signal values, measured in optical density, were considered to be relative plasma concentrations.

Extraction of H4k20me3-related Nucleosomal DNA from Antibody-bound Nuclesosomes in ELISA

We used the commercially available EpiQuik Global Tri-Methyl Histone Quantification Kit (Epigentek, Farmingdale, NY, USA), which enables ELISA-like measurements of H4K20me3. Nuclesomal DNA was, however, extracted from antibody-bound nucleosomes before the H4K20me3 measurement was completed. For this, 50 µL plasma was added into ELISA wells and H4K20me3-related nucleosomes were allowed to bind to the antibody for 1 hour. After several washing steps, bound DNA was extracted as follows: The extraction buffer [100 mM $NaHCO_3$, 1% SDS and proteinase K (250 µg/mL)] was added on H4K20me3-antibody complexes and incubated at 65 °C for 15 min leading to the disassociation of nucleosomal DNA from

immune complexes. Detached DNA was transferred into fresh tubes, diluted to 100 µL, and purified using spin columns. DNA was eluted in 50 µL elution buffer and stored at -20 °C after checking the purity.

Quantitation of Nucleosomal DNA by Quantitative Polymerase Chain Reaction (PCR)

We amplified two genomic sequences from the pericentric regions of chromosomes 1 and 16 because H4K20me3 is enriched in pericentric heterochromatin. The primers for chr16 allow the amplification of a single copy sequence and had the following sequences: 5'AATCCAATGGAATCATCGAGT-3' and 5'-GGTGATTTCATTCAAGTCCATTC-3'.

The primers for chr1 amplify at least 40-copies and were 5'-GGTTCCATTTGATGATGATTCC-3' and 5'-ATCGAGTGGAATCGAATGG-3'. LINE1 element, which is found in all genomic regions of the human genome and enriched near centromeres, was used as the reference sequence to normalize the target sequences. Primer sequences of LINE1 resulting in 148-bp amplicon were 5'-AAAGCCGCTCAACTACATGG-3' and 5'-TGCTTTGAATGCGTCCCAGAG-3'. Real-time PCR was conducted in a light-cycler 480 with SYBR green as the fluorescence molecule, according to the instructions. Reactions were run in double and relative concentrations of pericentric sequences were calculated using the ΔΔCt method.

Statistical Analysis

The study findings are presented as median, quartiles, and total ranges. Discrimination between the study groups was performed using the Mann-Whitney U test. P-values of <0.05 were considered statistically significant. Calculations were performed using the SPSS 21.0 statistical software (IBM Corporation, Armonk, NY, USA).

RESULTS AND DISCUSSION

All plasma samples taken from patients with colon cancer and controls had detectable levels of circulating nucleosomes with slightly higher levels in the cancer group (Figure 1A). Following the extraction of nucleosomal DNA from H4K20me3-ELISA immune complexes, DNA was present in all samples and used as a template for the amplification of pericentric sequences from Chr 1 and 16. The relative plasma level of H4K20me3-related nucleosomal DNA was lower in the patients with colon cancer than in the controls for both target sequences (Figure 1B and 1C). At the chr1-specific target, relative values of H4K20me3-related nucleosomal DNA were 2.74 and 3.2 (p=0.16) in the patients with colon cancer and those with polyps, respectively. At the chr16-specific pericentric region, the difference between the patients with colon cancer and the controls was statistically significant (relative values: 0.037 vs. 0.073, respectively; p=0.008). These findings show that H4K20me3-related circulating nucleosomal DNA is present in decreased levels in colon cancer compared to the premalignant state and confirm our previous findings when ChIP- or ELISA-like approaches were used[5,6]. Even if the number of patients in the study is very small, the patients with more advanced disease (n=7) had lower levels of both target sequences than those with localized disease (n=8) (2.26 vs. 3.4 for Chr1-specific target and 0.036 vs. 0.049 for Chr16-specific target, respectively). Overall, decreased plasma levels of H4K20me3-related nucleosomal DNA are likely to be a reflection of

Table 1. Patient characteristics

	Gender	Age	Tumor localization	T stage	Lymph node metastasis	CEA (ng/mL)	CA19-9 (u/mL)
Patient 1	M	50	Sigmoid colon	2	No	2.81	38.22
Patient 2	F	57	Sigmoid colon	3	No	2.49	8.1
Patient 3	M	60	Right colon	3	No	13.96	12.92
Patient 4	F	54	Right colon	3	Yes	3.25	0.84
Patient 5	F	72	Sigmoid colon	4A	No	23.4	24.42
Patient 6	M	55	Right colon	4A	Yes	11.5	13.4
Patient 7	F	58	Right colon	2	No	1.23	2.45
Patient 8	M	85	Right colon	4A	Yes	6.7	3.5
Patient 9	F	58	Right colon	4A	Yes	41.1	0.68
Patient 10	M	60	Right colon	4A	Yes	1.42	16.1
Patient 11	F	56	Right colon	4A	Yes	3.29	22.2
Patient 12	M	70	right colon	3	Yes	3.79	3
Patient 13	M	22	Right colon	3	Yes	3.11	17.1
Patient 14	M	58	Sigmoid colon	4A	Yes	1.98	8.91
Patient 15	M	56	Right colon	2	No	3.17	57.19

M: Male, F: Female

H4K20me3 loss in colonic tumors, which has been described as a hallmark of cancer[4,13].

To our knowledge, this is the first report to describe the applicability of extracting circulating nucleosomal DNA from ELISA immune complexes. Even though our study does not include a direct comparison of this approach with ChIP, this method may be superior to ChIP in preventing background noise signals because protein A/G agarose beads used in ChIP assays non-specifically bind DNA and non-target proteins[10-12].

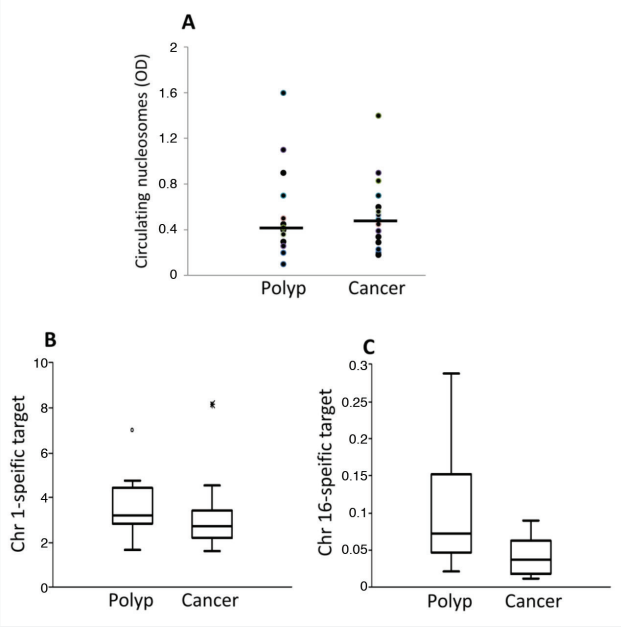

Figure 1. Plasma levels of circulating nucleosomes and nucleosomal DNA extracted from ELISA immune complexes in the study cohort. (A) Circulating nucleosomes were measured using a commercial kit according to the instructions. Mean signal values, measured in optical density, were considered to be relative plasma concentrations. The line mark (-) illustrates median values. (B) Relative plasma levels of extracted H4K20me3-related DNA in the pericentric region of Chr 1. Plasma was added into ELISA wells and H4K20me3-related nucleosomes were allowed to bind to the antibody for 1 hour. After several washing steps, bound DNA was detached from immune complexes using an extraction buffer (100 mM $NaHCO_3$, 1% SDS and proteinase K) and purified. A multiple-copy sequence from the pericentric region of Chr 1 was amplified using qPCR, which was normalized by the LINE1. (C) Relative plasma levels of extracted H4K20me3-related DNA in the pericentric region of Chr 16. After extraction of H4K20me3-related DNA from immune complexes, a single-copy sequence from Chr 16 was amplified. Shown are the 25th and 75th percentiles along with median values

ELISA: Enzyme-linked immunosorbent assays, qPCR: Quantitative polymerase chain reaction

The study of PTHM alterations in bodily fluids of individuals with disease represents a relatively new research field. Different techniques such as ChIP, ELISA-like measurement or more recently mass spectrometry have been used for the measurement of PTHMs in circulating nucleosomes[5-9], where cancer has been the main topic. The research conducted up to now has revealed the potential of liquid biopsy-based detection of PTHMs as surrogate cancer biomarkers. However, given the complexity in the number and the regulation of PTHMs, the lack of standardization of pre-analytics, and the method of their measurement, considerable research is required before validated markers become available for clinical use.

Study Limitations

The limitation of our study is the small number of patients. Further studies with higher number of patients are needed.

CONCLUSION

The detection of biomarkers in blood or other biologic fluids offers many advantages, including being minimally invasive and easily accessible. Here, we demonstrate the applicability of direct extraction of nucleosomal DNA from ELISA immune complexes, which represents a simplified method for the analysis of protein-DNA interactions and could provide an additional means in the assessment of PTHMs in liquid biopsy measurements.

Ethics

Ethics Committee Approval: The study was approved by the İstanbul University, İstanbul Faculty of Medicine Ethics Committee (approval no: 1095, date: 14.08.2018).

Informed Consent: Informed consent was obtained from each participant.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K., Concept: U.G., Design: U.G., Data Collection or Processing: E.Ö., E.E.Y., Analysis or Interpretation: E.Ö., E.E.Y., Literature Search: U.G., Writing: E.Ö., E.E.Y., M.K., U.G.

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

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An Evaluation of Mothers' Feeding Attitudes and Anxiety in Preschool Children

Okul Öncesi Çocuklarda Annelerin Besleme Davranışları ve Kaygı Durum Değerlendirilmesi

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ABSTRACT

Aim: The aim of this study was to determine the anxiety status as well as behaviors and attitudes of mothers about nutrition of their children.

Materials and Methods: Mothers of the children between 2 and 5 years of age, who thought that their children had feeding problems, were enrolled into the study. Beyond demographic characteristics, mothers were asked about their feeding behavior by using a child nutrition questionnaire. Body weight and body length measurements of the children were performed; body mass indexes (BMI) were calculated. Percentile (p) values depending on the age and gender were compared with the answers given to the questionnaire.

Results: The mean age of the two hundred and eighty-five children was 3.8±1.0 years and 127 (44.6%) of them were girls. Two hundred and seventy six (96.8%) children had weight between 3 and 97 p whereas 9 (3.2%) children had body weight above 97 p; 47 (16.5%) children were overweighed and 30 (10.5%) children were obese. The mean responsibility grade of mothers about feeding their children was 35 points for children whose bodyweight were between 3 and 97 p, and 30 points for those with bodyweight above 97 p (p=0.010).

Conclusion: It was detected that 27% of the children whose mothers thought that their child had a feeding problem had BMI over normal range. Mothers do not have sufficient information that may constitute positive samples while feeding their children, and they should be informed about feeding attitudes and habits by healthcare professionals by considering body measures of the child.

Keywords: Child, feeding, scale, obesity, overweight

ÖZ

Amaç: Bu çalışmada amaç, annelerin çocuklarının beslenmesi hakkındaki kaygı durumunu ve beslenme sırasındaki davranış ve tutumlarını belirlemektir.

Gereç ve Yöntem: Çocuğunda beslenme sorunu olduğunu düşünen, 2-5 yaş arasındaki çocukların anneleri çalışmaya alındı. Demografik özellikler dışında annelere beslenme davranışları hakkında çocuk beslenme anket soruları soruldu. Çocukların ağırlık ve kilo ölçümü yapıldı, vücut kitle indeksi (VKİ) hesaplandı ve bunların yaş ve cinsiyete göre hazırlanmış persentil (p) değerleriyle anket cevapları karşılaştırıldı.

Bulgular: İki yüz seksen beş çocuğun yaş ortalaması 3,8±1,0 yıl idi ve 127'si kız (%44,6) idi. Çocukların 276'sı (%96,8) ağırlıklarına göre 3-97 p arasında iken 9 (%3,2) çocuk ise >97 p idi. VKİ persentil değerlerine göre 47'sinin (%16,5) aşırı kilolu, 30'unun (%10,5) ise obez olduğu görüldü. Ağırlığa göre 3-97 p arasındaki çocukların annelerinde çocuklarını besleme konusunda gösterdikleri sorumluluklarının derecesinin ortalaması 35 puan, 97 p> olan çocukların annelerinde ise 30 puan olarak bulundu (p=0,010).

Sonuç: Çocuğunda beslenme sorunu olduğunu düşünen annelerin çocuklarının %27'sinin normalin üzerinde VKİ persentil değerleri olduğu görülmüştür. Annelerin çocuklarını beslerken olumlu örnek olabilecek davranışları tam olarak bilmedikleri ortaya koyulmuştur. Bu konuda sağlık çalışanlarının anneleri, çocuğun vücut ölçülerini de dikkate alarak beslenme tutum ve davranışları konusunda bilgilendirmeleri önerilmektedir.

Anahtar Kelimeler: Çocuk, beslenme, ölçek, şişmanlık, aşırı kilolu

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INTRODUCTION

The customs and traditions of each of the societies in the world, according to their cultures, affect the nutritional attitudes and behaviors in the family. Nutritional habits guided by various socioeconomic, cultural and educational influences are acquired in children in the early stages of life[1,2]. The personality of the child is shaped in the pre-school period, and the acquisition of nutritional habits is generally based on these years, and these habits can cause nutritional problems that may affect later in life and may arise in the future[2,3].

The most important reason for inadequate and unbalanced nutrition is the lack of access to food. In addition, wrong and ongoing nutritional attitudes and behaviors are also an important reason. Growing children are frequently affected by inadequate and unbalanced nutrition problems, especially in the pre-school period, and a tendency to obesity is observed. Wrong and inappropriate eating habits gained during this period also carry important risks related to obesity and cardiovascular diseases in the future[4-6].

In order for parents to evaluate their children's nutritional behaviors, various scales with objective characteristics have been developed for different age groups. Birch et al.[7] developed the child feeding questionnaire for children aged five to nine years, to determine the attitudes, behaviors and beliefs of parents while feeding their children. Baughcum et al.[8] applied a similar nutrition questionnaire for preschool children aged 2-5 years. These surveys are generally carried out to determine the parents' responsibility for their children's nutrition, eating pressure, restrictive behaviors, and perception levels of their children's weight[7-10].

The aim of this study is to determine the anxiety of mothers about the nutrition of their pre-school children and their behaviors and attitudes during feeding, and to reveal the potential physical negativities that could be caused by the incompatibility between the mother and the child about nutrition.

MATERIALS AND METHODS

This cross-sectional study was conducted by using face-to-face interview method among mothers with children aged 2-5 years, who applied to the pediatrics clinic of our hospital between October 2016 and December 2016 and thought that their children had a nutritional problem. A detailed history of the children was taken, their general examinations were carried out, weight and height measurements were performed, body mass index (BMI) was calculated, percentile (p) values were found and recorded for weight, height and BMI from tables created according to their age and gender. BMI <5 p was considered as underweight, BMI 5-85 p as ideal weight, BMI 85-95 p as overweight, and BMI >95 p as obese[11,12].

The demographic characteristics of the mothers, their education level, socioeconomic status, and family income as above or below the minimum wage were classified, and nutritional questionnaire questions were asked in addition to their status. Mothers of children who were born at term and had normal birth weight (>2,500 g), and height and weight not below normal for age and gender (>3 p), and who did not have a known chronic disease were included in the study after obtaining the consent for the questionnaire form. Mothers of children with chronic liver, kidney, heart and neuromuscular diseases, being on continuous drug use, being born prematurely and with low birth weight (<2,500 g), and with height and/or weight <3 p at the time of enrollment in the study and those who did not answer more than 10% questions in the questionnaire forms given to the mothers were excluded from the study.

Characteristics of the Questionnaire

The questionnaire we applied in our study included the feeding behaviors of mothers in preschool children and their evaluations about their children's nutrition. While 12 questions in the first part defined the demographic characteristics of the family, the remaining 33 questions (13-45) in the second part were about the nutritional survey. While 11 of these 33 questions were examining mothers' responsibilities in feeding their children, 7 of them were measuring the eating pressure that mothers put on their children. Two questions evaluated the degree of mothers' dietary restrictions on their children, one question assessed mothers' perceptions of their children's weight, and one question evaluated mothers' levels of interest in their children's weight. Two of the remaining 11 questions were about the children's choice of food, 3 questions about the child's misbehavior that would negatively affect the child's eating habits, 5 questions about non-mother factors affecting the child's nutrition, and 1 question about the place of snacks in the child's nutritional life (Table 1)[7-10]. While two of the questions, questions 17 and 28, were multiple-choice type, the answers to the remaining 31 questions were prepared in a five-point Likert scale type (1: Never, 2: Rarely, 3: Sometimes, 4: Often, 5: Always).

Approval for this study was obtained from the Ethics Committee of Health Sciences University Turkey, İstanbul Okmeydanı Training and Research Hospital (27.09.2016/523). Table 1 shows which scales the questions in the questionnaire belong to.

Statistical Analysis

The data obtained as a result of the research were evaluated in the SPSS 16.0 software. While evaluating the data collected with the survey questions, charts showing absolute and percentage numbers were prepared for each question. While

analyzing the study data, the conformity of the parameters to the normal distribution was evaluated with the Shapiro-Wilks test. In addition to descriptive statistical methods (mean, standard deviation, frequency), while comparing the quantitative data, the Student's t-test was used for the comparison of normally distributed parameters between two groups, and the Mann-Whitney U test was used for the comparisons of non-normally distributed parameters between two groups. The chi-square test or Fisher exact test statistics were used to compare categorical variables. The correlation between the variables was determined with the pearson or Spearman's correlation coefficient. Significance was evaluated at the $p<0.05$ level.

RESULTS

Five of the 305 mothers who initially participated in the study were excluded from the study because they did not complete the questionnaire. Fifteen cases with a weight or height <3 p after the measurements were also excluded from the study, and the remaining 285 children and their mothers were included in the study. The mean age of all children was 3.8±1.0 years,

Table 1. Nutritional questionnaire items and related scales

Item no	
13 (S)	Do you think your child does not eat as well as other children?
14(S)	Do you think you cannot feed your child well?
15 (S)	Do you feel worried that your child cannot be fed without you?
16 (F)	Do you think your child eats better when parents or siblings are together?
17 (S)	Does anyone other than you feed your child?
18 (F)	Do other people have similar problems while feeding your child?
19 (S)	Do you think you are not a good mother when your child does not finish his/her meal?
20 (A)	Even if the health professionals say that your child has normal weight, do you still think that he/she is insufficient?
21 (F)	Do you think your child has less appetite when angry or upset?
22 (C)	Does your child choose food?
23 (Y)	Do you turn on the TV for your child during meals?
24 (Y)	Do you play with your child to feed him/her?
25 (C)	Is it difficult to get your child to eat new foods?
26 (S)	Is it helpful to eat with father when starting new foods?
27 (S)	Have you ever prepared different foods when there is food he/she does not like?
28 (S)	How many different foods do you prepare in one meal when your child does not like it?
29 (S)	Do you feel successful when your child finishes his/her meal?
30 (S)	Do you help when your child does not finish his/her food?
31 (S)	During the meal, would you let your child choose from the foods that comes to the table?
32 (K)	Do you promise for after-meal dessert to get your child to eat healthy foods?
33 (K)	When your child is grumpy, do you give him/her food or sweets that he/she will like?
34 (D)	Would you let the elders come to the house to bring snacks for your child?
35 (B)	Do you force your child to eat more by punishing them or curtailing their privileges?
36 (F)	Do you think your child eats better when they are with other children?
37 (B)	Do you invite other children to the house so that your child can eat better
38 (F)	Do you think your child is better fed by people other than you?
39 (B)	Do you force your child to finish his/her meal so he can have dessert after meal?
40 (B)	Would you spoon-feed your child so he/she can finish what's on his/her plate?
41 (B)	When you can't get your child to eat, do other people in the house take on this task?
42 (Y)	Does your child sit at the table with you?
43 (B)	When your child leaves the table, do you follow him to finish his/her meal?
44 (B)	Do you let your child play with toys at mealtimes?
45 (A)	Do you think your child eats too much?

S: The degree of responsibility that mothers have for feeding their children, B: The degree of eating pressure put by mothers on children, K: The degree of mothers' behavior to restrict their children's food intake, A: Perceptions of mothers about their children's weight, C: Children's choice of food, Y: Misbehaviors that will negatively affect the child'ss eating habits during meals, F: Non-mother factors affecting the child's nutrition, D: The place of snacks in the child's nutritional life

3.7±1.0 years in girls and 3.8±1.0 years in boys. There was no statistically significant difference between the two groups in terms of mean age (p=0.328). While the number of children in families ranged from 1 to 6, the median number of children in families was 4. Demographic characteristics of the children included in the study and their mothers are given in Table 2.

While the number of mothers with two or less children was 176, the number of mothers with three or more children was 109. When these two groups were analyzed in terms of the distribution of children with 3-97 p and >97 p, no significant difference was found (p=0.758). Considering the income levels of the families, it was found that the income of 108 families (44.2%) was below the minimum wage, and the income of 177 families (45.8%) was above the minimum wage. There was no statistically significant difference between children with 3-97 p and >97 p in terms of weight according to their income level (p=0.889).

The weight, height and BMI percentile distributions of the children included in the study are shown in Table 3. Of the 285 children included in the study, 276 children (96.8%) were between 3-97 p in terms of weight, while the number of children >97 p was 9 (3.2%). Among the girls and boys, the distribution of those with 3-97 p and >97 p for weight was similar (p=0.522). Children included in the study were additionally classified according to their BMI percentile values. According to the BMI percentile values of the children, 30 (10.5%) were obese and 47 (16.5%) were overweight (Table 3).

Table 2. Demographic characteristics of the children and their mothers included in the study

	Mean±SD (min-max)	
Mean age of children	3.8±1.0	
Children's weight (kg)	15.9±3.4 (10.0-28.0)	
Height (cm)	100.1±9.0 (80-130)	
BMI (kg/m²)	15.8±2.1 (13-25)	
Mean age of mothers	34.0±6.5	
	n	**%**
Girl/boy	127/158	%44.6/55.4
Age group (according to the age to be turned)		
2 years	42	18.3
3 years	65	22.8
4 years	91	31.9
5 years	77	27.0
Educational status of mothers		
Illiterate	13	4.6
Primary school	114	40
Middle school	38	13.3
High school	77	27
University	43	15.1
Working status/housewife	72/213	25.3/74.7

BMI: Body mass index, SD: Standard deviation, min: Minimum, max: Maximum

Questionnaires were asked to the mothers about the nutrition of their children. While the number of mothers who let the child choose the food they wanted on the table was 217 (76.2%), 171 (60.0%) stated that they considered themselves successful when their children finished the food. 196 (68.8%) of them stated that they prepared different food when children did not like the food. 77.5% of them were helping their children when they did not finish their meal. Although health professionals said that their children were of normal weight, the number of mothers who thought that their children's weight was always insufficient was 14 (4.9%). Three (33.3%) of 9 mothers whose children had a weight of >97 p still thought that their children's weight was insufficient, contrary to what health professionals said. While the number of mothers who thought that their children ate more than necessary was 48 (16.9%), this perception was not present in 33.3% of those with obese children. 43.5% of the mothers stated that they did not punish their children for not eating more or they did not reduce their children's privileges.

Table 3. Weight, height and BMI percentile distributions of the children included in the study

Weight percentiles		
3-10	64	22.5
10-25	65	22.8
25-50	36	12.6
50-75	65	22.8
75-90	30	10.5
90-97	16	5.6
Height percentiles		
3-10	51	17.9
10-25	73	25.6
25-50	50	17.5
50-75	60	21.1
75-90	25	8.8
90-97	17	6.0
>97	9	3.2
BMI percentiles		
<5	41	14.4
5-15	20	7.0
15-25	36	12.6
25-50	56	19.6
50-75	36	12.6
75-85	19	6.7
85-95	47	16.5
>95	30	10.5

BMI: Body mass index

65.6% of them promised their children for after-meal dessert in order to make them eat the food which would be good for their children, but the children did not want to eat. 53.0% of them stated that they often or always played games with their children in order to feed them, 56.2% of them stated that they often or always watched television while feeding their children. Regarding the place of snacks in the child's diet, 47.3% of the mothers stated that these snacks were included in the child's diet every day.

Eleven of the questionnaire questions examined mothers' responsibilities in feeding their children. This scale reflected the mother's control over the child's nutrition and her feeling responsible for the portions and for preparing a healthy diet. The degree of mothers' responsibility for feeding their children (11 questions; 55 points in total), the degree of eating pressure that mothers placed on their children (7 questions; 35 points in total), child nutrition and non-mother factors (5 questions; total 25 points) were evaluated among working and non-working mothers. While there was no statistically significant difference between the mean level of non-maternal factors, it was observed that the mean levels of eating pressure and mother's responsibility scales were statistically lower in working mothers (p=0.027, p=0.021, respectively) (Table 4).

It was evaluated whether the mothers' responsibility and eating pressure scales were different in the mothers of children with a weight of 3-97 p and >97 p. The total score of the degree of mothers' responsibility in feeding their children was found to be lower in mothers having children with >97 p than in the other group and this was statistically significant (p=0.010), but no statistical significance was found in the comparison of the total score of the degree of eating pressure that mothers put on their children (p=0.123) (Table 5).

A positive and moderate correlation was found between the total score of the mothers' responsibility scale to feed their children and the total score of the mothers' eating pressure scale on their children (r: 0.485; p=0.000).

Table 4. Comparison of eating pressure, mother's responsibility and non-mother factors in child nutrition according to mother's employment status

	Housewife		Working mother		
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	p
Eating pressure	18.8±4.8	19 (7-30)	17.3±4.9	17 (7-28)	**0.027**
Mother's responsibility	34.2±6.1	34 (16-91)	32.4±5.2	32 (22-44)	**0.021**
Non-mother factor in child nutrition	12.5±2.8	13 (4-20)	12.6±2.6	13 (7-19)	0.790

p: Independent group t-test, SD: Standard deviation, min: Minimum, max: Maximum

Table 5. Comparison of the median values of mother's responsibility and eating pressure scale scores according to the weights

	Weight (p)	Total scale score medium value	p
Mother's responsibility	**3-97 p**	34	**0.010**
	>97 p	30	
Eating pressure	**3-97 p**	18	**0.123**
	>97 p	16	

Table 6. Comparison of mother's responsibility and eating pressure scales with mother's education level and working status

	Mother's educational level	n	Total scale score medium value	p	Mother's working status	Total scale score medium value	p
Mother's responsibility	Literate	**13**	**36**	**0.003**	**Housewife**	34	**0.013**
	Primary school graduate	114	34				
	Secondary school graduate	38	35		**Working**	32	
	High school graduate	77	33				
	University graduate	43	31				
Eating pressure	Literate	13	21	**0.004**	**Housewife**	19	**0.049**
	Primary school graduate	114	18				
	Secondary school graduate	38	19		**Working**	17	
	High school graduate	77	17				
	University graduate	43	17				

When the mother's education level was compared with the mothers' responsibility scale total score for feeding their children and the mothers' eating pressure scale total score on children, it was found that the score decreased as the education level increased. It was seen that the group of mothers who felt most responsible and made pressure to eat were literate, while the group of mothers who felt the least responsibility and did not make pressure to eat were university graduates (p=0.003 and p=0.004, respectively). When the mother's occupation (housewife/working) and the mothers' responsibility scale total score for feeding their children and the eating pressure scale total score on their children were compared, it was found that both scale scores were lower in working mothers (p=0.013 and p=0.049, respectively) (Table 6).

Mothers' responsibility and eating pressure were evaluated in the groups formed according to BMI percentile values. While the median value of mothers' responsibility scale was 32.5 in the group with BMI >95 p, it was 35 in the group with BMI <5 p. In this paired comparison, the mean values of the mothers' responsibility scale were found to be statistically significantly higher in the underweight group than those in the obese group (p=0.044). No statistically significant difference was found in other pairwise comparisons (p>0.05).

Eating pressure, mother's responsibility and non-mother factors in child nutrition were evaluated according to weight, weight percentile, BMI and BMI percentiles. When non-mother factors in eating pressure and child nutrition were evaluated, there was no statistical significance (p>0.05). BMI was found to be statistically significant in the mother's responsibility scale (r: -0.12, p=0.046). However, the same significance was not found in the BMI percentile (r: -0.10, p=0.095) (Table 7).

DISCUSSION

In our study, according to BMI measurements of the children of mothers who thought their children had eating problems, it was determined that 10.5% of were obese, and 16.5% were overweight. In recent years, obesity and being overweight in children stand out as a remarkable problem at the rates between 1.5% and 20% in different countries and in age groups[13-16]. In a study conducted in the age group of 5-6 years in our country, the frequency of being overweight was detected to be 8.3% and the frequency of obesity was 10.1%[17]. In another study, it was reported that the prevalence of being overweight was 8.6% and the frequency of obesity was 6.6% in the age group of 1-5 years[18]. It is thought that being overweight and obesity have increased in the childhood age group in recent years.

It is suggested that the way parents feed their children has an effect on the development of obesity. One of the methods that examines parents' responsibilities regarding their children's nutrition, controlling habits, attitudes and beliefs is the parental feeding style questionnaire. The main purpose of this type of survey is to reveal what directs eating habits and why parents use the methods they use. The survey questions we used in our study, on the other hand, were prepared in line with the data obtained from studies conducted in this direction so far, and they tried to descriptively define mothers' feeding attitudes[7-10]. The parental feeding questionnaire developed by Wardle et al.[9] for children aged 2-9 years was also validated in our country[19]. Our study included different questions, the most important of which was the degree of responsibility that mothers showed in feeding their children. It was observed that mothers having children with a weight above normal had a lower mean of responsibility scale than mothers of children with a weight within the normal range.

In some studies, it has been shown that overweight children are under less pressure to eat, but children with low BMI are under more pressur to eat[15,20,21]. In our study, however, no significant difference was found in the comparison of the mean scores on the scale of eating pressure created by mothers on children. In some studies, it has been shown that excessive controlling and restrictive behaviors of parents in children's nutrition increase the risk of overeating and obesity in children. In a study, it was determined that childhood obesity was related to parental nutritional behaviors, controlling children's food choices (for example; restricting snack foods) had negative consequences, and children consumed these foods excessively when they had the opportunity. The theoretical relationship between child nutrition practices and body weight stems from the prediction that parents

Table 7. The correlation of mother's eating pressure, responsibility, and non-mother factors in child nutrition with the child's weight and BMI percentiles

		Weight (kg)	Weight percentile	BMI (kg/m²)	BMI percentile
Eating pressure	**r**	0.04	-0.01	0.04	0.01
	p	0.509	0.985	0.813	0.526
Mother's responsibility	**r**	-0.04	-0.07	**-0.12**	-0.10
	p	0.484	0.234	**0.046**	0.095
Non-mother factors	**r**	-0.01	-0.05	-0.08	-0.04
	p	0.993	0.427	0.153	0.438

r: Correlation coefficient; Spearman's correlation test, BMI: Body mass index

are likely to control the child's nutrition if they believe the child is at risk of gaining excess weight. In the literature, it is stated that excessive controlling behaviors of parents about nutrition lead to irregularities in caloric intake and weight gain in children. It is claimed that excessive control of nutrition causes the child to ignore hunger and satiety signs and increase the tendency to obesity[21,22]. It is thought that avoiding restrictive behaviors in the nutrition of the child will contribute to the prevention of the risk of being overweight and obesity[23].

Parents' inability to evaluate their children's weight correctly also prevents them from directing them to a healthy diet. In some studies, it has been shown that families are not aware of that their children's weight is above normal[24-26]. In our survey, when the mothers were asked whether they thought that their children's weight was still insufficient even if health personnel said that the weight of their children was within normal limits, 14 (4.9%) mothers still thought that their children's weight was insufficient despite the doctor's indication, and this rate was 33% in the mothers of obese children. It was thought that the perception disorders of these mothers regarding the weight of their children might be an important cause of obesity in children. It has been shown that parents are not aware of that their children are overweight and do not perceive this situation as a health threat[26]. In solving this problem, parents' visual perceptions of their children's weight are of great importance.

It is important in terms of proper nutrition whether the nutrition of the child is according to the wishes of the child or the wishes of the family. Families affect children's eating habits not only by the food they offer them, but also by the way they are fed[27]. Families trying to feed the child by playing games to feed their children, distracting the child with television or moving musical visual content, and giving rewards affect the child's eating habits in the opposite direction[28]. In our study, it was seen that the majority of mothers tried similar methods. In order for the child to eat healthy, it is recommended that the family sit at the table and eat together. It has been shown that allowing the child to make his/her own choices by enabling the child to plan his own meals is a necessity for the child's adherence to nutrition and proper eating habits that will be formed in the future, and it has been reported that these children consume less food when they take their own starter food at the meal[29].

It has been suggested that low income level and socioeconomic status cause the child to be fed with fast-consuming high-calorie foods and to be overweight[30]. It has been shown that the risk of obesity is lower for the children of parents with higher education levels[15]. In our study, it was observed that higher education level decreased mother's responsibility and eating pressure, and this was low in mothers with obese children. It has been reported that the children of mothers with longer working hours are also at higher risk of being overweight[31]. In our study, it was observed that the responsibility and eating pressure scale scores of working mothers were lower than those of non-working mothers. It is thought that the reason for this may be that the working hours were not long or that the children were fed by another adult at home.

Study Limitations

Our study includes several limitations. First, the children included in the study were not evaluated in terms of food allergies. Second, the weight, height and BMI of the families were not evaluated.

CONCLUSION

As a result, the foundations of future nutritional habits are largely provided by the gains in the age period of 2-5 years. The data obtained in our study show that mothers do not fully know the behaviors that can be positive examples while feeding their children and they continue to make common mistakes in the society, such as finishing the meal completely, replacing undesired meals with acceptable ones. The decrease in the excessive pressure for eating, which will negatively affect the future nutrition style, with the level of education, and the decrease in the awareness of the responsibility for feeding the child with the education level show that the society's awareness should be raised and they should be educated on this issue.

Ethics

Ethics Committee Approval: Approval for this study was obtained from the Ethics Committee of Health Sciences University Turkey, İstanbul Okmeydanı Training and Research Hospital (27.09.2016/523).

Informed Consent: Informed consent was obtained from the patient.

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Authorship Contributions

Surgical and Medical Practices: M.J., Concept: M.J., Y.T., H.D., O.Ö., Design: M.J., Y.T., H.D., O.Ö., Data Collection or Processing: M.J., Analysis or Interpretation: M.J., Y.T., H.D., O.Ö., Literature Search: M.J., Y.T., H.D., O.Ö., Writing: M.J., Y.T.

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The Role of RIFLE, AKIN and KDIGO Criteria in Determining the Relationship Between Acute Kidney Injury and Mortality in Intensive Care Patients

Yoğun Bakım Hastalarında Akut Böbrek Hasarı ve Mortalite İlişkisinin Belirlenmesinde RIFLE, AKIN ve KDIGO Kriterlerinin Yeri

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ABSTRACT

Aim: *Risk, injury, failure, loss, and end stage* (RIFLE); acute kidney injury network (AKIN) and kidney disease: Improving global outcomes (KDIGO) classifications are the most commonly used criteria for the diagnosis of acute kidney injury (AKI). The aim of our study was to determine the relationship between the mortality and the severity of AKI diagnosed by using RIFLE, AKIN, and KDIGO classifications in critically ill patients.

Materials and Methods: Data of 1,491 patients hospitalized in tertiary intensive care unit were retrieved from electronic medical records and patients diagnosed with AKI were included in the study. AKI severity was determined according to the RIFLE, AKIN, and KDIGO classifications.

Results: One hundred fifty-five patients were included in the study. The percentages of patients in risk, damage, and failure stages according to the RIFLE criteria were 14.8%, 40.0%, and 45.2%, respectively. The percentages in stage 1, 2 and 3 were 45.6%, 30.6%, and 23.8% according to the AKIN criteria and 18.7%, 21.7%, and 54.1% according to the KDIGO criteria, respectively. There was a difference in mortality between the stages of AKI determined according to the AKIN and RIFLE criteria. Mortality was found to be higher in patients in KDIGO stage 3.

Conclusion: These three classifications do not consider the etiology of AKI. Therefore, it may be possible that they do not accurately reflect the relationship between mortality and AKI severity. However, the KDIGO classification, which emerged with the need arising from the inadequacy of the classifications used before it, seems to be more valid in this respect.

Keywords: Acute kidney injury, AKIN, KDIGO, mortality, RIFLE, intensive care

ÖZ

Amaç: Akut böbrek hasarının (ABH) daha kesin biçimde tanımlanması ve takip sürecinin daha iyi yönetilmesi amacıyla çok sayıda sınıflama gündeme gelmiştir. Bunlar arasında en yaygın kabul görenler *risk, injury, failure, loss, and end stage* (RIFLE), *acut kidney injury network* (AKIN) ve *kidney disease: Improving global outcomes* (KDIGO) sınıflamaları olmuştur. Bu çalışmada, yoğun bakımda izlenen ve ABH tanısı alan hastalarda RIFLE, AKIN ve KDIGO kriterlerine göre ABH şiddeti ile mortalite arasındaki ilişkinin saptanması amaçlanmıştır.

Gereç ve Yöntem: Dahiliye yoğun bakım ünitesinde izlenen 1.491 hastaya ait veriler retrospektif olarak incelendi ve ABH saptanan hastalar çalışmaya dahil edildi. Tüm hastalar için RIFLE, AKIN ve KDIGO kriterlerine kullanılarak ABH şiddeti belirlendi.

Bulgular: Çalışmaya 155 hasta dahil edildi. RIFLE kriterlerine göre risk, hasar, yetmezlik evrelerinde yer alan hasta oranları sırasıyla; %14,8, %40,0, %45,2; AKIN kriterlerine göre evre 1, evre 2 ve evre 3'te yer alan hasta oranları sırasıyla; %45,6, %30,6, %23,8; KDIGO kriterlerine göre evre 1, evre 2 ve evre 3'te yer alan hasta oranları sırasıyla; %18,7, %21,7, %54,1 idi. AKIN ve RIFLE kriterlerine göre belirlenen ABH evreleri arasında mortalite oranları açısından farklılık saptanmazken, KDIGO evre 3'te yer alan hastalarda evre 1 ve evre 2 ABH gruplarına göre mortalite daha yüksek saptandı.

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Sonuç: Her üç tanı ve evreleme sistemi de ABH etiyolojisini dikkate almamaktadır. Bu nedenle mortalite ve ABH şiddeti arasındaki ilişkiyi doğru yansıtmamaları söz konusu olabilir. Bununla birlikte, kendisinden önce kullanılan evreleme sistemlerindeki eksikliklerden doğan ihtiyaçla ortaya çıkan KDIGO evreleme sistemi bu açıdan daha geçerli görünmektedir.

Anahtar Kelimeler: Akut böbrek hasarı, AKIN, KDIGO, mortalite, RIFLE, yoğun bakım

INTRODUCTION

Acute kidney injury (AKI), defined as sudden and progressive deterioration of kidney functions, is among the leading causes of mortality in hospitalized patients. The reported incidence of AKI in patients followed in the intensive care unit is between 20% and 50%, and this rate exceeds 70% in the presence of sepsis. A mortality rate of 15-70% has been reported in this group[1-4]. The lack of consensus on the definition of AKI and the presence of different definitions over 35 currently in use are likely reasons for the large variability in reported frequency and mortality rates. This situation made it difficult to make comparisons among studies on AKI, and caused inadequacy in the evaluation of prognostic indicators.

Based on the need for diagnostic standardization, risk, injury, failure, loss, and end stage (RIFLE) was defined in 2004 by the Bellomo et al.[1] Then, the classifications of Mehta et al.[5] acute kidney injury network (AKIN) in 2007 and Kellum et al.[6] kidney disease: Improving global outcomes (KDIGO) in 2012 were defined. Although they have some limitations, criteria for the diagnosis and staging of AKI are the most agreed and widely studied subjects in these classifications[2]. In all three classifications, serum creatinine and urine amount are taken into account and the severity of AKI is defined in 3 stages. In RIFLE staging, differently, there are 2 stages associated with the outcome as "L-loss" and "E-end-stage renal disease". Unlike the other two, in the AKIN classification, creatinine and urine changes in the 48-hour period are taken into account. KDIGO classification can be interpreted as an integrated version of AKIN and RIFLE classifications.

In the majority of related studies, it has been reported that all three classifications can be used to predict mortality and that mortality increases as the AKI stage increases[7-15].

In our study, it was aimed to determine the severity of AKI according to AKIN, RIFLE and KDIGO criteria and to evaluate its relationship with mortality in patients who were followed up in the internal medicine intensive care unit of a tertiary hospital and developed AKI.

MATERIALS AND METHODS

The information of 1,491 adult patients who were followed up in the tertiary internal medicine intensive care unit between August 2003 and May 2010 were analyzed through the hospital information system, patient files and the registry system of the intensive care unit. The study protocol was prepared in accordance with the Declaration of Helsinki and Ethics Committee approval of Dokuz Eylül University University Faculty of Medicine was obtained (approval number: 252/2009). Among 589 patients with elevated serum creatinine, those who stayed in the intensive care unit for less than 48 hours (n=185), those with a history of chronic kidney failure and kidney transplantation (n=128), those with insufficient diagnostic data (n=75), and those who had highest serum creatinine levels at admission to the intensive care unit but had regression in the follow-up (late AKI, n=46) were excluded and 155 patients were included in the study. Patients' age, gender, indication for intensive care unit, co-morbidities, presence of sepsis, acute physiology and chronic health evaluation score-II (APACHE-II) and simplified acute physiology score-II (SAPS-II) scores, duration of monitorization in intensive care, need for invasive mechanical ventilation, basal and peak serum creatinine values, need for hemodialysis following the diagnosis of AKI, and outcome data were recorded. Co-morbidities were grouped as cardiovascular disease (diabetes, hypertension, cerebrovascular event, coronary artery disease, heart failure), malignancy (metastatic or non-metastatic solid and hematological malignancies), chronic obstructive pulmonary disease, liver failure and other. APACHE-II and SAPS-II scores were calculated by considering the worst parameters in the first 24 hours of admission to the intensive care unit. Even if the serum creatinine value decreased to basal level, the outcome was accepted as "death" if death occurred during the follow-up period,

AKI stage was determined for all patients according to the RIFLE, AKIN, and KDIGO criteria (Table 1). The last 2 stages in the RIFLE classification were not included in the staging because they were related to patient outcome. When staging AKI according to the RIFLE criteria, the patient's basal serum creatinine value, if known, was used, and if it is unknown, the serum creatinine value corresponding to 75 mL/min/1.73 m^2 glomerular filtration rate (GFR) according to the modification of diet in renal disease formula was used[1,16]. RIFLE staging was performed according to the highest serum creatinine and lowest GFR value in the 7-day period following the trend of increasing serum creatinine level. While AKI was staging according to the AKIN criteria, the lowest serum creatinine value was determined as basal serum creatinine in the 48-hour period in which AKI was detected; AKI severity was determined at the time of diagnosis according to the highest serum

creatinine value[5]. Those who underwent hemodialysis within the first 48 hours of being diagnosed with AKI were classified as stage 3. When staging AKI according to the KDIGO criteria, the basal serum creatinine value of the patient, if known, and the lowest serum creatinine value before the development of AKI, if not known, was determined as the basal value. AKI severity was determined according to the highest serum creatinine value in the 7-day period after the serum creatinine level started to increase. Those who underwent hemodialysis within 7 days were classified as stage 3[6,16].

Since daily urine output data were not sufficient in all patients, the urine amount criterion was not used in the diagnosis and staging of AKI.

Statistical Analysis

Statistical Package for the Social Sciences (Windows, version 25) software was used for data recording and analysis. P value <0.05 was considered significant. The homogeneity of variances was tested by the Kolmogorov-Smirnov analysis. The groups were compared with the ANOVA analysis of variance, Kruskal-Wallis test, Mann-Whitney U test or chi-square tests according to whether they were parametric or non-parametric. Logistic regression analysis was used to determine the effect of variables on mortality.

RESULTS

General Characteristics of the Patients

In our study, in which the data of 1,491 intensive care patients were analyzed, the rate of AKI was found to be 18.5%. One hundred fifty-five adult patients with sufficient data were included in the study. The median age was 62 (18-89) years, and the female sex rate was 42.6% in patients with a mean follow-up period of 12.7 (2-105) days. The most common comorbidity was cardiovascular diseases (50.3%), and 43.6% of the patients had more than two comorbidities. In 59.4% of the patients, the indication for follow-up in the intensive care unit was sepsis or systemic inflammatory response syndrome. The rate of patients who needed invasive mechanical ventilation was 92.3%, and sepsis and respiratory failure were found to coexist in 45.8% of the patients. The mean APACHE-II score was 24.6±8.8 and the SAPS-II score was 55.9±19.7. The rate of patients who received hemodialysis at any time during the follow-up was found to be 33.5%. The mortality rate was 77.4% (Table 2). There was no significant difference in mortality between the patients who underwent and did not undergo hemodialysis (p=0.054). Logistic regression analysis, including age, AKI stages, APACHE-II and SAPS-II scores, hemodialysis need, and mechanical ventilator and vasopressor needs, did not reveal any mortality-related parameter.

Characteristics of AKI Stages According to RIFLE Criteria

Of the 155 patients included in our study, 14.8% were included in the risk stage, 40.0% in the damage stage, and 45.2% in the failure stage. Mortality rates in the stages of risk, damage and failure were determined as 65.2%, 77.4% and 81.4%, respectively. No significant difference was found between the stages in this respect. There was no significant difference between RIFLE stages in terms of age, comorbid diseases, presence of sepsis and need for mechanical ventilation. While there was no significant difference between the stages in terms of APACHE-II scores, the mean SAPS-II score of the risk stage was found to be lower than the other stages (p=0.049). In the intensive care follow-up after the diagnosis of AKI, hemodialysis was applied to 3 (13.0%) patients in the risk stage, 12 (19.4%) patients in the failure stage, and 37 (52.9%) patients in the damage stage (p=0.000) (Table 3).

Characteristics of AKI Stages According to AKIN Criteria

Of the 155 patients included in the study, 8 remained outside the definition of AKI. 45.6% of the patients were included in the 1st stage, 30.6% in the 2nd stage, and 23.8% in the 3rd stage. Mortality rates of AKIN stage 1, stage 2 and stage 3 were determined as 77.6%, 77.8% and 80.0%, respectively, and no difference was found between the stages in this respect. There was no significant difference between AKIN stages in terms of age, co-morbidity, presence of sepsis and need for mechanical ventilation. While there was no significant difference between

Table 1. RIFLE, AKIN, KDIGO criteria according to serum creatinine value

Stage	Serum creatinine		
	RIFLE*	AKIN**	KDIGO**
Risk/stage 1	≥1.5 fold increase	1.5-2 fold increase or ≥0.3 mg/dL increase	1.5-1.9 fold increase or ≥0.3 mg/dL increase
Damage/stage 2	≥2 fold increase	>2-3 fold increase	>2-2.9 fold increase
Failure/stage 3	≥3 fold increase or when 4 mg/dL, sudden increase of 0.5 mg/dL and over	>3 fold increase or when 4 mg/dL, sudden increase of 0.5 mg/dL and over	≥3 fold increase or >4 mg/dL

*In addition to the serum creatinine value, a 25-50% decrease in glomerular filtration rate is defined as "risk", a 50-75% decrease as "damage", and a decrease of 75% or more as "failure". **The need for dialysis corresponds to stage 3 acute kidney injury, regardless of other criteria. RIFLE: Risk, injury, failure, loss of kidney function; end-stage kidney disease,AKIN: Acute kidney injury network, KDIGO: Kidney disease: Improving global outcomes

the stages in terms of SAPS-II score, the mean APACHE-II score of AKIN stage 1 was found to be higher than the other stages (p<0.019). Hemodialysis was applied to 17 (25.4%) patients in stage 1, 13 (28.9%) patients in stage 2, and 20 (57.1%) patients in stage 3 during the intensive care follow-up after the diagnosis of AKI (p=0.004) (Table 3).

Characteristics of AKI Stages According to KDIGO Criteria

Of the 155 patients included in our study, 18.7% were classified as stage 1, 27.1% as stage 2, and 54.2% as stage 3. Mortality rates in stage 1, stage 2 and stage 3 were found to be 72.4%, 64.3% and 85.7%, respectively, and the mortality rate in stage 3 was higher than in other stages (p=0.02). There was no difference between KDIGO stages in terms of age, comorbid diseases, presence of sepsis, need for mechanical ventilation, APACHE-II and SAPS-II scores. In the intensive care follow-up after the diagnosis of AKI, there was no patient in need of dialysis in stage 1, while hemodialysis was applied in 9.5% of patients in stage 2 and 57.1% of patients in stage 3 (p=0.000) (Table 3).

Table 2. Clinical and demographic characteristics of the study group

Characteristics	Whole population (n=155)
Age (year)	62 (18-89)
Gender (female), n (%)	66 (42.6)
Duration of intensive care monitorization (day)	8 (2-2190)
APACHE-II	24.6±8.8
SAPS-II	55.9±19.7
Comorbidity, n (%)	
Cardiovascular difease	78 (50.3)
COPD	26 (16.8)
Cirrhosis	37 (23.9)
Malignancy	16 (10.3)
Sepsis, n (%)	92 (59.4)
Those with the need of vasopressor, n (%)	148 (95.5)
Those with the need of mechanical ventilator, n (%)	143 (92.3)
Those ubdergoing hemodialysis, n (%)	52 (33.5)
Mortality, n (%)	120 (77.4)
Basal creatinine (mg/dL)	
RIFLE*	0.85 (0.42-1.3)
AKIN**	0.98 (0.42-3.1)
KDIGO*	0.85 (0.42-13)
Highest creatinine (mg/dL)	
RIFLE*	2.39 (1-7.91)
AKIN**	1.9 (0.79-7.60)
KDIGO*	2.39 (1-7.91)

*The creatinine value corresponding to 75 mL/min/1.73 m^2 of glomerular filtration rate according to the modification of diet in renal disease study formula was accepted as the basal value in patients whose basal creatinine value was unknown. **The lowest value in the first 48 hours evaluated for acute kidney injury was accepted as the basal creatinine value. AKI: Acute kidney injury, APACHE: Acute physiology and chronic health evaluation, SAPS: Simplified acute physiology score, COPD: Chronic obstructive pulmonary disease, RIFLE: Risk, injury, failure, loss of kidney function, AKIN: Acute kidney injury network, KDIGO: Kidney disease: Improving global outcomes

DISCUSSION

In our study, 155 patients diagnozed with AKI were evaluated in terms of AKI severity and AKI-related mortality according to 3 different staging systems. According to the RIFLE criteria, the distribution rates in risk, damage and failure stages were determined as 14.8%, 40.0% and 45.2%, respectively. When similar studies were examined, the rates of 16.9-53% for the risk stage, 24.1-38.8% for the damage stage and 17.4-45.4% for the failure stage were reported and compared to the rates in our study, it was observed that the rates of patients in the risk stage were higher in most of these studies[4,7,9-11,14,17-20]. In our study, the distributions in the AKI stages determined according to the AKIN criteria for the same patients were 45.6%, 30.6% and 23.8% for stages 1, 2, 3, respectively, and it was seen that the number of patients was higher in the early AKI stages, compared to other classifications. In addition, 8 patients remained outside the definition of AKI. In similar studies, patient distribution rates were reported as 24.1-59.2% in stage 1, 12.5-20.7% in stage 2, and 27.2-48.5% in stage 3, and similar to our study, most of them were observed to be in the early stages[4,10,11,14,17-19,21]. When the KDIGO classification was used for AKI staging in the study group, the distribution rates of stage 1, stage 2, and stage 3 patients were found as 18.7%, 21.7% and 54.2%, respectively. In similar studies, the reported patient rates for stages 1, 2 and 3 were 19.5-70.9%, 11.7-28.3% and 12-45%, respectively[4,13,14,17-19]. In our study, distributions in KDIGO staging were generally similar to those in the literature. Although all of the aforementioned studies were intensive care reports, it was observed that the distribution of patients in AKI stages was different from each other due to some factors such as the use of the urine criterion, the consideration of different criteria in determining the basal creatinine value, the difference in the monitorization periods selected to determine the severity of AKI, and the different characteristics of the study groups in some studies. This situation makes it difficult to comment on which classification is more accurate in determining the diagnosis and stage of AKI.

In our study group, in which the rates of sepsis and mortality were very high, the rate of late-stage AKI was found to be higher in both when the KDIGO and RIFLE classifications were used. In the AKIN classification, the rate was higher in the early stage AKI group. Compared to KDIGO and RIFLE classifications, it was observed that the median of basal serum creatinine value was higher in the AKIN classification, and the median of the highest serum creatinine value that determined the AKI stage

was lower. Therefore, due to the small difference between these two values, more patients seem to be in the early stage AKI group. In addition, the limitation of AKIN staging to the 48-hour period ignores the possible increase in serum creatinine compared to the 1-week evaluation period in the RIFLE and KDIGO classifications. This may be another reason for why more patients are included in earlier AKI stages in AKIN-based classification compared to other staging systems. For similar reasons, was reported that when the AKIN classification was applied, more patients were not diagnosed with AKI compared to other classifications. There are studies in the literature with similar comments regarding the AKIN classification[10,13,14,17,18].

In our study, it was observed that mortality increased in parallel with the severity of AKI determined according to each of the 3 staging systems, but this increase was found to be statistically significant in favor of stage 3 only in the KDIGO classification. However, AKI stages were not found to be determinative for mortality in all three classifications. In most of the similar studies, correlation was found between AKI severity and mortality in all three classifications[8,13,15,17,18]. The patient groups in these studies are quite heterogeneous. On the other hand, in a study of Pereira et al.[4] that included 457 septic patients and compared the relationship of RIFLE, AKIN, and KDIGO classifications with mortality, although AKIN and KDIGO classifications were the predictors of mortality, no correlation was found between AKI stages and mortality. In another study in which 1.036 patients were evaluated, a correlation was found between stage 2 and 3 AKI and mortality in all three classification systems[19].

Study Limitations

The main limitation of our study is the small number of patients compared to similar studies in the literature. Moreover, we think that the similarity of hospitalization indications in our study group reduces the heterogeneity among patients. Another limitation of ours is the inability to use the urine criterion in

Table 3. Comparison of AKI stages determined according to RIFLE, AKIN and KDIGO classifications

Parameter	Stage			p
RIFLE	**Risk**	**Damage**	**Failure**	
Number of patients, n (%)	23 (14.8)	62 (40.0)	70 (45.2)	-
Age (years)	59 (26-87)	62 (18-88)	65 (18-89)	0.687
Comorbidity, n (%)	20 (87.0)	57 (91.9)	63 (90.0)	0.782
APACHE-II	23.4±9.1	23.7±8.5	25.8±9.0	0.319
SAPS-II	46.7±19.0	56.3±19.9	58.4±19.0	0.049
Sepsis, n (%)	12 (52.2)	39 (62.9)	41 (58.6)	0.659
Hemodialysis, n (%)	3 (13.0)	12 (19.4)	37 (52.9)	0.000
Death, n (%)	15 (65.2)	48 (77.4)	57 (81.4)	0.272
AKIN	**Stage 1**	**Stage 2**	**Stage 3**	
Number of patients, n (%)	67 (45.6)	45 (30.6)	35 (23.8)	-
Age (years)	61 (26-89)	61 (18-81)	60 (18-88)	0.581
Comorbidity, n (%)	61 (91.0)	42 (93.3)	30 (85.7)	0.699
APACHE-II	22.8±9.1	25.0±8.9	28.0±7.8	0.019
SAPS-II	51.4±18.4	58.4±20.6	59.6±19.7	0.068
Sepsis, n (%)	40 (59.7)	26 (57.8)	23 (65.7)	0.757
Hemodialysis, n (%)	17 (25.4)	13 (28.9)	20 (57.1)	0.004
Death, n (%)	52 (77.6)	35 (77.8)	28 (80.0)	0.763
KDIGO	**Stage 1**	**Stage 2**	**Stage 3**	
Number of patients, n (%)	29 (18.7)	42 (27.1)	84 (54.2)	-
Age (years)	62 (26-87)	62 (18-88)	65 (18-89)	0.709
Comorbidity, n (%)	24 (82.8)	40 (95.2)	76 (90.5)	0.216
APACHE-II	23.4±8.1	23.7±8.8	25.5±9.1	0.408
SAPS-II	48.1±18.5	56.1±19.6	58.4±19.6	0.055
Sepsis, n (%)	19 (65.5)	21 (50.0)	52 (61.9)	0.332
Hemodialysis, n (%)	0 (0)	4 (9.5)	48 (57.1)	0.000
Death, n (%)	21 (72.4)	27 (64.3)	72 (85.7)	0.020

*A p value of <0.05 was considered statistically significant. RIFLE: Risk, injury, failure, loss of kidney function, end-stage kidney disease, AKIN: Acute kidney injury network, KDIGO: Kidney disease: Improving global outcomes, AKI: Acute kidney injury, APACHE-II: Acute physiology and chronic health evaluation, SAPS: Simplified acute physiology score

the diagnosis and staging of AKI, since the urine output of all patients could not be followed closely and appropriately. Finally, our high mortality rate, possibly due to causes other than AKI, made it difficult to compare mortality rates between AKI stages.

CONCLUSION

AKIN staging seems to be more applicable than the RIFLE and KDIGO criteria since it eliminates the need for baseline creatinine, includes the need for hemodialysis in the diagnosis, and suggests a shorter time window for the timing of diagnosis. However, due to these reasons, it is possible to reflect the severity of AKI as lowere than it is. The last of the three staging systems, the KDIGO criteria, which have been reported to diagnose AKI with a higher frequency in comparative studies and to predict the relationship between AKI severity and outcomes more accurately, have been used more frequently in recent years. However, the etiology of AKI is ignored in all three diagnostic systems. Considering the etiology-related parameters and the presence of early histological changes in the AKI process, we think that the inclusion of biomarkers in the diagnostic criteria may significantly increase the validity of existing classifications and contribute positively to patient follow-up.

Ethics

Ethics Committee Approval: Ethics Committee approval of Dokuz Eylül University University Faculty of Medicine was obtained (approval number: 252/2009).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.B., S.E., A.Ç., C.Ç., T.Ç., A.S., Design: N.B., S.E., A.Ç., C.Ç., T.Ç., A.S., Data Collection or Processing: N.B., S.E., H.A.B., Analysis or Interpretation: N.B., A.S., Literature Search: N.B., H.A.B., Writing: N.B.

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Training Course Results of the Assessment of Modified Rodnan Skin Score in Scleroderma

Sklerodermada Modifiye Rodnan Cilt Skoru Değerlendirilmesinin Eğitim Kursu Sonuçları

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ABSTRACT

Aim: Modified Rodnan Skin Score (mRSS) is generally used to assess skin involvement in patients with scleroderma. There are conflicting data on the effectiveness of mRSS training courses in previous studies. The aim of this study to evaluate the effectiveness of a mRSS training course applied to rheumatology minor residents.

Materials and Methods: Ten rheumatology minor residents were included in the study. The participants were given a 1-hour theoretical training including skin involvement in scleroderma and mRSS evaluation, by three experienced rheumatology specialists. Then training continued with performing mRSS on 4 patients with scleroderma for 1 hour. Participants made scores on a paper form on 2 patients before and after the training, including 17 regions, and the total score was between 0 and 51. Inter- observer reliability for pre- and post-training was evaluated with intra-class correlation coefficient (ICC) analysis. Agreement was evaluated with Fleiss's kappa according to 12 different score regions before and after the training.

Results: The ICC was detected as 0.867 [95% confidence interval (CI): 0.625-1.00, p=0.05] before the training, and 0.905 (95% CI: 0.045-1.00, p=0.02) after the training. When the regions of mRSS were evaluated individually, an increase in agreement was observed in some of the scores while there was no change in one region and a decrease in agreement was observed in some.

Conclusion: This study has shown that mRSS is an effective scoring that can be easily conveyed with training courses.

Keywords: Scleroderma, skin thickening, modified Rodnan Skin Score, training

ÖZ

Amaç: Sklerodermalı hastalarda deri kalınlığının değerlendirilmesi için genellikle modifiye Rodnan Cilt Skoru (mRCS) kullanılmaktadır. Literatürde mRCS eğitim kurslarının etkinliğine dair farklı veriler mevcuttur. Çalışmamızın amacı romatoloji yan dal asistanlarına uygulanan mRCS eğitim kursunun etkinliğinin değerlendirilmesidir.

Gereç ve Yöntem: Çalışmaya 10 romatoloji yan dal asistanı dahil edildi. Katılımcılara skleroderma konusunda deneyimli 3 romatoloji uzmanı tarafından, deri tutulumu ve mRCS değerlendirilmesini içeren 1 saatlik teorik eğitim verildi. Ardından 1 saat süreyle 4 hasta üzerinde pratik eğitim uygulandı. Katılımcılar 2 hasta üzerinde eğitim öncesi ve sonrası 17 bölgeyi içerecek ve toplam skor 0-51 aralığında olacak şekilde kağıt form üzerinde skorlama yaptılar. Daha sonra eğitim öncesi ve sonrası mRCS için gözlemciler arası güvenilirlik, sınıf içi korelasyon katsayısı (intraclass correlation, ICC) analizi ile değerlendirildi. Eğitim öncesi ve sonrası 12 ayrı Rodnan skor bölgesine göre uyumun Fleiss's kappa ile değerlendirilmesi yapıldı.

Bulgular: mRCS skoru için ICC değeri eğitim öncesi 0,867 [%95 güven aralığı (GA): 0,625-1,00, p=0,05], eğitim sonrası 0,905 (%95 GA: 0,045-1,00, p=0,02) olarak hesaplandı. Tek tek Rodnan skor bölgelerine bakıldığında eğitim sonrasında bir kısmında uyumda artış gözlendi, bir bölgede değişim olmazken, bir kısmında ise uyumda azalma tespit edildi.

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Sonuç: Bu çalışma göstermektedir ki, mRCS, eğitim kursları ile kolaylıkla aktarılabilen etkin bir skorlamadır.
Anahtar Kelimeler: Skleroderma, deri tutulumu, modifiye Rodnan Cilt Skoru, eğitim

INTRODUCTION

Systemic sclerosis (SSc) is a chronic inflammatory connective tissue disease that is characterized by skin fibrosis and can involve many organs such as the heart, lung, kidney, and gastrointestinal system[1]. SSc is divided into two main categories as diffuse cutaneous SSc and limited cutaneous SSc. The diffuse cutaneous form is more associated with mortality and morbidity, and skin involvement may progress rapidly in this form of the disease[2].

It has been shown that the increase in skin thickness in patients with diffuse cutaneous SSc, reflecting the severity of the disease, is associated with the involvement of internal organs and increased mortality. In this respect, skin score measurement is important[3]. In addition, improvement in skin score is associated with better clinical outcomes[2]. One of the best ways to identify high-risk patients for clinical trials is to measure the severity and extent of skin involvement[4].

Modified Rodnan Skin Score (mRSS), which is a measure of skin involvement, is used as a primary outcome measure in multicenter clinical studies because its feasibility, reliability and validity have been demonstrated[5].

While creating the scoring, the skin thickness of the distal forearm in patients with SSc was first scored by skin palpation (using a scale of 0-4) and this value was compared with the weight of the skin punch biopsy taken from the same site. A good correlation has been demonstrated between the degree of clinical palpation and the weight of the biopsy specimen[6]. Subsequently, the clinical palpation score method was used to estimate skin thickness at 26 skin sites, and the first full description of this methodology was published in 1982 by Clements et al.[3] in a controlled study of D-penicillin in SSc. A few years later, this scoring was modified using a 0-3 scale across 17 body regions. Nine of the skin regions [neck (1), shoulders (2), breasts (2), back (1), waist (1), toes (2)] were excluded from the calculation due to high inter-observer variation. One of the first applications of mRSS was a randomized clinical trial comparing high dose versus low dose D-penicillin in the early stages of diffuse cutaneous SSc[7]. Although there are scoring systems developed by other researchers, it has been agreed over time that mRSS is the gold standard for measuring skin thickness in SSc[8].

Modified RSS stands out with its easy applicability. The aim of this study is to evaluate the effectiveness of a practically applied mRSS course given to rheumatology minor residents by means of pre-training and post-training scorings.

MATERIALS AND METHODS

Selection and Definition of Cases

Ten rheumatology minor assistants studying at different universities were included in the study. Minor residents had not previously attended a course on mRSS. The participants were given theoretical training on skin involvement and mRSS evaluation, which lasted for 1 hour, by 3 rheumatology specialists experienced in SSc. Then, practical training was applied on 4 patients for 1 hour. Participants scored on a paper form before and after the training, including 17 regions on 2 patients and the total score between 0 and 51 (Figure 1). The forms of the participants were collected and evaluated.

The study were approved by the Dokuz Eylül University of Ethics Committee (protocol number: E-36862155, date: 14.09.2021).

Technical Information

Modified Rodnan Skin Score Calculation

In the calculation of mRSS, which is applied based on the literature, a total value is obtained by squeezing the skin between the fingers in 17 different parts of the body (face, chest, abdomen, as well as right/left fingers, hands, forearms, arms, thighs, legs, feet) and scoring the skin thickness between 0 and 3 (0=normal, 1=mild thickness, 2=moderate thickness, 3=severe thickness) (total score 0-51). Finally, three more anatomical regions (neck, upper back and lower back) were included in the scoring system[5,9].

How to apply the skin thickness measurement during the training was also explained on the patient. For this measurement, the skin should be slightly rounded or pinched using the index finger and thumb, or as a second method, the fold formed on the skin by the laterals of both thumbs should be examined. During measurement, it is important to understand the relative distribution of subcutaneous adipose tissue and underlying musculoskeletal structures in different anatomical regions.

a. mRSS=0: normal skin,

b. mRSS=1: thickened skin,

c. mRSS=2: thickened and unable to pinch skin,

d. mRSS=3: thickened and unable to move skin[10].

Statistical Analysis

Inter-observer reliability for pre- and post-training mRSS was evaluated with intraclass correlation (ICC) analysis using the Statistical Package for the Social Sciences 15 software. Compliance was evaluated with the Fleiss's kappa according to 12 different Rodnan score regions before and after the training.

RESULTS

The ICC value for the total Rodnan score was 0.867 before training [95% confidence interval (CI): 0.625-1.00; p=0.05] and 0.905 (95% CI: 0.045-1.00, p=0.02) after training. When the mRSS score regions were examined separately, an increase in compliance was observed in some of them after the training. There was no change in one of the score regions, while a decrease in compliance was detected in some (Table 1).

DISCUSSION

In this study, it was determined that the intra-class correlation coefficient increased after the training in the pre- and post-training evaluation of a theoretical and practical mRSS course given to rheumatology minor residents. In addition to theoretical information, it is seen that a training with this method, which includes evaluating on patients, enables mRSS to be taught in a short time to residents who are receiving rheumatology minor education.

There are different mechanical devices such as durometer, t ultrasonography, elastometer, caliper, and tonometer that have been fully or partially validated to measure skin thickness[11-13]. However, mRSS is considered the most appropriate technique for assessing skin thickness. Various studies have shown that mRSS assessment is easily applicable and reliable, repeatable, accurate, and sensitive to change[5,9,12,14,15]. Evaluation of mRSS requires experience and careful learning[15]. The intra-observer correlation coefficient (ICC) may be low if scoring is done by inexperienced rheumatologists[16].

The European Scleroderma Trials and Research Group (EUSTAR) conducted a study to standardize the mRSS measurement. In the study, which aimed to develop an effective methodology for teaching mRSS, it was stated that the coefficient of variation and ICC improved significantly in the repeated course, although the results of the first course were not satisfactory. In the aforementioned study, while the ICC increased from 0.496 to 0.722 to the "good" level, the coefficient of variation decreased from 54% to 32%. These results were comparable to the two previous studies [18.3% standard deviation (SD) 4.6

	Right				Left			
Fingers	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Hands	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Forearms	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Arms (above the elbows)	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Face			0 ☐	1 ☐	2 ☐	3 ☐		
Anterior chest			0 ☐	1 ☐	2 ☐	3 ☐		
Abdomen			0 ☐	1 ☐	2 ☐	3 ☐		
Thighs	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Legs (below the knees)	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Feet	0 ☐	1 ☐	2 ☐	3 ☐	0 ☐	1 ☐	2 ☐	3 ☐
Column total								
Total								

0=Normal, 1=Mild thickening, 2=Moderate thickening, 3=Severe thickening

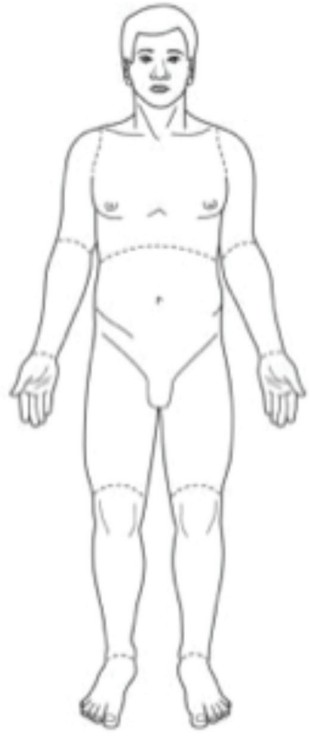

Figure 1. mRSS document used at the bedside in the study

mRSS: Modified Rodnan Skin Score

and 17.7% (SD 4.6), respectively][15,17]. With this study, it has been confirmed that mRSS is a suitable tool for measuring skin score, and it has been shown that it is possible to teach skin score measurement technique to many rheumatologists in a short time.

In a previous study, the interobserver variation of mRSS was reported to be approximately 50%[5]. The outcome of this course is also similar after repeated training. The initially good ICC did not increase among experienced rheumatologists, but increased from 0.50 to 0.72 in less experienced rheumatologists. This highlights the importance of the second training course for less experienced rheumatologists. It is encouraging that the intra-observer variability is around 20%. Although it had a low ICC value, it was observed that the second repeated teaching course was generally satisfactory and this was in line with previous experience[14]. For example, in the first EUSTAR/EULAR course, 100 young rheumatologists were trained and the intra-patient SD values were determined at a relatively good level (5,4)[5]. Most participants had low ICC results. Inter-observer variability after a single training still persisted despite experienced experts educating students, but after repeated training, inter-observer variability was significantly reduced. After the completion of the course, the intra-patient SD for interobserver variability was detected to be 5.5. A good ICC value was also obtained in the mixed patient population. Consequently, given the significant observer variability, it has been suggested that teaching courses be repeated at least twice for inexperienced rheumatologists[16].

There is also a study in the literature showing that good results of repetitive training courses can be stabilized. In this study, it was observed that the good initial ICC results obtained in two courses held 7 months apart continued to be similar. Therefore, the investigators suggested that training with 2 different subgroups of patients with a large number of diffuse-limited cutaneous SSc patients contributes to obtaining good results and a repeat course may not be necessary. However, it was stated that if there were significantly different scores between the trainer and the participant, a second training would be necessary. Higher coefficients of variation were obtained in patients with limited cutaneous SSc than in the diffuse cutaneous SSc subgroup[18].

Although it is accepted that the modified RSS tends to worsen in early disease and improve in late disease, the time when the peak value is reached has not been clearly determined. In early diffuse cutaneous SSc, rapid and severe skin thickness increase often develops 1-3 years after the onset of the disease. In the late stage, softening is observed as a result of the decrease in the extent and severity of induration of the skin[19,20]. One of the main goals of modified RSS teaching is to distinguish between the active period with skin thickening and the chronic period with skin atrophy.

Study Limitations

As a limitation of the study, our training was not repeated after a certain period of time, as suggested in previous studies, but was applied in a single step. For the optimization of the results,

Table 1. mRSS results by score regions before and after training

Region	Before training		After training	
	Fleiss's Kappa (95% CI)	Compliance, %	Fleiss's Kappa (95% CI)	Compliance, %
Right finger	0.38 (0.003, 0.73)	**53.3**	0.60 (-0.18, 1.00)	**70.0**
Left finger	0.42 (0.16, 0.68)	56.6	0.29 (0.29, 0.29)	46.6
Right hand	0.02 (0.02, 0.02)	**26.6**	0.11 (-0.06, 0.29)	**33.3**
Left hand	0.16 (-0.11, 0.42)	**36.6**	0.16 (-0.11, 0.42)	**36.6**
Right forearm	0.29 (0.29, 0.29)	46.6	0.02 (0.02, 0.02)	26.6
Left forearm	0.29 (0.29, 0.29)	46.6	0.02 (0.02, 0.02)	26.6
Right upper arm	0.11 (-0.06, 0.29)	33.3	-0.22 (-0.11, 0.06)	23.3
Left upper arm	0.24 (0.16, 0.33)	43.3	0.02 (0.02, 0.02)	26.6
Face	0.24 (0.16, 0.33)	43.3	0.02 (0.02, 0.02)	26.6
Chest	0.24 (0.16, 0.33)	43.3	0.16 (-0.11, 0.42)	36.6
Abdomen	0.16 (-0.11, 0.42)	36.6	0.11 (-0.06, 0.29)	33.3
Right upper leg	0.02 (0.02, 0.02)	**26.6**	0.29 (0.29, 0.29)	**46.6**
Left upper leg	0.02 (0.02, 0.02)	**26.6**	0.20 (-0.50, 0.90)	**40.0**
Right lower leg	0.38 (0.003, 0.73)	53.3	0.24 (0.16, 0.33)	43.3
Left lower leg	0.29 (0.29, 0.29)	46.6	0.20 (-0.50, 0.90)	40.0
Right foot	0.11 (-0.06, 0.29)	**33.3**	0.38 (0.003, 0.73)	**53.3**
Left foot	0.29 (0.29, 0.29)	**46.6**	0.42 (0.16, 0.68)	**56.6**

mRSS: Modified Rodnan Skin Score, CI: Confidence interval

it may be appropriate to increase the number of trainees and patients and the duration of the course in future studies.

CONCLUSION

This study shows that the training of a relatively inexperienced group by rheumatologists specialized in mRSS can be done effectively and simply. Considering that skin involvement of scleroderma is a good indicator of the course of the disease, it seems that a scoring such as mRSS has a place not only for use in studies but also for patient follow-up. For this reason, it seems appropriate to make it a part of the education through practically applied courses during rheumatology minor education.

Ethics

Ethics Committee Approval: The study were approved by the Dokuz Eylül University of Ethics Committee (protocol number: E-36862155, date: 14.09.2021).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.C., F.Ö., S.S.K., A.A., M.B., Design: G.C., F.Ö., D.S., A.A., M.B., Data Collection or Processing: G.C., A.K.A., S.B.K., G.K., Analysis or Interpretation: G.C., A.K.A., D.S., Literature Search: G.C., S.B.K., Writing: G.C., G.K.

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ORIGINAL ARTICLE

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The Effect of Vulvar Lichen Sclerosus on Urogynecological Functions in Postmenopausal Women

Postmenopozal Kadınlarda Vulvar Liken Sklerozun Ürojinekolojik Fonksiyonlara Etkisi

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ABSTRACT

Aim: To examine the relationship between urinary symptoms and vulvar lichen sclerosus.

Materials and Methods: This cross-sectional study was performed in our clinic between January 2019 and March 2020. One hundred and ten patients were included in the study. They were all postmenopausal women and were divided into two groups: the study group comprised of women diagnosed with lichen sclerosus (n=59), and the control group comprised of postmenopausal women who sought a routine gynecological examination (n=51). In the study group, all patients were dermatopathologically diagnosed with biopsy. Both groups completed two validated questionnaires: the Urogenital Distress Inventory (UDI-6) and the Incontinence Impact Questionnaire (IIQ-7). The total scores from questionnaires and sub-groups of UDI-6 and IIQ-7 were also analyzed in detail.

Results: There were no differences in demographic features or in time from the onset of menopause for both groups. Total UDI-6 scores were significantly higher in the study group ($p<0.05$). Scores in the sections of the UDI-6 that included irritative symptoms and urinary incontinence were also higher in the study group ($p<0.01$). Total IIQ-7 scores were also significantly higher in the study group ($p<0.05$). The IIQ-7 sub-scores regarding physical activity and travel were significantly higher in the study group ($p<0.01$).

Conclusion: To the best of our knowledge, this is the first study in the literature reporting the association between vulvar lichen sclerosus and urinary incontinence symptoms through validated objective tests. We demonstrated that both UDI-6 and IIQ-7 scores were significantly higher in patients with vulvar lichen sclerosus.

Keywords: Postmenopause, urogynecology, vulvar lichen sclerosus, UDI-6, IIQ-7

ÖZ

Amaç: Üriner semptomlar ile vulvar liken skleroz arasındaki ilişkiyi incelemektir.

Gereç ve Yöntem: Bu kesitsel çalışma Ocak 2019 ile Mart 2020 tarihleri arasında kliniğimizde yapılmıştır. Çalışmaya 110 hasta dahil edildi. Hepsi postmenopozal kadınlardan oluşan hastalar iki gruba ayrıldı. Çalışma grubu liken skleroz tanısı alan kadınlardan (n=59) ve kontrol grubu rutin jinekolojik muayene isteyen menopoz sonrası kadınlardan (n=51) oluşuyordu. Çalışma grubundaki tüm hastalara dermatopatolojik olarak biyopsi ile tanı konuldu. Her iki grup da valide edilmiş Ürogenital Sıkıntı Envanteri (UDI-6) ve İnkontinans Etki Anketi (IIQ-7) anketlerini doldurdu. UDI-6 ve IIQ-7'nin anket ve alt gruplarından alınan toplam puanlar analiz edildi.

Bulgular: Her iki grup için demografik özelliklerde veya menopoz başlangıcından bu yana geçen sürede fark yoktu. Toplam UDI-6 puanları çalışma grubunda anlamlı olarak daha yüksekti ($p<0,05$). Ayrıca, UDI-6'nın irritatif semptomlar ve üriner inkontinans bölümlerinin puanları da çalışma grubunda daha yüksekti ($p<0,01$). Toplam IIQ-7 puanları da çalışma grubunda anlamlı olarak daha yüksek bulundu ($p<0,05$). Fiziksel aktivite ve seyahat ile ilgili IIQ-7 alt puanları çalışma grubunda anlamlı olarak daha yüksekti ($p<0,01$).

Sonuç: Bildiğimiz kadarıyla çalışmamız, vulvar liken skleroz ile üriner inkontinans semptomları arasındaki ilişkiyi valide edilmiş objektif testler aracılığıyla bildiren literatürdeki ilk çalışmadır. Vulvar liken sklerozlu hastalarda hem UDI-6 hem de IIQ-7 skorlarının anlamlı derecede yüksek olduğu bulunmuştur.

Anahtar Kelimeler: Postmenopoz, ürojinekoloji, vulvar liken sklerozus, UDI-6, IIQ-7

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INTRODUCTION

Lichen sclerosus is a chronic, progressive dermatologic condition characterized by inflammation, thinning of the epithelium, and a variety of dermal changes, accompanied by symptoms of pruritus, pain, dyspareunia, and dysuria. This condition occurs in the anogenital region (85 to 98%). It can also be observed on any skin surface[1]. The majority of cases in women are the vulvar type of lichen sclerosus, which can occur at any age, but the most significant two peaks of onset are during the prepubertal and postmenopausal periods. Additionally, lichen sclerosus has been reported to occur in up to one in 30 elderly women and one in 59 women in a general gynecology practice[2]. The etiopathology of lichen sclerosus remains unknown. It is histopathologically defined as infiltration of T lymphocytes and hyalinization of the upper dermis[3].

Urinary incontinence is a highly prevalent disease, especially among postmenopausal women, and it causes social problems, psychological stress, and decreased quality of life. In the literature, studies on postmenopausal women with urinary incontinence and other medical problems are increasing. However, studies regarding the association between vulvar lichen sclerosus and urinary incontinence are limited in the literature. A few studies have suggested that vulvar lichen sclerosus can cause pelvic floor problems in addition to vulvar symptoms, causing bladder or bowel problems[4]. In 2018, Christmann-Schmid et al.[5] found that lower urinary tract symptoms are four times more frequent in women with vulvar lichen sclerosus than in those without. Urinary incontinence or overactive bladder syndrome is reported in patients with vulvar lichen sclerosus, but the specific clinical features of urinary incontinence and its effect on the quality of life have not been analyzed in detail.

This study aimed to assess the severity of incontinence and its effects on the quality of life in postmenopausal patients with vulvar lichen sclerosus, via validated questionnaires, namely, the Urogenital Distress Inventory (UDI-6) and Incontinence Impact Questionnaire (IIQ-7) tests.

MATERIALS AND METHODS

This cross-sectional study was conducted to examine the relationship between urinary symptoms and vulvar lichen sclerosus. Ethical approval was obtained from the Bakırköy Sadi Konuk Training and Research Hospital, Institutional Review Board and Local Ethics Committee (protocol number: 2019-07-11, date: 04.04.2019), and the study was conducted on the patients who came to the outpatient vulva clinic of our tertiary referral hospital, between January 2019 and March 2020.

Patients were divided into two groups: a study group comprising of patients who were diagnosed with vulvar lichen sclerosus (n=59) and a control group (n=51). All the cases of vulvar lichen sclerosus were dermatopathologically diagnosed via biopsy. Two dermatopathologic diagnostic criteria were determined for vulvar lichen sclerosus: the first criterion was the appearance of white sclerotic plaques with well-circumscribed atrophy; the second was the formation of histopathological findings including hyperkeratosis, epidermal atrophy, liquefaction degeneration, intradermal edema, lymphocytic infiltration, and hyaline homogenization of collagen fibers. The disease was confirmed as vulvar lichen sclerosus if it satisfies both criteria. However, the following diseases should be excluded: local scleroderma, chronic eczema, chronic dermatitis, vitiligo vulgaris, and lichen planus[6]. Therefore, all the biopsies were reviewed by a pathologist to confirm the diagnosis as containing these diagnostic criteria for our study.

The exclusion criteria were as follows: history or current use of hormone therapy locally, systemically, or both; chemotherapy or pelvic radiotherapy for cancer; thyroid disease; Cushing's disease; premature menopause occurring before the age of 40 years or surgically induced menopause. The exclusion criteria also included coexistent or present local scleroderma, chronic eczema, chronic dermatitis, vitiligo vulgaris, psoriasis, and lichen planus. As a reason, patients with lichen sclerosus and other vulvar dermatopathologies, which are pathologically similar, especially women with lichen planus, are prone to receiving an incorrect diagnosis of lichen sclerosus as well. Therefore, to maintain the integrity of our vulvar lichen sclerosus cohort, we chose to exclude women with other dermal diseases. In addition to dermal diseases, patients who were diagnosed with atrophic vaginitis, which may be associated with the urinary symptom scale, were not specifically included in the study. Additionally, to distinguish predisposing conditions for the development of urinary symptoms, women with neurological diseases (such as multiple sclerosis), diabetes, arterial hypertension, symptomatic pelvic organ prolapse, or previous operative vaginal deliveries and uterine myomas were excluded.

In this study, postmenopausal status was defined as having at least 12 consecutive months of amenorrhea with no other medical reason, and a level of follicle-stimulating hormone equal to or greater than 40 mIU/mL. The control group subjects, all healthy postmenopausal women scheduled for routine annual exams, were offered participation in the study at the time of registration in the clinic. Gynecological examinations of the healthy postmenopausal control patients were performed and those without vulvar and urogynecological findings were included in the study. These postmenopausal women were referred to specialists at our vulva outpatient clinic, and the study was explained to each patient. Therefore, from a total of 103 initially enrolled patients with vulvar lichen sclerosus, 59

patients were eligible for the final analyses during the study period, and 51 control group patients meeting the inclusion criteria were recruited to the study. Thus, after applying the inclusion and exclusion criteria, a total of 110 women were selected for inclusion in the study. Written informed consent was also obtained from each study patient.

The basic characteristics of the study population, such as age, gravidity, parity, and time period from the last menses, were recorded. The weight circumference and height measurements were obtained from the patients wearing light clothing and no shoes. The body mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m^2). Baseline demographic information of all study participants, such as age, gravidity, and parity, was analyzed.

The patients completed two validated questionnaires: the UDI-6, which screens for stress, irritative, and obstructive symptoms, and the IIQ-7, which reveals how these symptoms affect quality of life in terms of physical activity, travel, social relationships, and emotional health[7]. These are self-filled questionnaires that assess the degree of discomfort associated with urinary symptoms and assess their severity. These questionnaires were obtained after the diagnosis of vulvar lichen sclerosus and before its treatment. The total scores from the questionnaires and sub-groups of UDI-6 and IIQ-7 were also analyzed in detail.

Statistical Analysis

Data analysis was performed by using Statistical Package for the Social Sciences (SPSS) (version 20.0; SPSS, Inc., Chicago, IL, USA). Due to the cross-sectional study design and lack of pre-existing relevant validated objective test knowledge, the study by Christmann-Schmid et al.[5] was determined to be the most relevant study on the association between urinary tract symptoms and vulvar lichen sclerosus in the literature. A power analysis was performed to calculate the minimum sample size required for two independent study groups (alpha error=0.05 and 1-beta=0.95) by using the related study data, and at least 51 patients were required for each study group. All data were presented as the means and standard deviations. A one-sample Kolmogorov-Smirnov test was performed to analyze the distribution of the study variables. The Mann-Whitney U test was used to compare differences between nonparametric values. For all calculations, a p value of <0.05 was considered statistically significant.

RESULTS

Fifty-nine women with vulvar lichen sclerosus were included in our investigation. Demographic characteristics of these populations are shown in Table 1. There were no differences in mean age, gravidity, parity, BMI, or time from the onset of menopause between patients with lichen sclerosus and control patients.

Total UDI-6 scores were significantly higher in women with vulvar lichen sclerosus (p<0.05) (Table 2). The sections of the UDI-6 scores that included irritative symptoms and urinary incontinence were also higher in the lichen sclerosus group (p<0.01) (Table 2). Although the UDI-6 score of obstructive symptoms was higher in the vulvar lichen sclerosus group,

Table 1. Characteristics of patients with lichen sclerosus and control groups

	Lichen sclerosus (-) (n=51)	Lichen sclerosus (+) (n=59)	p value
	Mean±SD	Mean±SD	
Age (years)	57.53±6.66	60.19±10.62	0.13
Time since menopause onset (months)	10.18±5.31	10.97±7.31	0.91
Gravidity	3.37±3.54	3.51±3.21	0.24
Parity	2.55±3.51	3.01±1.95	0.21
BMI (kg/m^2)	28.86±3.53	29±4.29	0.93

Results are given in means±standard deviation. Student's t-test and Mann-Whitney U test were used for statistical analysis. A p value of <0.05 was considered significant.

BMI: Body mass index, SD: Standard deviation

Table 2. Symptom-related scores of the UDI-6 questionnaire in patients with lichen sclerosus and in controls

	Lichen sclerosus (-)	Lichen sclerosus (+)	p value
	Mean±SD	Mean±SD	
Irritative symptoms	1.37±1.86	3.02±2.36	**<0.01**
Urinary incontinence	0.71±1.28	1.59±2.02	**0.01**
Obstructive symptoms	0.37±0.77	0.59±1.49	0.97
UDI-6 total	2.45±3.44	5.21±5.18	**0.02**

Results are given in means±standard deviation. Student's t-test and Mann-Whitney U test were used for statistical analysis. A p value of <0.05 was considered significant for all bold values.

UDI-6: Urogenital Distress Inventory

Table 3. Symptom-related scores of the IIQ-7 questionnaire in patients with lichen sclerosus and in controls

	Lichen sclerosus (-)	Lichen sclerosus (+)	p value
	Mean±SD	Mean±SD	
Physical activity	0.76±1.35	2.08±2.33	**0.002**
Travel	0.41±1.01	1±1.52	**0.01**
Social relationship	0.24±0.58	0.39±0.89	0.56
Emotional health	0.06±0.23	0.22±0.91	0.56
IIQ-7 Total	1.47±2.82	3.69±4.93	**0.07**

Results are given in means±standard deviation. A p value of <0.05 was considered significant for all bold values.

IIQ-7: Incontinence Impact Questionnaire, SD: Standard deviation

there were no statistically significant differences between the study groups (p>0.05) (Table 2).

The IIQ-7 scores include physical activity, travel, social relationships, and emotional health questions. The total IIQ-7 scores were significantly higher in women with lichen sclerosus (Table 3). The IIQ-7 scores regarding physical activity and travel were significantly higher in the disease group (p<0.01) (Table 3). No statistically significant difference was seen in terms of the scores for social relationships and emotional health between the study groups (p>0.05) (Table 3).

DISCUSSION

To the best of our knowledge, this is the first study in the literature reporting the association between vulvar lichen sclerosus and urinary incontinence symptoms with validated objective tests. We demonstrated that both UDI-6 and IIQ-7 scores were significantly higher in vulvar lichen sclerosus patients.

When the scores were examined in more detail, the total UDI-6 scores for irritative, urinary incontinence, and obstructive symptoms were found to be higher in the vulvar lichen sclerosus group, and only the obstructive symptoms score was not significantly different between the study groups. Interestingly, the score for obstructive symptoms was lower than the scores for irritative symptoms and urinary incontinence. It is known that urinary incontinence symptoms can lead to irritative vulvar findings. Therefore, we think that urinary incontinence in vulvar lichen sclerosus patients may also affect the significant irritative symptoms score, which was determined in our study. There is a scarcity of data on the obstructive symptoms of vulvar lichen sclerosus due to erosions, labial fusions, and introital stenosis, all of which have a significant impact on the life of a woman[8,9]. Although it is thought that erosions, labial fusion, and stenosis, which may occur in vulvar lichen sclerosus patients, may increase obstructive symptoms, no significant difference was found in terms of the obstructive symptoms score between the groups in our study. Therefore, we may speculate that the pathological tissue changes of vulvar lichen sclerosus may explain the increasing irritative and urinary symptoms; however, more detailed studies should be performed in terms of the obstructive symptoms scale. Additionally, it is a known fact that the urinary system is negatively affected in the postmenopausal period. Our study significantly showed that patients with postmenopausal vulvar lichen sclerosus were predisposed to urinary incontinence. The coincidence of the vulvar lichen sclerosus natural age peak and the postmenopausal period, which particularly affects the urinary system, suggests the importance of the relationship between vulvar lichen sclerosus and the urinary symptoms.

The majority of data on vulvar lichen sclerosus focus on vulvar pruritus and irritation; however, this pathologic situation may affect urinary symptoms, and urologic symptoms may cause problems in physical activity, and in social and emotional health. The relationship of urinary symptoms, sexual dysfunction, and other social and emotional problems with vulvar lichen sclerosus is poorly understood, and only limited data are available regarding these symptoms in patients who have lichen sclerosus. Pinelli et al.[10] reported that sexual impairment could be a common consequence of lichen sclerosus, but further prospective, randomized studies are needed to delineate the management of this disease in postmenopausal women. Additionally, Burrows et al.[11] demonstrated the severity of emotional and sexual problems along with vulvar lichen sclerosus. When we analyzed the IIQ-7 scores, we observed that the physical activity and travel scores were significantly higher in women with lichen sclerosus. Interestingly, we found no statistically significant difference in the social relationship and emotional health scores between the study groups. However, Nieuwenhof et al.[12] reported that there were statistically more emotional issues than other problems in patients with vulvar lichen sclerosus. It is known that pelvic tissues are supported by an interconnected network that includes muscles, ligaments, endopelvic fascias, and skin. The structures that form this pelvic floor are in close anatomical and physiological association with each other. Unfortunately, the effect of vulvar lichen sclerosus on the pelvic floor is not fully understood. A study has found that vulvar lichen sclerosus can cause problems in organs near the pelvic floor, such as bowel and bladder[4]. Although these pathological changes may explain the urinary problems observed in vulvar lichen sclerosus patients, the effect on social and emotional problems is not clear. Therefore, social and emotional health issues linked to vulvar lichen sclerosus and the sociopsychological aspects of vulvar lichen sclerosus must be investigated by further studies.

Study Limitations

There are several limitations of our study. We had no access to the educational status of patients as a demographic variable. Additionally, mode of delivery type and history of episiotomy, which might affect urinary symptoms, were not included in the study analysis. The history of vaginal, vulvar, or urethral laceration during labor could not be analyzed because we could not get clear information from the anamneses of the postmenopausal patient group. Validated urogynecological tests such as UDI-6 and IIQ-7, which are widely used in studies, can create biases on total scores by further increasing irritative sub-scale scores in the progression of vulvar lichen sclerosus, which can lead to irritative symptoms by the very nature of the lichen sclerosus. Another major weakness of this study is the possibility of the coexistence of other vulvar pathologies, such as lichen planus or lichen chronicus, with

a proven lichen sclerosus diagnosis[13,14]. Future studies will be designed with a control group of different postmenopausal cohorts, on issues that might affect the urinary system and that could be analyzed, such as vulvar lichen planus, vulvar candidiasis, or atrophic vaginitis. Despite these limitations, our study highlights the need for increased attention to urinary symptoms when managing patients with vulvar lichen sclerosus.

CONCLUSION

In conclusion, our findings suggest that women with vulvar lichen sclerosus have more disturbed urinary symptoms than healthy women, and thorough management of vulvar lichen sclerosus requires a greater attention to these urologic symptoms. Additionally, the change in urogynecological problems after the routine treatment of vulvar lichen sclerosus must be investigated by further laboratory and clinical studies.

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Ethics

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Preoperative Low Serum Magnesium Level is a Significant Predictive Factor for Postoperative Hypomagnesemia in Patients Who Underwent Parathyroidectomy for Primary Hyperparathyroidism

Primer Hiperparatiroidi Cerrahisinde Ameliyat Öncesi Düşük Serum Magnezyum Düzeyi Postoperatif Hipomagnezemi için Önemli Bir Prediktif Faktördür

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ABSTRACT

Aim: Parathyroidectomy causes significant changes in mineral metabolism in patients with primary hyperparathyroidism (PHPT). Hypomagnesemia may be seen after parathyroidectomy with unknown mechanisms. Our study aimed to evaluate the severity of hypomagnesemia and its correlation with clinical and biochemical variables after the surgery.

Materials and Methods: A retrospective study was conducted in the patients with parathyroidectomy for PHPT between January 2017 and December 2020 in a single tertiary hospital. All consecutive patients with preoperative and postoperative magnesium levels (n=80) were included. Patients were divided into two groups according to the postoperative first-day serum magnesium levels: patients whose serum magnesium was <1.9 mg/dL (group HypoMg) and patients whose serum magnesium was ≥1.9 mg/dL (group NorMg). Demographic and clinical parameters and biochemical findings were recorded. The incidence of postoperative hypomagnesemia was the primary outcome.

Results: The mean age was 56.7±12.2 years. The female to male ratio was 3.21. There were 31 (38.8%) and 49 patients (61.2%) in group HypoMg and group NorMg, respectively. The groups were similar considering demographic and clinical parameters (p>0.05). There were no significant differences in the preoperative serum calcium, adjusted calcium, and parathyroid hormone levels between the groups (p>0.05). Preoperative magnesium levels were significantly lower in the HypoMg group (p<0.001). Postoperative serum magnesium levels were positively correlated with preoperative serum magnesium levels (r=0.719, p<0.001).

Conclusion: Postoperative hypomagnesemia may be seen after parathyroidectomy. It was significantly correlated with preoperative hypomagnesemia.

Keywords: Parathyroidectomy, primary hyperparathyroidism, magnesium, magnesium deficiency

ÖZ

Amaç: Paratiroidektomi, primer hiperparatiroidili hastalarda elektrolit metabolizmasında önemli değişikliklere neden olur. Açıklanamayan nedenlerle paratiroidektomi sonrası hipomagnezemi görülebilir. Çalışmamızda ameliyat sonrası hipomagnezeminin şiddetini, klinik ve biyokimyasal parametreler ile ilişkisini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Ocak 2017-Aralık 2020 tarihleri arasında hiperparatiroidizm nedeniyle paratiroidektomi ameliyatı yapılan hastalar retrospektif olarak değerlendirildi. Çalışmaya preoperatif ve postoperatif magnezyum düzeylerine ulaşılabilinen 80 hasta dahil edildi. Hastalar ameliyat sonrası birinci gün serum magnezyum düzeylerine göre iki gruba ayrıldı. Gruplar, serum magnezyum düzeyi <1,9 mg/dL olan (grup HypoMg) ve ≥1,9 mg/dL olan (grup NorMg) olarak belirlendi. Demografik, klinik parametreler ve biyokimyasal bulgular kaydedildi. Postoperatif hipomagnezemi ise birincil veri olarak değerlendirildi.

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Bulgular: Ortalama yaş 56,7±12,2 yıl idi. Kadın/erkek oranı 3,21 idi. Grup HypoMg'de 31 (%38,8) ve grup NorMg'de 49 (%61,2) hasta vardı. Gruplar demografik ve klinik parametreler açısından benzerdi (p>0,005). Gruplar arasında preoperatif serum kalsiyum, düzeltilmiş kalsiyum ve paratiroid hormon düzeyleri açısından anlamlı fark yoktu (p>0,05). Preoperatif magnezyum düzeyi ise HypoMg grubunda anlamlı olarak düşüktü (p<0,001). Postoperatif serum magnezyum değeri ile preoperatif değer arasında pozitif korelasyon vardı (r=0,719, p<0,001).

Sonuç: Paratiroid cerrahisi sonrası serum magnezyum eksikliği görülebilir. Bu klinik durum, özellikle preoperatif hipomagnezemi ile anlamlı derecede koreledir.

Anahtar Kelimeler: Paratiroidektomi, primer hiperparatiroidizm, magnezyum, magnezyum eksikliği

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a common endocrine disease. It occurs most commonly due to one or more autonomously functioning parathyroid adenomas. Parathyroid hyperplasia and rarely parathyroid carcinoma are other underlying pathologies for PHPT[1-3]. Hypercalcemia and high or inappropriately normal parathyroid hormone (PTH) levels are the major diagnostic criteria for the disease. Parathyroidectomy is regarded as the only opportunity for a definitive cure for PHPT[2]. The rapid decline in serum PTH and normalization or lowering of serum calcium following parathyroidectomy cause significant metabolic changes in the bone and mineral metabolism[2].

As the second most abundant intracellular cation and the fourth most abundant cation of the body, the metabolism of magnesium is directly related to that of calcium[2,4]. It is generally known that there is a positive correlation between calcium and magnesium levels[5]. Considering the possible pathophysiological mechanisms between magnesium, calcium, and PTH, it has been reported that magnesium levels regulate PTH secretion and induces an end-organ resistance to PTH[6-8]. Previous studies have shown that postoperative hypomagnesemia is linked to the onset of clinically relevant hypocalcemia following thyroidectomy[5,7-9]. In several case reports, it has also been mentioned that severe hypomagnesemia could be seen following parathyroidectomy for PHPT[10-12]. It seems very difficult to prove the exact mechanisms between serum magnesium and calcium levels in these patients[4,12].

Although there is no current guideline reporting serum magnesium measurements before and after parathyroidectomy, there may be a close relationship between postoperative serum levels of magnesium and calcium in PHPT patients after the surgery[2]. So, the influence of parathyroidectomy on serum magnesium levels in PHPT patients remains controversial. We hypothesized that there might be some degree of correlation between serum magnesium, calcium, and PTH levels in PHPT patients following parathyroidectomy.

This study aimed to investigate the possible relationships between preoperative and postoperative serum magnesium levels and parathyroidectomy.

MATERIALS AND METHODS

Study

A retrospective study of the patients who underwent parathyroidectomy for PHPT was conducted between January 2017 and December 2020 in a single tertiary hospital. The Institutional Review Board of Bağcılar Training and Research Hospital approved the study (protocol no: 2021.01.1.05.005, date: 15.01.2021). All procedures in this study were in agreement with the Declaration of Helsinki. We applied the institutional guidelines during data collection to ensure patient privacy and confidentiality. Written consent could not be taken from the patients due to the retrospective design of the study and unanimity of data.

Patients

All consecutive patients with parathyroidectomy for PHPT were recruited using the hospital information system. Diagnostic criteria for PHPT were as follows: albumin-corrected serum calcium >10.2 mg/dL and increased or inappropriately normal plasma PTH[1]. Patients with asymptomatic PHPT and simultaneous thyroidectomy were also included. The medical records of a total of 91 patients were analyzed. The patients without the measurements of preoperative and postoperative 1st-day serum magnesium levels (n=11) were excluded. Familial PHPT and lack of medical data were regarded as the other exclusion criteria. So, 80 patients were finally evaluated in the study.

The type of surgery was determined as minimally invasive, one-sided, and two-sided parathyroidectomy at the Endocrine Surgery Board based on the preoperative imaging findings. The surgical procedures were performed by the consultant surgeons of our endocrine surgery unit.

Patients were divided into two groups according to the postoperative 1st-day serum magnesium levels: patients whose serum magnesium was <1.9 mg/dL (group HypoMg) and patients whose serum magnesium was ≤1.9 mg/dL (group NorMg).

Variables

Demographic and clinical variables were collected using the medical records of the patients. Age, sex, history of osteoporosis, and medication use before or at the time of surgery (diuretics, proton pump inhibitors, bisphosphonates), operative details, and postoperative pathological diagnosis adenoma or double adenoma were recorded. Laboratory investigations included serum calcium and magnesium, serum adjusted calcium, PTH, and estimated glomerular filtration rate (eGFR) both preoperatively and on the postoperative first day. An intact PTH assay (Abbott Architect®, Illinois, USA) was used to measure the concentration of PTH (range 12-88 pg/mL). The EPI-CKD formula was used to calculate eGFR[1]. Patients were grouped based on the serum and plasma levels of calcium, adjusted calcium, and PTH as normal or high.

Statistical Analysis

The incidence of postoperative hypomagnesemia in the study group was the primary outcome. Postoperative hypomagnesemia with demographic, clinical, and laboratory parameters and the correlation of biochemical parameters with postoperative hypomagnesemia were the secondary outcomes.

Statistical analysis was performed using a statistical package (IBM, Statistical Package for the Social Sciences software, 21.0, Chicago, IL, USA). Descriptive statistics were given as mean±standard deviation and median with interquartile range (1-3) of 25% to 75% for continuous variables depending on their distribution. Numbers and percentages were used for categorical variables. The Kolmogorov-Smirnov test checked the normality of the numerical variables. In comparing two independent groups, the Independent Samples t-test was used where numerical variables had a normal distribution. In comparing the variables before and after the surgery, the paired t-test and Wilcoxon signed rank test were used. For variables without normal distribution, the Mann-Whitney U test was applied. To compare the differences between categorical variables, the Pearson chi-square and Fisher Freeman Halton tests were used in 2x2 tables. The Spearman and Pearson correlation coefficients depending on the type of distribution were calculated to analyze the relationships between numerical variables. Statistical significance was defined as $p<0.05$.

RESULTS

The mean age of all patients was 56.7±12.2 years. There were 19 (23.8%) male and 61 female patients (76.3%). There were 31 (38.8%) and 49 patients (61.2%) in group HypoMg and group NorMg. The groups were similar considering age and sex distribution (Table 1).

There were no significant associations between the postoperative hypomagnesemia and the incidence of osteoporosis and history of medications (diuretics, proton pump inhibitors, and bisphosphonates) between the groups ($p>0.05$).

The different types of parathyroidectomy showed almost equal distribution. Thyroidectomy was performed in 23 patients (28.8%). The types of parathyroidectomy and the incidence of coexisting thyroidectomy revealed no significant differences between the groups (Table 1).

In Table 2, the results of the preoperative biochemical investigations are given. The groups were similar regarding preoperative serum calcium, adjusted calcium, and PTH levels. The preoperative serum levels of magnesium were 1.76±0.26 mg/dL in the HypoMg group and 2.13±0.26 mg/dL in the NorMg group. The difference was statistically significant

Table 1. Demographic and clinical characteristics of the study groups

			Groups		p
		Overall (n=80)	HypoMg (n=31)	NorMg (n=49)	
Age (year)		56.7±12.2	58.9±10.6	55.3±13.0	0.200
Sex	Male	19 (23.8)	9 (29.0)	10 (20.4)	0.268
	Female	61 (76.3)	22 (71.0)	39 (79.6)	
Osteoporosis		24 (30.0)	7 (22.6)	17 (34.7)	0.184
Diuretic use		6 (7.5)	2 (6.5)	4 (8.2)	0.571
PPI		10 (12.5)	7 (22.6)	3 (6.1)	0.036
Biphosphonates		9 (11.3)	4 (12.9)	5 (10.2)	0.488
Surgery	Minimal invasive	28 (35)	9 (29.0)	19 (38.8)	0.542
	One-sided	28 (35)	13 (41.9)	15 (30.6)	
	Two-sided	24 (30)	9 (29.0)	15 (30.6)	
Thyroidectomy		23 (28.8)	10 (32.3)	13 (26.5)	0.380
Pathology	Adenoma	75 (93.8)	30 (96.8)	45 (91.8)	0.644
	Double adenoma	5 (6.3)	1 (3.2)	4 (8.2)	

(p<0.001). The incidence of the patients with low preoperative serum magnesium was significantly higher in the HypoMg group (p<0.001).

Considering the postoperative biochemical investigations, there were no significant differences in postoperative serum calcium, adjusted calcium, and PTH levels (Table 3). In 11 patients (13.75%), we detected low adjusted serum calcium levels. Postoperative magnesium levels were 1.7±0.2 mg/dL and 2.1±0.1 mg/dL in the HypoMg and NorMg groups (p<0.001).

Preoperative serum adjusted calcium, PTH, and magnesium levels reduced significantly after parathyroidectomy (p<0.0001, p<0.001, and p=0.009, respectively) (Table 4). The percentage of the reduction in serum magnesium levels in patients with osteoporosis [-2.7% (-6.1-2.1)] was not significantly different from the changes in patients without osteoporosis [-5.1 (-8.6-1.9)] (p=0.159).

Postoperative serum magnesium levels were positively correlated with preoperative serum magnesium levels (r=0.719, p<0.001) (Figure 1). Other parameters showed no significant correlations (Table 5).

DISCUSSION

This retrospective study showed that postoperative hypomagnesemia was significantly associated with preoperative hypomagnesemia. We found a significant positive correlation between preoperative and postoperative magnesium levels. We could not find any demographic, clinical, and laboratory variables predicting postoperative hypomagnesemia except

Table 2. Preoperative laboratory findings of the study groups

			Groups		p
		Overall (n=80)	HypoMg (n=31)	NorMg (n=49)	
Preop Ca		11.6 (11.1-12.3)	11.7 (11.0-12.6)	11.4 (11.1-12.0)	0.567
Adj. preop Ca		11.4 (10.8-11.9)	11.5 (10.8-12.5)	11.4 (10.8-11.7)	0.219
Preop Ca groups	Normal	6 (7.5)	3 (9.7)	3 (6.1)	0.429
	High	74 (92.5)	28 (90.3)	46 (93.9)	
Adj. preop Ca groups	Normal	8 (10)	2 (9.7)	6 (12.2)	0.512
	High	72 (90)	28 (90.3)	42 (87.8)	
Preop PTH		167.2 (130-326.8)	159.6 (122.4-300)	208.4 (145-366.3)	0.336
Preop PTH groups	Normal	3 (3.8)	2 (6.5)	1 (2.0)	0.332
	High	77 (96.3)	29 (93.5)	48 (98.0)	
Preop Mg		2.0±0.32	1.76±0.26	2.13±0.26	<0.001
Preop Mg groups	Low	31 (38.8)	26 (83.9)	5 (10.2)	<0.001
	Normal	49 (61.3)	5 (16.1)	44 (89.8)	
Preop eGFR		98.5 (88.0-106.8)	85.0 (97.0-104.0)	99.0 (89.0-111.0)	0.311

eGFR: Estimated glomerular filtration rate, Mg: Magnesium, PTH: Parathyroid hormone

Table 3. Postoperative laboratory findings of the study groups

			Groups		p
		Overall (n=80)	HypoMg (n=31)	NorMg (n=49)	
Postop Ca		9.0±0.9	9.1±0.8	9.5±0.7	0.596
Adj. postop Ca		9.3±0.8	9.5±0.7	9.2±0.9	0.127
Postop Ca groups	Low	28 (35)	11 (35.5)	17 (34.7)	0.726
	Normal/high	52 (65)	20 (64.5)	32 (65.3)	
Adj. postop Ca groups	Low	11 (13.75)	5 (16.1)	6 (12.2)	0.654
	Normal/high	69 (86.25)	26 (83.9)	43 (87.7)	
Postop PTH		26.6 (12.7-57.9)	25.6 (11.6-53.9)	32.7 (12.9-58.9)	0.632
Postop PTH groups	Normal	65 (81.3)	25 (80.6)	40 (81.6)	1.0
	High	15 (18.7)	6 (19.4)	9 (18.4)	
Postop Mg		1.9±0.3	1.7±0.2	2.1±0.1	<0.001
Postop eGFR		97 (88-106)	97 (89-100)	97 (84-109)	0.448

eGFR: Estimated glomerular filtration rate, Mg: Magnesium, PTH: Parathyroid hormone

for low preoperative serum magnesium levels. The absence of any correlation between calcium, PTH, and magnesium levels in patients who underwent parathyroidectomy was another striking finding in this study.

Several pathophysiological mechanisms are proposed to develop hypomagnesemia, including impaired intestinal absorption, increased gastrointestinal losses, or disturbances in renal magnesium handling[2,4]. Bowel preparation, blood transfusions, administration of catecholamines, and extracellular volume expansion are regarded as the other contributing mechanisms for hypomagnesemia[2]. We thought these contributing factors, except for a rapid decline in PTH and normalization or lowering of serum calcium levels after parathyroid surgery, are unlikely. So, postoperative hypomagnesemia might be directly related to postoperative PTH and calcium metabolism.

A highly significant negative correlation between serum calcium and magnesium levels in patients with PHPT was reported in a limited number of older studies[13,14]. Nevertheless, the association of serum magnesium, serum calcium, and PTH has been analyzed in patients with secondary hyperparathyroidism[4,6,15,16]. Fang et al.[4] studied the effect of PTH on serum magnesium levels in hemodialysis patients with secondary hyperparathyroidism. They found that hypermagnesemia was seen in 44% of the patients preoperatively, and there were significant decreases in serum magnesium levels immediately after the surgery. The severity was minimal at the first day, and gradually restored from the third day. Similar changes have been reported in patients with PHPT after parathyroidectomy[14,16]. They concluded that PTH might influence magnesium metabolism. Although magnesium is essential for PTH synthesis and release, it has also been mentioned that PTH increases gastrointestinal absorption and bone resorption of magnesium, leading to an increase in serum levels of magnesium[4]. In this study, we did not find significant correlations between calcium, PTH, and magnesium. So, we have difficulty showing a possible association between hypomagnesemia and a rapid decrease in PTH levels after parathyroidectomy, which necessitates prospective large-scale studies.

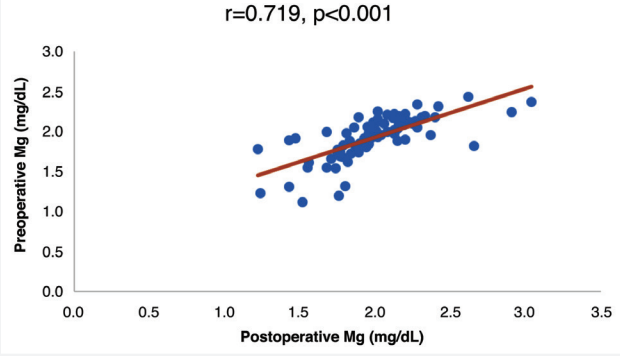

Figure 1. Correlation of preoperative and postoperative magnesium levels in the study group

Novodvorsky et al.[2] reported that a combination of hungry bone syndrome and hypoparathyroidism was the leading cause of early postoperative hypomagnesemia following parathyroidectomy. They found a rate of 7.1% for postoperative hypocalcemia in their parathyroidectomy series. There might be a shift of calcium and magnesium into the bones in these patients, which is the pathophysiological mechanism of the hungry bone syndrome[2]. In our study, low postoperative levels of adjusted calcium were seen in 13.75% of the cases without any clinical symptoms and signs of hypocalcemia. Contrary to Novodvorsky et al.[2] study, we could not detect a significant difference in the postoperative serum calcium levels depending on the postoperative magnesium levels.

Previous studies have commented that postoperative hypomagnesemia is associated with generalized bone disease[8,11,14]. King and Stanbury[14] reported that significant decreases in serum magnesium levels after parathyroidectomy were common. Besides, it was shown that it was sustained only in those with bone diseases and associated with prolonged postoperative hypocalcemia. Due to the retrospective design, we did not evaluate bone diseases in the study group. Investigation of severe bone diseases in PHPT patients may help to understand the pathophysiology of hypomagnesemia.

Study Limitations

Retrospective design and lack of postoperative follow-up period were the major limitations of this study. We

Table 4. Comparison of preoperative and postoperative levels of the laboratory variables

		95% CI		p
Postop vs. preop	Mean±SD	Lower	Upper	
Serum Ca	-2.83±1.33	-3.12	-2.53	<0.001
Adjusted Ca	-2.43±1.37	-2.73	-2.13	<0.001
PTH	-408.2±686.4	-561.0	-255.5	<0.001
Mg	-0.67±0.23	-0.12	-0.18	0.009
eGFR	0.15±13.6	-0.90	3.2	0.921

eGFR: Estimated glomerular filtration rate, Mg: Magnesium, PTH: Parathyroid hormone, SD: Standard deviation, CI: Confidence interval

Table 5. Correlation of postoperative serum magnesium levels

		Postop Mg
Age	r	-0.175
	p	0.123
Preop Ca	r	-0.870
	p	0.445
Adj. preop Ca	r	-0.132
	p	0.244
Preop PTH	r	0.023
	p	0.842
Preop Mg	r	0.719
	p	<0.001
Preop eGFR	r	0.097
	p	0.393
Postop Ca	r	-0.075
	p	0.508
Adj. postop Ca	r	-0.198
	p	0.078
Postop PTH	r	-0.054
	p	0.632
Postop eGFR	r	0.078
	p	0.497

eGFR: Estimated glomerular filtration rate, Mg: Magnesium, PTH: Parathyroid hormone

analyzed a single magnesium measurement taken on the first postoperative day. So, we could not contribute to trends in the postoperative magnesium levels. Due to the absence of preoperative or postoperative serum magnesium levels in some of our excluded cases, a selection bias might occur. We did not investigate these laboratory changes with clinical signs and symptoms of hypocalcemia and hypomagnesemia. Investigation of the clinical presentation of hypomagnesemia in these patients may help to increase the reliability of our results.

CONCLUSION

Hypomagnesemia may be seen after parathyroidectomy. It was significantly correlated with preoperative low magnesium levels. Prospective studies are needed to investigate the clinical importance of hypomagnesemia and its association with PTH and calcium metabolism.

Ethics

Ethics Committee Approval: The Institutional Review Board of Bağcılar Training and Research Hospital approved the study (protocol no: 2021.01.1.05.005, date: 15.01.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Evaluation of Disaster Medicine Knowledge Level and Educational Approaches of Future Health Professionals

Geleceğin Sağlık Profesyonellerinin Afet Tıbbı Bilgi Düzeyi ve Eğitim Yaklaşımlarının Değerlendirmesi

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ABSTRACT

Aim: This study aimed to determine the disaster medicine knowledge levels and educational approaches on disaster medicine of prospective healthcare students who are important factors of disaster response.

Materials and Methods: This cross-sectional descriptive study was conducted with final year students from the nursing department, the Emergency Aid and Disaster Management (EADM) Department, and the Medical Faculty at Tekirdağ Namık Kemal University in Tekirdağ, Turkey. The data were collected through a face-to-face administered questionnaire.

Results: Among the 159 study participants, 49% (n=78) of the participants had received disaster medicine education. The mean knowledge level of the EADM student group (78.96±10.56) was found to be higher than nurse (65.49±12.84) and medicine (72.33±10.56) student groups. Most of the students with high level of knowledge (n=56, 58.9%) participated in the disaster drill. Personal protective equipment (PPE) (n=30, 18.8%), decontamination (n=52, 32.7%) and triage (n=60, 37.7%) questions were respectively answered correctly with the lowest percentage. Students (n=82, 92.1%) who did not receive disaster medicine education stated that they wanted to receive disaster medicine education and most of students (n=115, 72.3%) preferred that disaster medicine courses be led by emergency medicine specialists.

Conclusion: Disaster medicine classes that address some special subjects like the use of PPE and decontamination procedures and triage should be included in the basic curriculum of health professions, and students' personal knowledge and competence perceptions on disaster medicine should be supported by reinforcing the learning outcomes with disaster drills.

Keywords: Disaster medicine, education, health profession students

ÖZ

Amaç: Bu çalışma, afet müdahalesinin önemli faktörleri olan sağlık öğrencilerinin afet tıbbı bilgi düzeylerini ve afet tıbbına yönelik eğitim yaklaşımlarını belirlemeyi amaçlamıştır.

Gereç ve Yöntem: Bu kesitsel tanımlayıcı tasarım çalışması, Türkiye'de Tekirdağ Namık Üniversitesi'ndeki Hemşirelik Bölümü, Acil Yardım ve Afet Yönetimi (AYAY) Bölümü ve Tıp Fakültesi son sınıf öğrencileri ile gerçekleştirilmiştir. Veriler yüz yüze uygulanan anket yoluyla toplanmıştır.

Bulgular: Araştırmaya katılan 159 kişiden %49'u (n=78) afet tıbbı eğitimi almıştır. AYAY öğrenci grubunun bilgi düzeyi ortalaması (78,96±10,5) hemşirelik (65,49±12,84) ve tıp (72,33±10,56) öğrenci gruplarına göre daha yüksek bulunmuştur. Bilgi düzeyi yüksek olan öğrencilerin büyük kısmı (n=56, %58,9) afet tatbikatına katılmıştır. Kişisel koruyucu ekipman (KKE) (n=30, %18,8), dekontaminasyon (n=52, %32,7) ve triyaj (n=60, %37,7) konularına ait sorular sırasıyla en düşük oranda doğru yanıtlanan sorular olarak tespit edilmiştir. Afet tıbbı eğitimi almayan öğrenciler (n=82, %92,1)

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afet tıbbı eğitimi almak istediklerini belirtmiş ve çoğu öğrenci (n=115, %72,3) afet tıbbı derslerinin acil tıp uzmanları tarafından verilmesini tercih etmiştir.

Sonuç: Sağlık mesleklerinin temel müfredatında KKE kullanımı, dekontaminasyon prosedürleri ve triyaj gibi konulara özel vurgu yapılan afet tıbbı derslerine yer verilmeli ve öğrenme çıktıları afet tatbikatları ile pekiştirilerek öğrencilerin afet tıbbı konusundaki kişisel bilgi ve yeterlilik algıları desteklenmelidir.

Anahtar Kelimeler: Afet tıbbı, eğitim, sağlık meslek öğrencileri

INTRODUCTION

Today's world has witnessed a steady increase in disasters, and greater populations of people are being directly or indirectly affected by these disasters[1-3]. This means, in effect, that there is a high possibility that at one point, other people will encounter disasters in their lifetime. Due to the random and unpredictable nature of most disasters, it is difficult to predetermine potential disaster victims and the teams that will be responsible for responding to disasters. Regardless of the professional field in which a doctor operates, they may be required to undertake an active role in patient care, field management, or incident command during disasters[4,5]. Students who are educated to be health professionals can also be assigned to provide health services in disasters as in various periods of history, or their voluntary participation in health service delivery can be encouraged as in the Coronavirus disease-2019 (COVID-19) pandemic process[6-11]. The American Medical Association, the Association of American Medical Colleges, and the World Association for Disaster and Emergency Medicine have all suggested that disaster medicine should be included in the medical education curriculum under various subjects[12-14]. Currently, disaster medicine is not at the desired level in global medical education curricula.

In examining medical education in Turkey in terms of disaster medicine training, it was found that the Undergraduate Medical Education National Core Education Program 2020 included only a few topics on disaster concepts under the title of Behavioral, Social, and Human Sciences Lists and that basic medical care practices included no courses on disaster medicine[15]. In other words, there is no standard disaster medicine education in medical faculties, and disaster medical care is not considered a professional medical field in Turkey. However, emergency medicine specialists receive education on the subject of disaster medicine as part of the "Emergency Medicine Education Core Curriculum"[16]. Like doctors, nurses also function as the foremost health care professionals responsible for providing healthcare services in disaster responses. Yet, despite the well-established, comprehensive content constituting the Nursing National Core Education Program in Turkey, the program lacks structured disaster medicine content[17]. The Emergency Aid and Disaster Management (EADM) program provides disaster management and undergraduate education on emergency situations in Turkey, as well as education on disaster medicine subjects and other fields of disaster. At the Tekirdağ Namık Kemal University, for students enrolled in the EADM department, the "Disaster Medicine I-II" course is mandatory; for nursing department students, the "Nursing Care During Disasters and First Aid" course is elective. There is, however, no structured disaster medicine course offered to students enrolled in the medical faculty.

The primary aim of this study is to determine the disaster medicine knowledge level and educational expectations for disaster medicine of prospective health practitioners, considering the important role they will play in the disaster responses of today and the future. The secondary aim of this study is to provide supporting evidence on the necessity of making disaster medicine education widespread in the curriculum of medicine and other health sciences by demonstrating how health practitioners' disaster medicine knowledge level can increase through the provision of disaster medicine education.

MATERIALS AND METHODS

This cross-sectional descriptive study was carried out in the 2019-2020 academic year with sixth-year students of the medical faculty, fourth-year students of the Nursing Department, and fourth-year students of the EADM Department at Tekirdağ Namık Kemal University in Tekirdağ, Turkey. Only final-year students (n=217) from these programs were chosen because their vocational curriculum was about to be completed, meaning that these students would likely have an important advantage insofar as taking a more holistic approach to disasters. The whole universe was included in the study without choosing a sample, and a questionnaire was applied to a total of 159 students who could be reached.

The participating students were asked to fill out a questionnaire consisting of 38 questions arranged under three sections (Appendix 1). The first section of the questionnaire includes seven questions addressing the students' demographics and education, such as their age, gender, department of study, disaster medicine education, and disaster drill experiences. The second section, disaster medicine knowledge level, includes 25 questions on topics such as basic disaster information, introduction to disaster medicine, fundamental principles of disaster management, decontamination, cardiopulmonary resuscitation, infectious diseases, public

health, and mental health. The third section has six questions, four of which are multiple-choice and open-ended questions about the educational approach toward disaster medicine education, and two of which involve self-assessment of competence and knowledge levels, where the participants rated themselves from 0 to 10. The total possible value of the disaster medicine knowledge level questions is four points, and there is only one correct answer to each of the four multiple choice questions. Scores of 70 points and higher, which fall within the 75th percentile on the scale of 100, indicate a high level of knowledge about disaster medicine, while scores below 70 indicate a low level of knowledge of disaster medicine.

The questionnaire was developed by the researchers based on previous studies and data published by the Centers for Disease Control and Prevention Disaster Preparedness and Response: Complete Course Facilitator Guide and the Didactical Course of the European Master Program in Disaster Medicine[18-23].

Prior to conducting the study, the participants were provided detailed information about its content and aim and they gave their written consent. This study was approved by the Tekirdağ Namık Kemal University Non-invasive Ethics Committee (decision date-no: 24.09.2019-2019.138.08.10).

Statistical Analysis

In this study, continuous data were analyzed as mean and plus/minus standard deviation, while categorical data were analyzed as a percentage (%). Normal distribution of the data was evaluated using the Shapiro-Wilk test, with normally distributed groups being compared using the one-way ANOVA in cases where the number of groups was three and higher. Results from the crosstab analysis performed were evaluated based on the Pearson chi-square test. All statistical analyses were carried out using the IBM Statistical Package for the Social Sciences (SPSS) Statistics 21.0 program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp.). Statistical significance was accepted as $p<0.05$.

RESULTS

Of the 217 students constituting the population, 73.2% (n=159) filled out the questionnaire. Only those students who fully completed the questionnaires were included in the study.

Participants' Demographic Data

Among the study participants, 45.9% (n=73) were studying in the medical faculty; 37.1% (n=59) in the nursing department; and 16.9% (n=27) in the EADM department. 64.5% (n=102) of the participants were female, 49% (n=78) reported that they had received education about disaster medicine, and 57.8% (n=92) reported that they had participated in a disaster drill before. Furthermore, only 3 of the participants (1.8%) reported that they were not interested in receiving disaster medicine education. Table 1 presents the participants' demographic data.

Knowledge Level

Table 2 presents brief information about the questions used in the knowledge level evaluation and the students' responses. The mean score was 72.33±10.56 (40.00-96.00) for the medical faculty students, 65.49±12.84 (24.00-84.00) for the nursing students, and 78.96±10.56 (56.00-96.00) for the EADM students. Of all the participants, 62 (38.9%) had a low knowledge level, and 97 (61%) had a high knowledge level. In terms of the student groups, it was found that 69.8% (n=51) of the medical faculty students, 40.6% (n=24) of the nursing department students, and 81.4% (n=22) of the EADM students had a high knowledge level. The results further showed that for all three student groups, higher disaster medicine knowledge levels corresponded to female gender and participation in a disaster drill. Moreover, having received disaster medicine education was associated with higher knowledge levels in students who were studying in the nursing department and those who were in the EADM department. However, there was no relationship between being a student in the medical faculty and high knowledge level. None of the participants answered all the questions correctly, nor was there one question answered correctly by all the participants. The question that was most correctly answered by the participants (n=152 95.5%) was Q1, about medical attention priority, while the question with the lowest correct answer rate (n=30 18.8%) was Q4, about the use of personal protective equipment (PPE), and Q1, Q2, and Q19 were correctly answered by all EADM students. The medical faculty students had lower correct answer rates on the questions related to the use of PPE, decontamination procedures, and triage.

Educational Approach Toward Disaster Medicine

Most of the students (58.4%) stated that education on disaster medicine should be carried out through "video conference." The least (n=42) preferred (26.4%) method for receiving disaster medicine education was "online web-based courses." One hundred-fifteen participants (72.3%) felt that disaster medicine education should be provided by "emergency medicine" specialists and that the duration of education should be "an academic year or a specifically designated period" (n=82) (Table 3). A student enrolled in the medical faculty stated, "the disaster medicine education should be provided during the sixth academic year in place of elective internships". Another participant suggested, "disaster medical care education should be provided by categorizing disaster types and spreading them out across academic years".

Table 4 shows the participants' average knowledge level and competence level according to their own estimations. EADM students' estimations of their personal knowledge level and competence level in all subjects were higher than those of students from other departments.

When participants were asked about the subject that they wished to receive disaster medicine education, "earthquakes" was the most (n=121, 76.1%) and "disaster epidemiology" was the least (n=40 25.1%) preferred response (Figure 1).

DISCUSSION

This study evaluated the disaster medicine knowledge levels and expectations for disaster medicine education of final-year students in the medical faculty, the nursing department, and the EADM department of Tekirdağ Namık Kemal University. Although this study was carried out with a lower number of participants compared to that seen in similar studies in the literature, it is unique in terms of the variety of the participants constituting the sample in Turkey[18,20,24-26]. This study found that most of the students who received disaster medicine education did so during their undergraduate years. Moreover, most of the students who reported that they had not received disaster medicine education during their undergraduate education were interested in receiving education on this subject. Similar to the study of Wunderlich et al.[24], the participants stated that they wanted to receive training in disaster medicine. The fact that the participants who had not received disaster medicine education before wanted to receive this education is a promising indicator for that these future health practitioners are aware of the fact that they may have to work during disasters and in disaster environments, and they want to be prepared for this. Undergraduate education forms the backbone of health professionals' occupational knowledge, skill acquisitions, their future in-service trainings, and lifelong learnings[27,28]. As Markenson et al.[29] stated, healthcare students need to be trained so as to be capable of undertaking tasks during disasters, as these tasks are an integral part of their profession. A common national disaster medicine education curriculum that is structured based on up-to-date developments and needs, which supports interdisciplinary cooperation, and that can be adapted according to the competencies of disciplines can help future health professionals to be more safely and systematically prepared for disaster response roles[30,31].

Table 1. Participants' personal information

	Medicine (n=73)	Nursing (n=59)	EADM (n=27)	p
Age (year)				
Mean±SD	24.918 (2.350)	22.966 (2.742)	22.222 (1.805)	<0.001[1]
Range	23.000-33.000	20.000-36.000	20.000-29.000	
Gender (%)				
Female	42 (57.5)	47 (79.7)	13 (48.1)	0.005[2]
Male	31 (42.5)	12 (20.3)	14 (51.9)	
Have you ever received education about disaster medicine? (%)				
Yes	11 (15.1)	40 (67.8)	27 (100.0)	<0.001[2]
No	58 (79.5)	16 (27.1)	0 (0.0)	
I do not know	4 (5.5)	3 (5.1)	0 (0.0)	
3. If so, where did you receive your disaster medicine education? (%)				
Undergraduate education	6 (60.0)	34 (85.0)	27 (100.0)	0.012[2]
Non-governmental organizations	2 (20.0)	2 (5.0)	0 (0.0)	
Internet	1 (10.0)	0 (0.0)	0 (0.0)	
Other resources	1 (10.0)	4 (10.0)	0 (0.0)	
3. If no, do you want to receive disaster medicine education? (%)				
Yes	60 (92.3)	21 (91.3)	1 (100.0)	0.671[2]
No	3 (4.6)	0 (0.0)	0 (0.0)	
I have no idea	2 (3.1)	2 (8.7)	0 (0.0)	
Have you ever participated in a disaster drill? (%)				
Yes	31 (42.5)	43 (74.1)	18 (69.2)	<0.001[2]
No	42 (57.5)	15 (25.9)	8 (30.8)	

[1]Linear Model ANOVA, [2]Pearson's chi-square test

EADM: Emergency Aid and Disaster Management, SD: Standard deviation

Contrary to the study of Arslan et al.[26], the rate of participation in disaster drills of the students was high. Disaster drills provide unique opportunities for future health professionals to, at the very least, experience the chaotic environments of disasters and to be prepared for their tasks in disasters. Previous studies show that drills can serve to develop health professionals' competence in practice-based subjects, such as incident command, triage, patient care, evacuation, and decontamination procedures[31-35]. Healthcare students should be given the opportunity to participate in disaster drills, and their participation should be encouraged. Conducting these drills with the simultaneous participation of multiple disciplines in undergraduate education would provide students the opportunity to experience the cooperative working environment and prepare them to quickly act together when necessary.

The participants' disaster medicine knowledge levels varied between groups. The fact that participants who had participated in disaster drills had higher knowledge levels suggests that drills foster disaster awareness and familiarity with basic information about disasters. Contrary to expectations and the current literatures, the high level of disaster medicine knowledge seen in the medical students, despite the lack of a standard structured disaster medicine curriculum, could be attributed to the fact that this subject is scattered throughout the curriculum of different clinical branches[18,24,25,36]. In this context, it would be fairly easy to simply gather the existing disaster medicine subjects and additional related subjects under the title of "disaster medicine" and add this to the medical education curriculum. This would be an encouraging step for students, educators, and education content planners alike in terms of fostering disaster medicine education. At the same time, the authors also recognize that a national study may produce also different results. The low knowledge level results of the nursing students are worrying. It is likely that these results are a consequence of disaster medicine and nursing subjects being provided in a single academic term as an elective course. Making disaster medicine a mandatory course in the nursing curriculum could be motivating for both educators and students and lead to an increase in students'

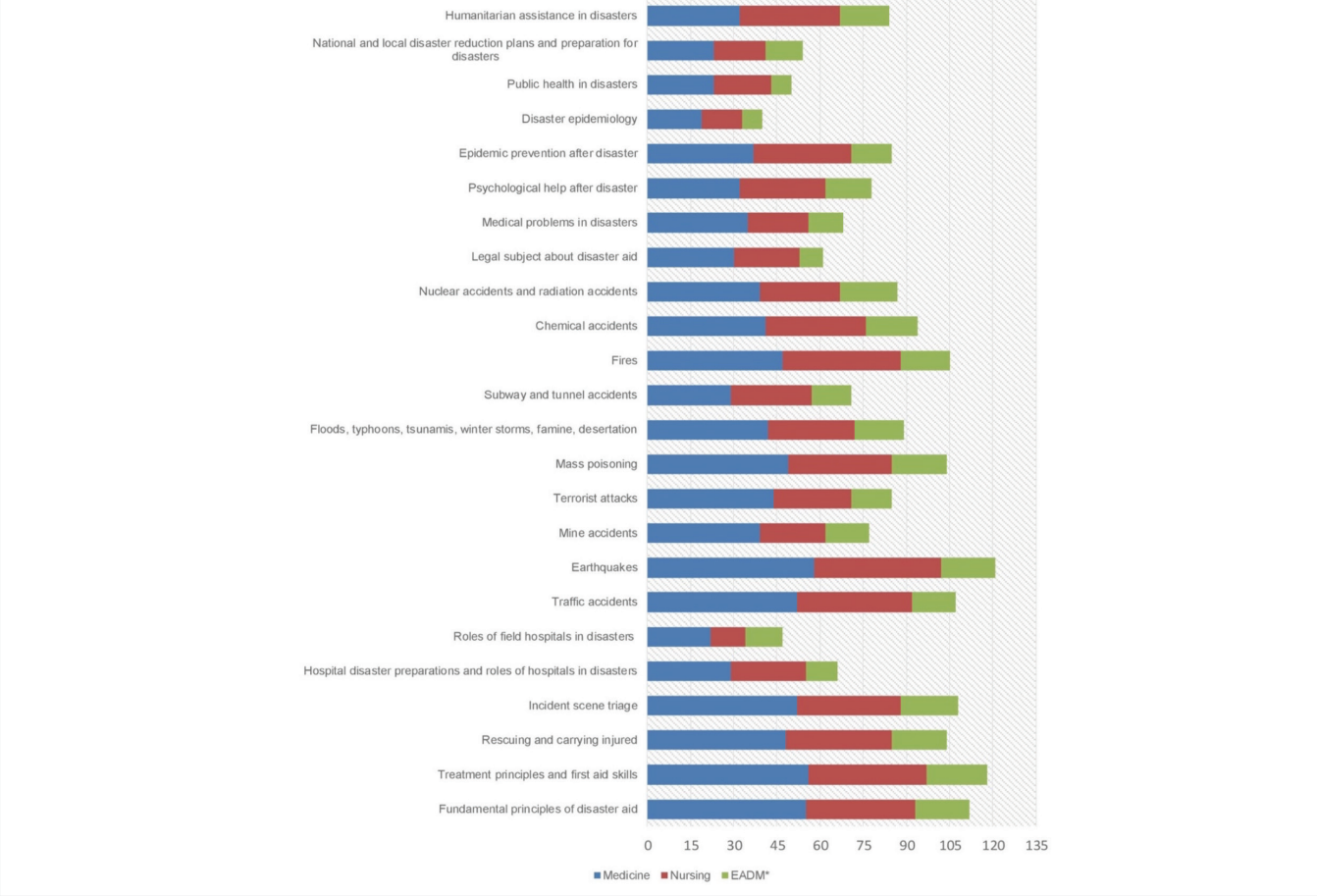

Figure 1. Number of students who want to receive education on various disaster medicine subject

EADM: Emergency Aid and Disaster Management

knowledge levels and the confidence they have in their personal knowledge and competence.

The medical faculty students reported that their knowledge levels and self-competence levels were low, despite proving to have high knowledge levels. This lack of confidence could lead to setbacks in patient care, critical decision-making, and incident command stages, and thereby increase the mortality and morbidity of victims of disasters. Being provided a disaster medicine course could positively contribute to improving the confidence that medical students have in their personal knowledge and competence levels. The fact that the EADM students received disaster medicine education likely played a big role in their high knowledge levels and their higher self-perceptions of personal knowledge and competence on the subject, as compared to other student groups. The preparedness and competence of healthcare professionals in extraordinary events, such as disasters, are valuable insofar as these would enable them to assume a managerial role and perform their duties in a focused/professional manner when disasters occur in their communities[28].

Knowledge about the use of PPE was low in all three groups. This is concerning considering how important it is that the personnel can efficiently use PPE to protect themselves and other patients in healthcare environments. It has been well established that with appropriate trainings, the inaccurate usage of PPE can be prevented[37]. A study related to the COVID-19 pandemic in Wuhan, China, which investigated the healthcare professionals responsible for the care of COVID-19 patients, demonstrated the importance of appropriate PPE usage, in that it found there to be no incidences of infection in the study participants despite the high exposure risks[38].

Contrary to the study by Barrimah et al.[21], this study revealed that participants preferred to receive disaster medicine training via videoconferencing during an academic year or during a certain period. Students were most interested in receiving education about how to respond to earthquakes,

Table 2. Number and percentage of students who correctly answered the disaster medicine knowledge level questions according to their departments

Knowledge level questions		Total (%)	Medical (%)	Nursing (%)	EADM (%)
Q1	Medical attention priority	152 (95.59)	70 (95.9)	55 (94.8)	27 (100)
Q2	Triage coding	147 (92.45)	62 (84.9)	58 (98.3)	27 (100)
Q3	Knowledge about decontamination procedures	52 (32.7)	32 (43.8)	8 (13.6)	12 (44.4)
Q4	Knowledge about the use of PPE	30 (18.86)	15 (26.3)	6 (11.3)	9 (33.3)
Q5	Management of biological agents	145 (91.19)	71 (97.3)	49 (83.1)	25 (96.2)
Q6	Cardiopulmonary resuscitation procedures	119 (74.84)	61 (83.6)	32 (54.2)	26 (96.3)
Q7	Knowledge about disaster management steps	149 (93.71)	70 (95.9)	53 (89.8)	26 (96.3)
Q8	Knowledge about public health in disasters	132 (83.01)	63 (87.5)	44 (74.6)	25 (92.6)
Q9	Knowledge about infectious diseases	95 (59.74)	35 (50.7)	38 (65.5)	22 (81.5)
Q10	Crush syndrome management	93 (58.49)	50 (68.5)	19 (32.2)	24 (88.9)
Q11	Knowledge about post-traumatic stress disorder	135 (84.9)	56 (76.7)	54 (91.5)	25 (92.6)
Q12	Psychological first aid	145 (91.19)	69 (94.5)	55 (93.2)	21 (80.8)
Q13	Use of resources in disasters	122 (76.72)	58 (86.6)	41 (70.7)	23 (85.2)
Q14	Chemical disaster response	112 (70.44)	46 (63.0)	45 (76.3)	21 (77.8)
Q15	Knowledge about incident command system	110 (69.18)	53 (73.6)	32 (55.2)	25 (92.6)
Q16	Media and public relations	141 (88.67)	66 (90.4)	53 (89.8)	22 (81.5)
Q17	Knowledge about disaster management steps	143 (89.93)	66 (91.7)	52 (91.2)	25 (92.6)
Q18	Trauma patient management	115 (72.32)	59 (81.9)	32 (56.1)	24 (88.9)
Q19	Basic disaster knowledge	131 (82.38)	62 (86.1)	42 (73.7)	27 (100)
Q20	Legal legislation knowledge about disasters	108 (67.92)	54 (78.3)	41 (69.5)	13 (48.1)
Q21	Knowledge about decontamination procedures	75 (47.16)	29 (43.9)	26 (44.1)	20 (74.1)
Q22	Communication in disasters	125 (78.61)	58 (80.6)	48 (82.8)	19 (73.1)
Q23	Simple Triage and Rapid Treatment triage knowledge	60 (37.73)	20 (31.2)	30 (50.8)	10 (37.0)
Q24	Management of mass deaths	103 (64.77)	49 (71.0)	33 (58.9)	21 (77.8)
Q25	Radioactive disaster response	80 (50.31)	46 (64.8)	20 (33.9)	14 (51.9)

Q: Question, EADM: Emergency Aid and Disaster Management, START: Simple Triage and Rapid Treatment

Table 3. Students' disaster medicine education choices

	Medicine n (%)	Nursing n (%)	EADM n (%)	p
Disaster medicine education method				
Classical classroom lectures/lecture presentations	30 (41.1)	28 (47.5)	23 (85.2)	<0.001
Video conference	40 (54.8)	41 (69.5)	12 (44.4)	
Video, podcast	26 (35.6)	22 (37.3)	11 (40.7)	
Online web-based courses	26 (35.6)	11 (18.6)	5 (18.5)	
Personal textbooks, brochures	26 (35.6)	14 (23.7)	5 (18.5)	
I have no idea	9 (12.3)	4 (6.8)	0 (0.0)	
Disaster medicine education duration				
An academic year or a specific period	27 (38.6)	31 (52.5)	24 (88.9)	0.238
3-5 days of workshops/conferences before graduation	27 (38.6)	14 (23.7)	2 (7.4)	
Two hours a week during the final academic year	13 (18.6)	12 (20.3)	0 (0.0)	
Other	3 (4.3)	2 (3.4)	1 (3.7)	
The branch of the teacher who will provide disaster education				
Emergency medicine	51 (69.9)	40 (67.8)	24 (88.9)	0.450
Family practice	0 (0.0)	1 (1.7)	0 (0.0)	
Public health	17 (23.3)	12 (20.3)	2 (7.4)	
Any clinical branch	0 (0.0)	1 (1.7)	0 (0.0)	
I have no idea	5 (6.8)	5 (8.5)	1 (3.7)	

EADM: Emergency Aid and Disaster Management

Table 4. Participants' averages regarding self-assessment of their personal knowledge level and competence level according to the 0–10-point visual analog scale

	Personal knowledge level estimation			Personal competence level estimation		
	Medicine	Nursing	EADM	Medicine	Nursing	EADM
Chemical incidents	3.10	3.35	4.70	2.96	2.75	4.19
Biological incidents	3.66	3.95	5.00	3.74	3.49	4.15
Radiological incidents	2.90	2.86	4.63	2.46	2.25	3.65
Nuclear incidents	2.49	2.82	4.70	2.21	2.42	3.50
Natural disasters	4.37	5.53	7.04	4.26	5.12	6.38
Epidemics	4.92/10	5.25/10	5.48/10	4.54	4.75	5.42

EADM: Emergency Aid and Disaster Management

which is not surprising considering that much of Turkey's geography lies on high-risk areas for earthquakes, and that earthquakes are a disaster type that causes the most loss of life and property in Turkey[39]. The students further indicated that they most preferred disaster medicine education to be provided by emergency medicine specialists. This preference likely stems from emergency medicine expertise being seen as a clinical branch that involves a holistic approach toward complex and extraordinary health issues, and from the ability of emergency medicine specialists to organize their clinics like a disaster manager during their routine emergency service operations. Considering these matters in the planning of content and methods for disaster medicine courses will provide key contributions to achieving educational goals.

Study Limitations

The main limitation of this study is that it was a single-center study. Including students from earlier academic years could yield different results. Secondly, the self-evaluations of the participants about their knowledge and competence levels may not be fully reliable. Studies carried out in multiple centers with broad sample sizes could provide more valuable data.

CONCLUSION

This study has revealed that future health professionals are interested in receiving disaster medicine education. It is recommended that disaster medicine courses be generalized with special emphasis on certain special subjects, such as the use of the PPE and decontamination procedures, in order

to increase knowledge level and personal knowledge, and competence level perception and to reinforce those gains with disaster drills. The results from this study can contribute to the design of disaster medicine courses that should be added to the basic curricula of health professions.

Ethics

Ethics Committee Approval: This study was approved by the Tekirdağ Namık Kemal University Non-Interventional Research Ethics Committee on 28.09.2019 with the protocol number 2019.138.08.10 and decision number 10.

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.B., S.B., M.Ç., Design: N.B., S.B., M.Ç., Data Collection or Processing: S.B., M.Ç., Analysis or Interpretation N.B., S.B., M.Ç., Literature Search: N.B., Writing: N.B., S.B.

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Appendix 1. Evaluation of disaster medicine knowledge level of future health practitioners

Questionnaire

1. PERSONAL INFORMATION

1) Age: .....

2) Gender

☐ **A.** Female ☐ **B.** Male

3) In which department are you enrolled?

☐ **A.** Medicine ☐ **B.** Nursing ☐ **C.** Emergency Aid and Disaster Management

4) Have you ever received any educational training on disaster medicine?

☐ **A.** Yes ☐ **B.** No ☐ **C.** I do not know

5) 4. If your answer is yes, where did you receive the educational training on disaster medicine?

☐ **A.** Undergraduate Education
☐ **B.** Non-governmental organizations
☐ **C.** Internet
☐ **D.** Other resources

6) 4. Soruya yanıtınız hayır ise; Afet tıbbı eğitimi almak ister misiniz?

☐ **A.** Yes ☐ **B.** No ☐ **C.** I do not know

7) Have you ever participated in a disaster drill?

☐ **A.** Yes ☐ **B.** No

2. KNOWLEDGE LEVEL

1) You will give medical attention to the injured and other survivors after a mining accident at the scene of the accident. Which group of patients will you prioritize for medical attention?

☐ **A.** Those who are severely injured, dying and unable to benefit from resuscitative interventions
☐ **B.** Those with fatal injuries but possibly able to survive if immediate treatment is applied
☐ **C.** Those who are in stable condition but have serious injuries and need treatment
☐ **D.** Those whose injuries are not serious

2) You are assigned as triage personnel after a bombing at a festival. One of the injured is a 25-year-old female patient, who has a left leg transfemoral amputation and a 4 cm-laceration over the right eyebrow. RR (respiration rate): 31 / min, capillary refill time: 4 sec; she makes meaningless sounds and cannot answer your questions. Which triage color will you choose to code this patient?

☐ **A.** Green
☐ **B.** Yellow
☐ **C.** Red
☐ **D.** Black

3) How would you decontaminate survivors who were exposed to leakage of a chemical substance in a factory?

- ☐ **A.** By washing them with warm water and soap.
- ☐ **B.** By wiping them with 5%-hypochlorite solution.
- ☐ **C.** By identifying the chemical substance and using its specific antidote.
- ☐ **D.** I do not know.

4) What level of personal protective equipment is recommended for use in decontamination procedures at hospitals?

- ☐ **A.** A
- ☐ **B.** B
- ☐ **C.** C
- ☐ **D.** D

5) What would your thoughts be about a patient who was admitted to the health institution you work at with high fever, fatigue, headache, pain in the muscles and joints, and rashes on their skin, and you find out from their medical history that they just returned from a West Africa trip?

- ☐ **A.** I would think that they have an upper respiratory infection. I would suggest that they take their medication and rest.
- ☐ **B.** I would suspect they had an allergic reaction and recommend they take an allergy test.
- ☐ **C.** It could be viral hemorrhagic fever (e.g. Ebola). I would consult the relevant specialist.
- ☐ **D.** I do not know.

6) What is the first stage of cardiopulmonary resuscitation?

- ☐ **A.** Applying defibrillation with the highest energy dosage (200&360j) of the defibrillator
- ☐ **B.** Administrating oxygen
- ☐ **C.** Applying chest compression
- ☐ **D.** Administrating adrenalin

7) At which stage of the disaster cycle should medical equipment and essentials be stocked?

- ☐ **A.** Preparation
- ☐ **B.** Damage reduction
- ☐ **C.** Intervention
- ☐ **D.** Improving

8) Which of the following public health practices is correct after a disaster?

- ☐ **A.** After a disaster, temporary toilets should be installed against the wind near water sources.
- ☐ **B.** The nutritional needs of children, pregnant women, and breastfeeding mothers are the same as for other disaster victims.
- ☐ **C.** Water resources likes streams, lakes, or dams can be used to meet the water needs of disaster victims during nuclear disasters.
- ☐ **D.** Boiling or chlorination of water can be used for disinfection of water in disasters.

9) Which of the following is not true about infectious diseases that can develop after disasters?

- ☐ **A.** Encouraging disaster victims to wash their hands and use soap is one of the most important steps for preventing fecal-oral infections.
- ☐ **B.** Insecticide spraying and insecticide-treated nets can be used to protect indoor environments from vectors.
- ☐ **C.** The bodies of those who died during disasters are one of the main causes of the emergence of infectious diseases.
- ☐ **D.** Post-traumatic wound care, tetanus prophylaxis and use of anti-inflammatory medication are important to prevent post-traumatic diseases.

10) You are assigned to an earthquake disaster region with a lot of debris. During the ongoing search and rescue operations of a 10-story wrecked building, an injured person whose right half of their body is trapped under a column was found. The person was male, 34 years old, and conscious, with TA: 113/65 mmHg, Pp: 108 /minutes, SD: 24 /minute Sat: 94%. The search and rescue personnel reported that the rescue operation would continue for at least 1.5 hours. How would you handle this patient at the scene?

- ☐ **A.** I would only give him oxygen.
- ☐ **B.** I would start a fluid treatment by opening a wide vascular access.
- ☐ **C.** I would wait for the patient to be rescued from the wreckage.
- ☐ **D.** I do not know.

11) A 42-year-old woman, who was among the victims of a fire that occurred in a large area and caused the death of 83 people, started to suffer two weeks after the fire from anorexia, sleeping disorder, palpitations, loss of energy, and recurring images of the fire in her mind. These conditions had been ongoing for two months. Which of the following is the most probable diagnosis for this patient?

- ☐ **A.** Acute stress disorder
- ☐ **B.** Panic disorder
- ☐ **C.** Dissociative disorder
- ☐ D. Post-traumatic stress disorder

12) Which of the following should not be said or done by post-disaster psychological first-aid care providers?

- ☐ **A.** Respect privacy.
- ☐ **B.** Provide accurate information
- ☐ **C.** React to the feelings of a person with statements such as "you should not feel like this" or "you should feel lucky to be alive".
- ☐ **D.** Show that you understand a person's feelings and losses and important events they talk about with a statement such as, "I am so sorry, I can only imagine how painful this is for you."

13) Which of the following is not correct in the allocation of resources to be used in the case of a disaster?

- ☐ **A.** It should be transparent
- ☐ **B.** It should be proportionate
- ☐ **C.** It should vary based on individuals
- ☐ **D.** It should be consistent

14) A rig carrying a chemical load has an accident near your house when you are at home. The officials report that there is a chlorine gas leakage because of the accident. Which of the following is the first step of emergency response?

- ☐ **A.** I would remain in areas where there is an upwind
- ☐ **B.** If I am inside the house, I would close all doors, windows and openings.
- ☐ **C.** I would quickly consult the nearest emergency service.
- ☐ **D.** I have no knowledge about this subject.

15) You are assigned to provide and manage medical care for the injured after a flood. In which unit do you work in the incident management system according to this information?

- ☐ **A.** Logistics department
- ☐ **B.** Finance department
- ☐ **C.** Operations department
- ☐ **D.** Planning department

16) Press and public statements will be made by the relevant people about this flood. Which of the following is appropriate?

- ☐ **A.** The statements are made by any personnel whose workload is not excessive at the time
- ☐ **B.** Use easily understandable language that does not include medical terms
- ☐ **C.** Do not disclose the real number of losses
- ☐ **D.** Give detailed, technical information about the incident

17) Which of the following is correct for disaster management?

- ☐ **A.** Disaster management steps can only be applied in disasters occurring in urban areas.
- ☐ **B.** It includes the damage reduction, preparation, response, and recovery periods of disasters.
- ☐ **C.** Drills and training operations are included in the response period.
- ☐ **D.** Logistics management is not included in disaster management.

18) Which of the following is correct for trauma patients?

- ☐ **A.** Reduction of open fractures should be carried out at the scene of incidents.
- ☐ **B.** The first method to be used is the tourniquet application for the control of bleeding in extremities.
- ☐ **C.** For patients suspected of spinal fractures, complete spinal immobilization should be provided.
- ☐ **D.** If a cervical brace is used, it should be a soft one.

19) Which of the following includes oil spills, nuclear explosions, and bioterrorism?

- ☐ **A.** Natural disasters
- ☐ **B.** Hydroclimatological disasters
- ☐ **C.** Human-made disasters
- ☐ **D.** Social disasters

20) Which of the following constitutes legislation that determines the general framework of the provision of health services during disasters in Turkey?

- ☐ **A.** Emergency Health Services Regulation
- ☐ **B.** Hospital Disaster and Emergency Plan Regulation
- ☐ **C.** Ambulance Services Regulation
- ☐ **D.** Directive on Duties and Working Principles of Disaster Health Services Unit and National Medical Rescue Teams

21) Which of the following is correct for decontamination procedures?

- ☐ **A.** It is recommended to hold the head back during body wash.
- ☐ **B.** Basic and advanced life support practices should be carried out after the decontamination procedure is completed.
- ☐ **C.** In extremely cold climate conditions, dry decontamination alone is sufficient for decontamination procedure.
- ☐ **D.** Contaminated clothes should be removed by taking them off over the head.

22) Which of the following is not correct in terms of disaster communication principles?

- ☐ **A.** There is no designated frequency to be used during disasters by the Ministry of Health, so any frequency can be used for communication.
- ☐ **B.** For radio communication, communication should be conducted using the lowest possible power that allows communication.
- ☐ **C.** Radio conversations should be short, clear, and understandable.
- ☐ **D.** To start the transmission and make sure that the conversation is fully understood by the target station from the start, you should wait a brief moment (approximately for 3 sec.) after pressing the radio latch before starting to talk.

23) In the "START" triage method, when and by which method is the conscious state of patients evaluated?

- ☐ **A.** Before the circulation step, by asking the patient's name
- ☐ **B.** Before the respiration step, by asking the moment of the incident
- ☐ **C.** In the first encounter with the patient, by asking the month and day
- ☐ **D.** After the circulation step, by asking them to perform a simple instruction, such as "hold my hand"

24) Which of the following is not true about mass death management?

- ☐ **A.** Dead bodies can be stored for 6 months in areas with a temperature of 2.7-5.5 °C
- ☐ **B.** Except in rare cases, infectious microorganisms cannot survive longer than 48 hours in dead bodies.
- ☐ **C.** Most mass deaths occur in hospitals.
- ☐ **D.** In cases when the morgue and storage capacities are exceeded, temporary underground burials can be performed.

25) Which of the following methods can be used to minimize unwanted radioactivity exposure and related damage?

- ☐ **A.** Taking oral iodine tablets
- ☐ B. Using personal protective equipment
- ☐ **C.** Shortening the exposure time, increasing the distance from the source, and covering the source
- ☐ **D.** Applying decontamination procedures multiple times

3. EDUCATIONAL APPROACH TOWARD DISASTER MEDICINE

1) How do you think disaster medicine education should be administered?
You can mark multiple options.

- ☐ **A.** Classical classroom lectures/lecture presentations
- ☐ **B.** Video conferencing
- ☐ **C.** Video, podcast
- ☐ **D.** Online web-based courses
- ☐ **E.** Individual textbooks, brochures
- ☐ **F.** I have no idea.

2) What do you think the duration of disaster medicine education should be?

- ☐ **A.** An academic term or a specifically designated period
- ☐ **B.** 3-5 days of workshops/conferences before graduation
- ☐ **C.** Two hours a week during the final academic year
- ☐ **D.** Other. Please explain. .....

3) Which branch should provide disaster medicine education?

- ☐ **A.** Emergency medicine
- ☐ **B.** Family practice
- ☐ **C.** Public health
- ☐ **D.** Any clinical branch
- ☐ **E.** I have no idea.

4) How would you evaluate your personal knowledge level on a scale of 0-10 (from the lowest to the highest) in each of the following situations?

- ☐ **A.** Chemical incidents
- ☐ B. Biological incidents
- ☐ **C.** Radiological incidents
- ☐ **D.** Nuclear incidents
- ☐ **E.** Natural disasters
- ☐ F. Epidemics

5) How would you evaluate your personal knowledge level on a scale of 0-10 scale (from the lowest to the highest) about giving medical attention in each of the following situations?

- ☐ **A.** Chemical incidents
- ☐ **B.** Biological incidents
- ☐ **C.** Radiological incidents
- ☐ **D.** Nuclear incidents
- ☐ **E.** Natural disasters
- ☐ **F.** Epidemics

6) About which of the following subjects would you like to receive disaster medicine educational training?

- ☐ **A.** Fundamental principles of disaster aid
- ☐ **B.** Treatment principles and first-aid skills
- ☐ **C.** Rescuing and transport of the injured
- ☐ **D.** Incident scene triage
- ☐ **E.** Hospital disaster preparations and roles of hospitals in disasters
- ☐ **F.** Roles of field hospitals in disasters
- ☐ **G.** Traffic accidents
- ☐ **H.** Earthquakes
- ☐ **I.** Mine accidents
- ☐ **J.** Terrorist attacks
- ☐ **K.** Mass poisoning
- ☐ **L.** Floods, typhoons, tsunamis, winter storms, famine, desertification
- ☐ **M.** Subway and tunnel accidents
- ☐ **N.** Fires
- ☐ **O.** Chemical accidents
- ☐ **P.** Nuclear accidents and radiation accidents
- ☐ **Q.** Legal issues regarding disaster relief
- ☐ **R.** Medical problems in disasters
- ☐ **S.** Psychological aid after disasters
- ☐ **T.** Epidemic prevention after disasters
- ☐ **U.** Disaster epidemiology
- ☐ **V.** Public health in disasters
- ☐ **W.** National and local disaster reduction plans and preparation for disasters
- ☐ **X.** Humanitarian assistance in disasters

ORIGINAL ARTICLE

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The Prognostic and Predictive Value of DR-70 Immunoassay, A Novel Fibrin-Associated Biomarker, in Patients with Advanced Gastrointestinal Cancers

İleri Evre Gastrointestinal Kanserli Hastalarda Fibrin ile İlişkili Yeni Bir Biyobelirteç Olan Serum DR-70 Düzeyinin Prognostik ve Prediktif Değeri

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ABSTRACT

Aim: DR-70 is a newly developed immunoassay that detects fibrin degradation products in blood. We aimed to evaluate ability of DR-70 in monitoring treatment response in advanced gastrointestinal (GI) cancers.

Materials and Methods: We prospectively enrolled patients with advanced GI cancers treated with different lines of systemic therapies. Imaging studies, DR-70 and conventional tumor markers [carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9] were analyzed at baseline and on the third month of treatment.

Results: A total of 142 patients diagnosed with colorectal (52.1%), esophago-gastric (32.4%) and pancreaticobiliary cancer (15.5%) were enrolled. Most patients were getting first-line treatment (56.3%). Second blood sampling was performed in 57% of patients. Among patients with esophago-gastric cancer, DR-70 response correlated well with treatment response (p=0.007) and low baseline DR-70 level was significantly associated with longer overall survival (p=0.02). There was a positive but weak correlation between pre-treatment DR-70 and CEA levels (p=0.03, r=0.244) in patients with colorectal cancer, while a moderate positive correlation was present between pre-treatment DR-70 and CA 19-9 levels in esophago-gastric and pancreaticobiliary cancers (p=0.01, r=0.402 and p=0.04, r=0.515, respectively). More than 25% reduction in DR-70 concentration was associated with better overall and progression-free survival.

Conclusion: DR-70 is a strong predictor of treatment response and survival, particularly in esophago-gastric cancer.

Keywords: Tumor markers, gastrointestinal cancers, treatment response, biomarker, prognosis

ÖZ

Amaç: DR-70, kandaki fibrin yıkım ürünlerini tespit eden yeni geliştirilmiş bir testtir. Bu çalışmada ileri evre gastrointestinal (GI) kanserlerde DR-70'in tedavi yanıtını izlemedeki etkinliğini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya farklı serilerdeki sistemik tedaviler ile tedavi edilen ileri evre GI kanserli hastalar dahil edildi. Görüntüleme çalışmaları, DR-70 ve geleneksel tümör belirteçleri [karsinoembriyonik antijen (CEA), karbonhidrat antijeni (CA) 19-9] başlangıçta ve tedavinin üçüncü ayında tekrarlandı.

Bulgular: Çalışmaya kolorektal (%52,1), özofagogastrik (%32,4) ve pankreatikobiliyer kanser (%15,5) tanısı konan toplam 142 hasta alındı. Hastaların çoğu birinci basamak tedavi alıyordu (%56,3). Hastaların %57'sinde ikinci kan örneği alındı. Özofagogastrik kanseri olan hastalarda, DR-70 yanıtı tedavi yanıtı ile iyi korelasyon gösterdi (p=0,007) ve başlangıçta düşük serum DR-70 düzeyi, daha uzun genel sağkalım ile anlamlı şekilde ilişkiliydi (p=0,02). Kolorektal kanserli hastalarda tedavi öncesi DR-70 ile CEA düzeyleri arasında pozitif fakat zayıf bir korelasyon (p=0,03, r=0,244) varken,

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tedavi öncesi DR-70 ile CA 19-9 arasında özofagogastrik ve pankreatikobilier kanserlerde orta düzeyde pozitif bir korelasyon vardı (sırasıyla p=0,01, r=0,402 ve p=0,04, r=0,515). DR-70 konsantrasyonunda %25'ten fazla azalma, daha iyi genel ve progresyonsuz sağkalım ile ilişkiliydi.

Sonuç: DR-70, özellikle özofagogastrik kanserde tedaviye yanıtı ve sağkalımı ön gören güçlü bir belirteçtir.

Anahtar Kelimeler: Tümör belirteçleri, gastrointestinal kanserler, tedavi yanıtı, biyobelirteç, prognoz

INTRODUCTION

Recently updated Global Cancer Statistics revealed that gastrointestinal (GI) tract cancers, including colorectal, gastric, liver, pancreatic and esophageal cancers, represent one of the most important public health problems, with an estimated 5 million new cases worldwide[1]. Survival rates are unsatisfyingly low, particularly in advanced stages; thereby discovering effective tools to use in early detection and follow-up period has received much attention over the last years. Serum tumor markers are one of those tools that have screening, diagnostic and monitoring roles[2].

Carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, CA 125 and alpha-fetoprotein are well-known and routinely used biomarkers shown in the literature with different diagnostic, prognostic and monitoring power[3]. However, controversies still exist regarding the value of these traditional markers for all above-mentioned roles. Therefore, development of new biomarkers that could be easily implemented in routine clinical practice is still of interest to many researchers.

In the presence of cancer, coagulation and fibrinolytic systems are known to be activated regardless of the type of tumor cells. DR-70 immunoassay was developed to detect fibrin and fibrin degradation products (FDPs) in human blood samples[4]. A growing body of literature has evaluated the relationship between FDPs and tumor growth and highlighted that patients with cancer have elevated FDPs in plasma[5-7]. Numerous studies have reported the diagnostic and screening performance of DR-70 immunoassay in different types of cancer[8-15], while only a few have also evaluated its role in prognosis[13,14]. However, only one study focused on the clinical impact of DR-70 on monitoring treatment response[16].

The aim of the present study was to evaluate the clinical efficacy of novel biomarker DR-70 to predict treatment response in metastatic GI cancers. We also investigated the correlation between traditional tumor markers and DR-70 at the time of enrollment. Lastly, the association between baseline DR-70 level and DR-70 change following the treatment and survival outcomes were analyzed.

MATERIALS AND METHODS

Patient Selection

We prospectively enrolled patients with advanced GI cancer at the time of initiating any lines of systemic therapy, after obtaining an informed consent. The study group mainly included patients with colorectal, esophagogastric and pancreaticobiliary cancers. All patients were evaluated with chest and abdominal computed tomography (CT) or ^{18}F-fluorodeoxyglucose positron emission tomography/CT (PET/CT) at the time of admission. Blood samples were collected for both DR-70 examination and other tumor markers such as CEA and CA 19-9 at the same time. Patients then received the treatment of physician's choice for 3 months. At the end of this period, response evaluation was performed based on the Response Evaluation Criteria in Solid Tumors (RECIST) or PET Response Criteria in Solid Tumors (PERCIST) with the identical imaging method previously used. DR-70, CEA and CA 19-9 were also reanalyzed. The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date of approval: 1 June 2018, protocol code: 09.2018.423).

DR-70 Immunoassay

A 5 ml of peripheral blood sample was drawn from each participant. After standing at room temperature for about half an hour, the blood was centrifuged at 1500 rpm for 10 minutes. All serum samples were then frozen and preserved at -80 ^{o}C until DR-70 level was analyzed. Serum concentration of DR-70 (µg/mL) was measured using AMDL DR-70 kits (AMDL, Inc., Tustion, CA, USA) according to the manufacturer's instructions. This is an enzyme-linked immunosorbent assay based serological test that was developed to quantify serum levels of FDPs.

Response Evaluation

We used RECIST (version 1.1) and PERCIST based on the imaging method to evaluate response to the therapy. We categorized patients into two groups, imaging responders and non-responders. Non-responders included patients whose disease progression was confirmed by imaging while responders included patients with complete response, partial response and stable disease. Regarding DR-70 response, we only analyzed patients with DR-70 level above 0.8 µg/mL at the time of admission, as this level was accepted as a threshold to

identify low risk patients for detecting cancer cell in previous studies[4,15]. Since there has been no established percentage change in DR-70 that is associated with disease progression in advanced GI cancer, we used the same threshold defined by Hung et al.[16] and divided patients into two groups, concerning DR-70 change, as follows: more than 20% elevation in DR-70 level defined non-responders, on the other hand remaining were considered as responders.

Statistical Analysis

Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as median (range) for continuous variables, and as percentages for categorical variables. After the normality of the distribution of continuous variables was tested by the Kolmogorov-Smirnov test, the Kruskal-Wallis test and Mann-Whitney U test were used to make inter-group comparisons for parameters that did not indicate a normal distribution. Correlation coefficient and its significance were calculated using the Spearman's rank correlation test. The Fisher's exact test was performed to highlight the relation between DR-70 response and imaging response. Survival analysis was performed with the Kaplan-Meier method and log-rank test. Confidence interval (CI) was selected as 95% and $p<0.05$ was accepted as the level of significance.

RESULTS

Clinical Characteristics of the Study Population

A total of 142 patients with advanced GI cancer were enrolled between July 2018 and January 2019. The median age was 60 (range 30-83) years. The majority of the patients were male (65.5%, 93 of 142 patients). More than half of the patients were evaluated with PET-CT (55%). In total, 3 groups of tumor types were represented among enrolled patients, most commonly colorectal cancer (52.1%), followed by esophagogastric (32.4%) and pancreaticobiliary cancers (15.5%). Most of the patients were enrolled just before first-line treatment (56.3%), the remaining had previously received one or more lines of treatment. Second blood sampling for DR-70 was performed in 81 of patients (57%), the remainder could not be evaluated due to loss of follow-up or death. The characteristics of participants are presented in Table 1.

The Relationship Between DR-70 and Clinical Characteristics

The median DR-70 levels of first and second blood sampling were 1.27 µg/mL (range 0.2-10) and 0.84 µg/mL (range 0.18-10), respectively. There were no significant differences between the median DR-70 levels in terms of tumor type ($p=0.37$), sex ($p=0.32$), and age ($p=0.42$). The number of previous lines of treatment also did not affect DR-70 level ($p=0.25$).

The Correlation Between Pre-treatment Tumor Markers and DR-70

For all study group, there was no correlation between pre-treatment DR-70 and CEA levels ($p=0.12$); however, a weak positive correlation was present between DR-70 and CA 19-9 levels ($p=0.001$, $r=0.287$). Considering tumor subtypes; there was a positive but weak correlation between DR-70 and CEA levels ($p=0.03$, $r=0.244$) in patients with colorectal cancer, while no correlation was seen between DR-70 and CA 19-9 levels ($p=0.16$). Concerning patients with both esophagogastric and pancreaticobiliary cancers, there was no correlation between DR-70 and CEA levels ($p=0.38$ and $p=0.70$, respectively). Nevertheless, a moderate positive correlation was present between DR-70 and CA 19-9 levels ($p=0.01$, $r=0.402$ and $p=0.04$, $r=0.515$, respectively).

Assessment of Treatment Response

Initially, we compared DR-70 response and imaging response after 3 months of therapy in all study group with a baseline DR-70 level higher than 0.8 µg/mL. Among 44 available patients, 25 were both DR-70 and imaging responders. On the contrary, 11 patients were non-responders for both DR-70 and imaging studies. We found a significant correlation between DR-70 response and imaging response based on the RECIST/PERCIST criteria by performing a Fisher's exact test ($p<0.001$). Then, we made the same comparison in subgroups regarding tumor type;

Table 1. Clinical characteristics of study population

	All patients (n=142)
Median age, years (range)	60 (30-83)
Sex, n (%)	
Male	93 (65.5)
Female	49 (34.5)
Tumor type, n (%)	
Colorectal	74 (52.1)
Esophagogastric	46 (32.4)
Pancreaticobiliary	22 (15.5)
Treatment line, n (%)	
1	80 (56.3)
2	35 (24.6)
≥3	27 (19.1)
Median pre-treatment DR-70 level (µg/mL), (min-max)	1.27 (0.20-10)
Colorectal	1.00 (0.20-10)
Esophagogastric	1.79 (0.24-10)
Pancreaticobiliary	1.28 (0.29-10)

min-maks: Minimum-maksimum

a significant correlation between DR-70 and clinical image response was only found in patients with esophagogastric cancer (p=0.007). Table 2 presents the detailed analysis of the correlation between DR-70 and imaging response.

The Association Between Baseline DR-70 and Survival Outcomes

We only analyzed the data of patients treated with first-line therapy for overall survival (OS) outcomes (80 patients). The median pre-treatment DR-70 level was used as cut-off for each tumor type. Only patients with esophagogastric cancer lived significantly longer in the low DR-70 group (14 months, 95% CI: 7.7-20.2) when compared to the high DR-70 group (4 months, 95% CI: 1.0-8.6) (p=0.02). No significant difference was observed in progression free survival (PFS) between low and high DR-70 levels in tumor subtypes. The Kaplan-Meier curves showing OS stratified by DR-70 level in each tumor type are presented in Figure 1.

The Association Between DR-70 Change (ΔDR-70) and Survival Outcomes

We set two different cut-off values for ΔDR-70 after the treatment: ΔDR70 ≥10% decrease and ΔDR70 ≥25% decrease. Among 81 patients with two blood samples, more than 25% reduction in DR-70 was related to significantly longer PFS (8.6 months vs. 5.8 months, p=0.01) irrespective of treatment line. Among 51 patients who received first-line therapy and had two samples of DR-70, more than 25% reduction in DR-70 was found to be associated with significantly longer OS (22.4 months vs. 15.3 months, p=0.03).

DISCUSSION

A close relationship between cancer and thrombosis has been recognized for more than a century. Four-to seven-fold increased risk of thromboembolism has been reported in cancer patients[17]. FDPs are over produced in cancer patients as a result of activation of tumor-induced degradation pathways. The novel tumor marker DR-70 is a polyclonal anti-FDP antibody-based immunoassay, which has been developed to detect the full complement of FDP[4]. This simple, rapid and non-invasive biomarker has been investigated in several trials as a screening and diagnostic tool, and was found to be promising in various malignant tumors such as colorectal, prostate, lung, gastric, tongue and liver[8-14]. However, there seemed to be insufficient data about monitoring role of this promising biomarker.

To the best of our knowledge, the current study is the first to evaluate the monitoring ability of DR-70 immunoassay in different types of advanced GI cancer treated with systemic therapy. Results of our study demonstrated a significant correlation between DR-70 and imaging response only in patients with esophagogastric cancer. This valuable finding is consistent with previous results of Hung et al.[16], which included a total of 51 patients with gastric cancer. Besides showing high sensitivity and specificity, an ideal tumor marker should have the potential to predict treatment response, which might actually save physicians from frequent and unnecessary imaging studies leading to financial toxicity as well as protect patients from waste of time and risk of radiation. DR-70 seems to be a promising marker to be used for treatment response evaluation in patients with esophagogastric cancer.

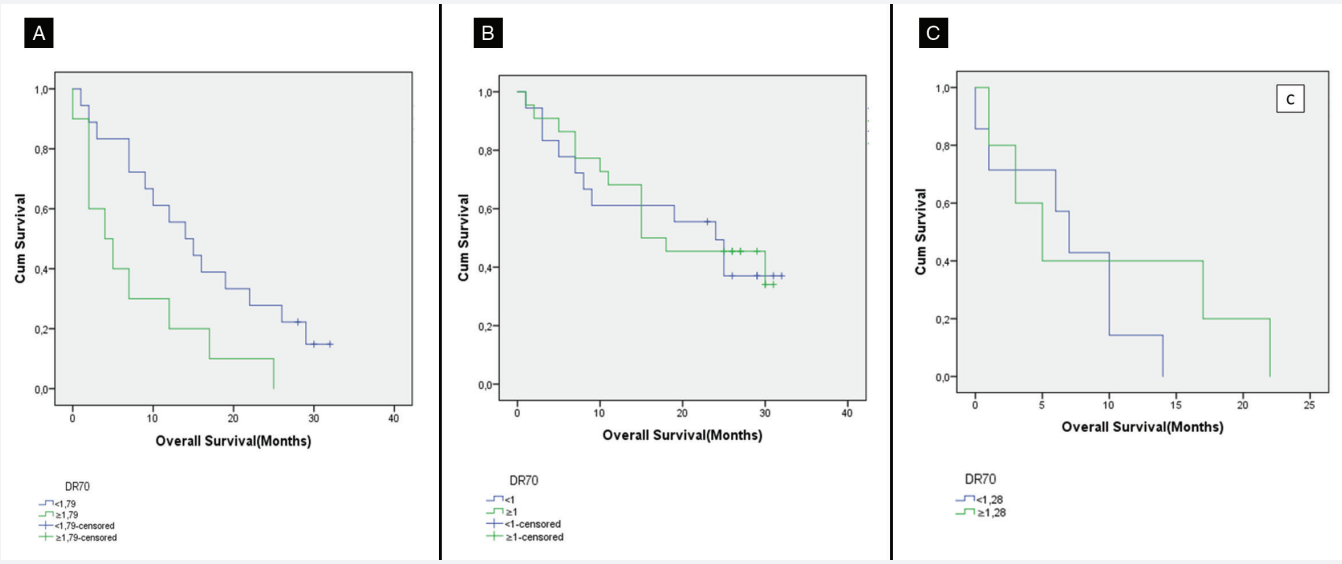

Figure 1. Kaplan-Meier curves showing overall survival stratified by DR-70 level in each tumor type: A) Esophagogastric cancer, B) Colorectal cancer, C) Pancreaticobiliary cancer

Table 2. The correlation between DR-70 and imaging response after treatment				
All group (n=44)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	25	3	28	
DR-70 non-responder	5	11	16	<0.001
Total	30	14	44	
Colorectal (n=26)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	14	2	16	
DR-70 non-responder	5	5	10	0.069
Total	19	7	26	
Esophagogastric (n=13)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	8	1	9	
DR-70 non-responder	0	4	4	0.007
Total	8	5	13	
Pancreaticobiliary (n=5)	**Imaging responder (n)**	**Imaging non-responder (n)**	**Total**	**p value***
DR-70 responder	3	0	3	
DR-70 non-responder	0	2	2	0.10
Total	3	2	5	

*Fisher's exact test

We further investigated the correlation between pre-treatment tumor markers and DR-70. In this context, we examined conventional tumor markers, such as CEA and CA 19-9, which are commonly used in GI cancers. In our findings, there was a positive but weak correlation between DR-70 and CEA levels in patients with colorectal cancer, while a moderate positive correlation was present between DR-70 and CA 19-9 levels in both esophagogastric and pancreaticobiliary cancers. Previous studies suggested to use DR-70 in combination with CEA and CA 19-9 to increase the sensitivity in patients with gastric cancer[11,16].

The prognostic performance of DR-70 was discussed only in few studies. Lin et al.[14] showed a good correlation between DR-70 level and OS in patients with hepatocellular carcinoma. The concentration of DR-70 in serum was also found to be significantly associated with 3-year survival in patients with tongue carcinoma[13]. On the other hand, no significant difference in either OS or PFS was observed between high or low DR-70 in patients with gastric cancer[16]. We analyzed the data of 80 patients treated with first-line therapy for OS outcomes and our results significantly differed from the findings of Hung et al.[16]. We only found a significant difference between the low and high DR-70 groups in terms of OS in esophagogastric cancer. There was no difference between the groups regarding PFS.

We also analyzed the association between DR-70 change during treatment and survival outcomes. More than 25% reduction in DR-70 concentration was found to be associated with longer OS and PFS. The utility of ΔDR-70 was thus highlighted for the first time.

Finally, a number of potential limitations need to be considered. First of all, in the present study, approximately 1 out of 3 patients who were actually considered to have a life expectancy more than 3 months died within this period. Therefore, second blood sampling could not be obtained from this group of patients in addition to the patients who were lost to follow-up. This unexpected situation unfortunately led to decreased number of samples, which may negatively affect statistical analyses. Second, we included patients with advanced GI cancer in different treatment lines which caused actually a heterogeneous group; however, we only analyzed patients treated with first-line therapy for OS outcomes to overcome this bias. Third, since there have been no established thresholds for DR-70 in different types of cancers, we used median levels or previously defined cut-off values for detecting cancer cell in the literature.

CONCLUSION

We conducted this pilot study to provide preliminary evidence on the clinical efficacy of the DR-70 immunoassay in different types of advanced GI cancers. DR-70 seems to be a good candidate to be used as a tumor marker in advanced esophagogastric cancer. The immunoassay correlates well with treatment response and OS. However, further large-scale studies are needed to confirm our findings.

Ethics

Ethics Committee Approval: The study was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date of approval: 1 June 2018, protocol code: 09.2018.423).

Informed Consent: Informed consent form was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.A.T., N.A.B., Ö.A., R.H., S.K., T.B., F.D., Concept: T.A.T., N.A.B., P.F.Y., Design: T.A.T., N.A.B., P.F.Y., Data Collection or Processing: T.A.T., S.H., E.T.Ş., Analysis or Interpretation: Ö.A., M.A.Ö., Literature Search: T.A.T., Ö.E., Writing: T.A.T.

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ORIGINAL ARTICLE

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Evaluation of Coronavirus Disease-2019 Patients with Nailfold Capillaroscopy

Koronavirüs Hastalığı-2019 Hastalarının Tırnak Dibi Kapilleroskopisi ile Değerlendirilmesi

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ABSTRACT

Aim: Microvasculopathy is one of the suspected complications in Coronavirus disease-2019 (COVID-19). Nailfold capillaroscopy is a noninvasive method used to evaluate microvascularity. It can be a guide in detecting endothelial dysfunction and microvasculopathy in COVID-19 patients.

Materials and Methods: Severe acute respiratory syndrome-CoV-2 polymerase chain reaction positive 54 patients were evaluated. The 2nd-5th digits of both hands were investigated by nailfold capillaroscopy. Capillary density, capillary architecture and capillary morphology were recorded. Patients with abnormal and normal nailfold capillaroscopy findings were compared in terms of COVID-19 clinical symptoms.

Results: Of the patients included in the study, 72% were male and the mean age was 35.6±11.6 years. In total, 22 patients (41%) had at least 1 abnormal capillaroscopy change. Diffuse capillaroscopic abnormalities were as follows: pericapillary edema 43%, enlarged and dilated capillaries 24%, and tortiosteal capillaries 22%. Hyperinflammatory response was observed in 17% of the patients and intensive care was required in only 1 patient. The frequency of hyperinflammatory response, anticytokine use and thrombosis increased in patients with abnormal capillaroscopy.

Conclusion: Abnormal capillaroscopy findings were found to be frequent in COVID-19 patients. Higher rates of the hyperinflammatory response and anticytokine drug use in patients with abnormal nailfold findings suggest that there may be a relationship between hyperinflammation and microvasculopathy in COVID-19. Further studies are needed to evaluate the clinical relevance of nailfold abnormalities with clinical manifestations of COVID-19 disease.

Keywords: COVID-19, microangiopathy, nailfold capillaroscopy

ÖZ

Amaç: Mikrovaskülopati, Koronavirüs hastalığı-2019 (COVID-19) hastalığı komplikasyonlarında rol alan mekanizmalardan biridir. Tırnak dibi kapilleroskopisi (TDK) mikrovasküleriteyi değerlendirmede kullanılan non-invaziv bir yöntemdir. COVID-19 hastalarında endotel disfonksiyonu ve mikrovaskülopatinin saptanmasında yol gösterici olabilir.

Gereç ve Yöntem: Şiddetli akut solunum yolu sendromu-CoV-2 testi pozitif çıkmış 54 hastaya TDK yapıldı ve hastalar kapiller yoğunluk, mimari ve morfoloji açısından değerlendirildi. Anormal ve normal kapilleroskopi bulguları olan hastalar COVID-19 klinik semptomları açısından karşılaştırıldı.

Bulgular: Çalışmaya alınan hastaların %72'si erkek, yaş ortalaması ise 35,6±11,6 idi. Toplamda 22 hastada (%41) anormal kapilleroskopik değişikliklerden en az 1 tanesi vardı. Kapilleroskopik yaygın anormallikler ise sırasıyla, perikapiller ödem (%43), genişlemiş ve dilate kapil (%24), tortiyozite kapiller (%22) oldu. Hastaların %17'sinde hiperenflamatuvar yanıt görüldü ve 1 hastada yoğun bakım ihtiyacı oldu. Anormal kapilleroskopik değişliği olan hastalarda hiperenflamatuvar yanıt, antisitokin kullanımı ve tromboz sıklığı artmıştı.

Sonuç: COVID-19 hastalarında anormal kapilleroskopik bulgular sıklıkla gözlenmiştir. Anormal kapilleroskopik bulguları olan hastalarda hiperenflamatuvar yanıt ve antisitokin ilaç kullanımı sıklığının artması hiperenflamasyon ile mikrovaskülopati arasında bir ilişki olabileceğini düşündürmektedir. TDK'nın, COVID-19 hastalığı klinik tutulumları ile ilişkisini değerlendirmek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: COVID-19, mikroanjiyopati, tırnak dibi kapilleroskopi

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INTRODUCTION

Severe acute respiratory syndrome- coronavirus-2 (SARS-CoV-2), as the causative agent of Coronavirus disease-2019 (COVID-19), caused a global pandemic following its first detection in Wuhan, China in December 2019[1]. The clinical course of COVID-19 may be asymptomatic or may progress with severe pneumonia by affecting the lower respiratory tract. In some patients, it can cause morbidity and mortality by causing disease in multiple organ systems[2]. The frequency of thrombosis has increased in the course of COVID-19[3]. Thrombosis in COVID-19 patients may be due to hyperinflammatory response, hypoxic injury, endothelial dysfunction, hypercoagulability and/or increased platelet activity[4]. Even in the early stages of COVID-19 disease, viral inclusion bodies can cause organ damage by causing apoptosis, inflammatory cell infiltration and microvascular thrombosis in endothelial cells. This microvasculopathy process in the course of COVID-19 may be responsible for the major thrombotic events[5,6].

Nailfold capillaroscopy (NFC) is a noninvasive method commonly used to evaluate microvasculopathy, especially in autoimmune connective tissue patients[7]. NFC is a bedside, easily applicable method which may also be helpful in detecting dysfunctional endothelial activation and microvasculopathy in patients with COVID-19[8,9].

In this study, we aimed to evaluate the nail fold of the fingers in hospitalized COVID-19 patients by NFC and compare the COVID-19 clinical course in patients with and without NFC changes.

MATERIALS AND METHODS

We included 54 consecutive COVID-19 patients, who were hospitalized for COVID-19 in Ankara City Hospital Infectious Diseases Clinic between 01 and 30 April 2020. Patients' SARS-CoV-2 tests were evaluated by reverse transcriptase-polymerase chain reaction taken from nasopharyngeal or oropharyngeal swabs and all patients gave informed consent. Patients under the age of 18 years, pregnant and having any comorbidity or using chronic medication were excluded from the study. Age, gender and smoking status of the patients, as well as the clinical features, complications, treatments, laboratory and radiological results of the COVID-19 disease, were recorded. Approval from Ankara City Hospital Ethics Committee was obtained for the study, along with the permission of the Ministry of Health and an informed consent form (no: E1-20-679, date: 30.09.2020).

All patients included in our study were examined by NFC (Dino-Lite Premier AM4113T) between the 3rd and 5th days of their hospitalization. As defined in previous studies, patients were rested at room temperature (22-25 °C) for at least 15 minutes prior to the examination[7]. All fingers of both hands except thumbs were examined by NFC by AE. Capillaroscopic changes were evaluated in terms of capillary density, capillary architecture, and capillary morphology.

Capillary density was recorded as the mean number of capillaries calculated from two areas in each finger examined (dividing the number of capillaries at 1 mm distance from the middle of the nail fold to each side) by two. The presence of at least 9 capillaries per 1 mm was considered normal capillary density[10].

All elongated, curved, dilated, giant capillaries and the presence of hemorrhages were noted. Avascular areas were defined as the loss of at least two consecutive capillaries in the dermal papilla. The presence of branching, bushy capillaries or ramified capillaries was classified as neoangiogenesis. The percentage of curved and elongated capillaries was determined by evaluating the same areas used to determine capillary density[10,11].

To identify abnormal NFC examination findings in COVID-19 patients, we used the definitions proposed by Ingegnoli et al.[12]. If more than 1 morphological abnormality (giant capillary or >50% convoluted or >10% elongated capillary or hemorrhage area or angiogenesis or avascular areas plus the presence of another capillaroscopy abnormality) was defined in at least 2 different fingers, the patient's NFC examination was accepted as abnormal.

Statistical Analysis

Statistical analysis was performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA). The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Shapiro-Wilk test). Continuous data were defined as mean [±standard deviation (SD)] or median [interquartile range (IQR)] and categorical variables as percentages. The chi-square test was used to compare categorical variables. The Student's t-test or Mann-Whitney U test was used to compare continuous variables. A p value of <0.05 was considered statistically significant.

RESULTS

In this study, a total of 54 COVID-19 patients were included. Of them, 72% patients were male and the mean age was 35.6±11.6 years. Of the patients, 17% were active smokers and 6% were ex-smokers. The median (IQR) hospital stay of patients was 7 (4) days. All patients had a thorax computed tomography when hospitalized and ground glass opacity was observed in 85%, focal patchy infiltration in 4%, and normal findings in 11%.

COVID-19 symptoms of the patients were as follows: Cough 69%, fever 43%, shortness of breath 32%, myalgia 30%, sore throat 24%, arthralgia 20%, headache 15%, anosmia 13%,

nausea/vomiting 11%, diarrhea 7%, dysgeusia 6%, fatigue 6%, and abdominal pain 4%. Patients' COVID-19 treatments involved hydroxychloroquine 96%, azithromycin 57%, favipiravir 26%, glucocorticoid 2%, tocilizumab 6%, anakinra 6%, acetylsalicylic acid/dipyridamole 8%, colchicine 7%, and low molecular weight heparin 63%.

During the follow-up period, hyperinflammatory response was developed in 17% of the patients. Only 1 patient was admitted to the intensive care unit. In total, 2 thrombosis events, 1 being pulmonary thromboembolism and 1 being sinus vein thrombosis, developed in our COVID-19 patients during hospital stay. All patients with hyperinflammatory response and thrombosis were in the abnormal NFC group. No acute respiratory distress syndrome, myocarditis, sepsis, mechanical ventilation need, or mortality was seen in any of the COVID-19 patients.

NFC findings of all patients were shown in Table 1. In total, 22 patients (41%) had at least 1 of the described abnormal capillaroscopic changes. The evaluation of patients with and without abnormal NFC in terms of the COVID-19 disease course was shown in Table 2. Images of the NFCs of the patients with COVID-19 disease were shown in Figure 1.

DISCUSSION

In our study, 41% of 54 hospitalized COVID-19 patients had abnormal NFC findings. We found that the frequency of hyperinflammatory response and anticytokine drug use was increased in COVID-19 patients with abnormal NFC. This suggests that there may be a relationship between hyperinflammation and microvasculopathy in COVID-19 patients, but we could not find any association between abnormal NFC and mortality, and the need for mechanical ventilation.

Table 1. Nailfold capillaroscopy examination findings of patients with COVID-19 disease

Median (IQR) capillary density, number of capillaries/1 mm	9 (2)
Enlarged and dilated capillary, n (%)	13 (24)
Capillary tortuosity, n (%)	
≤50%	12 (22)
>50%	12 (22)
Elongated capillary, n (%)	
≤10%	7 (13)
>10%	11 (20)
Hemorrhage, n (%)	5 (9)
Pericapillary edema, n (%)	23 (43)
Branching capillary, n (%)	3 (6)
Bushy capillary, n (%)	0
Ramified capillary, n (%)	0
Avascular area, n (%)	0
Abnormal nailfold capillaroscopy, n (%)	22 (41)

IQR: Interquartile range, COVID-19: Coronavirus disease-2019

NFC examination is a simple method that may be used to demonstrate microvascular changes in capillaries. In clinical practice, it is mostly used as a diagnostic method in patients with systemic sclerosis and dermatomyositis with Raynaud's syndrome[7,13]. In the literature, abnormal NFC have also been found in primary vasculitides such as Behçet's disease, Henoch-Schönlein purpura, Takayasu arteritis and granulomatous polyangiitis[14-17]. It is also important that NFC may detect NFC changes at an early stage, especially in patients with systemic sclerosis[18].

The frequency of thrombosis has been increased in the course of COVID-19 disease[3]. Thrombosis in COVID-19 disease may be due to hyperinflammatory response, hypoxic injury, endothelial dysfunction, hypercoagulability and/or increased platelet activity[4,8]. Even in the early stages of COVID-19 disease, viral inclusion bodies can cause organ damage by causing apoptosis, inflammatory cell infiltration and microvascular thrombosis in endothelial cells. The microvasculopathic changes may be responsible for the major thrombotic events in the COVID-19 disease[5,6].

In the literature, there are not enough studies about the associations between abnormal NFC and COVID-19 disease yet. In our study, the median capillary density of all COVID-19 patients was found to be normal. Enlarged/dilated capillary was present in 13 patients and capillary hemorrhage was observed in 5 patients. Apart from these, 12 patients had >50% abnormal capillary tortuosity (total increased capillary tortuosity in 24 patients) and 10 patients had >10% elongated capillaries (total elongated capillaries in 18 patients).

No bushy capillary, ramified capillary or avascular area was detected in the NFC examinations. Branching capillary, which is one of the other common minor NFC changes, was observed in 3 patients and pericapillary edema was observed in 23 patients. In our study, as in the study of Natalello et al.[9], the frequency of minor NFC changes increased and the most common change was pericapillary edema. Giant capillary resembling scleroderma was not observed in any of the patients with COVID-19 disease. In this study, the incidence of pericapillary edema was 100% in the acute period of COVID-19 disease, while the rate of the cured patients was 70%. In another study, it was stated that papillary edema might indicate disease activity in Henoch-Schönlein purpura patients[15]. So pericapillary edema, being the most common finding in the early phase of the disease, may be considered as an indicator of active disease in our study.

In our study, comparing the patients with normal and abnormal NFC, there was no difference in terms of gender, age, and smoking status. The median capillary density of 22 patients

Table 2. Comparison of COVID-19 patients with and without capillaroscopic abnormalities			
	Abnormal capillaroscopy, (n=22)	**Normal capillaroscopy, (n=32)**	**p**
Male, n (%)	17 (77)	22 (69)	0.492
Age, year, mean±SD	38.5±13.1	33.4±10.1	0.104
Smoking, n (%) **Current** **Ex-smoker** **Never**	 4 (18) 2 (9) 16 (73)	 5 (16) 1 (3) 26 (89)	0.744
Capillary density, mean±SD	8.5±1.4	9.5±1.3	**0.022**
COVID-19 clinical features			
Fever, n (%)	12 (55)	11 (34)	0.141
Cough, n (%)	15 (68)	22 (69)	0.965
Dyspnea, n (%)	11 (50)	6 (19)	**0.015**
Arthralgia, n (%)	5 (23)	6 (19)	0.721
Myalgia, n (%)	6 (27)	10 (31)	0.753
Headache, n (%)	1 (5)	7 (22)	0.122
Sore throat, n (%)	3 (14)	10 (31)	0.199
Anosmia, n (%)	3 (14)	4 (13)	0.903
Dysgeusia, n (%)	2 (9)	1 (3)	0.560
Stomachache, n (%)	1 (5)	1 (3)	0.786
Nausea/vomiting, n (%)	3 (14)	3 (9)	0.671
Diarrhea, n (%)	1 (5)	3 (9)	0.638
Laboratory results			
Lymphocyte, median (IQR)	1450 (958)	1475 (800)	0.881
Hemoglobin, median (IQR)	14.0 (1.5)	14.7 (1.8)	0.659
Platelets, median (IQR)	213000 (115500)	214000 (68250)	0.685
Creatinine, median (IQR)	0.78 (0.33)	0.80 (0.26)	0.805
Aspartate aminotransferase, median (IQR)	22 (31)	19 (11)	0.062
Alanine aminotransferase, median (IQR)	24 (32)	27 (15)	0.359
Lactate dehydrogenase, median (IQR)	254 (121)	206 (63)	**0.010**
Creatinine kinase, median (IQR)	101 (209)	83 (49)	0.097
C-reactive protein, median (IQR)	18 (34)	7 (12)	0.369
Ferritin, median (IQR)	152 (331)	115 (113)	0.233
Fibrinogen, median (IQR)	3.7 (1.5)	3.3 (1.3)	0.279
D-dimer, median (IQR)	0.4 (0.5)	0.3 (0.3)	0.058
INR, median (IQR)	1.0 (0.1)	1.1 (0.1)	0.582
Troponin I, median (IQR)	2.5 (2.3)	2.5 (0.4)	0.578
COVID-19 clinical features			
Thorax computed tomography, n (%) **Ground glass opacity** **Focal infiltration/opacity** **Normal findings**	 19 (91) 0 2 (9)	 25 (81) 2 (6) 4 (13)	0.444
Hyperinflammatory response, n (%)	5 (23)	0	**0.008**
Thrombosis, n (%)	2 (9)	0	0.161
Length of stay in hospital, day, median (IQR)	8 (4.5)	7 (3.0)	0.131
Clinical complete recovery, day, median (IQR)	30 (52)	20 (48)	0.749

Tablo 2. Continued

	Abnormal capillaroscopy, (n=22)	Normal capillaroscopy, (n=32)	p
COVID-19 treatments			
Hydroxychloroquine, n (%)	21 (96)	31 (97)	0.786
Azithromycin, n (%)	9 (41)	22 (69)	**0.042**
Favipiravir, n (%)	9 (41)	5 (16)	**0.037**
Glucocorticoid, n (%)	1 (5)	0	0.407
Tocilizumab, n (%)	3 (14)	0	0.062
Anakinra, n (%)	3 (14)	0	0.062
Colchicine, n (%)	4 (18)	0	**0.023**
Acetylsalicylic acid/dipyridamole, n (%)	4 (18)	0	**0.023**
Low molecular weight heparin, n (%)	18 (82)	16 (50)	**0.023**

IQR: Interquartile range, COVID-19: Coronavirus disease-2019, SD: Standard deviation, INR: International normalized rate

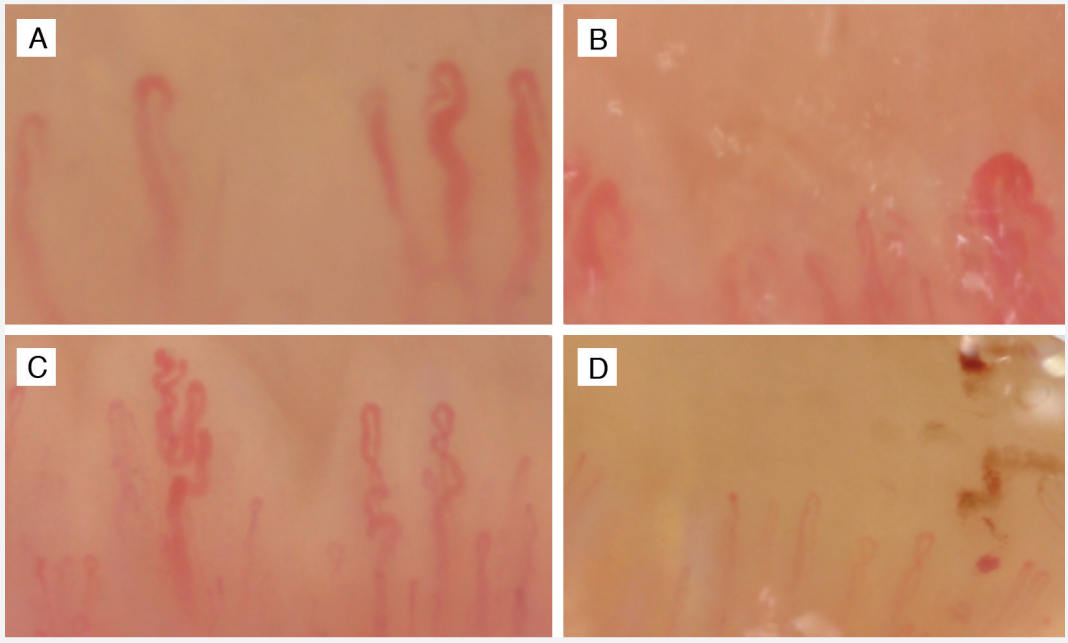

Figure 1. A) Elongated, dilated capillary and periungual edema, B) Dilated capillary, C) Capillary tortuosity, D) Capillary hemorrhage

with abnormal NFC was lower than that of 32 patients with normal NFC. Decreased capillary density, enlarged capillary and capillary tortuosity may also be seen in patients with diabetes mellitus and hypertension[10,19]. The exclusion of these comorbidities in our study suggests that the NFC changes detected could be due to COVID-19 disease.

In comparison of the COVID-19 clinical and laboratory features at the time of diagnosis, only the frequency of dyspnea and median lactate dehydrogenase levels were found to be higher in the group with abnormal NFC. In a meta-analysis review, elevated lactate dehydrogenase levels were associated with an increased incidence of COVID-19-related thrombosis[20]. In our patients, pulmonary-parenchymal involvement was similar between patients with and without abnormal NFC.

In the hospital follow-ups of the patients, a hyperinflammatory response was observed in 5 patients who were all in the abnormal NFC group. Thrombosis developed in 2 of these 5 patients (one sinus vein thrombosis and one pulmonary thromboembolism). In patients with normal NFC, there was no hyperinflammatory response or thrombosis. Apart from this, sepsis, acute respiratory failure or mortality, which can be a complication of COVID-19 disease, was not observed in any of the patients. Although the median hospital stay and clinical complete recovery time were higher in the group with abnormal NFC, this difference did not reach statistical significance. As a result of our findings, more hyperinflammatory response and thrombosis in COVID-19 patients with abnormal NFC changes is consistent with microvascular thrombotic processes that can be seen in the course of COVID-19 disease[21-23].

In our study, patients with abnormal NFC required more anticytokine, antiaggregant and antithrombotic drugs during the COVID-19 course. After the viremic phase at the onset of COVID-19, the excessive immune system activation increases the severity of the COVID-19 disease and reveals the need for anticytokine therapy[24]. Although the pathogenetic mechanisms are still unclear, the developing hyperinflammatory state may be directly related to the pathogenetic role of the viral agent causing endothelial activation in the early stages of the disease[25]. The need for more anticytokine therapy in the treatment management of our patients, who underwent a NFC examination in the first days of their hospitalization and who had abnormal NFC, is compatible with that in the severe COVID-19 disease course.

Study Limitations

There were several limitations in our study. The small number of patients and the absence of a control group are among the limitations of our study. That no NFC examinations were performed before the COVID-19 infection was another limitation of our study. Excluding patients with comorbidities and chronic drug use provides a homogeneity in our study but the lack of the objective COVID-19 severity scales is an important limitation.

CONCLUSION

In conclusion, increased abnormal NFC findings were detected in hospitalized COVID-19 patients in our study. The frequency of hyperinflammatory response and anticytokine drug use is increased in COVID-19 patients with abnormal NFC findings. In the light of these data, an association could be between hyperinflammation and microvasculopathy in COVID-19 patients. On the other hand, no association was observed between the presence of abnormal NFC findings in COVID-19 patients and mortality, and the need for mechanical ventilation. Therefore, in order to better demonstrate the clinical effect of abnormal NFC findings in COVID-19 disease and to confirm our findings, similar studies should be conducted with more patients and these patients should be followed up for a longer period of time.

Ethics

Ethics Committee Approval: Approval from Ankara City Hospital Ethics Committee was obtained for the study, along with the permission of the Ministry of Health and an informed consent form (no: E1-20-679, date: 30.09.2020).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ö., A.E., Concept: O.K., A.O., Design: B.A., O.K., A.O., A.E., Data Collection or Processing: B.Ö., A.A.A., E.A., Ö.K., Analysis or Interpretation: B.A., S.C.G., Literature Search: Ö.K., S.C.G., Writing: B.A., B.Ö.

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ORIGINAL ARTICLE

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Normal Distance Measurements Between the Origins of the Major Branches of the Abdominal Aorta on Computed Tomography Angiography in Children

Çocuklarda Bilgisayarlı Tomografi Anjiyografisinde Abdominal Aortun Ana Dallarının Orijinleri Arasındaki Normal Mesafe Ölçümleri

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ABSTRACT

Aim: The aim of the present study was to determine the normal distances between the origins of the major branches of the abdominal aorta, and their distances to the aorta at the diaphragmatic region and iliac bifurcation on multidetector computed tomography (MDCT) angiography in pediatric patients.

Materials and Methods: The MDCT angiography scans obtained from 245 children aged between 0 and 18 years (mean age±standard deviation, 8.48±5.14 years) were retrospectively re-evaluated. The distances between the origins of the celiac trunk (CTR), superior mesenteric artery (SMA), right renal artery (RRA), left renal artery (LRA), and inferior mesenteric artery (IMA) were measured. The distance measurements between the aorta at the diaphragmatic region, iliac bifurcation, and the origins of the major branches (CTR, SMA, RRA, LRA, IMA) were performed as well.

Results: The distances between the abdominal aorta and its branches were reported to vary in the age groups. All the distance measurements increased significantly with increasing age ($p<0.0001$). The distances between the aorta at the diaphragmatic region and the origins of the major branches were significantly higher in girls ($p<0.05$). The origin of the RRA was higher than the LRA in 51.8% of the study population.

Conclusion: This study is the first to provide data on the distances between the abdominal aorta and its branches in children. The present results may contribute to enhance the efficacy and safety of the endovascular and surgical procedures in the abdominal region.

Keywords: Abdominal aorta, major branches, computed tomography angiography, pediatrics

ÖZ

Amaç: Çok dedektörlü bilgisayarlı tomografi (ÇDBT) anjiyografi ile pediatrik hastalarda abdominal aort ana dallarının orijinleri ile diyafragmatik aort ve iliak bifurkasyon arasındaki normal mesafelerinin ölçülmesi amaçlandı.

Gereç ve Yöntem: Yaşları 0-18 yaş arasında değişen 245 çocuktan (ortalama yaş±standart sapma, 8,48±5,14 yıl) elde edilen ÇDBT anjiyografi tetkikleri retrospektif olarak değerlendirildi. Çölyak trunkus (ÇT), superior mezenterik arter (SMA), sağ renal arter (RRA), sol renal arter (LRA) ve inferior mezenterik arter (İMA) orijinleri arasındaki mesafeler ölçüldü. Diyafragmatik aort, iliak bifurkasyon ve ana dalların orijinleri (ÇT, SMA, RRA, LRA, İMA) arasındaki mesafe ölçümleri de yapıldı.

Bulgular: Abdominal aort ile dalları arasındaki mesafelerin yaş grupları arasında değiştiği tespit edildi. Yaş ilerledikçe tüm mesafe ölçümlerinin anlamlı olarak arttığı gözlemlendi ($p<0,0001$). Kızlarda diyafragmatik aort ile majör dalların orijinleri arasındaki mesafe anlamlı olarak daha uzundu ($p<0,05$). Çalışma popülasyonunun %51,8'inde RRA'nın orijini LRA'dan daha yüksek yerleşim göstermekteydi.

Sonuç: Bu çalışma, çocuklarda abdominal aort ile dalları arasındaki mesafeler hakkında veri sağlayan ilk çalışmadır. Mevcut sonuçların, karın bölgesindeki endovasküler ve cerrahi prosedürlerin etkinliğinin ve güvenliğinin artırılmasına katkıda bulunacağını umuyoruz.

Anahtar Kelimeler: Abdominal aort, ana dallar, bilgisayarlı tomografi anjiyografi, pediatri

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INTRODUCTION

The widespread availability of low dose, contrast-enhanced multidetector computed tomography (MDCT) has significantly reduced the need for invasive conventional angiography providing useful information on the vascular anatomy of the human body[1].

Several cadaveric and radiological series in adults have reported that the distances between the major branches of the abdominal aorta are highly variable in relation to age, gender, and ethnicity. Knowing the distances between the anatomical landmarks is important especially for the vascular surgeons and interventional radiologists to avoid complications during the surgical and endovascular procedures in the abdominal region[2,3]. However, to the author's knowledge, no previous study has examined the distances between the abdominal aorta and its branches in children of various age groups.

The objective of the present study was to determine the normal distances between the origins of the major branches of the abdominal aorta, and their distances to the aorta at the diaphragmatic region and iliac bifurcation on MDCT angiography in pediatric patients.

MATERIALS AND METHODS

Patients

A descriptive cross-sectional and retrospective study design was adopted. From March 2014 to February 2019, a total of 827 children who underwent abdominal CT examinations for various reasons were retrospectively evaluated. The exclusion criteria were listed as follows: Subjects over 18 years of age; CT examinations performed without intravenous contrast agents (n=300); vascular distortion caused by the presence of abdominal tumor mass or lymphadenopathy (n=87); massive ascites (n=31); active bleeding caused by polytrauma (n=7); supernumerary renal arteries (n=127); severe vertebral scoliosis or kyphosis (n=16); and CT images of insufficient quality for analysis (n=14).

The MDCT angiography images of 245 children with normal body weight for chronological age were finally enrolled in the current study. The study flowchart is shown in Figure 1.

CT Protocol and Analysis of The CT Findings

The abdominopelvic contrast-enhanced CT examinations were performed with a 32 multi-slice CT scanner (SOMATOM go. Now, Siemens, Erlangen, Germany). The non-ionic iodinated contrast agent (Iobitridol, 300 mg I/mL) was injected intravenously by using a power injector (dose: 1-2 mL/kg, injection rate: 2 mL/s). To adhere to as low as reasonably achievable (ALARA) principle, the CT scans were made at a tube voltage of 80-120 kV and tube current of 20-350 mAs depending on the patient's weight. The CT parameters were as follows: Tube rotation time, 0.3-0.5 s; pitch, 1; and reconstruction interval, first multiplanar data reconstruction with a slice thickness less than or equal to 3 mm was performed to identify the abdominal aorta and its branches.

A single board-certified radiologist (European Board of Radiology) performed all the measurements for this study. The maximum distance measurements between the abdominal aorta and its branches were obtained in the arterial phase from sagittal-oblique sagittal and coronal-oblique coronal CT images. The curve of the abdominal aorta was taken into consideration. To measure the distance between the origins of the major branches, a line parallel to the abdominal aorta was drawn between the inferior margin of the cranially situated vessel and the inferior margin of the caudally positioned vessel (Figure 2A). The distance between the aortic hiatus and the origins of the major branches was measured between the inferior margin of the vessel and the horizontal line, which represents the diaphragmatic level (Figure 2B). The length between the iliac bifurcation and the origins of the major branches was measured between the inferior border of the vessel and the horizontal line, which passes through the bifurcation level (Figure 2C).

The distances between the origin of the celiac trunk (CTR) and superior mesenteric artery (SMA); CTR and right renal artery (RRA); CTR and left renal artery (LRA); and CTR and inferior mesenteric artery (IMA) were measured. The distance measurements between the SMA-RRA, SMA-LRA, SMA-IMA, IMA-RRA, and IMA-LRA were performed as well. Moreover, the distances between the aorta at the diaphragmatic region, aortic bifurcation, and the origins of the major branches (CTR, SMA, RRA, LRA, IMA) were reported.

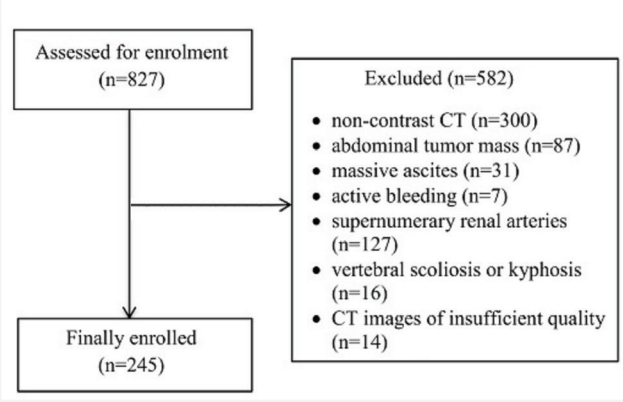

Figure 1. Flowchart for study selection

CT: Computed tomography

Ethical Statement

The study protocol was approved by University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Ethics Committee (protocol number: 2019/285, date: 11.04.2019). The declaration of Helsinki was preserved. The need for informed consent was waived by the ethics committee.

Statistical Analysis

The normality of continuous data was assessed visually using histograms and Q-Q plots for each measurement. The variables had a normal distribution and were expressed as mean±standard deviation (SD), and the categorical variables were presented as frequencies and percentages. The chi-square and one-way ANOVA tests were used to compare categorical and continuous data, respectively. Significant correlations were determined by the Pearson correlation test. Statistical Package for the Social Sciences (SPSS) software (version 20.0, SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The level of significance was set at a p value of less than 0.05.

RESULTS

Demographic Data

The study population consisted of 134 (54.7%) boys and 111 (45.3%) girls aged between 0 and 18 years (mean age±SD, 8.48±5.14 years; 95% confidence interval 7.83-9.13 years). The subjects were classified into six groups according to their age (Table 1).

Distances Between the Origins of the Major Branches

The mean distances between the origins of the CTR-SMA, CTR-RRA, CTR-LRA, and CTR-IMA were 12.8±4.2 (range; 4.7-27.2), 22.1±6.6 (range; 6.4-45.1), 23.0±6.6 (range; 6.2-48.3), and 67.0±17.8 (range; 16.6-116.1) mm, respectively. The mean distances between the origins of the SMA-RRA, SMA-LRA, and SMA-IMA were 9.3±5.4 (range; 0.9-32.1), 10.3±5.3 (range; 1.0-29.6), and 54.2±15.2 (range; 11.9-99.8) mm, respectively. The mean distances between the origins of the IMA-RRA and IMA-LRA were 45.8±14.4 (range; 9.6-87.5) and 44.6±13.9 (range; 9.5-84.5) mm, respectively.

Table 1. Distribution of study population according to age and gender

Groups (%)	Age	Male (n)	Female (n)	Total (n)
Group 1 (7.3)	0-12 month	12	6	18
Group 2 (13.9)	1-3 year	20	14	34
Group 3 (19.2)	4-6 year	31	16	47
Group 4 (24.5)	7-10 year	31	29	60
Group 5 (17.1)	11-14 year	21	21	42
Group 6 (18.0)	15-18 year	19	25	44
Total	0-18 year	134	111	245

n: Number of patients

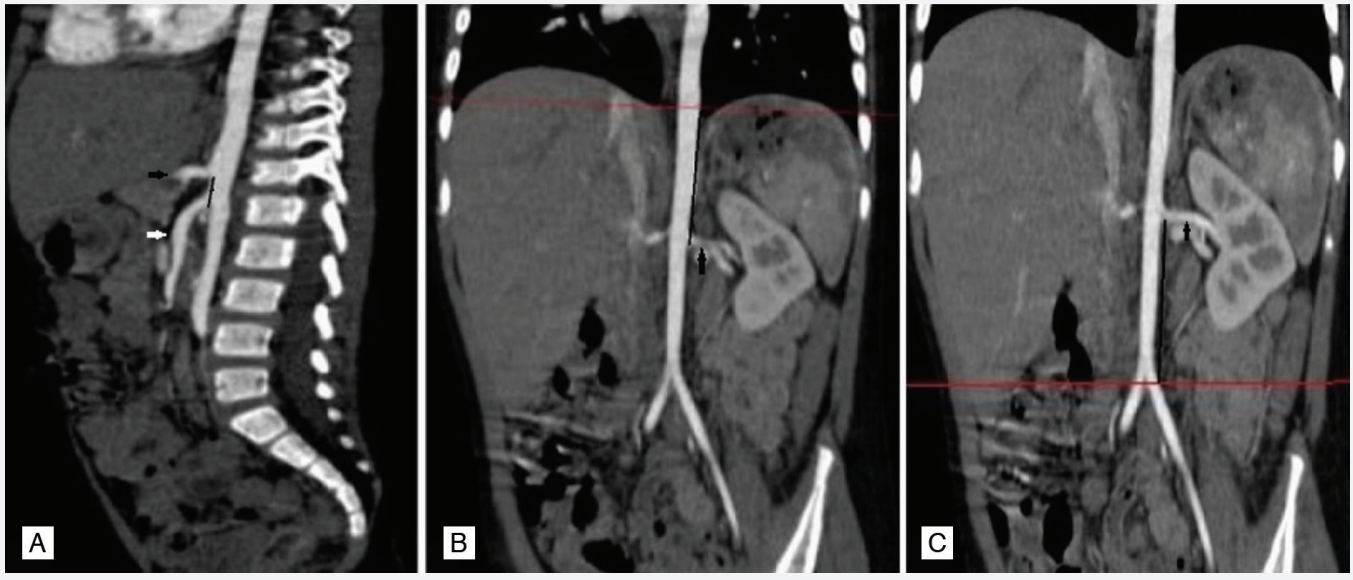

Figure 2. Computed tomography angiography of the abdominal aorta of a 4-year-old male patient. The oblique sagittal image demonstrates the distance measurement between the celiac trunk (black arrow) and superior mesenteric artery (white arrow) (A). The oblique coronal image shows the distance measurement between the left renal artery (black arrow) and the line that represents the aorta at the diaphragmatic region (B). The oblique coronal image demonstrates the distance measurement between the left renal artery (black arrow) and the line that represents the aortic bifurcation level (C)

The origin of the RRA was higher than LRA in 127 (51.8%) patients. The origins of the renal arteries were at the same level in only 50 (20.4%) cases. The origins of the renal arteries according to age groups were as follows: Group 1 [RRA higher than LRA (n=8); LRA higher than RRA (n=4); RA at same level (n=6)]; group 2 [RRA higher than LRA (n=14); LRA higher than RRA (n=8); RA at same level (n=12)]; group 3 [RRA higher than LRA (n=25); LRA higher than RRA (n=14); RA at same level (n=8)]; group 4 [RRA higher than LRA (n=31); LRA higher than RRA (n=16); RA at same level (n=13)]; group 5 [RRA higher than LRA (n=25); LRA higher than RRA (n=10); RA at same level (n=7)]; and group 6 [RRA higher than LRA (n=24); LRA higher than RRA (n=16); RA at same level (n=4)].

The distances between the origins of the major branches increased significantly with increasing age (p<0.0001 for all). Gender was not associated with any of these measurements (p>0.05 for all). Data for the distances between the origins of the major branches according to age and gender are summarized in Table 2.

Distances Between the Aorta at the Diaphragmatic Region and the Origins of the Major Branches

The mean distances between the aortic hiatus and the origins of the CTR, SMA, RRA, LRA, and IMA were 38.8±13.7 (range; 6.4-101.0), 51.5±15.8 (range; 11.1-116.5), 58.4±17.0 (range; 16.6-115.5), 60.2±17.3 (range; 16.3-117.7), and 104.0±27.7 (range; 26.2-180.9) mm, respectively. The distances between the aorta at the diaphragmatic region and the origins of the major branches showed a significant positive correlation with age (p<0.0001 for all). These measurements were higher in girls than boys and this gender difference reached the statistically significant level (p<0.05 for all).

The distances between the aortic hiatus and the origins of the major branches according to age and gender are reported in

Table 2. The mean distances between the origins of the CTR, SMA, RRA, LRA, and IMA according to age groups and gender

Distances (mm)		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	p values
CTR-SMA	F	8.7±1.6	10.0±1.5	11.9±2.6	13.1±3.8	16.7±4.7	14.5±3.7	p<0.001
	M	6.4±1.1	9.5±2.2	10.9±1.5	12.4±2.9	14.9±3.6	18.4±2.2	
	T	7.2±1.7	9.7±1.9	11.3±1.9	12.7±3.4	15.8±4.2	16.2±3.6	
CTR-RRA	F	12.7±2.6	17.9±3.1	22.9±8.0	24.3±5.0	25.3±5.8	24.3±3.9	p<0.001
	M	12.1±3.3	15.6±3.8	20.3±4.7	22.6±4.3	25.2±7.0	29.5±5.8	
	T	12.3±3.0	16.6±3.7	21.2±6.1	23.4±4.7	25.2±6.3	26.5±5.4	
CTR-LRA	F	13.8±3.3	18.4±3.2	23.4±7.0	25.1±4.6	27.1±3.6	25.4±3.6	p<0.001
	M	13.1±5.1	16.2±3.6	20.7±4.9	23.9±5.3	27.8±6.7	28.9±6.5	
	T	13.3±4.5	17.1±3.6	21.6±5.8	24.5±5.0	27.4±5.3	26.9±5.3	
CTR-IMA	F	40.3±6.7	50.8±5.0	64.1±12.1	70.2±9.9	80.6±15.6	76.9±12.1	p<0.001
	M	34.7±9.2	49.1±6.4	59.6±9.5	69.1±9.1	81.9±14.8	88.1±13.9	
	T	36.6±8.7	49.8±5.8	61.1±10.6	69.7±9.5	81.2±15.0	81.8±13.9	
SMA-RRA	F	3.9±1.4	7.9±4.2	10.9±7.9	11.2±5.5	8.6±6.9	9.8±5.5	p<0.001
	M	5.7±2.6	6.1±3.8	9.4±4.8	10.1±4.0	10.3±5.6	11.0±4.9	
	T	5.2±2.4	6.9±4.0	9.9±5.9	10.7±4.8	9.4±6.3	10.3±5.2	
SMA-LRA	F	5.1±2.7	8.4±4.0	11.5±6.9	12.0±4.8	10.4±6.0	10.9±4.7	p<0.001
	M	6.7±4.3	6.7±3.5	9.7±4.9	11.4±4.6	12.8±5.2	10.4±6.6	
	T	6.2±3.8	7.4±3.8	10.3±5.7	11.7±4.7	11.6±5.7	10.7±5.5	
SMA-IMA	F	31.5±6.2	40.8±5.4	52.1±12.2	57.2±8.8	63.9±14.3	62.5±12.0	p<0.001
	M	28.3±8.5	39.6±6.6	48.7±9.9	56.7±8.5	66.9±12.8	69.7±13.1	
	T	29.4±7.8	40.1±6.1	49.9±10.7	56.9±8.6	65.4±13.5	65.6±12.9	
IMA-RRA	F	28.0±5.7	33.5±3.7	39.9±4.8	46.9±8.0	57.9±15.9	54.9±9.2	p<0.001
	M	22.3±6.6	32.9±5.6	37.6±8.7	48.1±7.7	60.6±12.4	59.7±11.5	
	T	24.2±6.7	33.1±4.9	38.4±7.6	47.6±7.8	59.3±14.1	56.9±10.4	
IMA-LRA	F	25.8±5.0	33.2±3.2	39.7±6.7	46.2±8.3	55.8±16.5	52.3±10.7	p<0.001
	M	21.9±5.2	33.2±6.7	37.2±7.5	47.0±7.6	58.9±12.0	56.2±11.7	
	T	23.2±5.3	33.2±5.5	38.0±7.3	46.6±7.9	57.3±14.4	54.0±11.2	

Data are presented as mean±standard deviation. P values were obtained using One-Way ANOVA test; p value <0.001 was considered highly significant.

F: Female, M: Male, T: Total, CTR: Celiac trunk, SMA: Superior mesenteric artery, RRA: Right renal artery, LRA: Left renal artery, IMA: Inferior mesenteric artery

Table 3. The age-dependent distributions of these distances are presented in Figure 3.

Distances Between the Iliac Bifurcation and the Origins of the Major Branches

The mean distances between the iliac bifurcation and the origins of the CTR, SMA, RRA, LRA, and IMA were 89.7±24.5 (range; 31.6-142.1), 77.0±21.6 (range; 25.4-122.5), 70.2±21.0 (range; 21.4-118.2), 68.5±19.8 (range; 21.7-111.4), and 24.7±9.7 (range; 4.7-51.5) mm, respectively. The mean distance between the aorta at diaphragmatic region and iliac bifurcation was 128.7±33.3 (range; 38.0-207.5) mm.

Children's age was significantly associated with the distances between the aortic bifurcation and the origins of the main branches ($p<0.0001$ for all). Gender did not have a significant impact on these distance values ($p>0.05$ for all). The mean distances between the aortic bifurcation and the origins of the major branches according to age and gender are presented in Table 4. The age-dependent distributions of these distances are demonstrated in Figure 4.

DISCUSSION

The present study is the first to calculate the distances between the origins of the major branches of the abdominal aorta and their distances to the aorta at the diaphragmatic region and iliac bifurcation in children aged 0 to 18 years. Here, we emphasize that knowing the distances between the major branches of the abdominal aorta may contribute to enhancing the safety and quality of the endovascular procedures in the abdominal region. The contrast-enhanced MDCT allows comprehensive and fast imaging of the abdominal aorta and its branches, thus minimizing the need for sedation in children[2]. Moreover, low dose CT provides images with a radiation dose that is much lower than conventional CT[4]. The three-dimensional multiplanar reformations present unique viewing and measuring advantages as well[5]. As a result, MDCT has become a valuable tool in the evaluation of vascular anatomy and pathologies in children[3,6]. The distance measurements between the abdominal aorta and its branches have been reported in various radiological and cadaveric studies focusing only on adults or both adults and children. Thus, a comparison of the present results with those from previous studies was undertaken with caution due to the differences in the age of patients, methodological approach, and geographical location.

A cadaveric study, carried out by Ozan et al.[7], reported the mean distance between CTR and SMA, RRA, and LRA as 18.0, 30.0, and 33.2 mm, respectively. According to Lawton et al.[8], the average CTR-SMA, CTR-RRA, and CTR-LRA distances on MDCT were 16.7±5.0, 30.7±7.9, and 30.5±7.7 mm, respectively. Arslan and Karacan[9] reported the mean distances for CTR- SMA, CTR-RRA, CTR-LRA, and CTR-IMA as 15.08±4.36, 30.35±9.17, 33.49±7.80, and 89.00±1.42 mm, respectively. Most of the studies measured the mean CTR-SMA distance between 14 and 18 mm[10-12]. The origins of the RRA and LRA were located between 28.5-32 mm, and 27-36 mm below the CTR, respectively[10,12]. A recent MDCT angiography study by Ekingen et al.[13] reported the mean CTR-SMA and CTR-IMA distances as

Table 3. The mean distances between the aorta at the diaphragmatic region and the origins of the CTR, SMA, RRA, LRA, and IMA according to age groups and gender

Distances (mm)		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	p values
Aorta at diaphragmatic region-CTR	F	25.6±7.1	28.1±8.5	32.7±8.8	40.5±10.7	53.9±11.2	46.1±16.8	p<0.001
	M	20.7±7.4	30.3±7.3	35.5±9.0	40.7±9.0	41.5±12.5	46.6±16.4	
	T	22.3±7.5	29.4±7.8	34.5±8.9	40.6±9.8	47.7±13.3	46.3±16.4	
Aorta at diaphragmatic region-SMA	F	34.3±8.5	38.1±8.5	44.7±8.7	53.5±12.7	70.6±13.2	60.6±16.5	p<0.001
	M	27.1±8.1	39.8±7.1	46.4±9.0	52.7±9.0	56.4±12.9	65.0±15.8	
	T	29.5±8.8	39.1±7.6	45.8±8.8	53.1±10.8	63.5±14.7	62.5±16.1	
Aorta at diaphragmatic region-RRA	F	34.9±9.6	45.5±10.7	56.2±8.8	60.4±13.7	77.1±11.9	65.7±13.4	p<0.001
	M	31.5±12.6	47.4±11.4	54.3±9.7	57.7±10.9	66.5±15.2	71.9±19.5	
	T	32.6±11.5	46.7±11.0	54.9±9.3	59.0±12.3	71.8±14.5	68.3±17.8	
Aorta at diaphragmatic region-LRA	F	38.6±8.3	45.8±9.8	56.7±7.6	62.8±13.1	79.5±12.0	67.4±15.5	p<0.001
	M	32.8±13.4	46.5±10.6	55.1±10.1	59.5±10.4	67.9±14.4	78.0±18.4	
	T	34.8±12.0	46.2±10.1	55.6±9.3	61.1±11.8	73.7±14.3	72.0±17.4	
Aorta at diaphragmatic region-IMA	F	66.3±9.8	78.5±8.9	92.9±9.5	108.8±18.1	133.5±22.8	122.7±20.9	p<0.001
	M	54.5±18.0	73.9±12.0	91.1±14.6	107.3±12.5	122.6±12.8	137.1±17.9	
	T	58.5±16.5	75.8±10.9	91.7±13.0	108.0±15.3	128.0±19.0	128.9±20.7	

Data are presented as mean±standard deviation. P values were obtained using one-way ANOVA test; p value <0.001 was considered highly significant.

F: Female, M: Male, T: Total, CTR: Celiac trunk, SMA: Superior mesenteric artery, RRA: Right renal artery, LRA: left renal artery, IMA: Inferior mesenteric artery

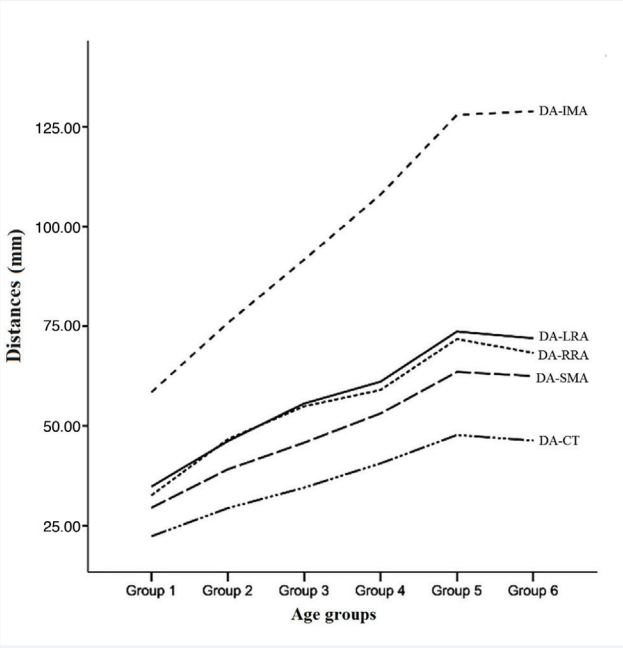

Figure 3. The graph shows the age-dependent distribution of the distances between the aorta at the diaphragmatic region (DA) and the origins of the celiac trunk (CTR), superior mesenteric artery (SMA), right renal artery (RRA), left renal artery (LRA), and inferior mesenteric artery (IMA

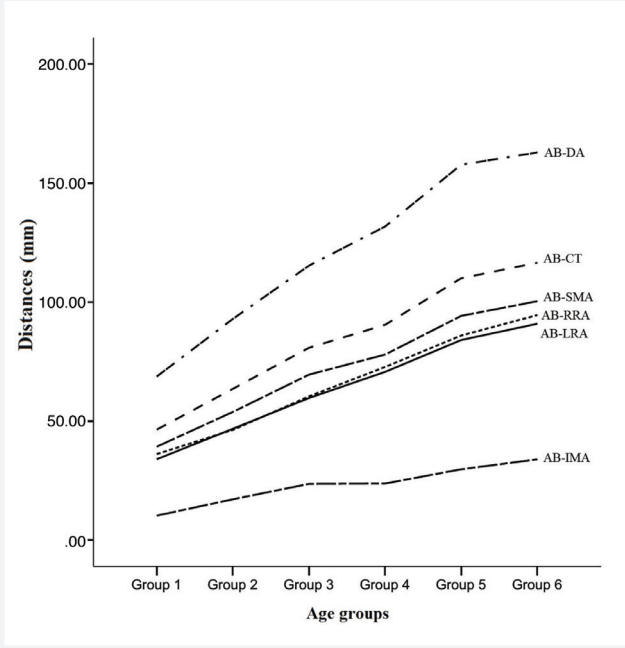

Figure 4. The graph demonstrates the age-dependent distribution of the distances between the aortic bifurcation (AB), aorta at the diaphragmatic region (DA), and the origins of the celiac trunk (CTR), superior mesenteric artery (SMA), right renal artery (RRA), left renal artery (LRA), and inferior mesenteric artery (IMA)

Table 4. The mean distances between the iliac bifurcation and the origins of the CTR, SMA, RRA, LRA, and IMA according to age groups and gender

Distances (mm)		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	p values
Iliac bifurcation-CTR	F	48.9±4.5	66.5±5.6	82.0±10.5	91.6±9.5	112.0±14.5	107.9±13.9	p<0.001
	M	45.2±13.2	61.5±8.6	80.2±10.9	89.5±13.5	108.1±14.9	128.1±9.9	
	T	46.4±11.0	63.6±7.8	80.8±10.7	90.5±11.7	110.1±14.7	116.6±15.9	
Iliac bifurcation-SMA	F	40.1±3.8	56.6±6.0	70.1±10.4	78.5±7.7	95.3±13.5	93.4±13.0	p<0.001
	M	38.8±12.3	52.0±8.9	69.3±11.4	77.5±11.5	93.2±13.4	109.6±8.5	
	T	39.3±10.2	53.9±8.0	69.5±10.9	78.0±9.8	94.2±13.3	100.4±13.8	
Iliac bifurcation-RRA	F	39.7±3.4	49.1±4.9	58.6±6.2	71.7±9.2	88.9±13.1	88.3±11.6	p<0.001
	M	34.4±8.3	44.3±8.6	61.4±10.0	73.8±8.1	83.1±13.6	102.8±10.6	
	T	36.1±7.4	46.3±7.6	60.4±8.9	72.8±8.6	86.0±13.5	94.6±13.2	
Iliac bifurcation-LRA	F	35.9±3.6	48.9±5.7	58.0±7.0	69.2±7.8	86.5±11.6	86.6±11.1	p<0.001
	M	33.0±7.1	45.3±8.7	60.6±9.5	72.1±8.8	81.7±12.6	96.6±8.8	
	T	34.0±6.2	46.8±7.7	59.7±8.7	70.7±8.4	84.1±12.2	90.9±11.2	
Iliac bifurcation-IMA	F	66.3±9.8	78.5±8.9	92.9±9.5	108.8±18.1	133.5±22.8	122.7±20.9	p<0.001
	M	54.5±18.0	73.9±12.0	91.1±14.6	107.3±12.5	122.6±12.8	137.1±17.9	
	T	58.5±16.5	75.8±10.9	91.7±13.0	108.0±15.3	128.0±19.1	128.9±20.7	
Iliac bifurcation-aorta at diaphragmatic region	F	74.5±9.8	94.7±10.4	114.7±7.7	132.1±16.1	165.9±19.8	154.0±21.6	p<0.001
	M	65.9±19.3	91.8±10.7	115.7±15.8	131.6±14.3	149.6±15.9	174.7±15.4	
	T	68.7±16.9	93.0±10.5	115.3±13.5	131.8±15.1	157.8±19.6	162.9±21.6	

Data are presented as mean±standard deviation. P values were obtained using One-Way ANOVA test; p value <0.001 was considered highly significant.

F: Female, M: Male, T: Total, CTR: Celiac trunk, SMA: Superior mesenteric artery, RRA: Right renal artery, LRA: Left renal artery, IMA: Inferior mesenteric artery

13.5±0.37 and 89.40±0.99 mm for males and 12.20±0.33 and 84.80±0.92 mm for females, respectively.

These studies were performed on adults and cannot be considered relevant for pediatric patients. The current study is the initial and unique imaging study to evaluate the vascular distances in children according to their age and gender. The mean distances between the origins of the CTR-SMA, CTR-RRA, CTR-LRA, and CTR-IMA were between 20% and 30% lower than those reported in adults[7-9,13]. Moreover, these distances increased significantly with age possibly related to aortic elongation with the age and body height of the children. Although not statistically significant, these distances were slightly longer among females probably due to the fact that girls in the pubertal and prepubertal periods tend to be taller than the boys. These findings were consistent with previously published results[8,14].

Sośnik and Sośnik[15] measured the SMA-RRA and SMA-LRA distances in 324 cadavers aged between 0 and 90 years. The average distance between SMA and renal arteries in children aged 0 to 20 years was 3.3±2.5 mm for the RRA and 4.1±3.7 mm for the LRA. The values reported by Sośnik and Sośnik[15] were nearly 60% lower than ours. The reasons for this discrepancy might be explained by the differences in the diagnostic tools, age of the study population, and geographical factors. Previous studies conducted on adult patients found that the origins of the RRA, LRA, and IMA were located between 2-84 mm, 3-50 mm, and 24-100 mm below the SMA, respectively[7,9,14,15], and were nearly 50% higher than the ranges reported in our study.

We found no data in the literature on the distances between IMA and renal arteries in the pediatric population. The mean distance between IMA and renal arteries in adults was 58.55±11.98 mm for the RRA and 55.10±11.38 mm for the LRA[9]. Our distance measurements were more than 20% lower than those reported in adults[9,14]. In the current study, the origin of the RRA was higher than LRA, which is in agreement with previous reports on adults[8,16].

Anamaria et al.[14] postulated that the origins of the CTR, SMA, RRA, LRA, and IMA in adults were located between 5.1-42.0 mm, 31.6-64.1 mm, 28.4-76.7 mm, 30.4-76.8 mm, and 107.4-153 mm below the level of the diaphragmatic aortic hiatus, respectively[14]. In the present study, the distances between the aorta at the diaphragmatic region and the origins of the major branches in children were between 40% and 65% lower than those reported by Anamaria et al.[14] Moreover, these distances were significantly longer among females, which is in disagreement with the previous study[14]. This evidence could be important in order to plan different diagnostic and treatment procedures in boys and girls; however, the clinical impact of this finding is still to be determined.

Yahel and Arensburg[10] measured the distances between aortic bifurcation and the origins of the unpaired splanchnic arteries in thirty-four adults and eight cadavers aged between 4 and 11 years. The average distances from the CTR, SMA, and IMA to the aortic bifurcation were 125.0±16.8 mm, 109.8±15.5 mm, and 41.8±6.9 mm, respectively[10]. Our distance measurements were approximately 35% lower than those reported by Yahel and Arensburg[10]. This discrepancy may have resulted from the selection bias, a small number of cadavers, and the differences in the diagnostic approach. These distances were not associated with gender, which is consistent with the findings reported by Yahel and Arensburg[10].

A previous MDCT study on adults showed that the origins of the RRA and LRA were located between 59.3-113 mm and 59.2-109 mm above the aortic bifurcation, respectively[14]. The distance from the aorta at the diaphragmatic region to the aortic bifurcation was between 135.3 and 183.0 mm[14]. These results were approximately 60% higher than those reported in the current pediatric study.

The normative data for the distances between the origins of the major branches of the abdominal aorta, and their distances to the aorta at the diaphragmatic region and iliac bifurcation in pediatric patients may show wide variations due to the differences in the geographical, racial, ethnic, and genetic factors. Thus, we recommend that reference data for the vascular distances should be determined on a national level.

Study Limitations

Our study had several limitations that should be acknowledged. The presented findings were compared with the results of similar studies on adults due to the lack of currently available standardized measures for children. There was also no "gold standard" measurement tool for confirming the CT results. We did not evaluate the diameters and the topographic location of the main branches of the abdominal aorta in relation to the vertebral bodies, which should be addressed in future studies. This was a single-center study including a limited number of patients in each age group, so that future multicenter studies of a larger pediatric population are recommended to verify our findings.

CONCLUSION

In conclusion, to the best of our knowledge, this is the first study to report the distances between the abdominal aorta and its branches in children up to 18 years of age. Our results might be used as a reference tool for determining the position of the major branches of the abdominal aorta which in turn may help to reduce the endovascular and surgical procedure-related complications in pediatric patients.

Ethics

Ethics Committee Approval: The study protocol was approved by University of Health Sciences Turkey, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Ethics Committee (protocol number: 2019/285, date: 11.04.2019).

Informed Consent: Retrospective study.

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How Effective is a Fecal Occult Blood Test to Detect Malignancy?

Maligniteyi Saptamak için Gaitada Gizli Kan Testi Ne Kadar Etkilidir?

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ABSTRACT

Aim: Cancer, an important public global health problem, is the second leading cause of death after cardiovascular diseases. We aimed to reveal the incidence of colorectal cancer (CRC) by the positive fecal occult blood test (FOBT), requested within the scope of periodic health examination (PHE) in patients applied to the family medicine outpatient clinic.

Materials and Methods: A total of 119 people aged between 50 and 70 years, who applied to the family medicine outpatient clinic of a university hospital for general health check-up, were included in the study. A questionnaire, in which socio-demographic data and CRC risk factors were questioned, was applied to the participants. The hemogram, FOBT, colonoscopy, and the pathology results of the patients were evaluated.

Results: Of 119 participants, 62 (52.1%) were female and 57 (47.9%) were male. The mean age of the participants was 61.0±7.6 years. FOBT was found to be positive in 65 (54.6%) of all participants. Five people (4.2%) who participated in the study were diagnosed with CRC according to the biopsy results obtained during the colonoscopy procedure. In our study, the rate of malignancy detection in all patients with positive FOBT including CRC diagnosis was found to be 7.7% (n=5). Our study detected statistically significant relationships between FOBT positivity and diagnosis of CRC.

Conclusion: Following the appropriate recommendations of PHE guidelines and CRC screenings in individuals with high-risk levels, FOBT contributes to early diagnosis and referral to treatment as soon as possible.

Keywords: Colorectal cancer, fecal occult blood test, cancer screening, periodic health examination

ÖZ

Amaç: Kanser, kalp damar hastalıklarından sonra ikinci önde gelen ölüm nedeni ve önemli bir halk sağlığı sorunudur. Aile hekimliği polikliniğine başvuran hastalarda periyodik sağlık muayenesi (PSM) kapsamında istenen gaitada gizli kan test (GGKT) pozitifliklerinde kolorektal kanser (KRK) görülme sıklığını ortaya koymayı amaçladık.

Gereç ve Yöntem: Çalışmaya bir üniversite hastanesinin aile hekimliği polikliniğine genel sağlık kontrolü için başvuran 50-70 yaş arasındaki 119 kişi dahil edildi. Katılımcılara sosyo-demografik verilerin ve KRK risk faktörlerinin sorgulandığı bir anket uygulandı. Hastaların hemogram, GGKT, kolonoskopi sonuçları ve patoloji sonuçları değerlendirildi.

Bulgular: Araştırmaya katılan 119 kişinin 62'si (%52,1) kadın, 57'si (%47,9) erkekti. Katılımcıların yaş ortalaması 61,0±7,6 yıl idi. Tüm katılımcılardan 65 kişinin (%54,6) GGKT'si pozitif saptandı. Araştırmaya katılan 5 kişiye (%4,2) kolonoskopi işleminde alınan biyopsi sonuç raporlarına göre KRK tanısı konuldu. KRK tanısı konan 5 kişinin GGKT sonucu pozitif olup, GGKT pozitif çıkan tüm hastalardaki malignite saptanma oranı, araştırmamızda %7,7 (n=5) olarak bulundu. Çalışmamızda GGKT pozitifliği ve KRK tanısı arasında istatistiksel olarak anlamlı bir ilişki saptandı.

Sonuç: Birinci basamak PSM kılavuzlarının uygun önerileri doğrultusunda ve risk düzeyi yüksek olan bireylerde KRK taramalarında, GGKT erken tanı koyması ve en kısa sürede tedaviye yönlendirmesine katkı sağlamaktadır.

Anahtar Kelimeler: Kolorektal kanser, gaitada gizli kan testi, kanser taraması, periyodik sağlık muayenesi

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INTRODUCTION

Cancer is the second leading cause of death and disease in Turkey after cardiovascular diseases as an important public health problem[1]. Cancer, in which environmental factors and genetic predisposition play a role together, differs from geographical, economic, social, and cultural reasons. In addition, epidemiological studies show that the frequency of colorectal cancer (CRC) increases in lifestyle changes such as obesity, red/processed meat consumption, tobacco and alcohol use. However, the pleasing part for some types of cancer is that they can be prevented by inexpensive and easily applicable methods[2].

CRC is the third most common cancer diagnosed in men and the second most common in women. Over the years, CRC grows slowly from premalignant lesions before transforming into a malignant form. CRC is suitable in screening for early diagnosis, as the colon and rectum are easy-to-scan organs in cancer screenings[3]. Studies have proven that CRC screening methods are effective in reducing cancer incidence and mortality. Screening methods of CRC include a fecal occult blood test (FOBT), rectosigmoid colonoscopy, colonoscopy, double-contrast barium enema, and computed tomography colonography. In particular, some studies have shown that FOBT can reduce mortality by 1/3[4].

Periodic health examination (PHE) is the evaluation of people who are not sick or have no signs of disease according to age, sex, and risk factors, through screening, examination, and tests with evidence-based structured, effective, specific, acceptable, and applicable follow-up plans[5]. PHE is recommended to follow-up and screen to detect precancerous lesions or early-stage tumors at regular intervals in non-patients in primary care. Thus, PHE aims to reduce the morbidity and mortality of healthy individuals by identifying the early stages symptoms and risk factors of preventable and treatable diseases. In our country, breast cancer, cervical cancer, and CRC are screened within the scope of PHE[6]. It is recommended that all men and women between the ages of 50 and 75 years, who are the target population, be screened for CRC once a year with FOBT and once every 10 years by colonoscopy[5].

Family physicians (FPs) are obliged to provide comprehensive and continuous personal preventive health services and primary diagnosis, treatment, and rehabilitative health services for everyone regardless of age, sex and disease. Therefore, FP fulfills one step of preventive health services with PHE[7]. In our study, we aimed to reveal the incidence of CRC in patients with FOBT positivity within the scope of PHE in patients who applied to the Family Medicine Outpatient Clinic.

MATERIALS AND METHODS

The study was prospectively planned in 119 volunteers who applied to a Tekirdağ Namık Kemal University Hospital Family Medicine Outpatients Clinic between October 2020 and March 2021, with the approval of the Ethics Committee (protocol no: 2020.168.07.01). People with a cancer diagnosis or colon disease and/or a history of upper gastrointestinal tract erosion or ulcer were not included in the study. In addition, people using antiaggregant, anticoagulant and non-steroidal anti-inflammatory drugs were among the exclusion criteria in our study. After getting informed consent, the healthy volunteers between the ages of 50 and 70 years were tested for FOBT within the scope of PHE. The FOBT was performed with the commercial FOB rapid test cassette (Feces)/citest kit. The occult blood was measured by rapid chromatographic card test and qualitative lateral flow immunoassay. It measures the occult blood in the stool over 50 ng/mL or 6 ug/g with the double sandwich technique. The test line has anti-hemoglobin (Hb) antibodies. Colored line formation indicates a positive result. The stool sample was collected in a clean container and studied within 6 hours. After mixing the buffer with the stool, the result was evaluated in 5 minutes. Relative sensitivity was 95% [95% confidence interval (CI): 91-97.6%], relative specificity was 98.6% [95% CI: (97.7%-99.2%)], and precision within- and between-run was >99%[8].

The questionnaire prepared by the researchers, which questioned socio-demographic data of the patients, chronic diseases, treatments, risk factors, and common symptoms of CRC, was administered to the participants face-to-face. A FOBT sample was requested 3 times from each participant. The test is not affected by diet. People give samples without taking alcohol and aspirin or other drugs for 48 hours. Participants, who gave consent for CRC screening, were then referred to the endoscopy unit for colonoscopy. Colonoscopy procedures to the entire colorectal region of the patients were performed by a gastroenterology specialist. Biopsy was taken from the lesions during colonoscopy and transferred to pathological examination. The participants were evaluated for Hb value, colonoscopy, pathology, and FOBT results according to the questionnaire form.

Statistical Analysis

The Kolmogorov-Smirnov test was performed in all groups and parametric/non-parametric distribution of parameters was determined. To examine the difference in parameters between the groups, the Student's t-test was used for tests with parametric distribution and the Mann-Whitney U test was used for tests with the non-parametric distribution. The categorical variables were analyzed using the chi-square test.

All statistical analyses were performed with the SPSS 18.0 (SPSS İnc., Chicago, IL) program, and p-values less than 0.05 were considered statistically significant.

RESULTS

Out of a total of 119 people in the research group, 62 (52.1%) were female and 57 (47.9%) were male. The mean age of the study group was 61.0±7.6 years (the mean age was 60.82±7.22 years for women and 61.28±7.99 years for men). There was no statistically significant difference between male and female genders in 46.2% (n=55) of patients aged 60 years and younger and 53.8% (n=64) of patients over 60 years of age (p>0.05) (Table 1). Considering the FOBT results of the participants, the tests of 65 people (54.6%) were positive and the tests of 54 people (45.4%) were negative (Figure 1).

When the relationship between socio-demographic characteristics and occult blood tests were evaluated, there was no statistical significance between age, gender, exercise, smoking-alcohol use, occupational status, and income level of individuals, and FOBT positivity. When we looked at FOBT positivity according to the BMI of the participants, a statistically significant relationship was determined with the FOBT positivity of the group with overweight/obese (p=0.023). When we questioned the dietary habits of the participants, FOBT positivity was significantly higher in the group with high animal food consumption than in the low consumption group. (p=0.029) (Table 1).

As a result of the colonoscopy procedure applied to all participants, no pathology was detected in 54 people (45.4%) and the most common pathology was colon polyps in 29 people (24.4%). According to the results of the colonoscopy report after the procedure, four people (3.4%) were pre-diagnosed with tumors. When the results of pathology samples taken during colonoscopy were evaluated, five people (4.2%) received the main diagnosis of CRC (Table 2).

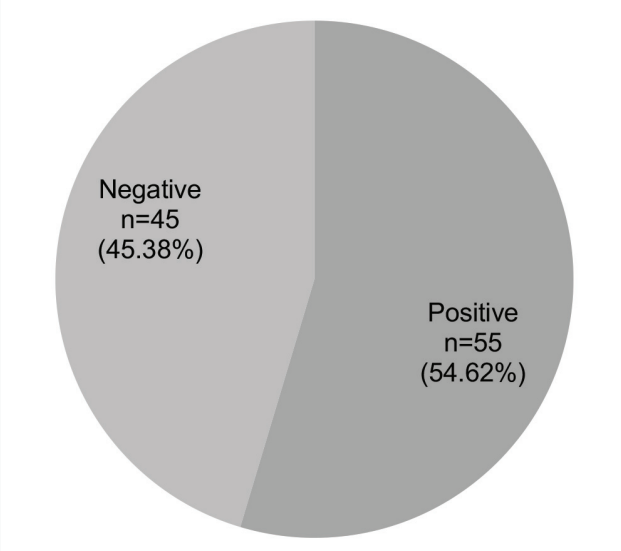

Figure 1. Fecal occult blood test positivity rate

Considering the colonoscopy report results and FOBT results of 119 patients who underwent colonoscopy, the incidence of FOBT positivity was statistically significantly higher in the group with lesions than in the group without lesions (p<0.001). FOBT positivity rates after colonoscopy were statistically significantly higher in the group with lesions compared to the group without lesions (p=0.017) (Table 3).

DISCUSSION

CRC is a largely treatable disease if diagnosed at an early stage. Early diagnosis of the disease is possible by screening people in the asymptomatic stage[9]. For this purpose, FOBT is an appropriate screening test in our country and reduces the need for colonoscopy[10].

According to the FOBT result applied to 119 people in our study, 65 people (54.6%) were tested as positive. In the study conducted by Levi et al.[11] on 16,359 people, of the 2.266 participants undergoing FOBT, 88 (3.9%) tests were positive. In many similar studies, FOBT positivity rates were found to be lower than in our study[12-14]. We can attribute the higher rate of FOBT positives in our results, compared to other studies, to the fact that our target group screened with PHE consisted of people aged 50 and over. However, epidemiological studies with larger participation are needed.

In our study, 50.8% of the participants who were positive for FOBT were female and 49.2% were male. In a similar study by Demiral et al.[15], 48.6% of those with positive FOBT results were female and 51.4% were male. In both studies, the rates of FOBT positivity and FOBT positivity according to gender were similar, and gender had no effect on the test result. Contrary to our study, in the study of Kara et al.[16], 60% of the 300 patients with positive FOBT were female and 40% were male, and they found the rate of test positivity higher in females.

In our study, no pathology was detected with colonoscopy in 21.5% of FOBT positive patients. In other similar studies, 75.7% of patients tested positive for FOBT were reported normal with colonoscopy, which was much higher than in our study[12,15,17]. In other studies similar to our findings, even pathological lesions were not detected in people with FOBT positive[18,19]. In our study, at least one lesion was detected in 78.5% of the patients in the colonoscopy performed on FOBT positive patients. The reason for the higher incidence of pathological lesions in our study can be attributed to the fact that the screened group was 50 years or older.

When we looked at the pathology results of our patients, whose FOBT results were positive, CRC was detected in 7.7% of the patients. Quyn et al.[20]. reported a 7.1% CRC detection rate in their series of 53,332 cases of FOBT positivity in the screening group, and our findings were similar to several similar studies[12,18]. There are also studies with a lower rate of CRC compared to our study[15,16,19].

In our study, CRC was detected in five people (4.2%) according to the colonoscopy results of all participants and no pathology was detected in 54 people (45.4%). In the study, conducted by Yaşar[21], CRC was found in 3.9% of people according to the results of colonoscopy, and no pathology was detected in 42.9%. When we compared our study with similar studies, the percentages of CRC were close to each other and our results were similar to the literature in this sense[22,23].We can attribute the differences in studies to the diversity of genetic and environmental factors, dietary changes, the frequency of familial CRC syndromes (such as FAP), and lower cancer screening rates in some countries.

Study Limitations

The research population is limited to 119 individuals applying to Tekirdağ Namık Kemal University Faculty of Medicine, Department of Family Medicine Outpatient Clinic.

CONCLUSION

In our study, FOBT was positive in patients diagnosed with CRC and there was a statistically significant relationship

Table 1. The relationship between the socio-demographic characteristics and habits of the participants and the results of the fecal occult blood test

Socio-demographic data		Fecal occult blood test		Total (%)	p
		Positive n (%)	Negative n (%)		
Gender	Female	33 (50.8)	29 (53.7)	62 (52.1)	0.750
	Male	32 (49.2)	25 (46.3)	57 (47.9)	
Age (years)	50-60	32 (49.2)	23 (42.6)	55 (46.2)	0.470
	61-70	33 (50.8)	31 (57.4)	64 (53.8)	
Body mass index	Normal	15 (23.1)	23 (42.6)	38 (31.9)	**0.023***
	Overweight/obese	50 (76.9)	31 (57.4)	81 (68.1)	
Exercise	Yes	27 (41.5)	21 (38.9)	48 (40.3)	0.769
	No	38 (58.5)	33 (61.1)	71 (59.7)	
Smoking	Yes	15 (23.1)	8 (14.8)	23 (19.3)	0.116
	No	31 (47.7)	36 (66.7)	67 (56.3)	
	Quit	19 (29.2)	10 (18.5)	29 (24.4)	
Alcohol use	Yes	7 (10.8)	6 (11.1)	13 (10.9)	0.913
	No	56 (86.2)	47 (87.0)	103 (86.6)	
	Quit	2 (3.1)	1 (1.9)	3 (2.5)	
Animal food consumption	Rarely	4 (6.2)	5 (9.3)	9 (7.6)	0.029*
	Sometimes	30 (46.2)	36 (66.7)	66 (55.5)	
	Often	31 (47.7)	13 (24.1)	44 (37.0)	
Family history of IBD CRC in family history	Yes	5 (7.7)	1 (1.9)	6 (5.0)	0.147
	No	60 (92.3)	53 (98.1)	113 (95.0)	

* p<0.05, IBD: Irritable bowel disease, CRC: Colorectal cancer

Table 2. Lesions detected in participants according to colonoscopy and pathology results

According to colonoscopy results	n (%)	According to the pathology results	n (%)
Polyp	29 (24.4)	Adenomatous polyp	11 (9.2)
Inflammation	16 (13.4)	Inflammation	19 (16.0)
Hemorrhoids	12 (10.1)	Hyperplastic polyp	7 (5.9)
Tumor	4 (3.4)	Malignancy	5 (4.2)
Angiodysplasia	2 (1.7)	Tubular adenoma	9 (7.6)
Ulcerated lesion	2 (1.7)	Adenomatous polyp	11 (9.2)

Table 3. The relationship between the colonoscopy and pathology results of the participants and the result of the fecal occult blood test

		Fecal occult blood test		Total (%)	p
		Positive n (%)	Negative n (%)		
Colonoscopy result	Polyp	18 (27.7)	11 (20.4)	29 (24.4)	0.000**
	Inflammation	14 (21.5)	2 (3.7)	16 (13.4)	
	Hemorrhoids	12 (18.5)	0 (0.0)	12 (10.1)	
	Tumor	4 (6.2)	0 (0.0)	4 (3.4)	
	Angiodysplasia	2 (3.1)	0 (0.0)	2 (1.7)	
	Ulcerated area	1 (1.5)	1 (1.9)	2 (1.7)	
	Natural	14 (21.5)	40 (74.1)	54 (45.4)	
Pathology result	Inflammation	17 (44.7)	2 (15.4)	19 (37.3)	0.017*
	Adenomatous polyp	8 (21.1)	3 (23.1)	11 (21.6)	
	Malignancy	5 (13.2)	0 (0.0)	5 (9.8)	
	Tubular adenoma	6 (15.8)	3 (23.1)	9 (17.6)	
	Hyperplastic adenoma	2 (5.3)	5 (38.5)	7 (13.7)	

*p<0.05, **p<0.001

between FOBT positivity and malignancy diagnosis. FOBT, which is evaluated in our country with the clinical findings and the age of the patient in cancer screenings carried out within the scope of PHE, has an important place in reducing mortality and morbidity by early diagnosis of CRC. Screening of the registered population by FPs in terms of risk factors and ensuring protection before disease occurs is the key for preventive medicine in terms of informing people about cancer screening programs. As FPs, we should provide education to the population we follow about cancer screenings and counseling on predisposing causes such as obesity, nutrition, smoking and alcohol use that play a role in the etiology of CRC.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Tekirdağ Namık Kemal University Ethics Committee (protocol no: 2020.168.07.01, date: 28.07.2020).

Informed Consent: It was planned prospectively on 119 volunteers who applied to the family medicine outpatient clinic of a university hospital between October 2020 and March 2021.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.A.Ş., E.Ç.G., R.M., Concept: T.A.Ş., E.Ç.G., Design: T.A.Ş., E.Ç.G., R.M., Data Collection or Processing: T.A.Ş., E.Ç.G., R.M., Analysis or Interpretation: T.A.Ş., E.Ç.G., Literature Search: T.A.Ş., E.Ç.G., Writing: T.A.Ş., E.Ç.G.

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Does the Pathology Education Received in the Undergraduate Preclinical Years Provide Benefits in the Clinical Education Period? A Survey on 5th Grade Medical Students

Temel Tıp Döneminde Alınan Patoloji Eğitimi Klinik Eğitim Döneminde Yarar Sağlıyor mu? 5. Sınıf Tıp Öğrencilerinde Yapılan Bir Anket Çalışması

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ABSTRACT

Aim: We aimed to evaluate the perceptions of 5th grade medical faculty students regarding the adequacy of the pathology education they received in the preclinical period and the benefit of it in clinical clerkship training,

Materials and Methods: A 14-question digital questionnaire consisting of open-ended and multiple choice questions was created and sent via e-mail and whatsapp to the students who were in the 3rd grade at our faculty of medicine in the 2018-2019 academic year and were in the 5th grade in the 2020-2021 academic year. The results were analyzed and statistically evaluated.

Results: 56% of the students found the pathology education they received sufficient. Microscopy and macroscopy training, visiting the pathology laboratory, case-based discussion and self-study of the resources suggested by the instructors had a significant contribution to students' learning ($p<0.05$). The medical education received in the 3rd grade offered a significant benefit during the clinical clerkship period ($p=0.02$). The greatest benefit was obtained from macroscopy education and the students strongly recommended macroscopy and case based learning. According to 75% of the students, the education they received helped to understand the disease mechanism.

Conclusion: The medical pathology education formed the basis of the clinical clerkship period and case-based learning and macroscopy training were the most effective methods. To be more useful, didactic lessons should be combined with different and up-to-date learning methods and the curriculum should be updated. Standardization of pathology education in medical faculties is also an issue that needs to be addressed.

Keywords: Undergraduate medical education, pathology, macroscopy training, case based learning, curriculum

ÖZ

Amaç: Bu anket çalışmasında tıp fakültesi 5. sınıf öğrencilerinin preklinik dönemde aldıkları patoloji eğitiminin yeterliliği ve klinik staj eğitimlerine faydası ile ilgili algılarının ölçülmesi ve değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Açık uçlu ve çoktan seçmeli sorulardan oluşan 14 soruluk bir dijital anket oluşturularak, 2018-2019 eğitim yılında tıp fakültemizde 3. sınıfı okumuş ve 2020-2021 eğitim yılında 5. sınıfta okumakta olan öğrencilere e-mail ve WhatsApp aracılığı ile gönderilmiştir. Sonuçlar incelenmiş ve istatistiksel olarak değerlendirilmiştir.

Bulgular: Öğrencilerin %56'sı 3. sınıfta aldıkları patoloji eğitimini yeterli bulmuştur. Mikroskopi ve makroskopi eğitiminin, patoloji laboratuvarı ziyaretinin, olgu örneği eşliğinde tartışmanın ve eğitmenin gösterdiği kaynakları kendi kendine çalışmalarının öğrencilerin patolojiyi öğrenmelerine anlamlı katkısı saptanmıştır ($p<0,05$). Öğrenciler 3. sınıfta alınan tıp eğitiminin klinik staj döneminde anlamlı yararı olduğunu düşünmektedir ($p=0,02$). En yüksek yararı makroskopi eğitiminden görmüş olup makroskopi ve olgu bazlı öğrenme çalışmaları yapılmasını önermektedirler. Öğrencilerin %73'ü aldıkları eğitimin hastalık mekanizmasını anlamakta yarar sağladığını düşünmektedir.

Sonuç: Fakültemizde alınan tıbbi patoloji eğitiminin klinik staj dönemine temel oluşturduğu ve kliniği öğrenmekte faydalı olduğu görülmüştür. Öğrenciler, olgu bazlı öğrenme ve makroskopi eğitimini en etkili yöntemler olarak görmekte ve ağırlık verilmesini önermektedirler. Didaktik derslerin

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daha yararlı olması için farklı ve güncel öğrenme yöntemleri ile kombine edilmesi ve müfredatın güncellenmesi uygun olacaktır. Ülkemizde tıp fakültelerinde patoloji eğitiminin standardizasyonu da üzerinde çalışılması gereken bir konudur.

Anahtar Kelimeler: Tıp eğitimi, patoloji, makroskopi eğitimi, olgu bazlı öğrenme, müfredat

INTRODUCTION

Pathology teaching in medical faculties that run an integrated program aims to enable medical students to gain competence in three basic areas. These are understanding of disease mechanisms, integration of mechanisms into organ system pathology, and application of pathology to diagnostic medicine[1-4]. Traditionally, classroom lectures and laboratory practice training are carried out together in pathology education. In the last 10 years of PubMed, Medline and ULAKBİM literature review, there is no national or international publication that measures the reflection of the pathology education given in the 2nd and 3rd grades to the clinical education years. In this survey-based study, it was aimed to measure and evaluate the perceptions of the 5th grade students of our faculty about the value of pathology and the reflection of the pathology education that they previously received on their clinical (internship-practice) education. It is thought that the results will contribute to the development of pathology education in medical faculties.

MATERIALS AND METHODS

In this study, among the students who had attended the 3rd grade in our medical faculty in the 2018-2019 academic year and who were studying in the 5th grade in the 2020-2021 academic year, those who gave their consent to participate in the survey were included. A 14-item questionnaire, created jointly by the Medical Training and Medical Pathology Departments and prepared with Google Forms, was sent to the students via e-mail or WhatsApp, and they were asked about the reflection of the pathology education they received during their basic education years on their clinical education. The questions were prepared as open-ended (2 questions), multiple-choice with single answer (2 questions), and multiple-choice with answer more than one (2 questions) and with Likert scale (8 questions) method (Figure 1).

This study sought answers to the following questions:

- Which learning methods do the students think contribute and will contribute positively to their success in clinical internships in the 3rd year?

- According to the students, what are the positive aspects of the pathology education given in the 3rd grade and which aspects need to be improved?

Statistical Analysis

The data were analyzed by applying the chi-square test with the SPSS 23.0 statistical software. In the chi-square test, $p<0.05$ was considered significant.

RESULTS

33 (47.8%) of 69 students attending the 5th grade participated in the study by giving their consent (13 girls/20 boys). The age distribution was between 21 and 27 years, with a mean age of 22.8 years. Six (18.1%) of the students found the education that they received insufficient, 8 (24.2%) partially sufficient, 11 (33.3%) sufficient and 8 (24.2%) very sufficient.

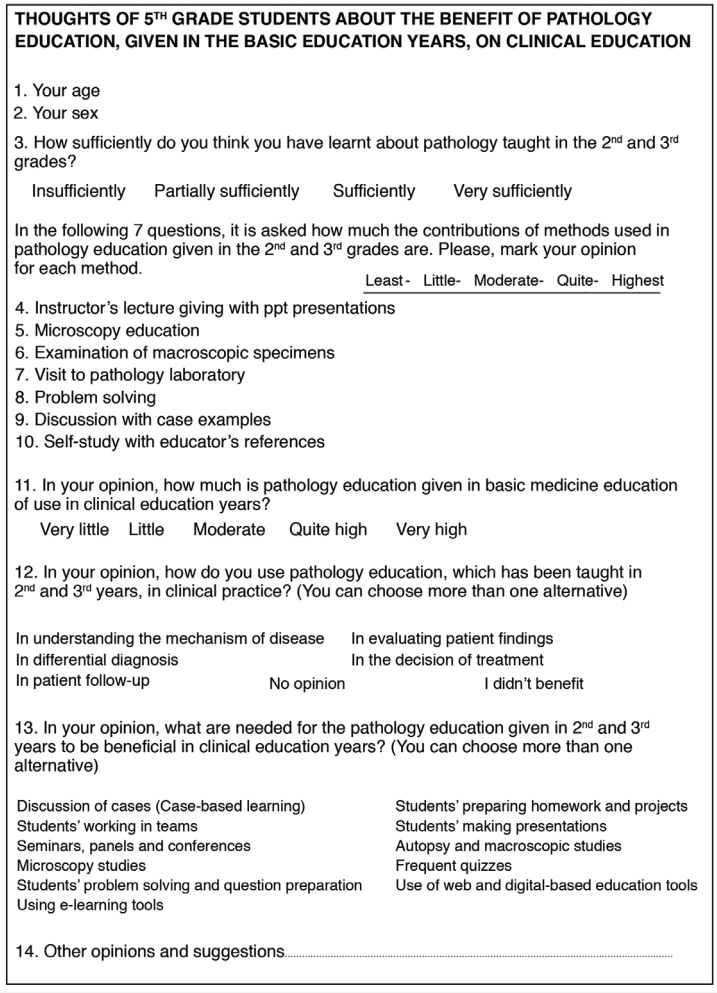
The evaluations of the contribution of the methods used in the pathology education to students' learning are given in Table 1. Students thought that microscopy/macroscopy training, pathology laboratory visit, discussion with case examples and self-study methods with the instructor's reference contribute more to their learning.

The students were asked, "How useful do you think the pathology education received in basic medical education is during the clinical education years?" and their answers to the question are shown in Figure 2. According to the results, there is a statistically significant relationship between the total adequacy of the education they received and its clinical usefulness ($p=0.02$). Approximately 68% of the students think that the education received will provide a lot of benefits in the clinic.

To the question "How do you think the pathology education you received in your 2nd and 3rd grades is useful for you in the clinic?", the students answered as "for understanding the disease mechanism" with the highest rate (72.7%, n=24). Other responses are shown in Figure 3.

The answers given to the question of what is needed for the pathology education received in the preclinical period to be useful during the clinical education years are given in Table 2. Accordingly, the most recommended education method is case-based learning (63.6%) and autopsy and macroscopic studies (63.6%).

An open-ended question for their other opinions and suggestions was asked to the students and some of the answers are presented in Table 3.



THOUGHTS OF 5TH GRADE STUDENTS ABOUT THE BENEFIT OF PATHOLOGY EDUCATION, GIVEN IN THE BASIC EDUCATION YEARS, ON CLINICAL EDUCATION

1. Your age
2. Your sex
3. How sufficiently do you think you have learnt about pathology taught in the 2nd and 3rd grades?

Insufficiently Partially sufficiently Sufficiently Very sufficiently

In the following 7 questions, it is asked how much the contributions of methods used in pathology education given in the 2nd and 3rd grades are. Please, mark your opinion for each method.

Least- Little- Moderate- Quite- Highest

4. Instructor's lecture giving with ppt presentations
5. Microscopy education
6. Examination of macroscopic specimens
7. Visit to pathology laboratory
8. Problem solving
9. Discussion with case examples
10. Self-study with educator's references

11. In your opinion, how much is pathology education given in basic medicine education of use in clinical education years?

Very little Little Moderate Quite high Very high

12. In your opinion, how do you use pathology education, which has been taught in 2nd and 3rd years, in clinical practice? (You can choose more than one alternative)

In understanding the mechanism of disease
In evaluating patient findings
In differential diagnosis
In the decision of treatment
In patient follow-up
No opinion
I didn't benefit

13. In your opinion, what are needed for the pathology education given in 2nd and 3rd years to be beneficial in clinical education years? (You can choose more than one alternative)

Discussion of cases (Case-based learning)
Students' working in teams
Seminars, panels and conferences
Microscopy studies
Students' problem solving and question preparation
Using e-learning tools
Students' preparing homework and projects
Students' making presentations
Autopsy and macroscopic studies
Frequent quizzes
Use of web and digital-based education tools

14. Other opinions and suggestions.....

Figure 1. Questionnaire

Table 1. "What is the contribution of the methods used in the pathology education you received in the 2nd and 3rd grades to your learning?"

	The least contribution (%)	Little contribution n (%)	Moderate contribution n (%)	Considerable contribution n (%)	The highest contribution n (%)
Instructor's lecture accompanied by ppt presentation	3 (9.0)	2 (6.0)	6 (18.1)	11 (33.3)	11 (33.3)
Microscopy education	5 (15.1)	1 (3.0)	7 (21.2)	8 (24.2)	12 (36.4)
Examination of macroscopic specimens	1 (3.0)	4 (12.1)	6 (18.1)	5 (15.6)	17 (51.5)
Pathology laboratory visit	3 (9.0)	6 (18.1)	6 (18.1)	9 (27.3)	9 (27.3)
Problem solving	10 (30.3)	4 (12.1)	5 (15.1)	4 (12.1)	10 (30.3)
Discussion with case example	6 (18.1)	3 (9.0)	3 (9.0)	5 (15.2)	16 (48.5)
Self-study with instructor's reference	8 (24.2)	3 (3.0)	6 (18.1)	8 (24.2)	8 (24.2)

n=33, ppt; PowerPoint

DISCUSSION

In our country, medical education includes 3 years of preclinical education, 2 years of clinical education and 1 year of internship. Medical Pathology, which is the branch of surgical sciences, and Medical Pharmacology, which is the branch of internal sciences, are also taught in the preclinical education years. Medical Pathology education aims to help doctor candidates to understand the pathogenesis of diseases and thus to comprehend diagnosis and treatment more easily.

Each medical school prepares a medical education curriculum according to the National Core Education Program (UCEP) objectives[4] and determines the pathology topics and course hours. Although the desired goal is the same, it is noteworthy that there is no standardization among medical faculties in our country regarding the classes and course hours in which medical pathology is given[5]. Another difference among medical faculties is the education methods applied[5-7]. Medical education cannot be considered as a priority issue because of the fact that the staff providing education in medical faculties are also those who provide routine diagnostic services and research, and it may not be possible to diversify education methods, benefit from modern methods or evaluate the success of education under heavy workload.

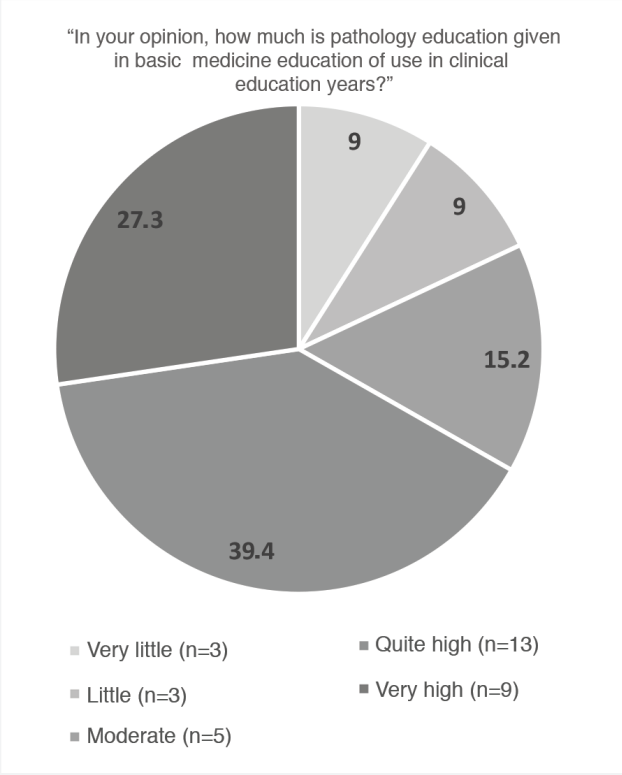


Figure 2. Responses to the question "How useful do you think the pathology education received in basic medical education is during the clinical education years?" (%)

Table 2. "What is needed for the pathology education you receive in the 2nd and 3rd grades to be useful during the clinical education years?"

Recommendations	n (%)
Discussion of cases (case-based learning)	21 (63.6)
Students' preparing homework and projects	12 (36.4)
Students' working in teams	7 (21.2)
Students' making presentations	12 (36.4)
Seminars, panels and conferences	3 (9.1)
Autopsy and macroscopic studies	21 (63.6)
Microscopy studies	14 (42.4)
Frequent quizzes	8 (24.2)
Students' problem solving and question preparation	9 (27.3)
Use of web and digital-based education tools	11 (33.3)
Using e-learning tools	7 (21.2)

Pathology education is given in the 2nd and 3rd grades in our medical faculty. In the second year, introduction to pathology about general pathology, terminology, cell damage and cell adaptation, inflammation and repair are covered, while in the third year, immunology, neoplasia and systems pathology courses are included in the integrated curriculum. In the group included in this study, theoretical lessons were taught with the traditional didactic method, and case examples were discussed during the lessons in order to integrate the pathology subjects with the clinic, and it was aimed to continue the lesson in an interactive manner by placing questions in Powerpoint presentations. However, education methods such as problem-based learning or teamwork were not used. The laboratory application part of the pathology courses was carried out with clinical discussions of case preparations prepared in light microscopy according to the number of students in the 2018-2019 academic year when this study was tested. In our faculty, in addition to microscopy, macroscopy specimens in the pathology museum are discussed. Moreover, visits are made to the pathology laboratory of the advanced training hospital, and the students receive information about the functioning of the pathology clinic from their representatives in each profession and see the process in place.

Up to 70% of the students who participated in the survey in this study stated that they benefited from the pathology training they received to a great extent during their clinical internship. They also stated that they benefited especially in understanding the mechanism of the disease, evaluating the patient's findings, and during differential diagnosis. These data suggest that the pathology education that we give has been successful and has achieved its main goals.

According to the results of our survey, 67% of the students, who thought that the pathology education they received was sufficient for them, benefited greatly from the macroscopic study. Consistently, the majority of students (64%) thought that training in macroscopy and autopsy is needed for pathology education to be useful during the years of clinical education. The benefits of macroscopic education are also emphasized in current publications on pathology education, but also the difficulties of establishing pathology museums are mentioned[8]. Moreover, there are publications on the use of digital techniques[9], video images, macroscopic specimens created via 3D printing[10], and mobile learning platforms[8]. Studies on

education methods in the last 10 years have focused on the importance of autopsy and cadaver in pathology education[11,12] and all have emphasized that this type of education is of great benefit despite the decreasing number of cadavers/autopsies. In our faculty of medicine, there is a large macroscopic archive of approximately 50 cases containing benign and malignant pathology samples of many organs such as thyroid, stomach, colon, breast, kidney and lung. The students who participated in this study and received the pathology education face to face had the opportunity to closely examine and discuss the specimens suitable for the committees, with their educators. As it is clearly seen in the student evaluations, it would be useful to be able to benefit from this archive, which we could not use during the pandemic period, as soon as possible. We think that the inclusion of autopsy observations in pathology practice trainings for the systemic evaluation of pathologies will also contribute a lot to education. After the pandemic process, we aim to speed up the planning and organization meetings that we started with the Forensic Medicine Institute. Although close observation will contribute more, we believe that autopsy training can also be done with up-to-date technologies and digital solutions.

Studies on which method contributes more to medical students in learning pathology show that educational methods in which students can participate interactively and learn by themselves are more beneficial[4,13,14]. According to our survey study, our students benefited from the case-based discussion sessions at a high level and reported that it was also useful in clinical internships (both, 64%). The benefit of integrated, case-based and problem-based learning is clearly seen in current medical education[3,15,16]. Students who have received digital microscopy laboratory education in our medical faculty since 2019 examine the cases together with their clinical, laboratory, radiological and pathological features, prepare in advance, present the cases during the laboratory education and discuss them with their educators. During the clinical internship years of these students, we plan to repeat the survey, which is the subject of this study, and discuss the results again.

The numbers of students who said that they benefited or did not benefit from the didactic education provided by the instructor, which is the most classical and common form of education, accompanied by powerpoint slides are close to each

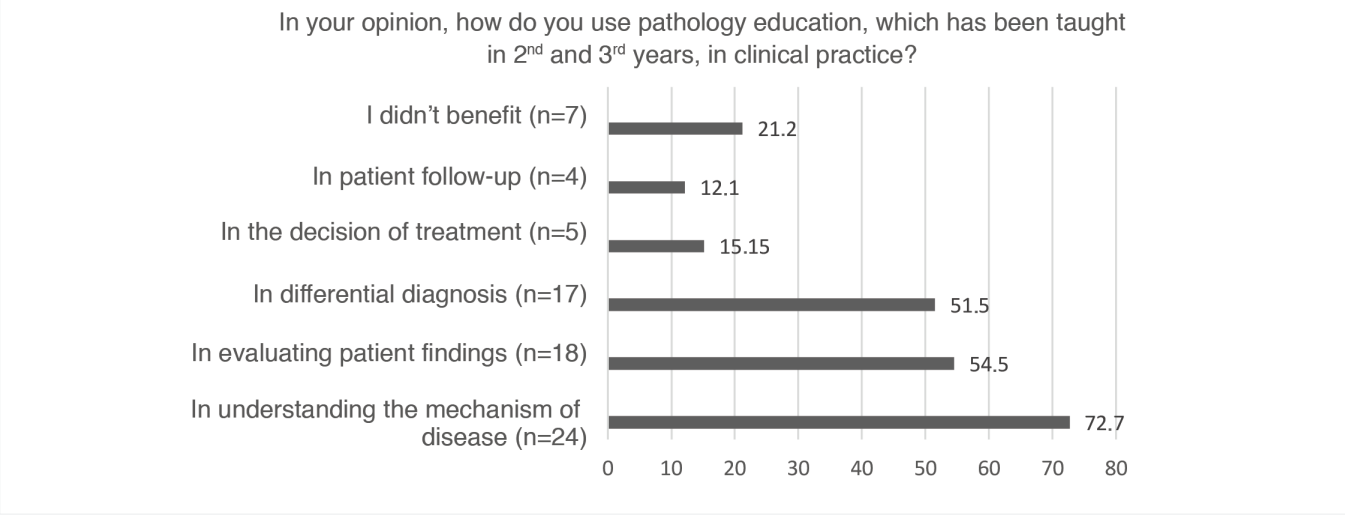


Figure 3. The answers given by the students to the question "how is the pathology education you receive in the 2nd and 3rd grades of use to you in the clinic?" (%)

Table 3. "What are your opinions and suggestions about the benefits of pathology education in clinical internships?"
We understand the role of pathology in understanding the disease, knowing its mechanism and planning the treatment for a physician, much better. We would love to receive pathology training again or a short repetition right now.
If the case-based lecture and the pathophysiology of the diseases are well understood, the work becomes a little easier in the clinic. For example, knowing the cardiomyopathies we have learned in cardiology internship from pathology made it easier for us in the oral and theoretical exams.
Carrying out a case-based and question-based education plan in pathology education will help students better understand both diseases and major concepts.
Pathology is one of the fields I mostly got use of in clinical internship. If I understood its pathophysiology, I mastered the diseas better.

other. Some arrangements can be made so that all students can get the maximum benefit from these courses, which require a long and detailed pre-work for the instructor. It is possible to enrich the traditional didactic lessons with various techniques, especially with case and problem-based learning, role-playing and team-based learning, and to make them useful and interesting[17-20]. Azer found 1061 publications, half of which were conducted in the last 4 years, in the search of Medline/HighWire databases for publications made between 1978 and 2003 with the keywords "good educator" and "mentor"[21]. The researcher stated that this result showed that the awareness about the training, development and monitoring of educators was increasing gradually and added that good educators were also good role models, affected students' career choices and enabled students to reveal their true potential[21]. It should also be considered that this issue should be handled on the basis of administrative approaches and planning, and that pathology specialists who choose to be educators should be evaluated in a different status.

The theoretical load of medical education is heavy in the 2nd and 3rd years of medical education, and pathology courses cover a wide area of the preclinical curriculum. The heavy curriculum makes it difficult to diversify education methods and reach students who learn in different ways. In this respect, it may also be beneficial to review the pathology curriculum together with the Department of Medical Education in line with the goals of UCEP. Moreover, methods such as flipped classroom, working in groups, peer education, and student presentation can reduce the number of didactic lessons. It would be appropriate for the educator to gradually turn from lecturer to a guide and facilitator[22].

There are also studies that mention the importance of an accelerated repetition of pathology during the internship period[23-25]. In these publications, it is stated that a rapid repetition of pathology-specific medical knowledge, basic skills and processes in anatomical pathology and laboratory medicine in the last year of medical school will be beneficial, and it is also thought that it will increase the orientation to pathology specialization. This issue can also be reviewed with the Department of Medical Education and also discussed on a national scale for curriculum standardization.

As Hortsh says in his article, "listening to the student will have its own rewards"[26]. We believe in the importance of student feedback in the development of learning materials and educational strategies and in order for the educator to be more effective. We think that a two-way, effective educator-student communication will make it easier for us to reach the educational goal we want to achieve. Therefore, we believe that such survey studies will contribute to the quality of education.

Study Limitations

One of the limitations of our survey study was the low participation in the survey (48%, 33 students). However, it was determined that the group had diversity suitable for the analysis. Another limitation is that the sample group consisted of students of our relatively young faculty, who were given pathology training for the first time. After the class chosen as an example for this survey study, Digital Microscopy education was started in the pathology laboratories in the 3rd grades in the 2019-2020 academic year. The next academic year (2020-2021), on the other hand, was conducted completely synchronously online due to the global pandemic. Despite the difficulties of the pandemic conditions, education has been tried to be diversified and updated even more with methods such as case-based education, teamwork, peer education, addition of digital opportunities, and panels in each new student group. It would be appropriate to learn the thoughts and evaluations of these student groups with a new questionnaire when they reach the 5th grade and to discuss them by comparing with the current results.

CONCLUSION

In summary, in this survey study, it was aimed to obtain data that would benefit education planning by asking 5th grade medical students how much and how they benefited from the pathology education they received in the 3rd grade during their clinical internships. According to the findings, the medical pathology education formed the basis of the clinical internship period and was useful in learning the clinic. We found that the two most effective methods on this subject in this group of students who received medical pathology training during the face-to-face education period were case-based discussion and macroscopy education. It is considered appropriate to combine didactic courses with different and up-to-date learning methods and to review the curriculum with the Department of Medical Education in order to be more useful. In our country, discussion and standardization of pathology education in medical faculties are among the subjects that need to be studied.

Acknowledgements

We thank the students who gave consent and joined the questionnaire for their valuable contributions to medical education.

Ethics

Ethics Committee Approval: The study was approved by the Human Research Ethics Committee of Istinye University (protocol no: 87, date: 15.09.2020).

Informed Consent: Informed consent is taken from all participants.

Peer-review: This survey study is externally peer reviewed.

Authorship Contributions

Practices: S.Ş., S.K., Y.S.G., N.E., Concept: S.Ş., S.K., Y.S.G., Design: S.Ş., S.K., Y.S.G., H.K., N.T.F., Data Collection or Processing: S.Ş., S.K., Y.S.G., Analysis or Interpretation: S.Ş., S.K., Y.S.G., H.K., N.T.F., Literature Search: S.Ş., S.K., Writing: S.Ş., S.K., Y.S.G., H.K., N.T.F.

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Cochlear Implant Surgery Experiences of a Tertiary Health Center in the Thrace Region

Trakya Bölgesinde Üçüncü Basamak Bir Sağlık Merkezinin Koklear İmplant Cerrahisi Deneyimleri

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ABSTRACT

Aim: Cochlear implants (CIs) aid in language and speech development through improved hearing in patients with bilateral severe or profound hearing loss. In this study, we evaluated the outcomes of our patients undergoing CI surgery.

Materials and Methods: Preoperative, perioperative and postoperative clinical and audiological findings, hearing loss etiology, surgical approach techniques, and complications were evaluated retrospectively in 31 patients (35 ears) undergoing CI surgery.

Results: Thirty one patients (13 adults and 18 children) were included in the study. After posterior tympanotomy following cortical mastoidectomy, electrodes were introduced through the round window in 21 ears and via cochleostomy in 14 ears. CIs with different number of electrodes (22, 16, 12) from 3 different companies were used. No postoperative complications were observed in any of the patients. The mean free field audiogram (FFA) was 95.2±19.13 dB preoperatively and 37.8±8.46 dB postoperatively in 24 patients who attended the control visits. Postoperative hearing gains were significantly different from the preoperative values (p<0.001). There was no significant difference between different devices (p=0.340). Electrodes were introduced through the round window or by cochleostomy, and comparison of these two groups revealed no statistically significant difference in terms of postoperative FFA values (p=0.425) or speech awareness threshold and speech reception threshold values (p=0.132).

Conclusion: The significant hearing gains in the postoperative period without any complications indicate the success of the surgical technique utilized in this study. It can be said that the difference in electrode insertion location and numbers does not affect the postoperative results.

Keywords: Cochlear implantation, postoperative complications, cochleostomy, round window, correction of hearing impairment

ÖZ

Amaç: Koklear implantlar (Kİ), iki taraflı ciddi veya ileri derecede işitme kaybı olan hastalarda işitmeyi iyileştirerek dil ve konuşma gelişimine yardımcı olur. Bu çalışmada, Kİ cerrahisi geçiren hastalarımızın sonuçlarını değerlendirdik.

Gereç ve Yöntem: Kİ cerrahisi uygulanmış olan 31 hastada (35 kulak); preoperatif, perioperatif ve postoperatif klinik ve odyolojik bulgular, işitme kaybı etiyolojisi, cerrahi yaklaşım teknikleri ve komplikasyonlar retrospektif olarak değerlendirildi.

Bulgular: Otuz bir hasta (13 yetişkin ve 18 çocuk) çalışmaya dahil edildi. Kortikal mastoidektomiyi takiben posterior timpanotomi sonrası 21 kulağa yuvarlak pencereden ve 14 kulağa kokleostomi ile elektrotlar yerleştirildi. Üç farklı firmadan farklı sayıda elektrotlu (22, 16, 12) Kİ'ler kullanıldı. Hiçbir hastada postoperatif komplikasyon görülmedi. Kontrole gelen 24 hastanın ortalama serbest alan odyogramı (FFA) ameliyat öncesi 95,2±19,13 dB, ameliyat sonrası 37,8±8,46 dB idi. Ameliyat sonrası işitme kazanımları ameliyat öncesi değerlerden anlamlı derecede farklıydı (p<0,001). Farklı marka cihazlar arasında anlamlı bir fark yoktu (p=0,340). Elektrotlar yuvarlak pencereden veya kokleostomi ile yerleştirildi ve bu iki grubun karşılaştırılmasında, postoperatif FFA değerleri (p=0,425) veya konuşma farkındalığı eşiği (SAT) ve konuşmayı algılama eşiği (SRT) değerleri (p=0,425) açısından istatistiksel olarak anlamlı bir fark görülmedi (p=0,132).

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Sonuç: Postoperatif dönemde herhangi bir komplikasyon olmaksızın elde edilen önemli işitme kazanımları, bu çalışmada kullanılan cerrahi tekniğin başarısını göstermektedir. Ayrıca elektrot yerleştirme yeri ve sayıları arasındaki farklılığın da ameliyat sonrası sonuçları etkilemediği söylenebilir.

Anahtar Kelimeler: Koklear implantasyon, postoperatif komplikasyonlar, kokleostomi, yuvarlak pencere, işitme bozukluğunun düzeltilmesi

INTRODUCTION

Cochlear implant (CI) is an electronic neuroprosthesis, applied in patients with bilateral severe or profound sensorineural hearing loss (SNHL) who do not benefit from conventional hearing aids, and it is effective in rehabilitation of prelingual and postlingual deafness[1]. CI allows children to improve their speech skills by giving them an opportunity to hear. In adults who develop deafness later in life, CI supports communication through regained hearing[2,3].

Most cases of deafness are caused by the absence or damage of cochlear hair cells. The defect in cochlear functions interferes with the transformation of mechanical acoustic signals into synaptic activity of the auditory nerve[4]. CIs are electronic devices that convert sound into electrical signals, bypass defective cells and directly stimulate the spiral ganglion cells. This allows the transmission of acoustic information to the central nervous system through direct electrical stimulation of cochlear nerve fibres[4].

The main components of a CI system include a microphone that collects the sound and converts it into an electrical signal, an external processor that processes these signals, an internal receiver-stimulator, and an electrode carrier fitted inside the cochlea to transmit electrical signals to spiral ganglion cells[5,6]. Devices of different brands have different features such as consisting of 32, 22, 16 or 12 electrodes, and containing singlechannel or dualchannel sound processors[7]. While the electrodes are often implanted through a round window, in some cases, they may need to be implanted through cochleostomy. Different techniques can also be used to place the CI receiver-stimulators in the skull. CI receiverstimulators are usually implanted in a special bony bed created by drilling the skull and fixed with sutures into the holes created in this area. However, rare intracranial complications and migration of receiverstimulators have been reported with this standard method[8]. In 2009, Balkany et al.[8] described the subperiosteal temporal pocket technique, which allows anchoring the receiverstimulator with anatomically compatible strong fixation points without an extra surgical procedure while preventing migration and dural complications.

In the present study, we wanted to share our experiences by evaluating our results as the first center to perform CI surgery in the Thrace region. We also investigated whether the differences of the electrode placement and the number of electrodes of the CIs had an effect on the postoperative results of our patients who underwent subperiosteal temporal pocket technique in our clinic.

MATERIALS AND METHODS

Preoperative, perioperative and postoperative clinical and audiological findings, hearing loss etiology, surgical approach techniques and complications were evaluated retrospectively in 31 patients (35 ears) aged 14 months to 57 years, who underwent CI surgery from December 2013 to September 2019 at Trakya University. Bilateral CI surgery was performed in 4 of these 31 patients in different sessions. Scientific Research Ethics Committee of the Trakya University Faculty of Medicine approved the procedures of the study (protocol number: 2018/282, date: 07.08.2018). All protocols adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from all subjects.

Selection of the Cases

During the review of patient files, the following were taken into account: the brand/number of electrodes in the device, the technique used to implant the CI receiver and the electrode; postoperative complications; preoperative tympanometry, audiometry, free field audiometry (FFA) and brainstem evoked response audiometry (BERA) tests; postoperative FFA, speech awareness threshold (SAT), speech reception threshold (SRT), speech discrimination score (SDS) and data on neural response telemetry (NRT) measurements performed by the relevant CI company postoperatively.

Eligible CI candidates were patients with bilateral severe or profound SNHL, who did not benefit from hearing aids and had an intact cochlear nerve and adequate internal ear development to allow electrode implantation as evidenced by magnetic resonance imaging (MRI) and/or high-resolution computed tomography (CT). Indications were established after an assessment by the CI committee, which included an earnosethroat (ENT) specialist, an audiologist, a pediatric audiologist and a psychologist. Behavioral and physiological evaluation results were compared in order to determine hearing sensitivity in patients who presented with a pre-diagnosis of hearing loss before cochlear implantation. For this purpose, pure tone audiometry in adult patients; airway hearing thresholds (250-8000 Hz), bone conduction hearing thresholds (500-4000 Hz), speech tests; SRT, SDS, immitansmetric evaluation, otoacoustic emissions (TEOAE, DPOAE) and BERA tests were applied. Behavioral test methods suitable for age, immitansmetric assessment (1000 Hz probe tone), otoacoustic

emissions (TEOAE, DPOAE) and BERA tests were applied in the behavioral evaluation of pediatric cases. Fine motor, personal-social development levels were evaluated and reported. Based on the results of the evaluation, the auditory rehabilitation process was initiated with a hearing aid, considering that people diagnosed with severe/profound hearing loss might be candidates for CIs. The cochlear implantation process was initiated for cases that could not gain sufficient level of hearing aid by evaluating the hearing aid thresholds after appropriate amplification. CI brand choice is made according to the rules determined by the Republic of Turkey Ministry of Health. Our clinic cannot make a decision on that matter.

Minimally invasive CI surgery technique was used in all patients. In this technique, a 4 cm Balkany et al.[8] incision is made 12 mm posterior to the retroauricular sulcus as the initial step. Posteroinferior-based periosteal flap follows the first step. Following a limited cortical mastoidectomy, posterior tympanotomy is performed and the electrode is introduced in the scala tympani through the round window after a vertical incision. In patients in whom the round window visualization is inadequate, the electrode is introduced in the scala tympani by means of cochleostomy. The processor is introduced and secured in the pocket created below the periosteum using Balkany et al.[8] subperiosteal temporal pocket technique. Subsequently, the number of electrodes in the cochlea is checked by NRT. Upon confirming an adequate number of electrodes in cochlea, electrodes are stabilized in the mastoid cavity with bone chips and tissue adhesive. The first telemetric measurements and programming of the device are usually performed in 1 month by the relevant implant company.

Statistical Analysis

The Shapiro-Wilk test was used to check the normal distribution. The paired dependent sample t-test was used for the comparison of dichotomous dependent groups. The paired independent sample t-test was employed to compare dichotomous independent groups. One-way analysis of variance was utilized in the comparisons of more than two independent groups. The means and standard deviations were presented as descriptive statistics. Level of significance was considered as $p<0.05$. All the statistical analyses were performed with the TURCOSA v.1.0 (Turcosa Analytics Ltd. Co., Kayseri, Turkey) statistical software.

RESULTS

Thirtyone patients, consisting of 13 adults and 18 children, were included in the study. The mean age was 19.2±20.3 years, and the median age was 7 years (14 months-57 years). While 11 of the children were in prelingual period (<4 years of age, mean 2.6±0.92 years), 7 were in the age group of 4-15 years (mean 9±3.87 years). The 4 prelingual patients underwent bilateral CI in different sessions within one year.

With regard to the etiological causes in these children, two patients had a history of meningitis (11 and 3 years before the operation), one had ototoxicity, one had Down syndrome, and one of the children had juvenile rheumatoid arthritis in addition to the history of SNHL with onset at 5 years of age. Another child had a history of spina bifida and chronic kidney failure, and another had chronic otitis media (COM) (previously operated from the same ear due to granular otitis). The remaining children had bilateral SNHL due to congenital hearing loss.

The etiological causes in adults included temporal bone fracture in one patient, brucellosis (7 years before the operation) in one patient, and COM in two patients. In one of the adult patients, left-sided temporal meningioma recurrence was suspected, and CI was therefore applied to the right ear. The remaining 8 adult patients were idiopathic.

According to the results of preoperative BERA and FFA with the device performed in all patients, 5 had bilateral severe SNHL and 26 had bilateral profound SNHL. None of the patients gained any benefit from the conventional hearing aid. The mean bone conduction threshold was 70±4.3 dB in 15 patients with implant, who underwent preoperative audiometry, and the mean airway threshold was 109.3±7.76 dB. The mean FFA with the device was 85.7±10.4 dB in the remaining 16 patients. Preoperative tympanograms were type A in all patients except the patients with COM. In preoperative BERA tests, 3 patients achieved the 5th wave at 100 dB, 2 at 90 dB and 5 at 80 dB, while the 5th wave was not achieved in BERA results of the other patients.

The preoperative radiological evaluations revealed a fracture line in the right temporal bone in a patient with posttraumatic bilateral severe SNHL and CI was therefore applied to the left ear. In a patient with facial canal dehiscence on the right and signs of chronic mastoiditis on the left, residual hearing was better in the right ear, and CI was applied to the right ear for this reason. Radiological images of another patient revealed type 1 incomplete partition findings. Atay and Sennaroğlu's[6] corktype electrode was used in this patient and no perilymphatic gusher or any other complication was observed. Postoperative changes were noted in the CT and MRI reports of 2 patients who underwent mastoidectomy and CI surgery in the same ear. The cranial MR report of a patient with a history of neurobrucellosis showed findings consistent with residual white matter alterations of neurobrucellosis in subcortical white matter. In this patient, the operation was terminated due to dura opening during the mastoidectomy procedure performed for the right ear, and CI was successfully completed in the left ear 2 months later. Since the imaging reports of a patient with a history of radiotherapy for left frontotemporal meningioma revealed findings in favor of the recurrence of the left frontotemporal meningioma, CI was applied to the right ear.

The electrodes were introduced through the round window in 21 ears and by cochleostomy in 14 ears. Cochleostomy was used to introduce the electrodes due to the absence of the round window in the visualized area in the ears of 8 patients (9 ears), ossification above or deep below the round window in 2 patients, several electrodes' being left out in the event of introduction through the round window in 2 patients, and the lack of round window development in 1 patient. While transmastoid approach was employed in 29 patients, combined surgical approach was applied in 2 cases due to high facial ridge. Transcanal approach was included in addition to the standard retroauricular approach in these patients.

Of the 35 ears operated in thirtyone patients, 23 were right and 12 were left ears. The ear with better residual hearing was operated first in 4 patients undergoing bilateral CI surgery. The 22electrode CI device of Cochlear® (Cochlear Co. Ltd., Sydney, Australia) was used in 7 ears while the 16electrode Advanced® (Advanced Bionics Co. Ltd., Santa Clarita, CA, US) CI was used in 4 ears and the 12electrode Medel® (Med-El GmBH, Innsbruck, Austria) CI device was implanted in 23 ears. Information on the brand and characteristics of the device could not be obtained in 1 patient.

In the perioperative measurements of 7 patients for whom twentytwo electrode devices were used, response was obtained in all electrodes in 6 patients while response was

Table 1. Number of electrodes with response in perioperative and postoperative measurements with the brand and models of devices in tabulated form

Patients undergoing implantation	Device brand/model	Number of electrodes with perioperative response	Number of electrodes with postoperative response
1	ADVANCED HIRES90K (HIFOCUS 1j Electrode - 16e)	16	16
2	ADVANCED HIRES90K (HIFOCUS 1j Electrode - 16e)	16	16
3	ADVANCED HIRES90K (HIFOCUS 1j Electrode - 16e)	16	16
4	ADVANCED HIRES90K (HIFOCUS 1j Electrode - 16e)	16	16
5	COCHLEAR NUCLEUS (CI24RE ST - 22e)	22	22
6	COCHLEAR NUCLEUS (CI24RE ST - 22e)	22	22
7	COCHLEAR NUCLEUS (CI24RE ST - 22e)	22	22
8	COCHLEAR NUCLEUS (CI24RE ST - 22e)	22	22
9	COCHLEAR NUCLEUS (CI24RE ST - 22e)	22	22
10	COCHLEAR NUCLEUS (CI24RE ST - 22e)	22	22
11	COCHLEAR NUCLEUS (CI24RE ST - 22e)	20	22
12	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	12
13	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	12
14	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	12
15	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	12
16	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	12
17	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	11
18	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	11
19	MEDEL OPUS 2 (SONATA TI100 - 12e)	12	9
20	MEDEL OPUS 2 (SONATA TI100 - 12e)	11	10
21	MEDEL OPUS 2 (SONATA TI100 - 12e)	11	9
22	MEDEL OPUS 2 (SONATA TI100 - 12e)	10	11
23	MEDEL OPUS 2 (SONATA TI100 - 12e)	10	10
24	MEDEL OPUS 2 (SONATA TI100 - 12e)	8	8
25	MEDEL OPUS 2 (SONATA TI100 - 12e)	8	7
26	MEDEL Synchrony (Standard - 12e)	12	12
27	MEDEL Synchrony (Standard - 12e)	12	12
28	MEDEL Synchrony (Standard - 12e)	12	12
29	MEDEL Synchrony (Standard - 12e)	12	10
30	MEDEL Synchrony (Standard - 12e)	9	8
31	N/A	n/a	n/a

obtained in 20 electrodes in 1 patient. However, postoperative controls revealed response in all electrodes in these 7 patients. Response was obtained in all electrodes both in perioperative and postoperative measurements in all 4 patients for whom 16 electrode devices were used. The measurement results obtained in 19 patients for whom 12 electrode devices were used are shown in Table 1.

No postoperative complications were observed in any of the patients. Postoperative imbalance complaint ongoing for 1 month was noted in the patient who underwent scala vestibuli cochleostomy due to lack of round window development.

There were 24 patients who attended the control visits regularly with a mean implant duration of 3.48±2.30 years (4 months-8 years). The mean preoperative FFA of the patients, recorded and averaged at 500-1000-2000-4000 Hz frequencies, was 95.2±19.13 dB and the mean postoperative FFA was 37.8±8.46 dB (range: 25-55). A significant difference was noted in terms of hearing gain in the comparison of preoperative and postoperative audiological findings of the patients under follow-up (p<0.001) (Table 2).

Although we had a limited number of patients, we evaluated the postoperative results of CIs with different electrode numbers. Of these twenty four patients, 6 had 22 electrode implants, 4 had 16 electrode implants, and 14 had 12 electrode implants. Comparison of their postoperative FFA values revealed no statistically significant difference between the devices (p=0.340). Again, no statistically significant difference was noted in terms of SAT/SRT values (p=0.862) (Table 3).

Electrodes were introduced through the round window in 15 of twenty four patients and by means of cochleostomy in the remaining 9 cases. In the round window group, the mean preoperative FFA value was 92.66±20.16 dB, while the mean postoperative FFA value was 38.89±8.65 dB. In the cochleostomy group, these values were 99.44±17.57 dB and 35.97±8.30 dB, respectively. A significant difference was observed between the preoperativepostoperative FFA values of patients in the round window group (p=0.0001). Similarly, there was a significant difference between the preoperative and postoperative FFA values of the patients in the cochleostomy group (p=0.0001). No statistically significant difference was noted between the two groups in terms of postoperative FFA values (p=0.425) and SAT/SRT values (p=0.132) (Table 3).

DISCUSSION

Rehabilitation of deafness may be possible after CI surgery performed in patients with bilateral severe or profound SNHL, who do not gain any benefit from conventional hearing aids. The results of the study presented herein show a significant hearing gain in patients undergoing CI surgery. Relevant information should be provided for the families of adult patients and pediatric patients, including the importance of training and the rules to be followed after surgery so that children may develop speaking skills comparable to their peers with normal hearing[9]. Geers et al.[10] emphasized the importance of postoperative training to ensure maximum benefit from the implantation in their study, showing a comparable level of producing and understanding the English language in more than half of 181 children aged 8-9 years who were CI users. In our clinic, although the necessary information is provided and followup is initiated from the time of identifying CI candidates, we encounter patients who do not continue their follow-up in the long term, as reflected in the results of the present study.

The round window pathway is used to introduce electrodes in all cases eligible for this approach at our clinic. If the

Table 2. Statistical comparison of preoperative and postoperative hearing thresholds of patients

	n (number of patients)	Mean	Standard deviation	p value
Preoperative FFA thresholds	24	95.2083	19.1379	<0.001
Postoperative FFA thresholds	24	37.8	8.4656	

FFA: Free field audiogram

Table 3. Statistical comparison of postoperative hearing test results of devices with different number of electrodes and postoperative hearing test results of electrodes inserted by different techniques

		Postoperative FFA			Postoperative SAT/SRT		
Device	**n (number of patients)**	**Mean**	**Standard deviation**	**p value**	**Mean**	**Standard deviation**	**p value**
Advanced (16e)	4	38.3750	10.2744	0.340	35	7.0711	0.862
Cochlear (22e)	6	33.3750	7.9667		34.1667	12.8128	
Medel (12e)	14	39.5321	8.1027		36.4286	7.1867	
Technique							
Cochleostomy	9	35.9722	8.3099	0.425	32.2222	7.5462	0.132
Round window	15	38.8967	8.6512		37.6667	8.6327	

FFA: Free field audiogram, SAT: Speech awareness threshold, SRT: Speech receipt threshold

round window cannot be visualized conveniently through the facial recess or if ossification is present, then the electrodes may be introduced by cochleostomy. Electrode introduction through the round window results in less acoustic trauma as fewer drills are performed[11]. Richard et al.[12] demonstrated less intracochlear trauma with the round window approach for the introduction of electrodes. Jiam et al.[13] concluded that the round window approach allowed implanting the electrodes in closer proximity to the cochlear neural elements. On the other hand, no superiority was seen between the round window approach and cochleostomy in the review by Havenith et al.[14] in 2013. Results from the study by Rajput and Nilakantan[15] did not reveal any difference between the round window approach and cochleostomy in terms of electrode positioning based on the postoperative speech skills and hearing level of the patients. Helms and Moser[16] evaluated communication skills in patients for whom CIs of two different brands were used and found that one brand was more successful than the other. In the present study, both CI positioning techniques significantly improved postoperative hearing thresholds of the patients. Furthermore, no difference was observed in terms of postoperative hearing gain in patients who received implants of different brands with different number of electrodes. Considering these findings, we may conclude that both techniques are applied successfully without leading to difference in postoperative hearing gains obtained with the CI brands included in this study.

Balkany et al.[8] introduced the temporal pocket technique to the literature in 2009. They used this technique, which does not require drilling to introduce the receiver, in 171 patients and did not observe device migration or any intracranial complication throughout the followup of at least one year. Jethanamest et al.[17] also utilized the subperiosteal pocket technique and observed no perioperative complication or postoperative migration in any of 63 patients. In our clinic, we use Balkany et al.[8] method and we have not encountered any postoperative complications in our patients.

There are different applications for patients previously operated due to chronic suppurative otitis media. Some surgeons clear the epithelium in the mastoidectomy cavity and perform the implantation in the same session in the absence of infection, while some others perform tympanoplasty or tympanomastoidectomy in the first session and perform CI in a second session[18-20]. Cevizci and Bayazit[21] applied the "canal wall up" tympanomastoidectomy technique without cavity obliteration and concluded that it was a safe surgical method as they did not observe any complications or recurrent cholesteatoma during long-term follow-up. In the present study, chronic suppurative otitis media was eradicated with an appropriate surgical approach in the first session in three patients with this condition, and CI surgery was performed after a follow-up of at least 6 months. We performed tympanomastoidectomy for one of the patients due to adhesive otitis and in another one for granular otitis. At six months, no discharge was noted in the ears of these patients, their tympanic membranes were intact, and no sign of recurrence was observed in tomography images. In another patient, cholesteatoma was detected and "canal wall down" mastoidectomy was performed for pathological clearance, followed by cul de sac and external ear canal closure. CI surgery was performed in the same ear 9 months later. No recurrence of cholesteatoma was observed during the follow-up.

Brucellosis is a zoonotic disease that is endemic in Mediterranean countries[22]. This condition may manifest as neurobrucellosis involving the nervous system in 5% of affected patients. Guneri et al.[23] published for the first time in 2009 that they successfully performed CI surgery in a patient with neurobrucellosis. Subsequently, Ocak et al.[24] from our country in 2015 and Bajin et al.[25] in 2016 published their cases with neurobrucellosis undergoing CI surgery. In 2015, we successfully performed CI surgery in a 45 year old patient with paraplegia, history of meningitis, bilateral SNHL and history of neurobrucellosis.

A high prevalence of SNHL is observed in patients with rheumatoid arthritis[26,27]. Dekker and Isdale published a case report of CI surgery in a patient with juvenile chronic arthritis and bilateral progressive SNHL[28]. We also applied a CI to a 7 year old patient with juvenile rheumatoid arthritis and bilateral profound SNHL, and achieved an increased hearing threshold.

In 2012, Yorgancılar et al.[29] performed CI surgery in 33 children and 3 adults and introduced the electrodes through the round window in nearly all cases. None of these patients had any postoperative complications. Furthermore, Şahan et al.[30] evaluated the outcomes of CI surgery in 144 patients and reported successful outcomes, low complication rates and notable improvement in post-CI speech perception scores as well as audiological performance. Based on the results of the present study, we concluded that the temporal pocket bed approach we used in CI surgery was an effective and safe method. Postoperative hearing gains are significant. It can be said that the difference between the electrode insertion location and numbers has no effect on postoperative results. We believe that our study is important as it evaluates the first cases operated with the temporal pocket approach in the Thrace Region and the results demonstrate that none of the patients operated with this technique developed complications such as implant rejection or migration.

Study Limitations

The most important limitation of our study is the limited number of patients in the groups stratified by the type of implants used for the procedure. Although this translates into a low level of reliability, our findings are consistent with those previously reported in the literature.

CONCLUSION

Although we have a limited number of patients, the significant hearing gains in the postoperative period without any complications show the success of the surgical technique utilized in this study. In terms of post-operative hearing gains, it can be said that the difference in electrode insertion location and numbers does not affect the results. We also believe that the surgeon's ability to master different approaches is essential for successful surgery.

Ethics

Ethics Committee Approval: The study were approved by the Trakya University Faculty of Medicine Scientific Research Ethics Committee (protocol number: 2018/282, date: 07.08.2018).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.G.G., C.U., Concept: S.G.G., C.U., M.T., E.D., Design: S.G.G., C.U., M.T., E.D., Data Collection or Processing: S.G.G., M.T., E.D., Analysis or Interpretation: S.G.G., C.U., M.T., E.D., Literature Search: S.G.G., M.T., E.D., Writing: S.G.G., C.U., M.T., E.D.

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CASE REPORT

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Round Pneumonia Management in COVID-19 Patient

COVID-19 Hastasında Round Pnömoni Yönetimi

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ABSTRACT

Round pneumonia is an infrequent entity in the adult population. Its clinical presentation and radiological features resemble to malignant tumoral lesions very much; therefore, it is often misdiagnosed as being malignant in nature. In patients with solitary pulmonary nodules, especially when clinical findings of pneumonia are present, plain radiography or computed tomography of the chest should be repeated after the treatment and follow-up time should be extended. Although radiological findings of Coronavirus disease-2019 (COVID-19) pneumonia have been described, it should be kept in mind that other respiratory tract infections may be observed in these patients as a co-infection to COVID-19.

Keywords: Pneumonia, viral pneumonia, COVID-19, neoplasms, computed tomography

ÖZ

Round pnömoni, yetişkin popülasyonda seyrek görülen bir durumdur. Klinik görünümü ve radyolojik özellikleri malign tümöral lezyonlarla oldukça benzerdir; bu nedenle, doğası gereği genellikle kötü huylu olarak yanlış teşhis edilir. Soliter pulmoner nodülü olan hastalarda, özellikle klinik pnömoni bulguları mevcutsa, tedaviden sonra direkt radyografi veya göğüs bilgisayarlı tomografisi tekrarlanmalı ve takip süresi uzatılmalıdır. Koronavirüs hastalığı-2019 (COVID-19) pnömonisinin radyolojik bulguları tanımlanmış olmakla birlikte, bu hastalarda COVID-19 koenfeksiyon olarak başka solunum yolu enfeksiyonlarının da görülebileceği akılda tutulmalıdır.

Anahtar Kelimeler: Pnömoni, viral pnömoni, COVID-19, neoplazmalar, bilgisayarlı tomografi

INTRODUCTION

Round pneumonia is a subtype of pneumonia that is frequently observed in the pediatric age group and very rarely in adults[1]. The pathology is normally attributable to the presence of immature Kohn pores and canals of Lambert in children, causing inflammatory consolidation to be limited to a round morphology[2]. In adults, on the other hand, inflammatory processes normally spread laterally to cause what is known as lobar pneumonia. However, developmental arrest, or faulty development of Kohn pores and the canals of Lambert duct, may frequently limit the consolidation in adults as well[2]. In such cases, *Streptococcus* is the most common cause of etiology, although *Coxiella burnetii* and coronaviruses may also present as round pneumonia[3]. Lately, we have encountered such an appearance in a patient with computed tomography (CT) findings and reverse transcription-polymerase chain reaction (RT-PCR) evidence of Coronavirus disease-2019 (COVID-19). Although many studies have been conducted to describe the typical, indeterminate, and atypical CT features of the disease, as of yet, round pneumonia has not been reported as a primary or accompanying finding of the disease[4].

CASE REPORT

A 42-year-old male hospital worker was admitted to the emergency department with the complaints of cough, sputum, shortness of breath, and pleuritic pain in the left side for the last two days. He was a heavy smoker (20 packs/year). Upon admission, his vital signs were as follows: body temperature: 36 °C, blood pressure: 125/81 mmHg, pulse: 102 bpm, and O_2 saturation: 96%. Laboratory findings were as follows:

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white blood cell count: 7x10^3/uL (4-10), C-reactive protein: 1.21 mg/dL (0-0.5), lymphocyte count 3.3 10^3/uL (0.80-4.00), D-dimer: 0.23 ug/mL (0-0.5), and ferritin: 92.96 ng/mL (22-275). Chest CT and naso-oropharyngeal swab were performed due to the patient's potential risk of exposure to COVID -19 patients in the hospital. Chest CT findings were normal except for a pleural-based 6.6×6.2 mm rounded mass with regular margins in the left upper lobe, which was interpreted as solitary pulmonary nodule (SPN) (Figure 1A). Due to the size of the nodule, short-term follow-up was planned. However, the patient was readmitted to the emergency department four days after his initial presentation. At that time, he had increased back pain and shortness of breath. His vital signs and oxygen saturation were normal. CT scan was repeated and it revealed a significant progression of the mass to 16.2×12.5 mm (Figure 1B) and structural findings (i.e., bilateral peripheral, posterior, ground-glass opacities) of COVID-19, although RT-PCR test was still negative (Figure 1B). However, in the light of typical CT findings and according to national management guidelines, the patient was diagnosed as mild COVID-19[5]. The SPN, on the other hand, was interpreted as an atypical finding of COVID-19 pneumonia, due to the rapid progression of the lesion and its temporal relationship with the latter. The patient received favipiravir regiment (2×1600 mg/day) on the first day as a loading dose, followed by a total of 1200 mg/day (2×600 mg/day) for 14 days. Although the parenchymal findings of COVID-19 disappeared on the fourteenth day (Figure 1C), the solitary lesion had significantly progressed in size and reached 27.5×26 mm in size and had irregular margins (Figure 1C). The lesion was thought to have a bacterial origin and a broad-spectrum antibiotic was empirically initiated. Fourteen days after the start of antibiotic treatment, the lesion had significantly regressed to 14×13 mm in size, with air bronchogram with pleural thickening (Figure 1D). Although the clinical findings of the patient had resolved, positron emission tomography (PET)/CT was requested by the clinician and the lesion was evaluated as benign in nature with low fluorodeoxyglucose uptake (Figure 2). Informed consent was obtained from the patient.

DISCUSSION

Round pneumonia is a type of pneumonia almost always seen in the pediatric age group. It is uncommon after eight years of age but it can also be encountered in adulthood. It may be an early stage of lobar pneumonia or related to developmental defect of inter-alveolar communications and collateral airways[1,2]. While they mostly occur in inferior lobes in children, in adult patients, superior lobes are dominant and air bronchograms are very rare (17%)[2]. The typical radiological feature of round

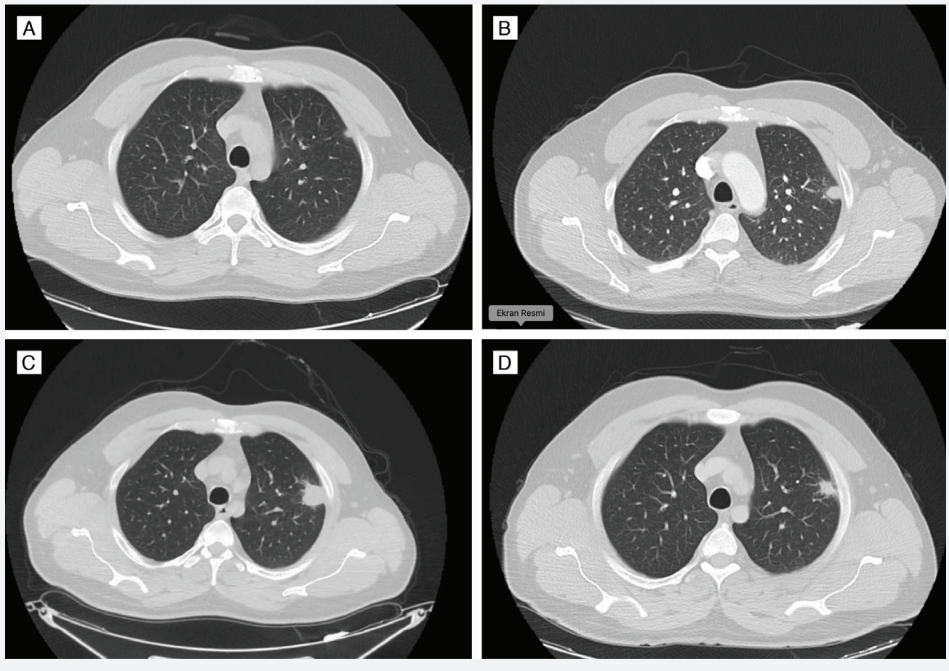

Figure 1. A) Chest computed tomography (CT) shows the round, 6.6×6.2 mm, pleural-based mass-like lesion in left upper lobe at initial presentation. There were no other findings. B). A repeat CT scan shows a significant progression of the mass to 16.2×12.5 mm in size and bilateral peripheral, posterior, ground-glass opacities after four days. C) The parenchymal findings of COVID-19 had disappeared fourteen days after favipiravir treatment was initiated but the solitary lesion had progressed to 27.5×26 mm in size and it had spiculated pattern. D) Fourteen days after the initialization of the broad-spectrum antibiotic, the lesion had regressed to 14×13 mm in size and it had air bronchogram with pleural thickening and spicules

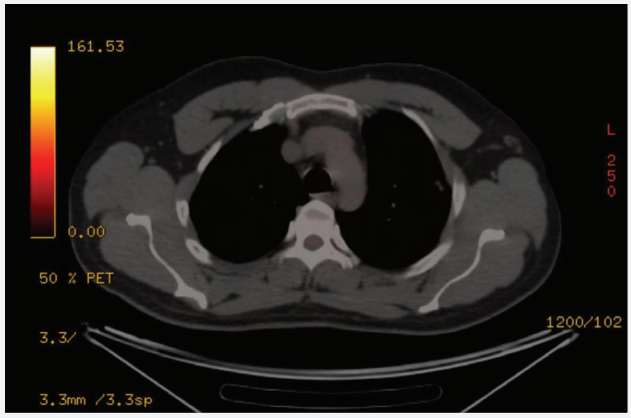

Figure 2. No significant FDG uptake was observed in the lesion in PET/CT, which was conducted ten days after the patient's last admission

pneumonia is a round, well circumscribed consolidation area with irregular margins[1]. They may have spicules, pleural thickness, and satellite nodules[6]. The differential diagnosis of round pneumonia from bronchogenic carcinoma can be difficult and is generally based on the lesion's response to antibiotics. However, there are cases where malignant obstructions may cause secondary infection and antibiotic treatment may result in temporary but not permanent regression in lesion size. In such cases, FDG-PET may attempt to provide clues, though seldomly as both lesions may show increased metabolism[7,8]. The definitive diagnosis is either by complete resolution of the lesion in due course or by tissue biopsy.

The histopathological features of COVID-19 pneumonia are parenchymal and pulmonary interstitial damage. This damage manifests as so-called "typical" findings that include but not limited to ground-glass opacities and consolidation[9]. Rarely, focal GGO or opacities may be encountered in round morphology in COVID-19 pneumonia[10]. It must be noted that other respiratory infections can also be observed in COVID-19 pneumonia patients as demonstrated in this report. There are no obvious guidelines for bacterial co-infection in COVID-19 but empiric antibiotics should be given if radiological atypical findings such as round pneumonia are present[11].

CONCLUSION

In conclusion, round pneumonia should be considered in the differential diagnosis of patients with nodular consolidation and should be evaluated in terms of malignancy after appropriate medical treatment.

Ethics

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.Y., Concept: G.Y., H.M.K., Design: G.Y., H.M.K., Data Collection or Processing: G.Y., Analysis or Interpretation: G.Y., Literature Search: G.Y., Writing: G.Y., H.M.K.

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ERRATUM

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Çalışkan T, Tunçkale T. The Impacts of Intraoperative Ultrasonography Use on Revision Rate in Cases of Brain Tumor. Nam Kem Med J. 2021;9:219-26.

The mistake has been made inadvertently by the author.

The title English on page 219 of the related article has been corrected by the author as below.

The incorrect English title

The Impacts of Intraoperative Ultrasonography Use on **Revision** Rate in Cases of Brain Tumor

The corrected English title

The Impacts of Intraoperative Ultrasonography Use on **Resection** Rate in Cases of Brain Tumor