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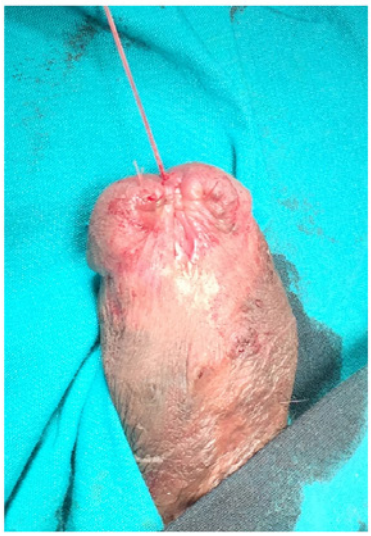

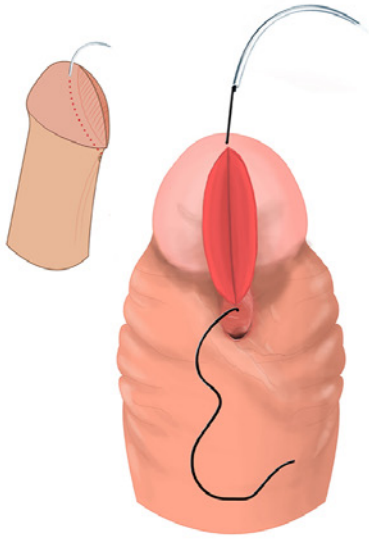

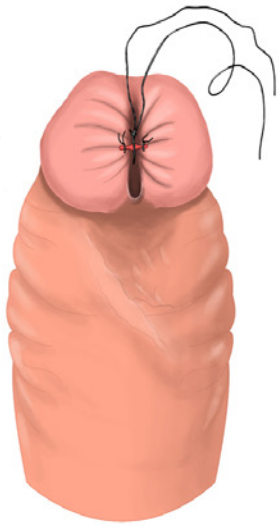

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Cover Art
Ulubay, Page 42, Fig. 4

CONTENTS ***Page***

Clinical Research

Pulmonary involvement in Behcet's disease: Definition of Tc99m-MAA lung scintigraphy perfusion patterns according to the affected pulmonary vascular levels

S. U. Semirgin, O. Uzun **29**

Comparison of patient-side capillary glucose measurements with autoanalyzer results

S. Sezik, H. A. Sezik **37**

Use of the penis's own tissue for urethral reconstruction in the treatment of complications developing following hypospadias repair

M. Ulubay **41**

The antibacterial and antifungal activities of commonly used herbal oils

M. Karameşe, D. Özgür **47**

Are serum lipid and androgen levels different in women with natural or surgical menopause?

C. S. Çalışkan, B. Şahin, S. Çelik, B. Şahin, Ş. Hatırnaz **53**

Case Report

Different types of kidney tumors together in the same kidney: Is it rare condition?

A. Karaköse, H. Öztürk **57**

Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Clinical Research

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Pulmonary involvement in Behcet's disease: Definition of Tc99m-MAA lung scintigraphy perfusion patterns according to the affected pulmonary vascular levels

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ABSTRACT

The aim of this study was to define lung perfusion scintigraphy findings in Behcet's disease (BD) patients with different variations of pulmonary involvement. Medical records and imaging findings of 23 patients with pulmonary involvement of BD were retrospectively reviewed. Before scintigraphic evaluation, patients were classified according to the affected pulmonary vascular level on CT angiography (CTA) as follows: Macroscopic pulmonary vascular involvement (Gr-1) [Pulmonary artery aneurysm without thrombosis (Gr-1a), pulmonary artery aneurysm with thrombosis (Gr-1b), pulmonary artery thrombosis without aneurysm (Gr-1c)]; microscopic pulmonary vascular involvement (Gr-2; no macroscopic CTA findings but with clinical diagnosis and/or scintigraphic abnormality). There were 18 patients in Gr-1 and five patients in Gr-2. Segmental/subsegmental perfusion defects were the most common perfusion pattern and no distinctive pattern was observed among all groups. In 12 patients with macroscopic disease and two patients with microscopic disease, perfusion defects were more extensive than involved vessels on CTA and/or also in the contralateral lung. There were 13 patients with scintigraphic follow-up findings. No change was observed in all Gr-1b and Gr-2 patients; there were heterogeneous changes in 6/7 patients in Gr-1c. No typical perfusion pattern could be demonstrated for a given macroscopic vascular category. In patients with microscopic disease, similarly no characteristic pattern could be defined. As almost all patients with microscopic disease showed perfusion anomalies, scintigraphy may be proposed as a first step examination in case of suspected pulmonary involvement in BD. Scintigraphic follow-up may be of value in pure thrombotic pulmonary involvement.

1. Introduction

Behcet's disease (BD) is a triad of recurrent oral and genital ulcers with relapsing uveitis and was first described in 1937 by Hulusi Behcet (Behcet, 1937). Skin, central nervous system, cardiovascular system, gastrointestinal tract and pulmonary system were defined as the main affected areas since its first description (Koç et al., 1992; Akpolat et al., 2002; Düzgün et al., 2002). Pulmonary involvement is a rare condition, usually represented by episodic hemoptysis. Pulmonary vessels may be affected macroscopically (pulmonary artery aneurysms and/or thrombosis) or microscopically (vasculitis of small sized vessels) in BD (Erkan et al., 2001; Uzun et al., 2005; Uzun et al., 2008; Uzun 2011). Differential diagnosis between these pulmonary artery pathologies is important, because clinical signs and symptoms were similar but the treatment and prognosis may be

different. CT angiography (CTA) and Technetium 99m-Macroaggregated Albumin lung perfusion scintigraphy (MAA lung scintigraphy) are used in the diagnosis and follow-up of pulmonary involvement. CTA may represent macroscopic pulmonary involvement, but has limited diagnostic value in small sized vessel vasculitis. MAA lung scintigraphy findings in pulmonary involvement of BD were presented in a limited number of studies (mostly case based) and as yet, no specific radiotracer accumulation pattern has been reported. The aim of this study was to define MAA lung scintigraphy findings in BD patients with different variations of pulmonary involvement.

2. Materials and methods

Medical records of 23 patients from 2006 to 2019 were retrospectively reviewed. Patient's data were reevaluated according to the criteria defined by International Study Group for BD (International Study Group for BD 1990). All patients underwent CTA and MAA lung scintigraphy with a maximum time interval of five days. Both CTA and MAA lung scintigraphy images were reevaluated. For MAA lung scintigraphy, 185 MBq of Tc-99m-MAA was injected intravenously. Standard anterior, posterior, right lateral, left lateral, right posterior oblique and left posterior oblique static images were obtained with either E-CAM (Siemens, USA) or Discovery NM 630 (General Electric, Hayfa, Israel) dual headed gamma camera equipped with high resolution collimators. Radiotracer uptake patterns on MAA lung scintigraphy images were evaluated visually in lung segments. CTA images were obtained either with X press/GX model TSX-002a (Toshiba, Tachigi-Ken) helical CT or Aquilion 16 system (Toshiba Medical Systems Corporation, Japan) multidetector CT scanner. Non-ionic intravenous contrast agent administered at a rate of 4 ml/s with a delay of 15–20 s before scanning. CTA images were obtained during suspended inspiration and were taken from the level of the aortic arch to 2 cm below the level of the diaphragm.

Patients were classified as follows according to the pathologies in the pulmonary arteries according to the CTA findings:

1. Macroscopic pulmonary vascular involvement (Gr-1)
   a. Pulmonary artery aneurysm without thrombosis (Gr-1a)
   b. Pulmonary artery aneurysm with thrombosis (Gr-1b)
   c. Pulmonary artery thrombosis without aneurysm (Gr-1c)

2. Microscopic pulmonary vascular involvement (Gr-2) (without any macroscopic CTA findings but having clinical diagnosis or MAA lung scintigraphy abnormality)

Immunosuppressive therapy was given to all patients and dose was adjusted according to the severity and extent of involved pulmonary arteries and also clinical parameters including massive hemoptysis. Most aggressive treatment with "pulse" corticosteroid (1-3 days) and cyclophosphamide were given to the patients with pulmonary artery aneurysm. Colchicine was used in all patients.

3. Results

MAA lung scintigraphy and CTA findings were reevaluated in 23 patients (17 males, 6 females). The mean age was 33±9 and the age range were 18-56. The most common symptom was oral ulceration (in 22 patients; 95.6%). Cough and hemoptysis were the most common pulmonary symptoms presented in 17 (73.9%) and 15 (65.2%) patients respectively. Pulmonary involvement was diagnosed simultaneously with BD in 17/23 patients (73.9%). During data analysis of this study, 22/23 (95.6%) patients were still alive, one patient was lost to follow-up after 47 months from the diagnosis of pulmonary involvement of BD. Patients were followed-up for a period of 107±46 months. Patient's symptoms and clinical features are presented in Table 1 and 2 respectively.

Table 1. Symptoms of 23 BD patients with pulmonary involvement.

Symptoms	**Number of patients (%)**
Oral ulceration	22 (95.6)
Genital ulceration	15 (65.2)
Ocular lesions	9 (39.1)
Skin lesions	10 (43.5)
Positive pathergy test	4 (17.4)
Pulmonary symptoms	
Cough	17 (73.9)
Hemoptysis	15 (65.2)
Hemoptysis (massive)	10 (39.1)
Dyspnea	11 (47.8)
Chest pain	10 (39.1)
Fever	12 (52.2)

CT angiography findings

Macroscopic pulmonary vascular involvement (Gr-1) was detected in 18 patients according to CTA findings; two patients had pulmonary artery aneurysm without thrombosis (Gr-1a), seven patients had pulmonary artery aneurysm with thrombosis (Gr-1b), nine patients had pulmonary artery thrombosis without aneurysm (Gr-1c). Microscopic pulmonary vascular involvement (Gr-2) was detected in five patients; four patients had normal findings and one patient had thinning of peripheral vascular branches on CTA.

Table 2. Clinical Features of 23 BD patients with pulmonary involvement.

Pt no	Age/ Sex	Presenting pulmonary symptom	BD and pulmonary involvement	Clinical follow-up (months)
1	36/M	Hemoptysis	15 yrs	142
2	33/F	Cough	4 yrs	152
3	21/F	Cough	2 yrs	62
4	49/M	Chest pain	Same time	78
5	56/M	Leg swelling	Same time	123
6	41/M	Hemoptysis*	Same time	141
7	34/M	Hemoptysis*	Same time	141
8	24/F	Hemoptysis*	Same time	52
9	24/M	Hemoptysis	Same time	57
10	36/M	Chest pain	Same time	102
11	40/M	Fever	5 yrs	118
12	32/M	Fever	Same time	130
13	41/M	Dyspnea	2 yrs	133
14	38/M	Fever	Same time	144
15	19/F	Dyspnea	Same time	153
16	42/M	Fever	Same time	150
17	23/M	Hemoptysis	Same time	164
18	29/M	Hemoptysis	Same time	24
19	32/F	Hemoptysis	Same time	47
20	34/M	Dyspnea	1 month	148
21	29/M	Hemoptysis*	Same time	31
22	18/F	Dyspnea	Same time	49
23	40/M	Chest pain	Same time	129

Perfusion lung scintigraphy findings

Group 1 (18 patients)

In Gr-1a (two patients) multiple segmental and subsegmental perfusion defects were detected in both right and left lungs in two patients. In Gr-1b (seven patients), multiple segmental and subsegmental perfusion defects in both right and left lungs in two patients and only in the lower lobe of right and/or left lung in four patients were determined. In Gr-1c (nine patients), multiple segmental and subsegmental perfusion defects in both right and left lungs in eight patients and only in the lower lobe of right and left lungs in one patient were observed. The extent of defective areas on MAA lung scintigraphy were larger and encompassed more segments than on CTA and/or perfusion defects in the contralateral lung with normal CTA findings were observed in one patient in Gr-1a, three patients in Gr-1b and seven patients in Gr-1c. MAA lung scintigraphy and CTA findings of a patient patient with pulmonary artery aneurysm without thrombosis (Gr-1a) were given in Fig.1 .

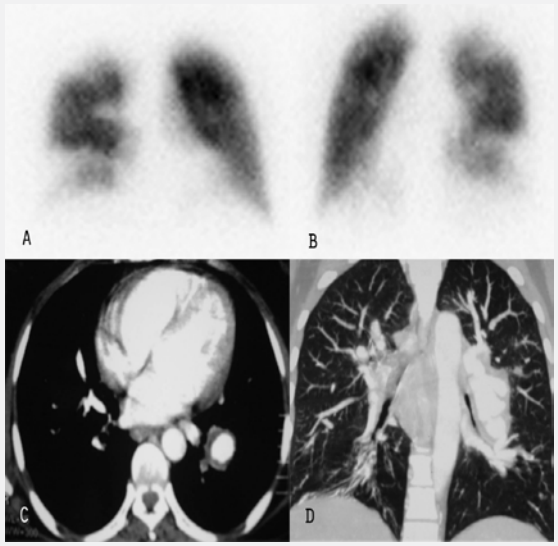

Fig. 1. Segmental and subsegmental perfusion defects in left and right lungs were observed on anterior (A) and posterior (B) perfusion lung scintigraphy images of a 33-year-old female patient. CT angiography of this patient shows non-occlusive huge aneurysmatic pulmonary arteries on axial (C) and coronal (D) images. This patient is an example of "pulmonary artery aneurysm without thrombosis" (Gr-1a). She received intense immunosuppressive treatment without anticoagulation. She is now asymptomatic after 10 years of first presentation with low-dose immunosuppressive after two exacerbation of pulmonary involvement when she was on remission without any treatment.

Group 2 (five patients)

Heterogeneous and decreased radiotracer accumulation in two patients, multiple segmental and subsegmental perfusion defects in two patients were observed. One patient had normal perfusion pattern.

MAA lung scintigraphy follow-up findings

There were 13/23 patients (56.5%) with MAA lung scintigraphy follow-up findings in a range of 1 to 153 months (seven patients in Gr-1c, three patients in Gr-1b and three patients in Gr-2). Perfusion findings were changed in the follow-up of 6/7 patients in Gr-1c. Perfusion changes were heterogeneous; there were newly formed and enlarged defects in some patients as well as patients with narrowed and completely resolved defective areas. A patient with multiple perfusion defects in both left and right lungs in Gr-1c had a completely normal follow-up MAA lung scintigraphy finding. Interestingly changes in follow-up studies were observed only in patients with pure thrombotic vascular involvement (Gr-1c). Fig. 2 represented MAA lung scintigraphy and CTA findings of a patient with newly formed and narrowed defects.

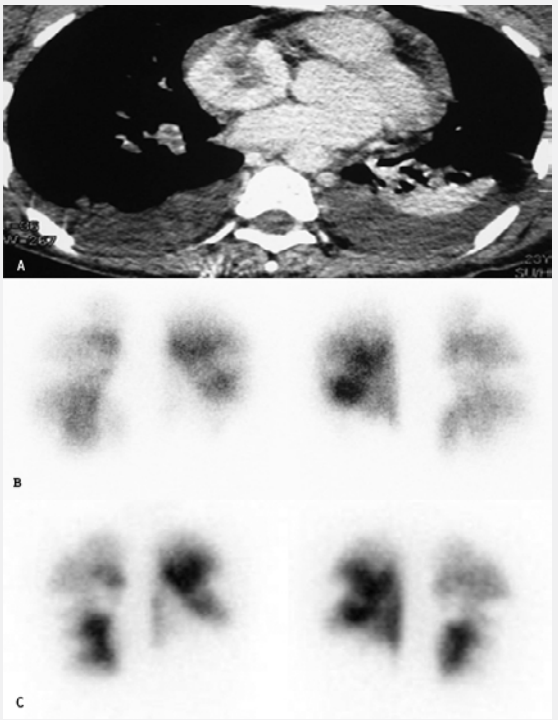

Fig. 2. On CT angiography of the 19 year-old female patient who presented with vena cava superior syndrome with severe dyspnea showed thrombotic occlusion of jugular veins, subclavian veins, vena cava superior, pulmonary arteries as well as right atrium (A). The patient went well without any symptom after 13 years of presentation with intense immunosuppressive treatment and anticoagulation lasting almost 5 years. Anterior and posterior perfusion lung scintigraphy images (B) of patient showed segmental and subsegmental perfusion defects in left and right lungs. After about 13 years of follow-up, newly formed and narrowed defects in the right and left lungs were determined (C). This patient is an example of "thrombotic pulmonary disease" (Gr-1b).

There was no change observed on MAA lung scintigraphy follow-up findings of all Gr-1b and Gr-2 patients. MAA lung scintigraphy follow-up and CTA images of a patient in Gr-2 were given in Fig. 3. Table 3 represented CTA, MAA lung scintigraphy and follow-up findings of patients.

4. Discussion

Technetium 99m-MAA lung scintigraphy has been reported to have high negative predictive value in terms of pulmonary involvement (Caglar et al., 2000). In this study, we classified patients as macroscopic or microscopic pulmonary vascular involvement and investigated the radiotracer accumulation patterns on MAA lung scintigraphy. To our knowledge, this was the first systematic study evaluating this issue.

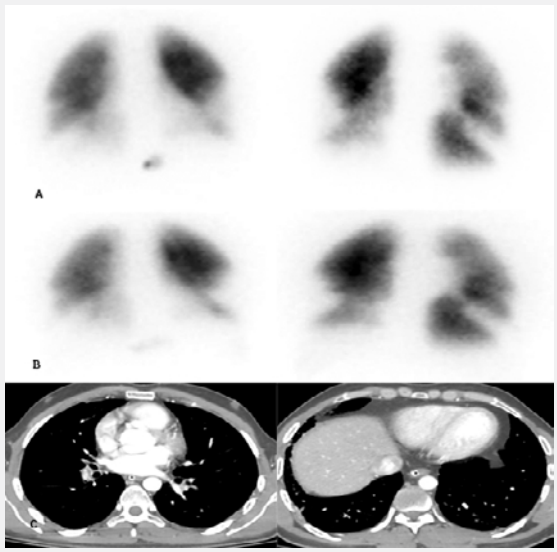

Fig. 3. Anterior and posterior perfusion lung scintigraphy images (A) of a 29-year-old male patient were revealed with multiple segmental and subsegmental perfusion defects in the lower lobe of left and right lungs. No significant change was observed on the perfusion lung scintigraphy images after 17 months of follow-up (B). CTA images displayed thinning in branches of the right and left lower lobe pulmonary artery without any obvious thrombotic lesion (C). This patient is an example of "macroscopic pulmonary vascular involvement" (Gr-2).

Although perfusion lung scintigraphy with 99mTc-MAA is the most frequently performed scintigraphic method in BD patients with pulmonary involvement, some other radionuclide agents were also used to demonstrate pulmonary vascular endothelial damage. Gumuser et al. showed faster clearance half time of 99mTc-DTPA radio-aerosols and decreased 99mTc-HMPAO lung clearance rate in BD patients. They reported the contribution of these findings to predict pulmonary involvement in the early stages of BD (Gumuser et al., 2008; Gumuser et al., 2011). In another study, prolonged lung retention of 123I-meta-iodobenzylguanidine found to be associated with disease severity (Unlu et al., 2001). In a small number of studies with PET tracers, it has been reported that increased accumulation of 18F-fluorodeoxyglucose demonstrates pulmonary artery inflammation (Denecke et al., 2007; Trad et al., 2013).

The results of our study showed that MAA lung scintigraphy alone cannot specify the affected vascular level in BD patients with pulmonary involvement. This was thought to be the result of vasculitis, vascular remodeling and in situ thrombosis causing nonspecific perfusion defects with the occlusion of pulmonary arteries on perfusion lung scintigraphy. A remarkable finding was that the affected vascular bed areas detected

Table 3. CT angiography, perfusion lung scintigraphy and follow up findings of patients.

Patient No	CT Angiography	Perfusion Lung Scintigraphy	Follow-up Perfusion Lung Scintigraphy	Time Interval (Months)
Group 1 (Macroscopic pulmonary vascular involvement)				
Group 1a: Pulmonary artery aneurysm without thrombosis				
1	Aneurysm in basal posterior branch of left pulmonary artery	*Multiple segmental and subsegmental perfusion defects in left and right lungs	-	-
2	Aneurysm in main pulmonary artery	Multiple segmental and subsegmental perfusion defects in left and right lungs	-	-
Group 1b: Pulmonary artery aneurysm with thrombosis				
3	Arterial aneurysm with thrombosis in right pulmonary artery	*Segmental and subsegmental perfusion defects in the lower lobe of left and right lungs	-	-
4	Arterial aneurysm with thrombosis in lower lobe branch of left pulmonary artery	Segmental perfusion defects in the lower lobe of left lungs	-	-
5	Arterial aneurysm with thrombosis in lower lobe branch of right pulmonary artery	Subsegmental perfusion defects in the lower lobe of right lung	Same findings	46
6	Arterial aneurysm with thrombosis in lower lobe branches of left and right pulmonary artery	*Multiple segmental and subsegmental perfusion defects in left and right lungs	Same findings	20
7	Arterial aneurysm with thrombosis in right pulmonary artery	*Multiple segmental and subsegmental perfusion defects in left and right lungs	Same findings	48
8	Arterial aneurysm with total thrombotic occlusion in left pulmonary artery	Perfusion defect in the lower lobe of left lung	-	-
9	Arterial aneurysm with thrombosis in lower lobe branches of left pulmonary artery	Subsegmental perfusion defects in the lower lobe of left lung	-	-
Group 1c: Pulmonary artery thrombosis without aneurysm				
10	Thrombotic occlusion in lingular branch of left pulmonary artery and laterobazal branch of right pulmonary artery	*Segmental and subsegmental perfusion defects in left and right lungs	-	-
11	Thrombotic occlusion in lower lobe branches of left and right pulmonary artery	*Multiple segmental and subsegmental perfusion defects in left and right lungs	-	-
12	Thrombotic occlusion in lower lobe branch of right pulmonary artery	*Perfusion defects in left and right lungs	Normal findings	126
13	Thrombotic occlusion in segmental branches of left and right pulmonary artery	Multiple segmental and subsegmental perfusion defects in left and right lungs	Defects with narrowing borders	119
14	Thrombosis in right ventricule, nodular density at right and left lower lobes	*Multiple segmental and subsegmental perfusion defects in left and right lungs	Expansion of defect borders in right lung	13
15	Thrombotic occlusion in lower lobe branches of left and right pulmonary artery	*Segmental and subsegmental perfusion defects in left and right lungs	Newly formed and narrowed defects	153
16	Thrombotic occlusion in lower lobe branches of left and right pulmonary artery	*Multiple segmental and subsegmental perfusion defects in left and right lungs	Expansion of defect borders	2
17	Thrombotic occlusion in left and right main pulmonary artery	Multiple segmental and subsegmental perfusion defects in left and right lungs	Newly formed defects	35
18	Thrombotic occlusion in lower lobe branches of left pulmonary artery	*Segmental and subsegmental perfusion defects in the lower lobe of left and right lungs	Same findings	1
Group 2 (Microscopic pulmonary vascular involvement)				
19	Normal	Normal perfusion	Same findings	41
20	Normal	Bilateral heterogenous and decreased perfusion	-	-
21	Thinning in branches of the right and left lower lobe pulmonary artery	Multiple segmental and subsegmental perfusion defects in the lower lobe of left and right lungs	Same findings	17
22	Normal	Bilateral heterogenous and decreased perfusion	-	-
23	Normal	Decreased perfusion in left lung; bilateral segmental and subsegmental perfusion defects	Same findings	31

*Patients with perfusion defects encompassed more segments than pathological vessels on CTA and/or in the contrlateral lung with normal CTA findings.

by scintigraphic evaluation were more extensive than pathological vessels detected by CTA in macroscopic pulmonary vascular disease. In some patients, perfusion defects were observed in contralateral lung with normal vessels on CTA which was probably due to microvascular changes not visible on CTA.

The detection of perfusion abnormality in patients with pulmonary artery aneurysm without thrombosis and in patients with microscopic pulmonary disease was the other important finding. For two decades CTA is widely accepted as a routine imaging tool serving in the amelioration of survival rates of pulmonary

involvement of BD. Hamuryudan et al. showed prominent survival advantage without any therapeutic change in pulmonary BD patients diagnosed by means of CTA after 1992 compared with the patients diagnosed previously (Hamuryudan et al., 2004). In a previous cumulative analysis of pulmonary BD patients, diagnosed and followed up primarily by CTA, 1 and 5-year survival rates of patients with pulmonary artery aneurysm were 57% and 39% respectively (Uzun et al., 2008). In the current study, no patient died during the mean follow-up period of 107±46 months and this finding supported the importance of early diagnosis of pulmonary involvement.

Caglar et al. previously mentioned that, scintigraphic evaluation was ideal for pulmonary involvement of BD patients and normal findings on perfusion lung scintigraphy ruled out pulmonary involvement with a 100% of negative predictive value (Caglar et al., 2000). Our findings mostly supported this detection, because 4/5 patients with microscopic vascular disease had perfusion abnormality on MAA lung scintigraphy. As microscopic involvement might be the earliest form of pulmonary BD, this was thought to be an important result of this study.

In most of the patients in Gr-1c, changes in the perfusion patterns in follow-up MAA lung scintigraphy were remarkable. There was no change observed in Gr-1b and Gr-2 patients on follow-up scintigraphy. We think that, newly formed or improved perfusion defects in the follow-up scintigraphy in Gr-1c patients supported the presence of an unorganized thrombus which represents an early disease and a dynamic process. The ongoing inflammation due to the activity of disease with the failure of compliance on treatment and/or inappropriate treatment caused newly formed defects and appropriate treatment might be the cause of improvement on MAA lung scintigraphy. Although this study was one of the largest case series evaluating MAA lung scintigraphy in pulmonary BD, we have some drawbacks. We have to remark that relatively low number of cases, retrospective evaluation of patients and unblinded nature of evaluation were weak aspects of this study. There was also no histopathological confirmation of pulmonary findings in this case series. This was mostly related to difficulties to perform a biopsy procedure in these severely bleeding cases and ethical issues in the less severe non-aneurysmatic cases who all need urgent immunosuppressive and anticoagulation therapy.

In conclusion, MAA lung scintigraphy findings alone could not differentiate macroscopic and microscopic pulmonary involvement in BD. More extensive vascular involvement was detected on MAA lung scintigraphy in patients with macroscopic pulmonary disease and could be additionally performed to determine the extent of the affected lung area in these patients. Although more patients with pulmonary thrombosis without aneurysm were diagnosed with CTA and longer survival rates had been achieved, we need prospective studies to accurately demonstrate microscopic vascular disease which might be an early form and precursor of thrombotic and aneurysmatic pulmonary vascular disease. In most of the patients with microscopic vascular involvement, multiple perfusion defects or decreased/heterogenous perfusion were observed on MAA lung scintigraphy and this technique can be used as an early diagnostic method in patients with pulmonary symptoms and normal CTA. Follow up studies with MAA scans may be of value in pure thrombotic (Gr-1c) pulmonary involvement, which necessitates further detailed investigation. As CTA provides detailed morphological information about pulmonary vascular pathologies and MAA lung scintigraphy gives additional findings in the evaluation of both macroscopic and microscopic pulmonary disease, these two diagnostic tests can be considered as complementary. However, since almost all patients with microscopic disease and normal CTA showed MAA scan anomalies, perfusion scintigraphy may be proposed as the first step examination in case of suspected pulmonary involvement in BD.

Conflict of interest

There is no conflict of interest to declare.

Ethical approval

The study was approved by the Ethics Committee of Ondokuz Mayıs University hospital (date 27.06.2019, No. 2019/504). The study was conducted in accordance with the principles of the Declaration of Helsinki.

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Comparison of patient-side capillary glucose measurements with autoanalyzer results

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ABSTRACT

Rapid diagnosis and treatment are essential issues for emergency department (ED) physicians. A glucometer is a biochemical measurement tool used for the rapid diagnosis and the detection of complications that can be lethal for patients with diabetes and differential diagnosis in the ED. Patients who were admitted to our ED between August 2014 and August 2015, had their finger-prick glucose values measured with a glucometer and their blood glucose levels checked simultaneously with an autoanalyzer in our biochemistry laboratory were enrolled in our study. In our study, the correlation coefficients for the capillary blood glucometer glucose versus the laboratory autoanalyzer blood glucose was found to be 0.9654 (95 % confidence interval (CI)). According to Bland-Altman analysis, glucose values were mostly within conformity limits. According to Error Grid analysis, 92.2 % of the participants were in the A zone, 6.7 % were in the B zone 0.97 % were in the D zone. Perhaps another important point is that a new biochemical autoanalyzer, that can yield values very similar to reference values within a short period and allows rapid decision making at the clinical level, needs to be developed.

1. Introduction

Rapid diagnosis and treatment are essential issues for emergency department (ED) physicians. A glucometer is a biochemical measurement tool used for the rapid diagnosis and the detection of complications that can be lethal for patients with diabetes and differential diagnosis in the ED. Doctors use these results as the basis for patients who require a quick response according to their glucose values; after that, these results are compared with those of the routine autoanalyzer, and then the treatment is re-evaluated. It takes much time to re-evaluate the treatment according to the results of the autoanalyzer. Therefore, conformance of the values measured with glucometers to the values considered, as the reference is required regarding the accuracy of the decision for clinical treatment. The 2013 version of ISO 15197 specifies that a bias of 15% might be present in 95% of measurements that are higher and lower than 100 mg/dl compared with reference measurements (ISO 15197, 2013). In addition, according to the American Diabetes Association criteria, bedside glucose results should not show a deviation of more than 5% compared with the reference autoanalyzer results (ADA, 1996). Therefore, bedside glucose measurements must be compared with the results of the autoanalyzer that are considered reference values.

According to the glucometer evaluation criteria of the Clinical and Laboratory Standards Institute, POCT 12-A3 January 2013, 95% of results should be within the ±12 mg/dl range at <100 mg/dl glucose concentrations and within the range of ±12.5% at >100 mg/dl glucose concentration. The number of results deviating more than 15 mg/dl from a glucose value <75 mg/dl and the number of samples deviating more than 20% at glucose concentrations >75 mg/dl should not be more than 2% of all results (Krouwer, 2013).

This study aimed to investigate the comparison of bedside capillary glucose measurements with autoanalyzer results and the accuracy of clinical acceptability.

2. Materials and methods

Our study is a retrospective cross-sectional study. Our hospital is a district government hospital that provides care to approximately 130.000 patients in the ED per year. Patients who were admitted to our ED between August 2014 and August 2015, had their finger-prick glucose values measured with a glucometer and their blood glucose levels checked simultaneously with an autoanalyzer in our biochemistry laboratory were enrolled in our study. In our study, 232 patient files were scanned. Patients with high blood glucose values too high to be measured with an autoanalyzer, glucometer and patients whom we could not obtain blood glucose values with an autoanalyzer were excluded. Data for our study were obtained from the hospital automation system. Blood glucose values and demographic information of patients and their diabetes diagnoses were recorded in our study form. A 'GlucoLeader® Yasee Diabetic Blood Glucose Meter-GLM76' was used in our ED for the measurement of blood capillary glucose levels and an Erba Mannheim® XL 1000 autoanalyzer was used in our laboratory for a blood examination.

Statistical analysis

Statistical analysis of our study was performed with the MedCalc Software program. Correlation and regression analyses, Bland-Altman analysis and conformity limits were determined with this programme. For the clinical confirmation of the study results, the error grid analysis developed in 1987 by Clarke et al. was used (Clarke et al., 1987). In this analysis, A, B, C, D and E zones are present and results falling into the A and B zones show clinical acceptability; results falling into the C, D and E zones show clinical unacceptability.

3. Results

In our study, 232 patient files were scanned. Since finger-prick glucose values of five patients were recorded as high and autoanalyzer results of 21 patients could not be obtained, our statistical values were calculated using 206 patients. The number of women enrolled in our study was 113 (54.9%) with an average age of 58.09±19.44 years, and the average age of the men was 61.26±16.14 years. There was no statistically significant difference between the average ages and sexes. In our study, a diabetes diagnosis for females was found to be significantly higher than for men (p=0.011). For our study, a comparison of glucose levels is provided in Table 1, and the correlation curve is shown in Fig.1. The regression analysis yielded the equation, y=0.9683+16.91. Fig. 2 shows that according to the Bland-Altman analysis, glucose values were mostly within conformity limits. Fig. 3 presents the results of the error grid analysis and shows that 92.2% of participants were in the A zone, 6.7% were in the B zone, and 0.97% were in the D zone.

Table 1. Comparison of blood glucose level autoanalyzer and capillary.

	N	BGMV	95 % CI	r
Autoanalyzer	206	207.21±134.22	0.9547-0.9736	0.9654
Capillary	206	196.52±133.82		

N: The number of participants, BGMV: Blood glucose mean values, r: Correlation coefficients

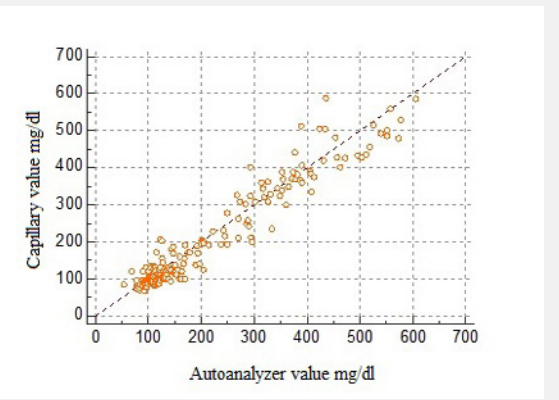

Fig. 1. Autoanalyzer-capillary correlation table.

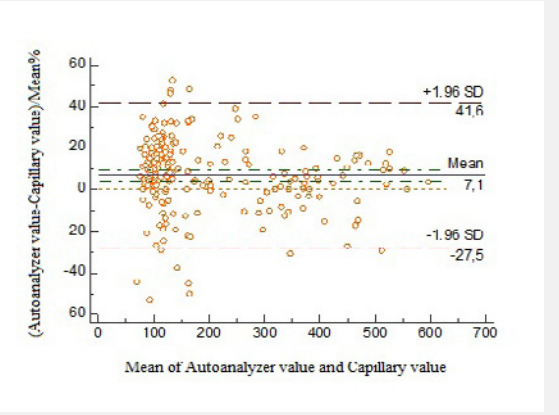

Fig. 2. Blant-Altman plot analysis.

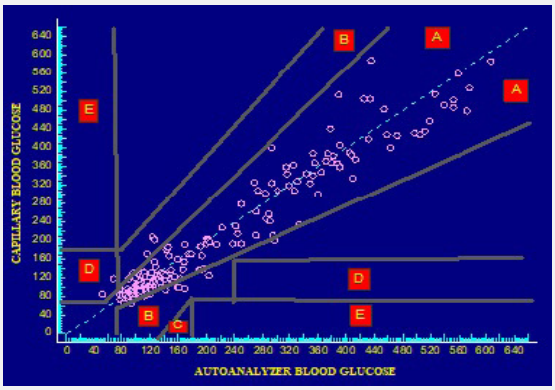

Fig. 3. Error grid analysis.

Limitations

Since our study is a retrospective file scanning study, our main constraint was the inability to standardise the collected samples. Capillary blood glucose levels detected from the patient files may be incorrectly recorded even with a low probability. While increased haematocrit levels reduced glucose measurements, decreased haematocrit levels result in higher glucose measurements. Although some new devices yield results by automatically correcting this issue, the bedside glucose measurement device that we use in our ED does not have this feature. In addition, the influence that the oxygenation state of the patient might have had on the glucometers and glucose measurement results and the presence of hyperlipidaemia could not be obtained from the files.

4. Discussion

In EDs, bedside capillary glucose measurement results are rapidly evaluated, and appropriate treatment is provided according to these results. The physician may look into the results of the autoanalyzer approximately an hour later and may evaluate the appropriateness of treatment. This period is too long for a patient in the ED. In addition, some patients require several additional glucose measurements to be performed until the results of the autoanalyzer are obtained. Therefore, it is important for bedside glucometers to yield relevant results. Furthermore, despite technological advances, autoanalyzer results are still obtained very late, and biochemical tools yielding rapid results are not found in EDs to a large extent. When we searched the literature related to this topic, we found a study by Boyd et al. in which they compared bedside glucose values in the ED (Boyd et al., 2005). The correlation coefficient between laboratory blood glucose values and capillary blood glucometer glucose levels was 0.97, and the correlation coefficient between venous blood glucometer glucose levels was 0.96. In the discussion section of this study, it states that 'although a good correlation is the norm between venous and capillary derived samples, caution must be exercised in accepting the results as equivalent or using either as substitutes for a laboratory blood glucose results.'(Boyd et al., 2005). In the study by Clarke et al. the correlation coefficient was 0.91, and regression analysis produced the equation, y=0.92x + 20.09 (Clarke et al., 1987). Yaraghi et al. measured glucose levels of comatose patients and found that the correlation coefficient between capillary and intravenous laboratory glucose measurements was 0.78 (Yaraghi et al., 2015). Nayeri et al. compared capillary blood glucose levels obtained with a glucometer to standard laboratory measurements (Nayeri et al., 2014). They found the sensitivity to be 83% and specificity to be 97.5% and stated that these values were acceptable. Thus, measurements performed with a glucometer were recommended as an appropriate diagnostic test (Nayeri et al., 2014).

In another study, Patel et al. compared glucose levels obtained by a glucometer and an autoanalyzer (Patel and Patel, 2015). They reported that measuring capillary blood glucose in diabetic patients and monitoring emergencies in non-diabetic patients are good alternatives to estimating venous plasma glucose (Patel and Patel, 2015). The study by Aral et al. compared the results obtained with a venous plasma autoanalyzer with those of capillary blood results, the correlation between the two methods was r=0.969 and regression was y=0.910x+7.008 (Aral et al., 2004). In the study by Chen et al., the regression was high, y=0.79x+50 and r=0.77 (Chen et al., 1998).

Other studies in the literature as well as our study report that measurements with a glucometer show a high correlation to a great extent, and it is reported to be an appropriate test. It is important to consider the clinical acceptability of the results that were found to be statistically positive. Aral et al. and Chen et al. stated this issue in their studies. They specified in their respective studies that having a high correlation is not sufficient for data to be evaluated clinically (Chen et al., 1998; Aral et al., 2004). Error grid analysis is being used for this purpose (Clarke et al., 1987). In error grid analysis, zones A and B specify clinical acceptability, whereas zones C, D and E specify unacceptability. In our study, 98.7% of measurements were found within the zones that are considered to be acceptable. In a study by Foss-Freitas et al., it was determined that a statistically significant difference was found between capillary and venous plasma values of non-glycaemic individuals during fasting (a period of 10-14 hours) (Foss-Freitas et al., 2010). However, no difference was found in diabetic patients, and capillary and venous plasma glucose levels were found to be statistically different in normoglycemic and diabetic patients (Foss-Freitas et al., 2010).

In an interesting study, Yang et al. compared venous and finger-prick glucose levels in healthy

volunteers (Yang et al., 2012). In this study, fasting and postprandial glucose levels of 12 healthy volunteers were compared, and no significant difference was detected during fasting. However, a significant difference was detected in postprandial measurements, and capillary blood glucose values were found to be 35% higher than venous blood glucose levels. Although the intergroup correlation coefficient was r=0.875, venous blood glucose levels are specified as being better indicators clinically. Since the fasting states of patients were not questioned in the ED, misleading results may be obtained when compared with this study. This problem can be eliminated by conducting additional studies that are performed in patients whose fasting states are known (Yang et al., 2012). In the blood glucose monitoring systems evaluation performed by Freckmann et al. according to DIN EN ISO 15197 standards; seven of 34 systems could not completely satisfy the requirement for minimal accuracy according to ISO standards. In this study, they stated that faulty systems result in risky treatment decision making and that glucometers and test strips should be evaluated regularly and standardised to be in accordance with the quality standards (Freckmann et al., 2012).

In conclusion, all hospitals should check the glucometers that they use as standardised and should compare them with the results obtained with laboratory methods. Perhaps another important point is that a new biochemical autoanalyzer that can yield values very similar to reference values within a short period and allows rapid decision making at the clinical level needs to be developed.

Conflict of interest

The author declares that there is no conflict of interest.

Ethical approval

The study was approved by the Ethics Committee of İzmir Katip Çelebi University Non-interventional Clinical Studies Institutionel Review Board (Date: 30.12.2015, No: 251). The study was conducted in accordance with the principles of the Declaration of Helsinki.

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Use of the penis's own tissue for urethral reconstruction in the treatment of complications developing following hypospadias repair

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ABSTRACT

The aim of the present study is to evaluate the results of the surgical technique applied in patients who have previously undergone several operations and who develop persistent distal hypospadias. A novel surgical technique was applied to 13 cases of persistent distal hypospadias presenting between November 2012 and January 2019. The mean age of these patients was 12.7 years (the youngest four and the oldest 21). In methodological terms, fistula formation was prevented by contracting the suture line inside the glans by means of sutures to an incision made between the glans apex and meatus. Thirteen patients underwent surgery, two of which involved complications developing in our clinic. The other 11 had been referred to our clinic from other centers. The common feature of the complications was that they were at the distal hypospadias level. Two patients had undergone two operations, and 11 more than two. Following our therapeutic technique, complete dehiscence was achieved in one patient (7%), external meatal stenosis was present in one (7%), and preoperatively existing urethral stenosis persisted in two cases. Positive results can be achieved in suitable cases of persistent distal hypospadias using the recommended technique.

1. Introduction

Despite an increase in surgical methods and technical materials used in hypospadias surgery, complication rates still range between 6% and 30% (Beuke and Fisch, 2007; Borer and Retik, 2007). Early complications include bleeding, hematoma, and infection. Late complications include fistulae, urethral stenosis, meatal stenosis, partial or complete dehiscence, permanent curvature, and a hairy urethra. The incidences of these vary depending on the type of operation and the clinic involved, although fistulae are the most common complication. Dehiscence (persistent hypospadias) is less common than fistula in treated hypospadias cases (Mousavi and Aarabi, 2014). Since the treatment of these cases is difficult and success rates are lower than with primary repair, accurate evaluation is required. This study involves cases indicated for the surgical technique applied. All consisted of secondary cases developing into persistent distal hypospadias with partial or complete dehiscence following hypospadias surgery.

2. Materials and methods

Thirteen were operated due to hypospadias between November 2012 and January 2019 becoming persistent distal hypospadias exhibiting partial or complete dehiscence was included in the study. The patients' mean age was 12.7 years, the youngest being four, and the oldest 21. Complete dehiscence was present in five patients (Fig. 1) and partial dehiscence in eight (Fig. 2).

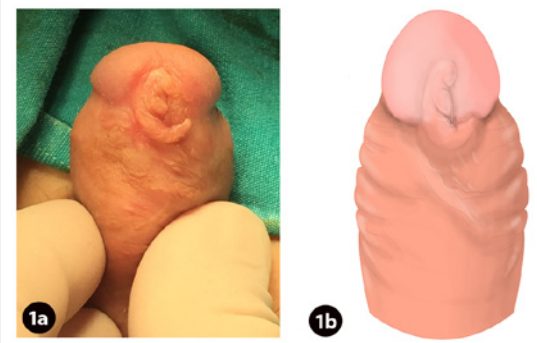

Fig. 1. Complete dehiscence was present in five patients.

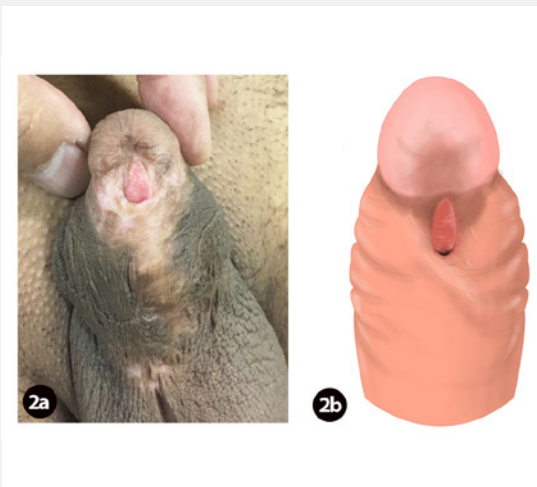

Fig. 2. Partial dehiscence was in eight patients.

General anesthesia was used in all cases. An incision of a suitable depth for urethral catheter installation was first made from the apex of the glans to the site of the meatus (Fig. 3). Next a 5/0 or 4/0 (depending on the size of the penis) polyglactin suture was applied to join the incision, meatus, and glans (Fig. 4). This suture was left long for subsequent urethral stent fixation. The dehiscence on either side of the glans were then sutured. A 10, 12 or 14 F urethral catheter was used, depending on the width of the urethra. A flap was prepared with the flip-flap technique to close the ventral dehiscence.

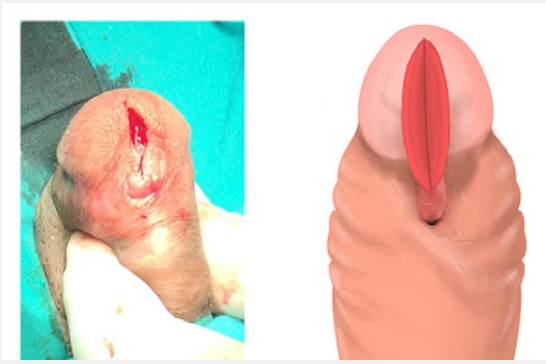

Fig. 3. An incision of a suitable depth for urethral catheter installation was first made from the apex of the glans to the site of the meatus.

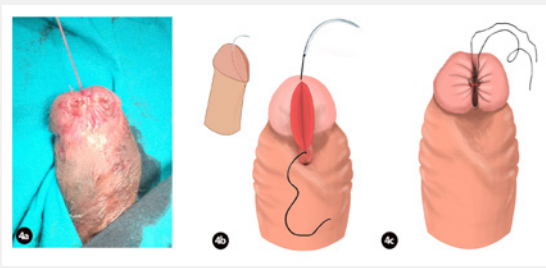

Fig. 4. A polyglactin suture (5/0 or 4/0, depending on the size of the penis) was applied to join the incision, meatus, and glans.

The circumcision line was completed as far as the dorsal aspect, and the penile skin was degloved (Fig. 5). Parallel longitudinal incisions were performed from the meatus to the distal aspect to form the posterior urethral wall (Fig. 6). In order to provide easier closure of the glans, the glandular wings were dissected as far as possible laterally. The flap was then reversed and sutured distally using 7/0 and 6/0 polyglactin, and the urethral tip was constituted by suturing to the dehiscence on the sides (Fig. 7).

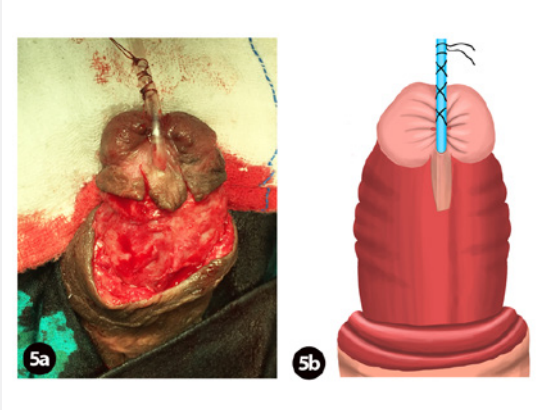

Fig. 5. A flap was prepared with the flip-flap technique to close the ventral dehiscence.

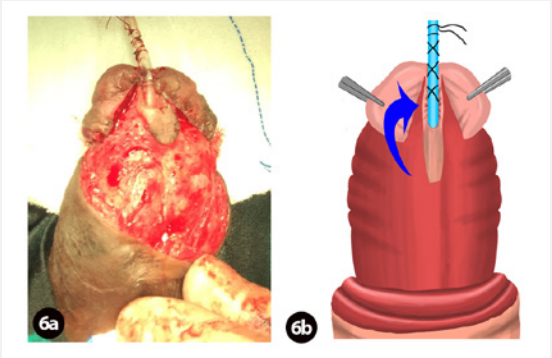

Fig. 6. Parallel longitudinal incisions were performed from the meatus to the distal aspect to form the posterior urethral wall.

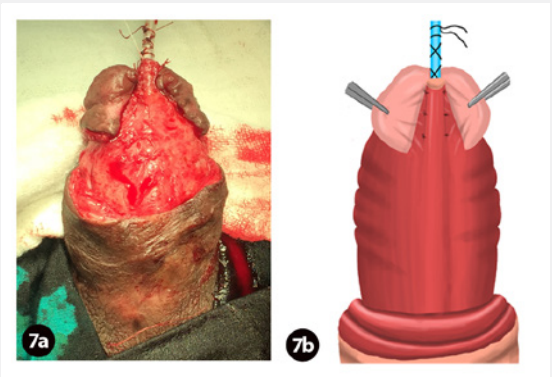

Fig. 7. The urethral tip was constituted by suturing to the dehiscence on the sides.

A sling suture was applied to the tip of the flap to create an external meatus. In order to close the glans, subcutaneous support sutures were first applied, followed by closure with a matrix suture with 5/0 and 4/0 polyglactin (Fig. 8).

Fig. 8. The urethral tip was constituted by suturing to the dehiscence on the sides.

The external meatus as formed with the help of sutures to the tip of the flap. The circumcision line was closed with 5/0 and 4/0 polyglactin. The penis was then covered with a self-adhesive bandage (Coban). In order to avoid compression of the ventral suture line, the urethral catheter was fixed to the abdominal wall with sticking plaster. Broad-spectrum intravenous antibiotic therapy was initiated one hour before surgery and maintained until the third day. Oral antibiotics were applied from the third day until removal of the urethral catheter. The bandages were opened on the third day postoperatively in the absence of bleeding or soakage. The urethral catheter was removed on day 10. Urine cultures were performed preoperatively and on the 50th day and first month postoperatively. Patients were followed-up for 20 months.

3. Results

Thirteen patients underwent surgery, two due to complications occurring in our own clinic, while the other 11 were referred due to complications from other centers. The common characteristic of the patients was distal hypospadias, and chordee was also present in two subjects. Two patients had previously undergone two operations, and the remaining 11 more than two. Moderate stenosis was observed in the penoscrotal region during urethral catheter insertion in two patients. Mean period of catheterization was eight days, and mean period of hospital stay was six days. Existing preoperative stenosis in two patients persisted in the postoperative period, and fistula developed in the proximal aspect of the previous stenosis in one case. External meatus stenosis also developed in one case (7%). Urethral dilation was applied to these patients. External meatotomy was subsequently required in the cases of external meatus stenosis. One case wasopened completely due to infection in the postoperative period (7%) in one patient, who was aged 16. One patient was re-treated one year subsequently using the same technique. One of the cases with chordee improved with resection. Dorsal plication was performed on the other. Although cosmetic results satisfactory to the family and the physician were achieved in the majority of cases; these were not obtained in one case. This was due to this patient having previously undergone a large number of operations. Penile reconstruction surgery was subsequently performed in this case, and the desired patient and physician satisfaction was achieved.

4. Discussion

The repair of hypospadias complications may be difficult due to the low quality of surrounding tissue arising from impairment of normal vascularity. The most commonly encountered complications are fistulae and urethral stenosis (Mousavi and Aarabi, 2014). Both single and multi-stage therapeutic methods are available for the repair of these. The inadequate nature of the surrounding tissue and mobilization problems in the repair of these complications make treatment using standard methods difficult (Beuke and Fisch, 2007; Mousavi and Aarabi, 2014). The repair of these complications is complex due to the absence of foreskin following first hypospadias repair or due to broad scar tissue formation in previous operations.

Approaches such as buccal mucosa, hairless skin grafts, the dartos layer flap, bladder mucosa, lyophilized human dura, and in vitro cultured urethral epithelial urethroplasty are employed in different centers in the treatment of these complications (Hendren and Crooks, 1980; Romagnoli et al., 1990; Garat and Villavicencio, 1991; Hübner et al., 1991; Olsen et al., 1992; Brock, 1994; Kinkead et al., 1994). The question of which method should be preferred to treat this difficult condition is still controversial. The general approach individualizes the patient following careful evaluation. However, the buccal mucosa or bladder mucosa is generally used. Barbagli et al. achieved an 82% success rate with the use of buccal mucosa (Barbagli et al., 2016), while another study reported 22% graft contracture in patients treated using buccal mucosa (Myers et al., 2012). Although hypospadias repair using the bladder mucosa graft is not popular, it can also be employed. Li et al. reported 87.6% success and a 12.4% complication rate in unsuccessful hypospadias patients (Li et al., 1995), while severe meatus stenosis, graft contracture, and fistula formation were observed in the long term with bladder mucosa use in another series (Romagnoli et al., 1990; Olsen et al., 1992; Kinkead et al., 1994; Ducket et al., 1995; Myers et al., 2012; Barbagli et al., 2016). Bladder mucosa is occasionally used today in contrast to buccal mucosa use. Since these cases have previously been operated, difficulties are sometimes experienced with standard methods because of insufficiency of surrounding tissue and mobilization problems caused by scar formation. The approach described in this study would seem to be the option of choice if appropriate penile skin conditions are established.

Tubularized incised-plate (TIP) urethroplasty, described by Snodgrass, is another technique involving the penile skin in these cases (Snodgrass, 1994; Li et al., 1995; Saleh, 2007; Mousavi, 2008). The TIP urethroplasty method is widely employed by several surgeons in treating primary hypospadias patients. However, due to its application in selected cases, there is still significant debate concerning its use in hypospadias complication repair (Snodgrass and Lorenzo, 2002). Urethral stenosis was reported following hypospadias complication repair with the TIP method, and urethral dilation was required. Meatal stenosis occurred in only one of our cases. Improvement was not achieved with urethral dilation in that case, and external meatotomy was performed. A very small fistula also developed proximally to the stenosis in one case with previous urethral stenosis. The urethral catheter was removed on the sixth day due to obstruction, and a fistula developed 20 days following catheter removal. The catheter was reinserted and left in place for ten days. The patient urinated normally following removal of the catheter. The glandular wings were dissected as far as possible laterally in order to prevent urethral and meatal stenosis. Care was taken during closure of the glans to ensure comfortable movement of the stent. The tip of the flap was used by being reversed over the glandular wings, producing an external meatus comparable to the original. The urethral catheter was left in place for ten days in these secondary cases.

Another important complication observed in the TIP method is urethral fistula. This was not observed in any of our cases, although complete dehiscence due to infection occurred in one case. This patient was successfully treated using the same method one year later. We attribute the absence of fistula in the 13 cases in which our method was applied to the short suture line. In order to reduce the suture line, we transferred the suture to the incision extending from the apex of the glans to the meatus and the suture line inside the glans. As a result, fewer sutures were applied to the lateral part of the flap we reversed. When the glans is closed, these sutures generally remain beneath it, and we observed that in the absence of severe infection no glans dehiscence occurred and no fistula developed. In addition, care was taken to ensure that the glans was of sufficient size in the cases in which we applied this technique. The aim in this proposed technique is to move the urethra inside the glans and to provide a cosmetically regular penis, and we think that this will not be possible in the case of a small glans.

Sufficient tissue for repair purposes is not present in the majority of these patients. One part of existing tissues may lack healthy vascularization and tissue supply. In addition, some tissues in which surgery has been applied are not sufficiently elastic. The penis may also assume a complicated appearance due to inadequate surgeon experience and skill. Care was taken to ensure a regular penile appearance in our cases, and this was successfully achieved in all but one case. In this problematic case, the penile skin had been very poorly used in previous operations. However, we achieved a cosmetic penile appearance by performing subsequent reconstruction.

The technique described here is quite simple. The suture applied to the incision between the apex of the glans and the meatus and the suitable wings formed on both sides of the glans permit comfortable stent insertion. The depth of this incision must be arranged so that the urethral stent can be easily inserted and also for easy closure of the glans. Closure of the glans in two layers prevents both dehiscence of the glans and also fistula development.

In conclusion, surgical procedures all have various advantages and disadvantages. Surgical difficulties and postoperative problems encourage surgeons to look for easier and practicable methods. Our experience shows that the proposed approach can achieve positive results in appropriate cases of persistent distal hypospadias.

Conflict of interest
The author declares his individual contribution to this paper. And the author declares that he has no conflicts of interest.

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent
Informed consent was obtained from individual participants included in the study.

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The antibacterial and antifungal activities of commonly used herbal oils

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ABSTRACT

The antibacterial and antifungal activities of herbal oils and their derivatives has been studied for several years; however, more studies are needed to develop alternative strategies to destroy pathogenic microorganisms due to increasing concerns about the development of antimicrobial resistance amongst them. In this study, our aim was to investigate the minimal inhibitory concentrations (MIC) of 23 different commercially available herbal oils on both yeasts and bacteria strains. Twenty three commercially available herbal oils including centaury, ginger, curcumin, eucalyptus, black cumin, cinnamon, sesame, rosemary, safflower, cardamom, argan, thyme, etc. were used to determine the antibacterial and antifungal activities on both yeasts and bacteria (standard ATCC strains). *Candida albicans, Candida parapsilosis, Candida glabrata* from yeasts, *Escherichia coli* from gram-negative bacteria, *Acinetobacter baumannii* from non-fermentative bacteria, and *Staphylococcus aureus* from gram-positive bacteria were selected. The effective MIC values of herbal oils were detected by using resazurin microtiter assay plate (REMA) technique. All herbal oils were effective on standard bacteria and yeast strains in different concentrations. The effective concentration ranges of herbal oils on each bacteria and yeast were as following; 15.625-31.25 µg/ml for *Candida parapsilosis* (ATCC 22019), 15.625-125 µg/ml for *Acinetobacter baumannii* (ATCC 49139), 31.25-62.5 µg/ml for *Candida albicans* (ATCC 14053), *Candida glabrata* (ATCC 15126), and *Staphylococcus aureus* (ATCC 29213), 62.5-125 µg/ml for *Escherichia coli* (ATCC 25923). In conclusion, antimicrobial capacities of some herbal oils that provide alternative solutions to pathogen microorganisms inhibition, which are made more difficult due to widespread resistance to antimicrobial agents, were evaluated in this study. We believe that this study will contribute to other related studies on the identification of herbal oil antimicrobial mechanisms of action.

1. Introduction

The percentage of infectious diseases causing human deaths is quite high. On the other hand, the widespread resistance to antimicrobials among pathogenic microorganisms poses a serious threat in the treatment of microbial diseases (Maurice et al., 1990; Faydalıoğlu and Sürücüoğlu, 2011). This resistance has led to a necessity for new strategies in the treatment or prevention of infectious diseases (Ozmen et al., 2015; Karamese et al., 2016). At that point, the use of plant extracts and herbal oils as a natural product source for combating resistant and/or non-resistant microorganisms offers alternative solutions (Prabuseenivasan et al., 2006). The World Health Organization (WHO) reported that the traditional medicine for primary healthcare has been preferred by the majority of the world's

population. Medicinal and aromatic plants are a major source of natural organic compounds widely used as medicine (Solmaz and Ata, 2009). Herbal oils which are natural, concentrated, volatile aromatic compounds isolated from plants have some preventive/therapeutic effects including antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. The number of plants used to provide an alternative solution against antibiotic resistance is quite high. In current literature, the most known and used commercially available herbal oils were obtained from *Pinus terebenthinae, Copaifera officinalis, Salvia officinalis, Cedrus libani, Aesculus hippocastanum, Hypericum perforatum, Santalum album, Foeniculum vulgare, Lavandula stoechas, Urtica dioica,* and *Citrus bergamia* plants (Burt, 2004; Kordali et al., 2005; Mickiene, 2011; Bilenler and Gökbulut, 2019). The bioactive compounds of these plants may involve multiple modes of antimicrobial action including changes in the synthesis of DNA and RNA, degradation of bacterial cell-wall, disruption the structure of cytoplasmic membrane, changes in the level of fatty acid and phospholipid constituents, and destruction of protein translocation. Hence, it is possible to use the herbal oils for antimicrobial effects against pathogenic microorganisms (Lambert et al., 2001; Shan et al., 2007; Witkowska et al., 2013).

In the present study, antimicrobial activity of 23 most known and used commercially available herbal oils was investigated against gram-negative, gram-positive, non-fermentative and yeast strains for minimal inhibitory activity.

2. Materials and methods

Oils, microorganisms, and culture conditions

The herbal oils were purchased from Biotama Natural Products (Biotama, Ankara, Turkey). The names of plants and oils used in this study are seen in Table 1.

The initial concentration of herbal oils with different concentrations obtained to 1mg/ml by the dissolving in Dimethyl Sulfoxide (DMSO) and filtered through 0.22 µM membrane filters. Reference microbial strains of American Type Culture Collection (ATCC, USA) were used in this study. The antimicrobial activity of Gram-positive bacterial strain [*Staphylococcus aureus* (ATCC 29213)], Gram-negative bacterial strains [*Escherichia coli* (ATCC 25923), *Acinetobacter baumannii* (ATCC 49139)] and yeast strains [Candida albicans (ATCC 14053), *Candida glabrata* (ATCC 15126) and *Candida parapsilosis* (ATCC 22019)] were investigated. The bacterial strains were stored at -80ºC until the experiment day. Blood Agar and Sabouraud Dextrose Agar (SDA) supplemented with 8% glucose were used for production bacteria and yeast, respectively. Mueller Hinton Broth (MHB) for bacteria and Tryptic Soy Broth (TSB) for yeast were used to determine the minimum inhibitory concentrations (MIC). The mediums were sterilized with autoclave at 121°C for 15-20 minutes and prepared according to the manufacturer's instructions.

Table 1. The names of plants and oils used in this study.

No	Plants	Oils
1	*Hypericum perforatum*	Centaury oil
2	*Cinnamomum verum*	Cinnamon oil
3	*Simmondsia chinensis*	Jojoba oil
4	*Carthamus tinctorius*	Safflower oil
5	*Eucalyptus globulus*	Eucalyptus oil
6	*Ocimum basilicum*	Basil oil
7	*Nigella sativa*	Black cumin oil
8	*Argania spinosa*	Argan oil
9	*Jasminum nudiflorum*	Jasmine oil
10	*Thymus vulgaris*	Thyme oil
11	*Sesamum indicum*	Sesame oil
12	*Rosa canina*	Rosehip oil
13	*Urtica dioica*	Nettle oil
14	*Ricinus communis*	Indian oil
15	*Cananga odorata*	Ylang ylang oil
16	*Rosmarinus officinalis*	Rosemary oil
17	*Curcuma longa*	Turmeric oil
18	*Lilium candidum*	Lily oil
19	*Elettaria cardamomum*	Cardamom oil
20	*Zingiber officinale*	Ginger oil
21	*Foeniculum vulgare*	Fennel oil
22	*Syzygium aromaticum*	Clove oil
23	*Cuminum cyminum*	Cumin oil

Inoculum and resazurin preparation

The stock bacterial and yeast suspensions used for inoculation were prepared at 105 CFU/ml by diluting fresh cultures at McFarland 0.5 density in sterile tubes. Suspensions of bacteria at McFarland density was diluted 1:20. Suspensions of the yeast at McFarland density was diluted 1:50 and 1:20 respectively. Resazurin sodium salt powder was used. Resazurin is a non-fluorescent blue dye used to test samples for bacterial and yeast contamination. It is also useful in sperm viability and semen quality test. Resazurin is applicable in cytotoxicity determination. A working solution was prepared at a 0.01% (w/v) concentration in distilled water and a 0.22 µM membrane filter was used for filtration and sterilization procedures.

Resazurin microtiter assay (REMA)

A sterile 96-well microplates were used for determination MIC. A volume of 100 µl of test medium (TSB for yeast and MHB for bacteria) was pipetted into the each well of the microplate. The stock concentration of oils (1 mg/ml) were added into the first well of microplates and two-fold dilutions were performed.

Serial dilutions were performed using multichannel pipette. Finally, 10 µl of bacterial suspension and 100 µl of yeast suspension was added to each well. Microplates including bacteria and yeast were covered with lids and incubated at 37°C for 24-48 hours. After incubation, 10 ml of freshly prepared 0.01% resazurin solution was added to each well and the plates were re-incubated at 37°C at 24 hours. A growth control containing any oils and a sterile control without bacteria and yeast were also used. Any color changes from purple to pink were considered as positive (Fig. 1). REMA was carried out in triplicate (Nateche et al., 2006).

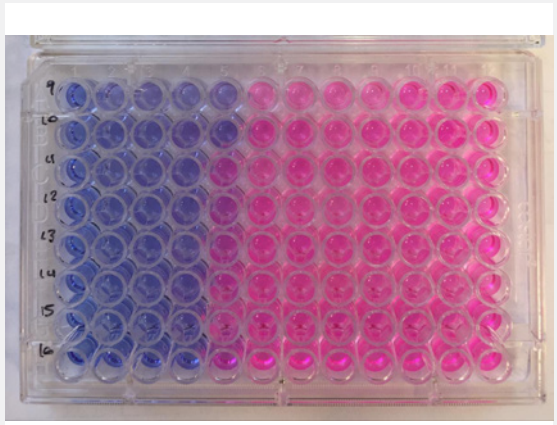

Fig. 1. Resazurin Microtiter Assay (REMA) performed in our study.

3. Results

The antibacterial and antifungal activities of 23 different herbal oils on six microorganisms are seen in Table 2. All herbal oils were effective on reference bacteria and yeast strains in different concentrations. The effective

Table 2. The MIC values of herbal oils on tested microorganisms.

	Minimal Inhibitory Concentrations (µg/ml) Microorganisms					
Herbal oils	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Candida albicans*	*Candida glabrata*	*Candida parapsilosis*
Hypericum perforatum (centaury oil)	62.5	31.25	62.5	31.25	62.5	15.625
Zingiber officinale (ginger oil)	62.5	62.5	62.5	31.25	31.25	15.625
Sesamum indicum (sesame oil)	125	31.25	62.5	31.25	31.25	15.625
Jasminum nudiflorum (jasmine oil)	125	62.5	62.5	31.25	31.25	15.625
Cuminum cyminum (cumin oil)	125	62.5	62.5	31.25	31.25	15.625
Ocimum basilicum (basil oil)	125	62.5	62.5	31.25	31.25	15.625
Nigella sativa (black cumin oil)	62.5	62.5	31.25	31.25	31.25	15.625
Cinnamomum verum (cinnamon oil)	62.5	62.5	31.25	31.25	31.25	15.625
Eucalyptus globulus (eucalyptus oil)	62.5	62.5	62.5	31.25	31.25	15.625
Rosmarinus officinalis (rosemary oil)	62.5	62.5	31.25	31.25	31.25	15.625
Argania spinosa (argan oil)	62.5	62.5	31.25	31.25	31.25	15.625
Foeniculum vulgare (fennel oil)	62.5	62.5	62.5	31.25	31.25	31.25
Rosa canina (rosehip oil)	62.5	62.5	62.5	31.25	31.25	31.25
Ricinus communis (indian oil)	62.5	62.5	62.5	31.25	31.25	31.25
Cananga odorata (ylang ylang oil)	62.5	31.25	62.5	31.25	31.25	31.25
Simmondsia chinensis (jojoba oil)	62.5	31.25	62.5	31.25	31.25	31.25
Carthamus tinctorius (safflower oil)	62.5	31.25	62.5	31.25	31.25	31.25
Curcuma longa (turmeric oil)	62.5	31.25	31.25	31.25	31.25	31.25
Elettaria cardamomum (cardamom oil)	62.5	31.25	31.25	31.25	31.25	31.25
Lilium candidum (lily oil)	62.5	31.25	31.25	31.25	31.25	31.25
Urtica dioica (nettle oil)	62.5	31.25	31.25	62.5	31.25	31.25
Syzygium aromaticum (clove oil)	62.5	15.625	62.5	62.5	31.25	31.25
Thymus vulgaris (thyme oil)	62.5	125	62.5	62.5	31.25	31.25

concentration ranges of oils on each bacteria and yeast were as following; 15.625-31.25 µg/ml for *Candida parapsilosis* (ATCC 22019), 15.625-125 µg/ml for *Acinetobacter baumannii* (ATCC 49139), 31.25-62.5 µg/ml for *Candida albicans* (ATCC 14053), *Candida glabrata* (ATCC 15126), and *Staphylococcus aureus* (ATCC 29213), 62.5-125 µg/ml for *Escherichia coli* (ATCC 25923). The most effective oils were centaury, ginger, sesame, jasmine, cumin, basil, black cumin, cinnamon, eucalyptus, rosemary, and argan oils for *Candida parapsilosis* (15.625 µg/ml); and black cumin, cinnamon, rosemary, argan, turmeric, cardamom, lily and nettle oils for *Staphylococcus aureus* (31.5 µg/ml). All tested herbal oils had antibacterial effects in different ranges on *Escheriehia coli* and *Acinetobacter baumannii*; however, this efficiency was less than the effect on other microorganisms. On the other hand, black cumin, cinnamon, rosemary, and argan oils were the most effective on all tested microorganisms (both bacteria and yeast strains).

4. Discussion

For centuries, herbal oils have been used extensively in different fields for the protection of foods, pharmaceuticals, medicine and natural therapeutic. In order to increase the quality in the field of health, it is essential to scientifically examine the herbal oils used in traditional medicine. Herbal oils have a high potential for the development of new antimicrobial agents. In our study, 20 herbal oils showed different rates of antimicrobial activity against six microorganisms (three bacteria and three yeasts).

In current literature, it has been reported that these herbal oils exhibit antibacterial activity on a variable scale against different microorganisms. Nostro et al., determined that plant extracts have inhibitory effects against some Gram (+), Gram (-) bacteria and yeast strains (Nostro et al., 2000). Another study reported that herbal oils obtained from eight different aromatic plants showed inhibitory effects on 11 different microorganisms (Sartoratta et al., 2004). Similarly, Witkowska et al. showed the efficiency of 30 different herbs and spices on *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas fluorescens* (Witkowska et al., 2013). They detected the MIC values of basil, cinnamon, cumin, fennel, ginger, rosemary, clove, thyme, and turmeric oils on *Escherichia coli* (40, 20-40, >40, >40, 20-40, 20-40, 5-10, 20-40, and 20-40 mg/ml-1, respectively) and *Staphylococcus aureus* (40, 20-40, >40, >40, >40, 20-40, 5-10, 20-40, and >40 mg/ml-1, respectively) standard bacteria strains. Our study shows similarity regarding current data about the antibacterial activities of herbal oils. In our study, the MIC value ranges were detected >40 µg/ml for *Escherichia coli*, and 31.25-62.5 µg/ml for *Staphylococcus aureus*.

Furthermore, two studies also reported the antimicrobial activities of rosemary, clove, cinnamon, cumin, eucalyptus, thyme, basil, fennel oils on 4 Gram (+) and 2 Gram (-) bacteria including *Escherichia coli* O157:H7 strain and expressed that the most effective ones were cloves, cinnamon, and rosemary oils (Ouattara et al., 1997; Roura et al., 2005). When compared to our study, the results were close to each other. Black cumin, cinnamon, rosemary, argan, turmeric, cardamom, lily and nettle oils were the most effective oils for Gram (+) bacteria.

On the other hand, the antifungal activity of herbal oils was also investigated in some researches. Çenet and Toroğlu showed antibacterial and antifungal activities of fennel, thyme, and ginger oils (Çenet and Toroğlu, 2006; Balkan et al., 2016). Another study performed in 2003 reported that some herbal oils had significant inhibitory effects on *Candida albicans* and *Candida vaginalis* yeast strains (Al-Howiriny, 2003). Furthermore, a group of researchers applied Kirby-Bauer disk diffusion test with herbal oils. They showed both antibacterial and antifungal effects of those; however, they observed that these oils were more effective in Gram (+) bacteria and yeast strains than Gram (-) bacteria (Dağcı et al., 2002). The centaury, ginger, sesame, jasmine, cumin, basil, black cumin, cinnamon, eucalyptus, rosemary, and argan oils were the most effective oils for *Candida* strains in our study. Similarly, Rabe and Staden studied the antimicrobial effects of 21 different herbal oils and reported that those were more effective on Gram (+) bacteria while no efficiency was detected on *Klebsiella pneumonia* (Rabe and Van Staden, 1997). Shan et al. performed a study about the same issue with 46 herbal oils on five food pathogen bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella anatum*) and detected the most of oils were more effective on Gram (+) bacteria (Shan et al., 2007).

Despite the widespread use of medical herbal oils in the fight against microorganisms, the mechanisms of antimicrobial action have not been fully defined. In literature, different approaches regarding the mechanism of action have been proposed; bacterial inhibition due to deterioration of membrane integrity, loss of cell content (molecules and ions) due to damage of selective permeable structure of membrane, secondary metabolites (phenolic compounds) in volatile oil composition causing damage to cell membrane, cell vital activities (energy production, protein synthesis) (Beyaz, 2014; Şengün and Öztürk, 2018; Bilenler and Gökbulut, 2019).

In conclusion, antimicrobial capacities of some herbal oils that provide alternative solutions to pathogen microorganism inhibition, which are made more difficult due to widespread resistance to antimicrobial agents, were evaluated in this study. We believe that

this study will contribute to other related studies on the identification of herbal oil antimicrobial mechanisms of action. For this reason, further detailed molecular studies should be performed.

Conflict of interest

The author declares that there is no conflict of interest.

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Are serum lipid and androgen levels different in women with natural or surgical menopause?

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ABSTRACT

Menopausal status is associated with the type of menopausal onset. The aim of this study was to investigate whether serum lipid and androgen levels are different in women with natural or surgical menopause. This retrospective case control study was conducted with 376 postmenopausal women with natural onset and 144 postmenopausal women with surgical onset. Each woman was assessed in terms of serum glucose, lipid and androgen levels. The mean serum glucose, cholesterol, triglyceride, HDL, LDL, VLDL and testosterone levels in the surgical menopause group were almost similar to those of the natural menopause group (p=0.510, p=0.873, p=0.807, p=0.950, p=0.807, p=0.972, p=0.086, p=0.778, respectively). The mean serum DHEAS levels in the natural menopause group were statistically higher in comparison with the surgical menopause group (p=0.044). Serum lipid levels in postmenopausal women are not different in terms of the type of menopause onset. Serum androgen levels were decreased more in surgical menopause with lower levels of DHEAS associated with surgical onset.

1. Introduction

Menopause is the cessation of menstrual periods either following a natural onset of gradual stopping for 12 months or following bilateral oophorectomy so that ovarian hormones are suddenly terminated (Landgren et al., 2004). As life expectancy has increased, women now live at least one third of their lives after menopause (Kulak et al., 2009). Metabolic changes following surgical and natural menopause differ and are associated with risk factors for cardiovascular disease, which is the leading cause of mortality in postmenopausal women (Ozdemir et al., 2009; Farahmand et al., 2015).

Natural menopause occurs at a median age of 51 years due to ovarian follicular depletion with a spontaneous

decline in ovarian hormone secretion. The removal of both ovaries at the time of hysterectomy or other pelvic surgery before the natural age of menopause is known as surgical menopause (Rodriguez and Shoupe, 2015). Although gynecological malignancies are certain indications for oophorectomy, in the setting of benign disease, the decision for removal of the ovaries at the time of hysterectomy is taken according to guidelines for premenopausal patients (ACOG, 2008).

The sudden withdrawal of estrogen, progesterone, and androgens in surgical menopause are associated with more severe, early onset and prolonged menopausal symptoms due to the acute reduction in ovarian sex steroid production (Davison et al., 2005). Hot flushes may even be apparent in the immediate postoperative period (Gallicchio et al., 2006). The intact ovary often maintains hormone production in the natural menopause, which is the transitional period known as the peri-menopause (Taylor et al., 2017). Although estradiol production dramatically decreases, testosterone production continues in the postmenopausal ovaries. The small amount of circulating testosterone seems to have beneficial effects on menopausal symptoms (Fogle et al., 2007). A prior oophorectomy affects lipid, lipoprotein, glucose, and insulin metabolism in women according to the changes in ovarian steroid production and secretion (Carr et al., 2000).

Early and surgical menopause are associated with an increased risk of CVD because of a decline in the natural ovarian hormones (Lobo et al., 2007). The aim of this study was to investigate whether serum lipid and androgen levels are different in women with natural or surgical menopause.

2. Material and methods

This retrospective, cross-sectional study was conducted with postmenopausal women who underwent annual examinations in the Outpatients Clinic of Samsun Women and Children's Health Research and Training Hospital between January 2016 and June 2019. Approval for the study was granted by the Local Institutional Review Board (decision no: GOKA/2019/3/9). The inclusion criteria were accepted as natural or surgical postmenopausal women aged between 40 and 65 years old, and not using hormone replacement therapy (HRT). The exclusion criteria were accepted as use of HRT, exogenous steroid therapy, or anti-lipid drugs, or a history of chemotherapy or pelvic radiotherapy because of a cancer.

Of a total of 550 postmenopausal women, 19 were excluded due to currently use of HRT to eliminate the possible implication of estrogen and progesterone in the relationship between menopause and lipid metabolism, and 11 because of chemotherapy for a malignancy. A total of 376 women with natural menopause and 144 women with surgical menopause were included in the study. Postmenopausal status was accepted as at least 12 consecutive months of amenorrhea with a follicle-stimulating hormone (FSH) level of 40 mIU/mL (Soules et al., 2001). Age, gravidity, parity, time since menopause onset and body mass index (BMI) were recorded as demographic characteristics. The height and weight measurements of women were obtained and BMI was calculated as the ratio of weight (kg) to the square of the height (m^2).

Blood samples were collected by venipuncture after a 12-hours overnight fast, and measurements for serum fasting plasma glucose (FPG), total cholesterol (TC), Triglyceride (TG), High density lipoprotein-cholesterol (HDL-C), very-low density lipoprotein-cholesterol (VLDL-C) testosterone, androstenedione and dehydro-epiandrostenedione-sulphate (DHEAS) were performed. These parameters were quantified using a 7600-110 Automatic Analyzer (Hitachi Inc., Tokyo, Japan). The low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.

Statistical analysis

Data obtained in the study were analyzed statistically using NCSS (Number Cruncher Statistical System) 2007 software (Kaysville, Utah, USA). Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used to evaluate the study data. The conformity of the quantitative data to normal distribution was tested using the Kolmogorov-Smirnov test, Shapiro-Wilk test and graphic evaluations. The Mann Whitney U test was used in the comparison of two groups of data which did not show normal distribution. The Pearson Chi-square test was used to compare qualitative data. Spearman's Correlation Analysis was applied to evaluate the relationships between variables. A value of $p<0.05$ was accepted as statistically significant.

3. Results

Evaluation was made of a total of 520 postmenopausal women, as 376 women in the natural menopause group and 144 women in the surgical menopause group. The age, gravidity, parity, abortus, time since menopause onset, and BMI values of the groups are presented in Table 1 ($p=0.065$, $p=0.011$, $p=0.021$, $p=0.069$, $p=0.696$, $p=0.611$, respectively).

The mean serum glucose, cholesterol, triglyceride, HDL, LDL, and VLDL levels in the surgical menopause group were almost similar to those of the natural menopause group ($p=0.510$, $p=0.873$, $p=0.807$, $p=0.950$, $p=0.807$, $p=0.972$, respectively). The mean serum testosterone and androstenedione were lower in the surgical menopause group, with no statistically significant difference between the groups ($p=0.086$, $p=0.078$, respectively). The mean serum DHEAS

levels in the natural menopause group were statistically significantly higher compared to those of the surgical menopause group (p=0.044) (Table 2).

Table 1. Characteristics of women with natural menopause and surgical menopause.

	Natural menopause (n=376)	Surgical menopause (n=144)	p value
Age (years) mean±sd	52.59±6.75	48.40 ±6.48	0.065
Gravidity min-max (median)	0-15 (4)	0-10 (3)	0.011*
Parity min-max (median)	0-12 (4)	0-7 (3)	0.021*
Aborts min-max (median)	0-6 (1)	0-5 (1)	0.069
Time since menopause onset (years) mean±sd	3.10±1.22	3.91±1.31	0.696
Body mass index (BMI, kg/m2) mean±sd	28.10±5.34	29.31±4.29	0.611

Mann Whitney U Test, *p<0.05.

Table 2. Laboratory test results of women with natural menopause and surgical menopause.

	Type of menopause		P value
	Natural menopause (n=376)	Surgical menopause (n=144)	
Glucose (mg/dl)	116.52±50.64	112.62±34.87	0.510
Cholesterol (mg/dl)	243.22±71.23	240.44±60.88	0.873
Triglyceride	179.93±81.91	183.28±90.21	0.807
HDL (mg/dl)	57.68±16.38	57.70±16.56	0.950
LDL (mg/dl)	168.06±64.22	165.31±66.3	0.807
VLDL (mg/dl)	45.27±25.65	46.67±28.44	0.972
Testosterone (mg/dl)	21.35±10.95	19.91±10.54	0.086
Androstenedione (mg/dl)	0.40±0.22	0.38±0.23	0.078
DHEAS (mg/dl)	100.24±66.72	87.13±60.97	0.044*

Mann Whitney U Test, mean ± sd *p<0.05.

4. Discussion

This retrospective, cross-sectional study investigated whether the serum lipid and androgen levels were different in women with natural or surgical menopause. The results indicated that serum lipid levels were not changed with natural or surgical onset in postmenopausal women. Serum androgen levels were higher in the natural menopause group but not to a statistically significant level. However, postmenopausal women with natural onset had higher serum DHEAS levels in comparison with postmenopausal women with surgical onset.

Menopausal transition is known to be associated with higher dense of LDL and higher LDL-C levels in comparison to premenopausal women (Wang et al., 2018). A history of oophorectomy is associated with increased lipid, lipoprotein, glucose, and insulin levels compared with intact ovaries in postmenopausal women (Yoshida et al., 2011). In the current study, there was no difference between the groups in respect of serum glucose, cholesterol, triglyceride, HDL, LDL, and VLDL levels. Nevertheless, close monitoring of lipids in routine examinations can be recommended for all postmenopausal women.

Surgical menopause results in a sudden reduction in ovarian sex steroid production and a complete absence of any steroid production with surgical removal of the ovary. However, intact postmenopausal ovaries often continue limited production of sex steroids, particularly of testosterone (Matsui et al., 2012). In the present study, serum testosterone and androstenedione levels were lower in women with surgical menopause than in women with natural menopause, but not to a statistically significant level. The removal of the premenopausal ovary and the sudden and significant reductions in testosterone and androstenedione are known to have a negative impact on sexual desire (Celik et al., 2009).

The decline in ovarian production of estrogen and increased adiposity may play a role in heightened estrogen synthesis in the adipose tissue by aromatase conversion of androgens after menopause (Castracane et al., 2006). In the present study, a statistically non-significant decline in testosterone and androstenedione levels was determined in postmenopausal women with surgical onset. It can be suggested that as the surgical menopause group comprised more over-weight women although it did not reach statistical significance, more adipose tissue may provoke more aromatization of testosterone and androstenedione. DHEAS is an important androgen and estrogen precursor in postmenopausal women with decreased ovarian production. Despite the low level of estrogen production, utilization of DHEAS is necessary in the postmenopausal period. Lower DHEAS has been associated with the development of atherosclerosis and insulin resistance depending on a higher BMI (Lasley et al., 2002). In the current study, decreased DHEAS levels were detected in the postmenopausal women with surgical onset.

The limitation of this study is that retrospective and cross-sectional nature containing the last three years, meaning that the results are not fully representative of the general population of postmenopausal women. Further prospective studies are needed.

In conclusion serum glucose, cholesterol, triglyceride, HDL, LDL, and VLDL levels are not different according to types of menopause onset in postmenopausal women. Serum testosterone and androstenedione levels were more decreased with surgical menopause although definitively decreased DHEAS levels were seen to be associated with surgical onset in postmenopausal women.

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Conflict of interest

The authors declare no conflict of interests.

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Case Report

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Different types of kidney tumors together in the same kidney: Is it rare condition?

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ABSTRACT

Renal cell carcinoma (RCC) is the most common solid lesion of the kidney and comprises 2-3% of all cancers. Bilateral synchronous benign and malignant renal tumors have been reported in some studies. However, unilateral concordance of malignant renal tumors of different histologic subtypes are very rare and only a few such cases have been reported involving different subtypes of malignant renal tumors arising within the same kidney. Herein, we describe a 59-year-old man with malign tumor of synchronous clear-cell and papillary subtypes RCC in the same kidney that were successfully treated with radical nephrectomy.

1. Introduction

Renal tumors which are usually categorized clear-cell, papillary, chromophobe, collecting duct, and unclassified carcinomas constitute 2% of general cancer mortalities and 3% of malignant tumors (Rothman et al., 2008). Two different types of synchronous malignant tumors are very rare. The most commonly reported renal cell carcinoma (RCC) subtype combinations are oncocytoma, angiomyolipoma and dissimilar histological subtype RCC (Klatte et al., 2007). There are only a few cases in which unilateral synchronous malignant tumors of different histologic subtypes have been reported (Klatte et al., 2007; Ustuner et al., 2014; Tele et al., 2015).

We report the case of a 59-year-old man with malign tumor of clear-cell and papillary subtypes of kidney tumors.

2. Case

A 59 -year-old man presented to our outpatient urology clinic with right flank pain with a duration of than three month. Physical examination and laboratory examination were normal and his serum creatinine is 1.1 mg/dl. An ultrasonographic examination revealed a mass in the right kidney. His magnetic resonance imaging (MRI) revealed a mass on the lower pole of right kidney with 3cm diameter, and with exophytic and solid character suggestive for malignancy. MRI scan also showed another 2.5 cm diameter mass on the upper pole of right kidney with solid character suggestive for malignancy (Fig. 1).

The tumor was clinically diagnosed as a right renal tumor and classified as T1aN0M0, according to tumor node metastasis system. Patient underwent right laparoscopic

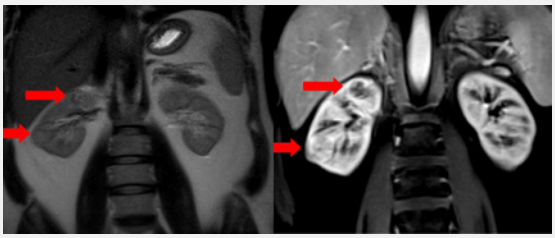

Fig. 1. Preoperative MRI image of the patient. Upper arrows show papillary RCC type 1. Lower arrows show clear cell type RCC.

radical nephrectomy (RN) and adrenalectomy. He was discharged at postoperative second day, without any complication. The patient was followed up. The patient was free from the disease according to sixth month and first year follow-up.

Pathology

Two different tumors on the lower and upper pole of the right kidney on macroscopic examination. The cut surface of the bigger one was at 3 cm diameter and its color was yellow. The smaller one was 2.5 cm diameter and its color was grey (Fig. 2).

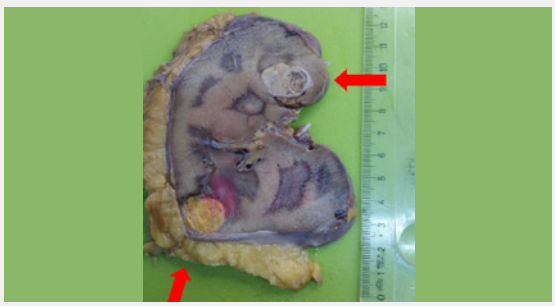

Fig. 2. Teo different tumors on the lower and upper pole of the right kidney on macroscopic examination. Upper arrow shows papillary RCC type 1. Lower arrow shows clear cell type RCC.

Microscopy revealed two different tumor patterns. The bigger tumor was diagnosed as clear cell type RCC with a diameter of 3 cm and smaller one was diagnosed as type 1 papillary RCC with a diameter of 2.5 cm on microscopic examination. The tumors which were restricted in the kidney did not invade the perihilar, perirenal, lymph vessels and blood. Pathologic examination revealed a normal adrenal gland.

Clear cell type of kidney tumors' region has composed of nests of cells with clear cytoplasm, sheets of carcinoma cells surrounded by plenty of blood vessels. The tumor's cell morphology was Fuhrman grade 2 with fine-grained chromatin containing small nucleoli which was not visible at 10x magnification (Fig. 3).

Papillary type of kidney tumors' region has tightly packed tubulopapillary structures. The Fuhrman nuclear grade was 2 (Fig.4).

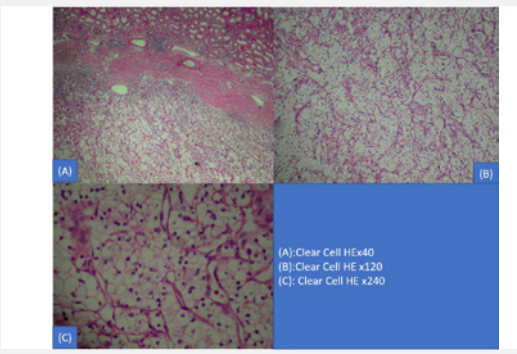

Fig. 3. Clear cell RCC, Fuhrman nuclear grade 2.

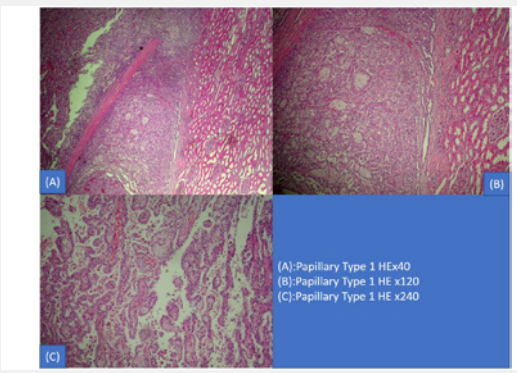

Fig. 4. Papillary RCC, type 1 with Fuhrman nuclear grade 2

3. Discussion

RCC accounts for 2-3% of all cancers. Smoking, obesity and hypertension are best known etiological factors for all types of RCC. Smoking depends on the dose to increase in risk with associated RCC (Hunt et al., 2005). The incidence of small asymptomatic renal masses has also increased over the last few decades. The widespread use of abdominal ultrasound and CT scan increased the diagnosis of renal tumor (Kuroda et al., 2011). Depending on the imaging modalities, renal tumors have cystic or solid character in generally. Radical or partial nephrectomy are recommended for all localized RCCs (Kang et al., 2010).

RCC has several subtypes with specific histopathology and genetic characteristics. Clear cell, papillary and chromophobe cell types are diagnosed mostly. Both clear cell type renal tumor and papillary type renal tumor are originated from the proximal tubules. Histopathological clear cell type renal tumor has clear cytoplasm with tubular, solid or cystic growth pattern. Papillary type renal tumor has two subtypes. Small cells and pale cytoplasm are called Type 1, and large cells and eosinophilic cytoplasm are called Type 2 (Ustuner et al., 2014).

There are some recent studies that describe benign and malignant tumors together in the same kidney. In a recent study the authors mentioned a case report containing clear

cell type renal tumors and angiomyolipoma at 86-year-old woman (Richstone et al., 2004). In another study on the subject the authors described two different types of clear cell carcinoma containing conventional clear cell and clear cell papillary type renal tumors. They reported a case of a 57-year-old male patient with a left renal tumor. They found three tumors in the same kidney after performing the left radical nephrectomy. The authors mentioned that clear cell papillary type is different from conventional clear cell or papillary type renal tumors after genetic evaluation (Kuroda et al., 2011). In another study the authors mentioned a 43-year-old female with the diagnosis of AML and suspicion of RCC who underwent left radical nephrectomy revealed multiple AML with chromophobe RCC and clear cell RCC (Kang et al., 2010). Richstone et al. reported a retrospective study focusing renal tumors in terms of multifocality (Richstone et al., 2004). They analyzed 1071 patients who underwent radical nephrectomy. According to their analysis they declared 57 cases of multifocality. Six of these cases had bilateral synchronous renal tumors. They also reported that papillary subtype RCC was significantly associated with the multifocality. However, they did not report any patient that had these different subtypes of tumors within the same kidney (Richstone et al., 2004).

According to the literature, as in our case, there are some studies reporting synchronous clear cell type and papillary type renal tumors in the same kidney (Capaccio et al., 2009; Simhan et al., 2013; Ustuner et al., 2014; Tele et al., 2015). In a recent study showing a 56-year-old male patient with coexistent clear cell RCC and papillary RCC in his left kidney (Tele et al., 2015). Ustuner et al. reported a 67-year-old male patient who had clear cell RCC and papillary RCC in his right kidney (Ustuner et al., 2014). According to a recent study, the author mentioned seven patients containing unilateral synchronous tumors with different subtypes. One of them had oncocytoma and one had clear cell RCC with synchronous AML (Capaccio et al., 2009). In another study,Simhan et al. analyzed the information of 97 patients who had multifocal RCC. The author declared eight patients with mixed renal tumors containing clear cell type and papillary type (Simhan et al., 2013). Radical nephrectomy was performed to treat these patients. In fact, there is not adequate information to evaluate the different types of renal tumors in the same kidney containing unifocal vs bilateral multifocal tumors. There is also not adequate data to compare these tumors in terms of survival or oncologic survey. On the other hand, there is no such data for unilateral synchronous different type of RCC (Ustuner et al., 2014).

In conclusion, we report a case of malign renal tumors containing clear cell type and papillary type which are rarely seen together in the same kidney. However, having different types of multiple kidney tumors in the same kidney does not affect the way of treatment. Radical nephrectomy is the best treatment option in these patients. Radiofrequency ablation therapy may be considered in selected cases for the treatment of more than one small kidney tumors (Karaköse et al., 2013; Yuksel et al., 2013).

Conflict of interests

None declared.

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Title page: The title page should contain the following items: (1) complete title; (2) full names of all authors; (3) complete affiliations of all authors; (4) the number of text pages of the whole manuscript and the number of figures and tables; (5) the name and complete address of the corresponding Author that includes telephone number, facsimile number and E-mail address to whom correspondence and proofs should be sent.

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Keywords: Provide at least **4-6 keywords** and avoiding general and plural terms and multiple concepts. These keywords will be used for indexing purposes.

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Research Reports should be divided into numbered sections headed by a caption (e.g. **Abstract**, **1. Introduction; 2. Materials and methods; 3. Results; 4. Discussion; Acknowledgements, References**).

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