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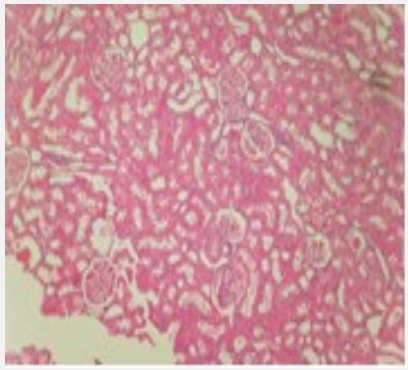

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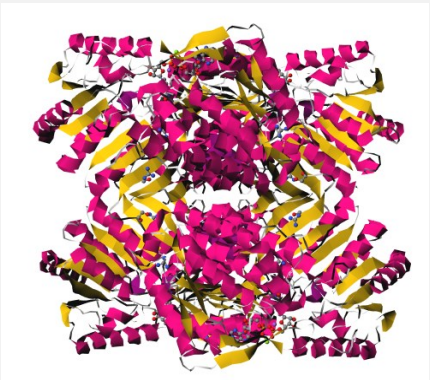

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Case Report

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Original Article
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Ultrasound-guided cannulation in placement of Hemodialysis Access Catheters in Predialysis Patients

Murat Yildar[1], Orcun Gurbuz[2], Gencehan Kumtepe[2], Murat Basbug[1], Omer Toprak[3]

Abstract

Aim: Ultrasound (USG)-guided hemodialysis catheter placement is known to be superior to and more reliable than catheter insertion guided by anatomical landmarks. USG is used for vascular mapping (VM) before catheter placement, or real time. This study investigated the effect on outcomes of USG techniques used in patients with hemodialysis catheters inserted through the internal jugular vein (IJV) due to emergency hemodialysis indication while being monitored for Predialysis Renal Failure (PRF).

Material and Methods: Fifty-nine patients with PRF undergoing USG-guided hemodialysis catheter placement between January 2012 and May 2014 at the Balıkesir University Health Practice and Research Hospital were evaluated retrospectively.

Results: Twenty-eight patients were male and 31 female. The right IJV was used in 57 patients and the left IJV in two. Success rate at first attempt in real time USG group was 91.3% (21/23), compared to 91.6% (34/36) in the VM group. Average number of puncture was similar (1.08±0.291 vs. 1.16±0.56). No complications occurred in either group.

Discussion: Use of USG in real time and for VM in patients with PRF gives similar results in terms of success and complication rates. We therefore recommend that USG only for vascular mapping be used solely in these patients in order to avoid time loss and increased costs.

Keywords: Ultrasound (USG) guided hemodialysis catheter placement

Introduction

The treatment modality of choice in end stage renal disease is kidney transplantation [1]. However, due to low donor numbers or graft dysfunction, patients may also require dialysis therapy [1, 2]. Recent years have seen an increase in centers providing medical therapy for patients with predialysis renal failure in order to delay hemodialysis [3]. One of the major problems for these patients is the requirement for urgent hemodialysis.

Vascular access with appropriate flow in the upper extremity, such as arteriovenous fistulae and grafts, are preferred for hemodialysis [4]. However, these have to mature in order to be capable of use. Vascular access in patients requiring hemodialysis can be temporarily established with a hemodialysis catheter inserted in the jugular, subclavian or femoral veins [5]. Potentially life-threatening complications may occur during hemodialysis catheter placement. Thrombosis associated with previous procedures, stenosis or abnormal vein position and congenital vein agenesis can all complicate the procedure [6, 7].

Ultrasound (USG) -guided catheter placement is recommended in order to minimize complications and increase success rates [7]. Several studies to date have compared the effectiveness of different forms of USG-guided catheter insertion for hemodialysis in patients with chronic renal failure (CRF) [6-8]. This study was planned to determine the difference between for pre-catheterization vascular mapping and real time use of USG

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Material and methods

Methods: Fifty-nine patients receiving hemodialysis catheter through the jugular vein using USG due to emergency hemodialysis indication during monitoring for PRF at the Balikesir University Health Practice and Research Hospital between January 2012 and May 2014 were included in this retrospective study. Computing system and patient medical files were reviewed, and patients were compared in terms of age, sex, body mass index (BMI), urea values, creatinine values and puncture number depending of method of USG use.

Operative technique: Catheter placed was performed by two surgeons, one using USG for pre-catheterization vascular mapping and the other in real time. Both surgeons had 5-years' experience of USG-guided catheter placement. Both surgeons performed USG-guided catheter insertion more than a hundred. The first surgeon used USG (ProSound Alpha 5 Aloka, Japan) to determine jugular vein anatomy before the catheterization to indicate the puncture site (pre- catheterization USG group). The second surgeon used USG both to assess vascular structures before the catheterization and in real time during it (real time USG group). On real time USG management, following local anesthesia with Prilokain hidroklorür (Citanest %2, Astra-Zenecca, Türkiye), in contrast to the other group, the USG probe was covered with a sterile sheath and inserted perpendicularly with the left hand into the vessels (Fig. 1). A 45 degree angle was established with the right hand and venous puncture was performed in real time. A 12.5 F hemodialysis catheter was inserted with the Seldinger technique. Patients were monitored using chest X-ray after the procedure in terms of catheter location and complications such as pneumothorax and hemothorax

Statistical Methods

Data are presented as the means and standard deviation, median, and percentage (range). The t test was used to compare normally distributed numerical data between the groups, and the Mann–Whitney U-test was used for non-normally distributed data. In all analyses, P < 0.05 was taken to indicate statistical significance. Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 21.0; SPSS Inc., Chicago, IL, USA).

Results

Twenty-eight of the 59 patients were men and 31 women. The Median age was 66 (range 26-86) years. Catheter insertion was performed by the first operator using pre- catheterization USG in 36 patients and by the second operator using real time USG in 23 patients. There was no significant difference between the groups in terms of age or gender.

Table 1: Patient characteristics and outcomes

Characteristics/Outcomes	VM (n=36)	RT (n=23)	P*
Age (years)	61.7±12.5	63.7±13.6	0,423
Sex (M/F)	19/17	9/14	
Mean BMI (kg/m^2)	26.4 ± 6,8	29.7± 7.9	0,131
Creatinine	6.9±3,5	5.55±1.8	0,227
Urea	180.1±73,7	161.2±77.2	0,501
Success rate at first attempt	34/36 (91.6)	21/23(%91.3)	0,561
Average number of puncture	1.16±0.6	1.08±0.3	0,078

Data are expressed as Mean±SEM or number (percentage).
* P values <.05 were considered statistically significant

Mean BMI was 26.38 ± 6.82 in the pre-catheterization USG group and 29,65± 7,90 in the real time group. Length of monitoring due to PRF was 546 days in the pre-catheterization USG group and 930 days in the real time USG group. No patient had a previous history of central venous catheter insertion. Urea and creatinine levels were similar in both groups. Patient groups' characteristics are shown in Table 1. Right internal jugular vein (IJV) was used in all pre-catheterization USG patients. Catheter was emplaced at first attempt in 34 patients. The first attempt success rate was 91.6%. The right IJV was used in 21 patients in the real time USG group and the left IJV in two. Catheter was inserted at the first attempt in 21 of these. The first attempt success rate was 91.3%. The difference between the groups was no significant. No catheter insertion failure occurred in any patient in either group. No complications were observed. Success rate at first attempt and average number of puncture are shown in Table 1.

Discussion

This study showed high success rates for USG-guided catheter insertion in patients with PRF. There was no difference in terms of success rates between real time or pre-catheterization USG.

Figure 1: Cannulation of the right internal jugular vein with using real time USG

Hemodialysis catheters are generally easily inserted using the anatomical landmark technique. However, puncture may need to be repeated due to reasons such as obesity, anatomical variations, previous catheterization procedures and inability to position the patient [7]. This not only prolongs the procedure, but increases complication rates and reduces success rates.

USG-guided IJV catheter placement is reported to have success rates close to 100% and to prevent carotid puncture [2, 6]. Success rates rise and complication levels also decrease in patients with CRF when catheter placement is performed using USG. Studies using USG in real time in hemodialysis catheter insertion have recorded first entry success rates of 56-85% and average number of needle puncture ranged between 1.17 and 1.75 [2, 8]. USG has been shown to be superior to the anatomical landmark technique in these studies. As distinct from other studies, we compared real time USG use with USG for vascular mapping. Similar success rates were observed in both forms of USG use. No carotid puncture was performed in any patient in either group, and no complications developed.

Real time USG use requires that the probe be covered with a sterile sheath. Some experts regard this as involving time and expense costs, and so perform vascular mapping first and real time USG if this is unsuccessful. Patients requiring hemodialysis catheter placement during monitoring for PRF did not receive previously a central venous catheter, and this had a positive effect on the results of USG use solely for pre-catheterization vascular mapping. No significant difference was observed in terms of success and complications between real time USG and vascular mapping use in this patient group. We therefore think that pre-catheterization vascular mapping may be a good alternative to real time USG in order to prevent costs and time loss in patients with PRF

Conflict of Interest

The authors declared that they had no conflicts of interest.

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Examination of echocardiographic parameters for the early diagnosis of cardiac dysfunction in beta thalassemia major patients

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Abstract

Objective: This study aimed to determine the utility of tissue Doppler, pulse-wave Doppler, and conventional echocardiography for the detection of cardiac dysfunction in the early period in beta thalassemia patients.

Methods: Forty beta thalassemia major patients undergoing regular blood transfusion and chelation therapy and 30 healthy controls with similar demographic factors were included in the study. Both the patient and control groups were examined with, tissue Doppler, pulse-wave Doppler, motion-mode echocardiography. Ferritin values and T2* magnetic resonance measurements were recorded for those in the patient group.

Results: Diastolic dysfunction indicators like early ventricular filling rate, atrial contraction rate, mitral A wave time, and reverse pulmonary venous flow velocity were higher in the patient group ($p<0.001$, $p=0.003$, $p<0.001$, $p=0.006$, respectively), the deceleration time and pulmonary venous systolic flow velocity ($p=0.035$, $p=0.033$, respectively) were lower. Moreover, in thalassemia major patients, there were significant increases in the ratios of early ventricular filling rate to early diastolic myocardial rate in the lateral, basal and midseptal walls ($p<0.001$, $p=0.001$, $p=0.012$, respectively). While the difference between the ferritin values and echocardiographic data was not significant in patient, it was significant between patients with severe and no cardiac iron load regarding an increase in left ventricular enddiastolic thickness and a decrease in left ventricular ejection fraction values ($p=0.045$, $p=0.049$, respectively).

Conclusions: In beta thalassemia major patients, tissue Doppler, pulse-wave Doppler and conventional echocardiography can be used to detect cardiac dysfunction in the early periods in cases where T2* magnetic resonance is not available.

Keywords: Beta thalassemia major, cardiac dysfunction, pulse-wave Doppler echocardiography, tissue Doppler echocardiography, conventional echocardiography, T2* magnetic resonance

Introduction

Thalassemia is a genetic disorder characterized by ineffective hematopoiesis, which iscaused by insufficient production of globin subunits and increased hemolysis. Patients with this disorder require frequent blood transfusions (1,2), which can cause iron accumulation in the heart that can lead to severe cardiac complications (3). Despite the improvements in chelation therapy, cardiac complications remain the main cause of mortality and morbidity in thalassemia major patients (4). Several procedures have been used to detect the iron load in the heart. Echocardiography is a non-invasive, repeatable, and relatively inexpensive technique that is capable of the absolute diagnosis of heart diseases (5). Tissue Doppler imaging is a recently developed echocardiographic technique that can be used to obtain global and local measurements of myocardial systolic and diastolic velocities. Tissue Doppler echocardiography provides better definition of the left ventricular functions than does conventional echocardiography (6,7). Magnetic

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resonance imaging (MRI) systems, which can detect myocardial iron load and myocardial functions, revolutionized the therapy of beta thalassemia major patients. An application known as T2* magnetic resonance is a powerful technique that detects the iron load by measuring myocardial relaxation time (8). T2* magnetic resonance allows for the preclinical detection of myocardial iron load, the prospective categorization of the cardiac risks, and the observation of the response to changes in chelation therapy (9). However, its use in developing countries is limited because it is not cost-effective or easy to access.

In this study, we aimed to examine the efficiency of conventional echocardiography, tissue Doppler, and pulse-wave Doppler echocardiography, which are easy to access and economical compared to T2* magnetic resonance, for the detection of cardiac dysfunction at an early phase (i.e., before systolic dysfunction occurs) in beta thalassemia major patients.

Materials and Methods

Forty beta thalassemia major patients undergoing regular blood transfusion and chelation therapy (mean age: 12.8 ± 3.34 years) in Dr. Behcet Uz Children's Hospital and 30 healthy controls with similar demographic factors (e.g., age and sex) (mean age: 11.9 ± 3.17) were included in the study. Height, weight, systolic and diastolic blood pressure, heart beat rate and hemogram values were recorded in both the patient and control groups. The patients were categorized into three groups according to their ferritin values as follows: <1000 ng/dl, 1000-2500 ng/dl and >2500 ng/dl. Ethical approval was obtained from the hospital ethical commission and parental consent forms were collected.

Echocardiographic studies were performed at least 24 hours after blood transfusion. Motion - mode, two dimensional imaging, tissue Doppler imaging and Doppler (pulse-wave, continuous-wave) echocardiographic examinations were performed with a Vivid 3 (GE Healthcare, Milwaukee, WI) ultrasound system using a 3-5 MHz transducer on both the patient and control groups. Images were taken from the 3rd or 4th intercostal space while the patient was lying down or at a slight left lateral decubitus position. The measurements were recorded according to the American Society of Echocardiography guidelines (10).

Motion-mode echocardiography: The systolic functions of the left ventricle were evaluated using motion-mode echocardiography as follows: Left ventricle internal diameter at end-diastole, interventricular septum diastolic thickness, left ventricular posterior wall thickness at end diastole, left ventricular diastolic mass, left ventricular diastolic mass index, fractional shortening and left ventricular ejection fraction.

Pulse-wave Doppler echocardiography: Mitral valve flow rates and diastolic functions were evaluated via pulmonary pulse Doppler as follows: Early ventricular filling rate, atrial contraction rate, the ratio of early ventricular filling rate to contraction rate, mitral valve A wave time, deceleration time, pulmonary venous systolic flow velocity, pulmonary venous diastolic flow velocity and reverse pulmonary venous flow velocity. The ratios of pulmonary venous systolic flow velocity to pulmonary venous diastolic flow velocity were calculated.

Tissue Doppler echocardiography: Diastolic functions were evaluated by recording the myocardial signals as follows: Systolic myocardial velocity, early diastolic myocardial velocity, late diastolic myocardial velocity, pulmonary venous systolic flow velocity, pulmonary venous diastolic flow velocity, the ratio of early ventricular filling velocity to diastolic myocardial velocity, isovolumic relaxation time, and isovolumic

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contraction time. Myocardial performance index was calculated. All measurements of the patient and control groups were performed by the same pediatric cardiologist who was blinded to the T2* magnetic resonance results.

T2* magnetic resonance: All cardiac magnetic resonance examinations were performed with a 1.5 T magnetic resonance (Symphony, Siemens, Erlanger, Germany). Myocardial iron load was evaluated by the myocardial T2* measurement. Images were obtained from the late diastolic phase of the cardiac cycle by electrocardiographic synchronization. The myocardial T2* measurement was performed using a multiecho technique when a cardiac event was triggered with a single-breath-hold (FOV, 400 mm; TR,135; TE, 2.6-22.3 (8 echo sequence) with the following settings: flip angle, 20; section thickness, 10mm; matrix, 192x75, NEX,1; Band width (Hz/pixel)).All magnetic resonance analyses were conducted by a radiologist who was blinded to the echocardiography results. The T2* analysis was performed by Thalassemia tools (Thalassemia Tools, Cardiovascular Imaging Solutions, London, UK).The region of interest was selected on the interventricular septum. The signal intensity of this region was measured using a signal intensity-echo time curve (curve formula= $y = Ke^{-TE/T2^*}$). Cases with a T2* value >20ms were considered to have no iron accumulation, cases with a T2* value between 10 and 20ms were accepted as having intermediate iron accumulation, and those with a T2* value ≤10 ms were considered to have severe iron accumulation.

Patients with left ventricular systolic dysfunction (Left ventricular ejection fraction <%54, fractional shortening <%27), congenital or acquired valve disease, hypertension, and patients aged younger than 8 years or older than 18 years were not included in the study.

Table 1: Clinical and demographic data of the patient and control groups.

	Study Group (n: 40)	Control Group (n: 30)	p value
Male/Female	19/21	13/17	0.811
Age (years)	12.8 ± 3.34	11.9 ± 3.17	0.253
Height (cm)	140.03 ± 12.48	145.43 ± 15.24	0.108
Weight (kg)	35.76 ± 9.53	40.78 ± 11.83	0.054
BMI (kg/m^2)	17.96 ± 2.86	18.89 ± 2.87	0.184
Systolic tension (mm/Hg)	114.55 ± 9.46	117.50 ± 8.87	0.189
Diastolic tension (mm/Hg)	68.22 ± 8.38	71.17 ± 6.13	0.109
Heart beat (/min)	98.33 ± 11.79	96.03 ± 12.95	0.443
Hemoglobin (gr/dl)	12.98 ± 1.67	12.39 ± 1.23	0.866

Statistical Methods

Statistical evaluations were performed using statistical tool SPSS 15.0 for Windows. A Chi-Square test or t-test was used to compare the characteristics of the two groups a Mann-Whitney U test was used to compare the echocardiographic parameters between the two groups. The ferritin and T2* values were compared to the echocardiographic parameters by a Kruskal-Wallis test.P values less than 0.05 ($p < 0.05$) were accepted as statistically significant.

Results

The basic characteristic features of the beta thalassemia major patients and the healthy controls are summarized in Table 1. Conventional echocardiographic data including left ventricle internal diameter at end-diastole, inter ventricular septal diastolic thickness, left ventricular diastolic mass, and left ventricular diastolic mass index were higher in the patient group than in the controls ($p=0.009$, $p=0.009$, $p=0.026$, $p<0.001$, respectively) (Table 2). While diastolic dysfunction indicators

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early ventricular filling rate, atrial contraction rate, mitral valve A wave duration and reverse pulmonary venous atrial velocity measured by pulse-wave Doppler echocardiography were higher in the patient group ($p<0.001$, $p=0.003$, $p<0.001$, $p=0.006$, respectively), deceleration time and pulmonary venous systolic flow velocity values were lower ($p=0.035$, $p=0.033$, $p<0.001$, respectively) (Table 3). Systolic myocardial velocity lateral, early diastolic myocardial velocity midseptal and the ratio of early ventricular flow velocity to early diastolic myocardial velocity (lateral, basal and midseptal) data measured by tissue Doppler echocardiography were higher in the patient group ($p=0.031$, $p=0.019$, $p<0.001$, $p=0.001$, $p=0.012$, respectively) while late diastolic myocardial velocity lateral, isovolumic contraction time lateral and isovolumic contraction time midseptal values were lower ($p=0.032$, $p=0.021$, $p=0.011$, respectively) (Table 4).

The mean ferritin value of patients was 2595.46 ng/dl (±1929.85). There were 10 patients with <1000 ng/dl ferritin, 14 patients with 1000-2500 ng/dl ferritin and 16 patients with >2500 ng/dl ferritin. There were no significant correlations between the ferritin values and the conventional, pulse-wave Doppler or tissue Doppler echocardiographic data. The mean cardiac T2* value was 23.13 ms (± 10.18). There were 26 patients with no cardiac iron load (T2*>20 ms), nine patients with intermediate iron load (T2*= 10-20 ms) and five patients with severe cardiac iron load (T2*<10 ms). There was no correlation between the T2* values and the PW Doppler or tissue Doppler echocardiography data ($p>0.05$). There was a statistically significant difference between patients with severe cardiac iron load and those with no iron load with respect to the increase in left ventricle internal diameter at end-diastole values and the decrease in left ventricular ejection fraction values detected by conventional echocardiography ($p=0.045$ and $p=0.049$, respectively).

Discussion

One of the main causes of mortality and morbidity in beta thalassemia major patients is iron accumulation due to blood transfusions (4). Therefore, the ability to track cardiac dysfunction and cardiac iron load in these patients is vital. The early ventricular filling rate value measured by tissue Doppler echocardiography is an indicator of ventricular relaxation and diastolic function (11). Spirito et al. examined diastolic abnormalities in 32 beta thalassemia major patients with tissue Doppler echocardiography and found that the increase in early ventricular filling rate value was significant as was the decrease in deceleration time value (12). In our study, we also reported that the early ventricular filling rate values of the patients were significantly higher than those of the controls (1.21 ± 0.1 m/s, 0.99 ± 0.15 m/s, $p<0.001$, respectively) and that the deceleration time values of the patients were significantly lower than those of the controls (157.05 ± 39.1 vs 170.57 ± 34.93 $p=0.035$, respectively).

Table 2: Conventional echocardiographic data of the patient and control groups.

	Study Group (n: 40)	Control Group (n: 30)	p value
LVIDd (cm)	4.06 ± 0.43	3.76 ± 0.46	**0.009**
IVSd (cm)	0.86 ± 0.16	0.77 ± 0.09	**0.009**
LVPWd(cm)	0.82 ± 0.14	0.84 ± 0.15	0.494
LVd Mass (gr)	106.46 ± 32.29	88.58 ± 27.56	**0.026**
LVd MI (gr/m2)	92.00 ± 24.78	69.31 ± 16.97	**<0.001**
FS (%)	36.31 ± 5.81	37.50 ± 4.60	0.427
EF(%)	66.17 ± 7.61	68.08 ± 5.68	0.373

LVIDd: Left ventricle internal diameter at end-diastole, IVSd: Inter ventricular septal diastolic thickness, LVPWd: Left ventricular posterior wall thickness at end diastole, LVd Mass: Left ventricular diastolic mass, LVd MI: Left ventricular diastolic mass index, FS: Fractional shortening, EF: Left ventricular ejection fraction

Deceleration time abnormality accompanying early ventricular filling rate was considered to be an indicator of diastolic dysfunction. Parale et al. evaluated the left ventricular functions by pulse-wave Doppler echocardiography in beta thalassemia major patients and reported a significant increase in early ventricular filling rate / early diastolic myocardial velocity value and a significant extension of isovolumic relaxation time (13). In our study, we found that the early ventricular filling rate / early diastolic myocardial velocity measured from the lateral, basal and midseptal walls in beta thalassemia major patients were significantly higher than those of the controls (p<0.001, p=0.001, p=0.012, respectively). Also, there was a significant increase in systolic myocardial velocity (lateral) and early diastolic myocardial velocity (midseptal) and a significant decrease in late diastolic myocardial velocity (lateral) as measured by tissue Doppler echocardiography in beta thalassemia major patients as compared to the controls (p=0.031, p=0.019, p=0.032, respectively).

The pulmonary venous flows, which are other indicators of diastolic function,were also measured in all groups. The disruption of ventricular relaxation might increase the atrial load, which in turn reduces the left atrial compliance and disrupts atrial relaxation. As a result, pulmonary venous systolic flow velocity often decreases while pulmonary venous diastolic flow velocity and reverse pulmonary venous atrial velocity wave amplitude and duration increase (14). In a study by Kremastinos et al.,early ventricular filling rate and atrial contraction rate values as well as pulmonary venous systolic flow velocity and pulmonary

Table 3: Pulse-wave Doppler echocardiographic data in the patient and control groups.

	Study Group (n: 40)	Control Group (n: 30)	p value
E (m/s)	1.21 ± 0.1	0.99 ± 0.15	**<0.001**
A (m/s)	0.76 ± 0.37	0.62 ± 0.11	**0.003**
E/A	1.73 ± 0.42	1.61 ± 0.29	0.206
MVA (ms)	129.23 ± 34.94	101.89 ± 38.74	**<0.001**
DT (ms)	157.05 ± 39.1	170.57 ± 34.93	**0.035**
PVS (ms)	0.66 ± 0.17	0.77 ± 0.17	**0.033**
PVD (ms)	0.63 ± 0.17	0.67 ± 0.16	0.805
PVS/PVD	1.08 ± 0.42	1.17 ± 0.25	0.233
PVAR (m/s)	0.45 ± 0.13	0.39 ± 0.06	**0.006**

E: Early ventricular filling rate, A: Atrial contraction rate, E/A: The ratio of early ventricular filling rate to atrial contraction rate, MVA: Mitral valve A wave duration, DT: Deceleration time, PVS: Pulmonary venous systolic flow velocity, PVD: Pulmonary venous diastolic flow velocity, PVAR: Reverse pulmonary venous atrial velocity.

venous diastolic flow velocity flow velocities were higher in beta thalassemia major patients, while there were no significant differences in the ratio of early ventricular filling rate to atrial contraction rate, deceleration time and isovolumic relaxation time values between patients and the control group (15). In our study, we found that the pulmonary venous systolic flow velocity values were lower in beta thalassemia patients when compared to the control group (0.66 ± 0.17 ms vs 0.77 ± 0.17 ms, p=0.033) while there were no differences in pulmonary venous diastolic flow velocity values. There was a significant increase in reverse pulmonary venous atrial velocity values (0.45 ± 0.13 m/s vs0.39 ± 0.06 m/s, p=0.006) in then patient group when compared to the controls.

Myocardial performance index is a Doppler index that enables the combined evaluation of left ventricular systolic and diastolic functions. It is a numerical value derived by dividing the sum of the isovolumic contraction time and isovolumic relaxation time by the left ventricular ejection fraction value (16). Ucar et al. reported an evident increase in myocardial performance index as measured by tissue Doppler echocardiography in 36 beta thalassemia patients with normal conventional echocardiographic parameters when compared to the control group (17). In our study, we could not detect a significant difference between the two groups with regards to the myocardial performance index as measured by tissue Doppler echocardiography.

Table 4: Tissue Doppler echocardiographic data in the patient and control groups.

	Study Group (n: 40)	Control Group (n: 30)	p value
Sm- lateral (ms)	0.11 ± 0.12	0.10 ± 0.02	**0.031**
Sm- basal (ms)	0.08 ± 0.01	0.09 ± 0.02	0.438
Sm- midseptal (ms)	0.07 ± 0.01	0.09 ± 0.15	0.455
Em- lateral (ms)	0.19 ± 0.02	0.19 ± 0.02	0.609
Em- basal (ms)	0.16 ± 0.09	0.15 ± 0.02	0.992
Em- midseptal (ms)	0.13 ± 0.02	0.12 ± 0.01	**0.019**
Am- lateral (ms)	0.07 ± 0.02	0.08 ± 0.01	**0.032**
Am- basal (ms)	0.06 ± 0.01	0.06 ± 0.01	0.531
Am-midseptal (ms)	0.06 ± 0.01	0.05 ± 0.01	0.481
IVRT- lateral (ms)	56.49 ± 7.57	58.42 ± 8.81	0.549
IVRT- basal (ms)	56.00 ± 8.33	57.59 ± 8.27	0.288
IVRT-midseptal (ms)	54.23 ± 6.22	56.76 ± 7.16	0.077
IVCT- lateral (ms)	62.64 ± 9.11	66.64 ± 7.78	**0.021**
IVCT- basal (ms)	60.85 ± 11.78	66.23 ± 9.55	0.146
IVCT-midseptal (ms)	60.32 ± 10.69	66.03 ± 8.15	**0.011**
E/Em- lateral	6.37 ± 1.07	5.21 ± 0.69	**<0.001**
E/Em- basal	8.34 ± 1.21	6.65 ± 1.36	**0.001**
E/Em- midseptal	8.99 ± 1.62	8.07 ± 1.35	**0.012**
MPI	0.46 ± 0.04	0.47 ± 0.05	0.772

Sm: Systolic myocardial velocity, Em: Early diastolic myocardial velocity, Am: Late diastolic myocardial velocity, PVS: Pulmonary venous systolic flow velocity, PVD: Pulmonary venous diastolic flow velocity, E/E_m:The ratio of early ventricular flow velocity to early diastolic myocardial velocity, IVRT: Isovolumic relaxation time, IVCT: Isovolumic contraction time, MPI: Myocardial performance index.

Conventional echocardiography is an economical, practical and frequently used technique for the evaluation of heart diseases (18). Stakos et al. found that the left ventricular mass and expansion in the left atrium as measured by conventional echocardiography was significantly increased in beta thalassemia major patients as compared to a control group, although there was no increase in cardiac iron load (19). In our study, left ventricle internal diameter at end-diastole, inter ventricular septal diastolic thickness, left ventricular diastolic mass and left ventricular

diastolic mass index values as measured by conventional echocardiography were significantly higher in the patient group than in the control group (p=0.009, p=0.009, p=0.026, p< 0.001, respectively).

While the serum ferritin level has a limited prognostic value for reflecting iron stores in iron overloaded patients, it is still valuable as an indirect indicator of iron stores. Moreover, it is a widely used and cost-effective method (20). Silvilairat et al. examined the relationship between the serum ferritin values and tissue Doppler echocardiography parameters in 31 thalassemia patients with normal left ventricular functions. High ferritin levels (>5000 ng/ml) were correlated with a decrease in deceleration time values and an increase in the ratio of early ventricular flow velocity to early diastolic myocardial velocity values (7). In a study examining the diastolic index and ferritin values of 29 thalassemia patients with normal systolic functions, Ashena et al. reported an increase in the early ventricular filling rate and atrial contraction rate values of patients >15 years old compared to patients <15 years old (21). In our study, the early ventricular filling rate and atrial contraction rate values were significantly higher in the patient group when compared to the controls (1.21 ± 0.1 vs0.99 ± 0.15 p<0.001, 0.76 ± 0.37 vs0.62 ± 0.11 p=0.00, respectively). The studies of Ashena et al and Parale et al. reported no correlation between ferritin values and diastolic indexes. In accordance with these recent studies, we also did not find a correlation between serum ferritin levels and diastolic dysfunction indexes as measured by both pulse-wave Doppler and tissue Doppler echocardiography, or with systolic dysfunction indexes as measured by conventional echocardiography. In regards to the correlation between body iron load and cardiac iron accumulation, it has been reported that it is still possible to observe cardiac iron accumulation despite low ferritin levels and the accumulation of iron in the liver (22). This may explain the lack of correlation between serum ferritin levels and diastolic dysfunction indexes.

Currently, cardiac MRI is the gold standard for myocardial tissue analysis in various cardiac diseases. However, its use in developing countries is limited due to its high cost and restricted access (8). In a prospective observational study of thalassemia major patients, it was demonstrated that patients with increased cardiac iron load (T2*<10 ms) carry a high risk of cardiac insufficiency (23). In a study conducted in patients with a mean age of 18±6 years (6-31 years), Aypar et al. examined the correlation of tissue Doppler echocardiography parameters with T2*magnetic resonance values. They found that the regional myocardial dysfunction was more evident in patients with T2*≤20 ms (86% of the patients). In addition, there was a significant correlation between midseptal Sm and Em values and cardiac iron load [24].

Vogel et al. evaluated 52 thalassemia major patients with the mean age of 29.2 years (14-43 years) with tissue Doppler echocardiography and cardiac magnetic resonance and found a significant correlation between a cardiac iron load of T2*<20m (73% of the patients) and myocardial rates (early diastolic, atrial and systolic myocardial velocities), which are diastolic dysfunction indicators [25]. In our study, we did not detect a significant correlation between T2* values and pulse-wave Doppler and tissue Doppler echocardiography indexes. This might be due to the lower mean age (12.8±3.3 years) of our patients compared to those of the previous studies and the low number of patients with T2* magnetic resonance values <20 ms (35% of the patients). Nonetheless, there was a weak correlation between the decrease in left ventricular ejection fraction value and the decrease in T2* value and between

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the increase in left ventricular diastolic thickness value and the decrease in T2* value (p=0.049, p=0.045, respectively). Left ventricular ejection fraction in these three groups (T2*>20 ms, T2*=10-20 ms, T2*<10 ms) were 68.00%, 63.08%, and 61.82%, respectively, and left ventricular posterior wall thickness at end diastole values were 4.03± 0.39 cm, 4.14± 0.41 cm, and 4.45± 0.03 cm, respectively.

In conclusion, the ratio of early ventricular flow velocity to early diastolic myocardial velocity (lateral, basal and midseptal) values as measured by tisse Doppler echocardiography, early ventricular filling rate, atrial contraction rate, mitral valve A wave duration, reverse pulmonary venous atrial velocity, deceleration time, and pulmonary venous systolic flow velocity values as measured by pulse-wave Doppler echocardiography, and left ventricle internal diameter at end-diastole, inter ventricular septal diastolic thickness, left ventricular diastolic mass, and left ventricular diastolic mass index values as measured by conventional echocardiography show distinct differences between beta thalassemia major patients and healthy controls. In these patients, tissue Doppler, pulse-wave Doppler and conventional echocardiography could be used as economical and easily-accessible alternatives for the observation of cardiac dysfunction due to iron load where T2* magnetic resonance is not available.

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Aldehyde dehydrogenase-1 expression and prognosis in triple-negative breast cancer

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Abstract

Objective: Understanding of other biological characteristics of Triple-negative breast cancer, the development of new therapeutical approaches and identification of new markers is necessary due to the lack of biological markers like ER, PR and HER2. The purpose of this study was to investigate the prognostic effect of the expression of aldehyde dehydrogenase-1 (ALDH1) in TNBC and its relationship with the clinico-pathological features

Methods: In this study 87 patient files were searched for clinico-pathological data obtained from the files and paraffin blocks. The prognostic value of these clinical data and ALDH1 positivity were evaluated by determining disease-free survival.

Results: TNM stage I vs III (p=0.03), vascular invasion (p=0.05), chemotherapy indication (p=0.02) were significantly associated with DFS. Multivariable analyses didn't demonstrate any statistically significant relationship between ALDH1 (p=0.61) and DFS.

Conclusions: We didn't find any statistically significant relationship between ALDH1 positivity and DFS. There was no correlation between ALDH1 expression and tumor's pathological features.

Keywords: aldehyde dehydrogenase 1, immunohistochemistry, triple-negative breast cancer

Introduction

Triple-negative breast cancer is defined by absent expression of the estrogen receptor [ER], progesterone receptor [PR], and the human epidermal growth factor receptor 2 [HER2] negative status [1]. Given the lack of these biologic markers endocrine therapy and HER2-directed therapies are not recommended in the treatment of TNBC [18-20].Although the same general principles used in the diagnosis and treatment of non-triple-negative breast cancer generally apply to TNBC, it has a lot of differences in risk factors, molecular and histologic features, clinical behavior and chemotherapy sensitivity [2-6].TNBC defines a heterogeneous mix of breast tumors [7]. TNBC generally has a poorer prognosis among patients with breast cancer [21]. ER, PR and HER2 are the important biologic markers used in targeted treatment of breast cancer [22]. Understanding of other biological characteristics of TNBC, the development of new therapeutical approaches and identification of new markers is necessary due to the lack of biological markers like ER, PR and HER2.CD44+, CD24-/low and ALDH1+ are the most consistently used biomarkers to identify the breast cancer stem cell groups [16-17].The clinical importance of ALDH1 in TNBC is a less investigated issue. The purpose of this study was to investigate the prognostic effect of the expression of aldehyde dehydrogenase-1 (ALDH1) in TNBC and its relationship with the clinico-pathological features

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Materials and Methods

In this study we included 87 patients followed between 2000 and 2012 in Cerrahpaşa Faculty of Medicine, Department of Medical Oncology Clinic diagnosed with TNBC. Formalin-fixed, paraffin-embedded tissues of these patients were retrieved from the Department of Pathology, in the same hospital.

Table 1: Patients general characteristics

Number of patients	87 total
Age	52.7years (28-80)
Family history	67 total
Yes	28 (41.8%)
No	39 (58.2%)
Height	159cm (134-177)
Body weight	73.5kg (51-159)
BSA	1.7m^2 (1.42-2)
Chemotherapy treatment	
Neoadjuvant	14 (16.1%)
Adjuvant	59 (67.8%)
Metastatic	13 (14.9%)
No treatment	1 (1.1%)

The patient files were searched retrospectively for age, sex, comorbidities, the presence of family history of cancer, breast cancer detection methods, pregnancy/number of children, height, weight, BSA, ECOG, biopsy date, whether or not operated, type of surgery, tumor size / TNM stage and localization, axillary nodal status, the presence of the tumor in the border of surgery, pathologic evaluation, the first chemotherapy option: adjuvant, neoadjuvant or metastatic, chemotherapy regimen series, number of cycles, the start/end dates and all were recorded.

The surveillance after treatment for early stage breast cancer was performed each 3 months for the first two years, every 6 months for the next three years and then every year after five years. Patients with metastatic disease were followed with a surveillance routine after receiving 3 and 6 cycles of chemotherapy.

Formalin-fixed, paraffin-embedded tissues was cut into 3 µm thick sections, deparaffinided, stained with hematoxylin-eosin and

Table 2: Tumor characteristics

Tumor size (cm)	3.1 cm (0.7-10)
Primary Tumor (T)	87 patients
T1	17 (19.5%)
T2	45 (51.7%)
T3	10 (11.4%)
T4	15 (17.2%)
Regional Lymph Nodes (N)	87 patients
N0	38 (43.7%)
N1	35 (40.2%)
N2	12 (13.8%)
N3	2 (2.3%)
Anatomic Stage	87 patients
IA	11 (12.6%)
IIA	28 (32.2%)
IIB	19 (21.8%)
IIIA	8 (9.2%)
IIIB	6 (6.9%)
IIIC	2 (2.3%)
IV	13 (14.9%)
Dissected axillary lymph node	12.6 (1-38) (83 patients)
Positiveaxillary lymph node	2.7 (0-23) (83 patients)
Nuclear grade	78 patients
grade 1	9 (11.5%)
grade 2	30 (38.5%)
grade 3	39 (50%)
Histological type	86 patients
Invasive ductal carcinoma	73 (84.9%)
other histological subtypes	13 (15.1%)
Lymphaticinvasion	70 patients
Yes	32 (45.7%)
No	38 (54.3%)
Perineural invasion	69 patients
Yes	6 (8.7%)
No	63 (91.3%)
Vascularinvasion	68 patients
Yes	7 (10.3%)
No	61 (89.7%)
Multicentric	76 patients
Yes	8 (10.5%)
No	68 (89.5%)
Histological grade	86 patients
Grade 1	2 (2.3%)
Grade 2	23 (26.7%)
Grade 3	61'i (70.1%)
Tumorlocalization	87 patients (129 localizations)
upper outer quadrant	69 (53.5%)
upper inner quadrant	21 (16.3%)
lower outer quadrant	23 (17.8%)
lower inner quadrant	14 (10.8%)
Periareolar	2 (1.6%)

immunohistochemically with ALDH1 (ALDH1a1: Biocare assay kit) then examined by light microscopy.

The prognostic value of these clinical data and ALDH1 positivity were evaluated by determining disease-free survival and tumor characteristics from the clinicopathological data obtained from the files and paraffin blocks.

Immunohistochemical staining ofALDH1 was classified as positive when tumor cells showed cytoplasmic positivity and negative when tumor cells showed cytoplasmic negativity.

The prognostic value of these clinical data and ALDH1 positivity were evaluated by determining disease-free survival and tumor characteristics from the clinicopathological data obtained from the files and paraffin blocks. Among 87 files included in the present study, the metastatic ones and those who received neoadjuvant therapy but found to be metastatic before receiving adjuvant therapy were excluded. Statistical analyses include 71 patients.

The DFS (disease-free survival) was defined as the time from the diagnosis to the date of breast-cancer-derived relapse/metastasis.

DFS for patients who received adjuvant therapy (month): (the date of relapse or last visit date for those without relapse –operation date)/30, for those who received neoadjuvant therapy (month): (the date of relapse or last visit date for those without relapse – operation date after neoadjuvant therapy)/30.

Statistical Analysis

Statistical analysis was performed using the SPSS (SPSS 16.0, SPSS Inc. Chicago, Illinois). DFS was calculated with the Kaplan–Meier technique, analyses of the prognostic factors and DFS were performed with the log-rank test. Multivariable analyses were conducted for the factors statistically significant in the Cox Proportional Hazards model.

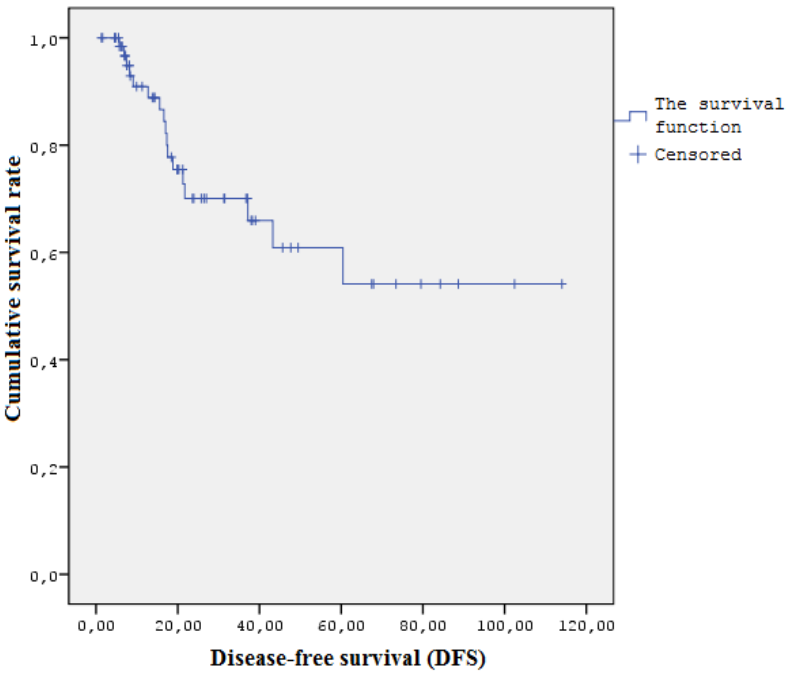

Figure 1: DFS for non-metastatic group

Results

Study population: The age on the patient files included in the present study ranged from 28 to 80 years old (mean of 52.7 years). 62 patients had no comorbidity, 5 had diabetes mellitus, 17 had essential hypertension, and 2 had hypothyroidism. The family history was not noted in 20 percent of the patient files. 39% of the patients had no family history, 28% of them had cancer diagnosed in their family. Based on the diagnostic evaluation of suspected breast cancer one of the patients was diagnosed after physical examination, one during routine screening and all others (n: 85) were suspected by their self-examination. Height ranged from 134 to 177cm (mean of 159 cm), body weight 51 to 159 kg (mean of 73.5kg), Body Surface Area (BSA) ranged from 1.42 to 2m^2(mean of 1.7 m^2).

Tumor characteristics: Among all the patients13 (14.9%) were metastatic at the beginning, 14 (16.1%) received neoadjuvant treatment, 59 received adjuvant treatment, 1 (1.1%) didn't receive any treatment. Tumor size was mean of 3.1 cm (0.7-10 cm). 17 of the cases (19.5%) were Tl, 45(51.7%) T2, 10 (11.4%) T3, 15 (17.2%) T4. 38 of the cases were N0, 35 Nl, 12 N2, 2 N3.

Of the 78 patients with known nuclear grade 9 were (11.5%) grade 1, 30 (38.5%) grade 2 and 39 (50%) grade 3. According to the histologicalgrade2.3% grade 1, 26.7% grade 2 and 70.1% grade 3. According to the anatomic stage 11 IA, 28 IIA, 19 IIB, 8 IIIA, 6 IIIB, 2 IIIC, 13 IV.

Of all patients included in the present study, 84.9% of cases were invasive ductal carcinoma, 15.1% the other histological types.

45.7% of the tumors showed lymphatic invasion whereas 54.3% showed no invasion. 8.7% of the tumors showed perineural invasion whereas 91.3% showed no invasion. 10.3% of the tumors showed vascular invasion whereas 89.7% showed no invasion. 10.5% of the tumors were multicentric whereas 89.5% were unicentric.Among87 patients,

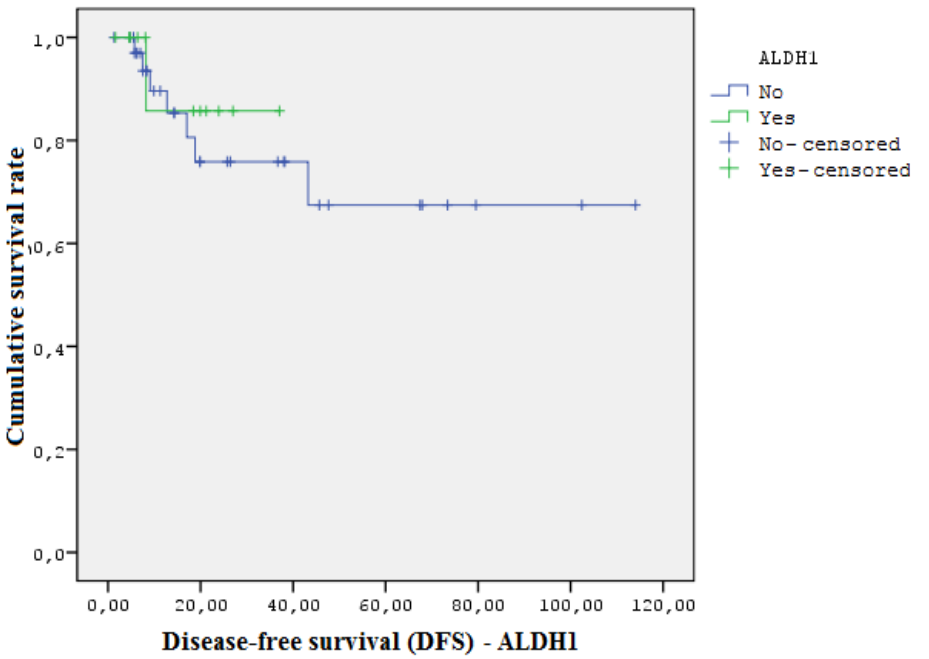

Figure 2: DFS according to ALDH1 expression

51 had tumor on their right breast, 36 on their left breast. Of the 129 localizations seen (knowing than some of the tumors locate in more than one quadrant) 69 (53.5%) were located upper outer, 21 (16.3%)upper inner, 23 (17.8%)lower outer, 14 (10.8%)lower inner, 2 (1.6%) periareolar.

Surgicaltreatment: Among 70 patients undergoing surgery,6 Mastectomy and Sentinel lymph node biopsy (SLNB), 23Breast-conserving surgery (BCS) and Axillary lymph node dissection (AD), 11 Breast-conserving surgery (BCS) and Sentinel lymph node biopsy (SLNB), 27 Modified radical mastectomy (MRM), 2 Roll excision, 1 Total mastectomywas performed.

Medicaltreatment: 87 patients included in this study received chemotherapy, 59 of them adjuvant (8 AC, 27 FEC/FAC, 6 FAC+Taxane, 11 AC+Taxane, 5 CMF, 1 Cyclophosphamide + Taxane), 14 neoadjuvant (10 FAC+Taxane, 1 FEC/FAC, 1 AC+Taxane, 1 Cyclophosphamide + Taxane, 1 Taxane), 13 metastaticdisease chemotherapy regiments, 1 patient didn't accept the treatment.

Disease-free survival (DFS) results: A total of 18 patients (%25.4)had relapseduring the follow-up. The median follow-upperiod until relapse was 18.64 months (0.4 - 46.7). The median follow-up period for all patients was714 days (31-2645). DFS of non-metastaticgroup is shown on Figure 1. A total of 11 patients were found to be ALDH1-positive. Analysis of the DFS showed no difference among ALDH1-positive and ALDH1-negative tumors (p=0.61) (Figure 2)

Multivariable analyses were conducted for the factors that were associated with DFS in the univariable analyses. Multivariable analyses didn't demonstrate any statistically significant relationship between ALDH1 (p=0.930), other prognostic factors and DFS. There was no correlation between ALDH1 expression and tumor's pathological features.

Discussion

Breast cancer is a heterogeneous disease with a lot of histological special types showing different molecular, histologic and clinic features [15]. We achieved a significant reduction in breast cancer mortality with the screening programs and treatment facilities in adjuvant treatment of early stage breast cancer. The mortality rates are being reduced even more by the use of targeted therapy. TNBC accounts for 15% to 20% of breast cancers [3].Endocrine therapy and HER2-directed therapies are not used in the treatment of TNBC since it is characterized by the lack of biological markers like ER, PR and HER2[23]. TNBC generally has a poorer prognosis compared to patients with other breast cancer subtypes. Considerable effort has been made to develop new therapeutical approaches in TNBC [8].

The classical pathological variables such as tumor grade, lymph node status, and tumor size, are the most important prognostic factors in breast cancer.Ki67 status, tumor grade, lymphatic, perineural, vascular invasion are also independent prognostic factors that can affect the therapy choice [9, 11].

Based on the diagnostic evaluation of suspected breast cancer one of the patients was diagnosed after physical examination, one during routine screening and all others (n:85) were suspected by their self-examination. These findings support the fact that TNBC will more likely present as an 'interval cancer' between two screenings. These findings also show that TNBC screening and diagnosis need to be improved [12-14].

In our study 14 patients (16.1%) received neoadjuvant therapy because of locally advanced disease, 59 patients (67.8%) received adjuvant

therapy. DFS of patients receiving neoadjuvant therapy was statistically significant short than others(p=0.02, 24 months DFS; %31.2 vs %80.5). This finding maybe reflective of locally advanced disease, but also specially inTNBC may suggest that neoadjuvant chemotherapy may have limited effect and delaying of the surgical treatment may affect the prognosis.

A total of 11 patients were found to be ALDH1-positive and this positivity doesn't seem to be statistically significant alone. ALDH1as a biomarker to identify the breast cancer stem cell groups that can lead to targeted therapies in breast cancer was not found to be a prognostic value alone. Further research combining with other biomarkers and with a greater number of patients is necessary to confirm the role of ALDH1 in TNBC.

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Conflict of Interest: The authors declared that they had no conflicts of interest.

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Impairments of Diabetes Mellitus related Nephropathy and blood biochemicals by the Thymus Vulgaris L. and Thymbra Spicata L.

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Abstract

Objective: Diabetes mellitus (DM) is a group of metabolic diseases with high blood glucose, which cause the damage and dysfunction of some of the organ systems. Although various oral drugs are used to treat DM, they do not prevent the development of DM related diseases such as nephropathy and retinopathy and glucose dependent vascular diseases. Thus novel strategies for the prevention and treatment of DM are urgently needed. This research aimed to reveal the effects of Thymus Vulgaris Lamiaceae (TVL) and Thymbra Spicata Lamiaceae (TSL) on the damaging effects of DM.

Methods: **P**repared TVL and TSL aqueous extracts were studied in the streptozocin induced experimental diabetic rat model. Blood glucose, body weight, and blood biochemical alterations were measured. Rat kidney for nephropathy were enucleated and fixed in paraffin blocks to examine histopathological changes.

Results: Briefly, impaired blood glucose, and weight loss of Diabetic rats were significantly improved by TVL in dose dependent manner ($P<0.01$). Impaired Blood Uric acid, Urea, and liver enzymes were significantly improved and adjusted to the control group values ($P<0.001$). The histological analyses of the kidney in TVL and TSL groups revealed the findings of significant healing properties, which was similar to the normal structures of kidney.

Conclusions: In this study, strong biochemical, physiological, and histological improvements in the DM related disorders, such as neuropathy and nephropathy were obtained by TVL treatment.

Keywords: Diabetes mellitus, Streptozocin, Diabetic rat, Thymus Vulgaris L., Thymbra Spicata L., AST, ALT, UREA, Nephyropathy, Nephyron,

Introduction

Diabetes mellitus (DM) is the most common metabolic disease referring to hyperglycemia due to the corruption of insulin secretion, insufficient insulin sensitivity, or both. The prevalence of DM continues to increase worldwide, and it predisposes to significantly increased complications. DM can affect almost every organ system in the body, and the level of the damage is particularly related to the severity and duration of the disease [1, 2] Type 1 DM (T1D) is an autoimmune disease characterized by the destruction of pancreatic ß-cells. Exposure of environmental harmful substances during neonatal period is accused of leading to initiation of immune process underlying the destruction of ß-cells and the development of disorder [3] Patients with Type 1 Diabetes (T1D) are presented with absolute insulin deficiency, and multiple types of insulin formulations have been developed for the treatment of T1D in the last three decades [4] Type 2 Diabetes (T2D), non-insulin-dependent type of DM, is mainly an adult disease and associated with insulin resistance. The incidences of T2D rapidly

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increase due to the decrease in physical activity, sedentary lifestyle, and aging population. T2D is characterized by insulin insensitivity as a result of insulin resistance, decreased insulin production, and finally pancreatic ß-cell failure. Patients with T2D should receive lifestyle alteration recommendations and diet modification. Pharmacological agents including oral antidiabetics are frequently required. Although novel drugs are being developed, no cure is currently available [5, 6] DM may lead to the microvascular complications, such as nephropathy. Diabetic nephropathy (DN) is characterized by initial microalbuminuria following persistent albuminuria, [7] DN is characterized by distal symmetric polyneuropathy. Thymus vulgaris L. (Local name: Izmir Kekigi) (TVL) is a species of flowering plant in the mint family Lamiaceae and native to the west of Turkey. Thymbra Spicata L (Local name: Karabas Kekik or Zahter) (TSL) is also a member of the Lamiaceae family, and the leaves of this plant have recently gained much popularity as a remedy to combat hypercholesterolaemia [8].

TVL and TSL have been arbitrarily and widely used in the west region of Turkey for the treatment of various diseases, such as diabetes mellitus, diabetes dependent urinary system disorders, in folk medicine. However, no previous scientific report is available regarding the influences of TVL and TSL on DM related damage and impairment in urinary, and blood biochemical systems. Therefore, the present study was created to examine the possible effects of TVL and TSL on the DM associated disorders in some of the organ systems, such as nephropathy, and nephropathy related blood biochemical

Materials and Methods

Animals and experimental groups

Male Wistar albino rats weighing 305.2± 4.33 g, 60 days old, were obtained from the Yüzüncü Yıl University experimental animal unit. Rats were maintained on 12-h dark/light cycle at 22 °C, housed in groups of seven, and fed with a standard commercial rodent chow. The care of the animals and this experimental animal study were conducted with the approval of the Institutional Animal Care and Use Committee of the Yuzuncu Yıl University Experimental Animal Unit and Ethic Committee (YUHADYEK).

DM induction

To examine the effects of TVL and TSL treatment on DM, fifty six Wistar albino rats were divided into seven equal groups. Group 2, 3, 4, 5, 6 and 7 were induced to DM. Severe DM was induced in the animals by intraperitoneal injection of streptozocin (STZ; Sigma–Aldrich, St. Louis, MO) that was dissolved in 0.1 M citrate buffer solution (0.1 M, pH 4.5) at the dose of 50 mg/kg body weight (BW). Animals were fasted overnight for 12 h prior to STZ administration. Water and food were available immediately after dosing. The development of DM was determined by observing hyperglycemia (>300 mg/dl) as measured by an Accu-Chek Go glucometer (Roche, Mannheim, Germany). Body weights and blood glucose levels were recorded once a week throughout the study. At end of the 5 weeks, Blood samples were collected, and the tissue samples of kidney were enucleated and embedded to paraffin blocks.

Extraction of TVL and TSL:

The species of TVL and TSL were collected from Aegean region (Aydın and Izmir cities). Taxonomic identification was performed by Associate Prof. Dr. Fevzi Ozgokce. The collected plants were dried in an oven at 40 °C and then ground into a powder. For extraction, the decoction method and distilled water as solvent were used [8]. For the decoction method, 20 g of dried powder was extracted with 100 ml of distilled water at 100 °C for 30 min in a water bath.

Subsequently, it was filtered, and the water was evaporated to dryness. The residue was weighed to obtain the extractive yield, and it was in air tight bottle at 4 °C. The yield of dried extract were found % 5.9 and 5.85, respectively. The extracts were prepared daily. Resolved in 100 and 200 mg/ml distilled water and orally administered to the rats daily.

The creation of groups and the assessment of the effects of TVL and TSL treatment on blood glucose level (BGL) and body weight changes:

Diabetic rats were treated by the agents of Glibenclamide, TVL and TSL aqueous extracts. Both BGL and body weight alterations were compared with the result of control group. The groups were created as; (1) no additive; (2) 50 mg/kg STZ only; (3) 50 mg/kg STZ plus 5 mg/kg glibenclamide; (4) 50 mg/kg STZ plus 100 mg/kg TVL; (5) 50 mg/kg STZ plus 200 mg/kg TVL; (6) 50 mg/kg STZ plus 100 mg/kg TSL; (7) 50 mg/kg STZ plus 200 mg/kg TSL. Body weight and were measured once in a week through 5 weeks. Rats were treated with glibenclamide/TVL/TSL single daily dose.

Blood biochemicals

The biochemical parameters measured included glucose, urea, uric acid, total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), and creatine kinase (CK). These parameters were measured by using Randox kits (Randox Laboratories Ltd, United Kingdom), and read by spectrophotometry.

Histological assessments

The samples were fixed in 10% formalin solution for 48 h. After washing, they were embedded in paraffin using routine embedding procedure. The 5 micron sections were taken from all blocks, and they were stained with hematoxylin-eosin (HE) using routine procedure for histochemical analyses.

Statistical analysis for blood glucose measurements, body weight changes

One-way Analysis of Variance (ANOVA) and Tukey-Kramer Multiple Comparisons tests were applied to the variables, and each result was reported as mean ± S.E.M. and a, p value less than 0.05 was accepted as statistically significant. (*P<0.05, **P<0.01 and ***P<0.001)

Statistical analysis for blood parameters

Descriptive statistics for biochemical parameters; mean, standard deviation (SD), minimum and maximum values were stated. Kruskal-Wallis test were used in order to determine whether there were differences between the determined parameters of groups. The level of statistical significance was taken as 5%, and SPSS 17.0 program was used for the statistical analyses.

Results

Blood glucose level of the control group was measured in an average of 86.7 mg/dl. BGL were elevated to the average of 422.3 mg/dl by the single dose STZ (50 mg/kg) administration. BGL remained stable in both Control and STZ groups for 5 weeks. BGL slightly decreased in Glibenclamide administered group. The most significant results over the increased BGL were obtained with TVL100 mg/kg and TVL 200 mg/kg treatments ($p<0.01$) (Table 1). The mean body weight was 305.2 gr, and it was decreased to 208.3 gr by the administration of STZ at the end of 5th week. TVL treatment reduced body weight loss in STZ administrated diabetic rats ($p < 0.01$) (Table 2). As a result of the biochemical analyses; it was found that almost all of the biochemical parameters were affected by the STZ administration that induced the impaired blood glucose. Particularly the urinary system parameters of urea, uric acid, and liver

Table 1: The effects of different doses of aqueous extract of TVL and TSL on fasting blood glucose level (mg/dl) in streptozocin induced diabetic rats. Values given represent the mean±SD; One-way Analysis of Variance (ANOVA) was applied to results *P<0.05, **P<0.01 and ***P<0.001.

	Blood glucose (mg/dl)				
	1st week	2nd week	3rd week	4th week	5th week
CRs	86,70±10,82	94,40±14,72	94,70±14,11	96,00±16,81	94,80±11,22
DRs	425,22±133,01	409,56±80,43	423,67±108,99	436,43±68,62	416,63±110,64
DRs+Gli	340,50±89,37	410,00±118,89	341,00±107,59	332,33±191,85	345,11±140,73
DRs+TVL100	312,10±53,71	286,11±66,04	298,78±85,85	237,56±100,37	322,75±182,56
DRs+TVL200	299,10±83,55	293,70±52,23	278,40±118,16	288,40±116,48	261,67±144,76
DRs+TSL100	303,50±76,57	431,40±91,14	367,00±140,82	345,50±115,92	425,33±156,48
DRs+TSL200	406,80±81,68	395,89±110,72	355,22±113,39	359,88±166,87	348,25±143,99
CRs/versus	P value				
DRs	***	***	***	***	***
DRs+Gli	***	***	***	**	**
DRs+TVL100	***	***	**	*	*
DRs+TVL200	***	***	**	**P>0.05**	**P>0.05**
DRs+TSL100	***	***	***	***	***
DRs+TSL200	***	***	***	***	**

Table 2: Effects of different doses of aqueous extract of TVL and TSL on body weight (g) changes of streptozocin induced diabetic rats. Values given represent the mean±SD; One-way Analysis of Variance (ANOVA) was applied to results. *P<0.05 **P<0.01 and ***P<0.001.

	Body weight (in g)				
	1st week	2nd week	3rd week	4th week	5th week
CRs	296,50±30,28	299,00±27,67	302,50±27,00	308,00±25,73	320,00±18,71
DRs	261,22±34,65	231,11±35,07	226,11±33,61	208,33±36,66	195,56±29,10
DRs+Gli	272,00±24,40	263,50±26,46	259,00±40,33	243,00±37,51	235,50±53,51
DRs+TVL100	259,00±17,29	251,22±30,80	247,22±34,01	247,78±30,83	246,67±42,43
DRs+TVL200	274,50±12,57	259,10±26,55	244,00±40,61	253,50±40,83	265,50±43,11
DRs+TSL100	266,00±14,49	241,00±29,61	228,50±34,56	211,50±44,91	206,11±52,49
DRs+TSL200	277,00±21,11	256,67±19,36	258,89±37,48	248,33±41,16	251,11±50,17
CRs/versus	P value				
DRs	**P>0.05**	***	***	***	***
DRs+Gli	**P>0.05**	**P>0.05**	**P>0.05**	**	***
DRs+TVL100	**P>0.05**	**	*	*	**
DRs+TVL200	**P>0.05**	*	**	*	**P>0.05**
DRs+TSL100	**P>0.05**	***	***	***	***
DRs+TSL200	**P>0.05**	**	**P>0.05**	*	*

enzymes including ALT, AS, and GGT were disrupted after the induction of DM by the STZ administration.

Although some of the biochemical parameters were affected by the glibenclamide treatment, most of the parameters were resistant to the glibenclamide treatment ($p<0.01$) (Figure 1, Table 3). The measured values of the blood glucose in STZ induced diabetic rats were significantly higher than in the control. The glucose levels were significantly declined by TVL100 and TVL200 mg/kg treatments as compared with just STZ and control groups. Some alterations in the enzymatic activities were also determined. The increased ALT level subsequent to the STZ administration was reduced by TVL100 and TVL200 mg/kg, and the reduction

was more distinct by the administration of TVL 200 mg/kg. The AST and GGT readings were also reduced by the presence of TVL ($p<0.01$) (Figure 1, Table 3).

was treated by TVL 200 mg/kg. Therefore, we concluded that TVL 200 mg/kg provided more posivitive impacts on the damaging effects of DM on the structure of glomerules (Table 4).

Table 3. The effects of TVL and TSL on the blood biochemical parameters and electrolyte concentrations of diabetic rats. Descriptive statistics of the studied variables. Values given represent the mean and S.E.M.

	CRs	DRs	DRs Gli	DRs TVL100	DRs TVL200	DRs TSL100	DRs TSL200	p
GLU	167,0 ± 36,2c	419,0 ± 165,3ab	513,5 ±107,9a	464,6 ±218,2a	277,0 ±140,1bc	573,8 ± 47,5a	479,3 ± 167,5a	0,001
URE	33,5 ± 5,5d	75,0 ±30,4ab	84,4 ±19,8a	50,3 ±15,1d	53,8 ±26,9bcd	63,2 ±11,5abc	58,7 ±16,3bc	0,003
KOL	60,5 ±12,6	61,1 ±11,4	67,4 ±12,6	70 ±13	71,5 ±12,6	72,4 ±10,5	59,4 ±10,8	0,200
TG	110,3 ± 44,2a	50,5 ±5,7b	70,7 ±17,9b	68,9 ±18,3b	73,3 ±17,5b	118,6 ± 31,4a	60,0 ±15,4b	0,000
HDL	21,5 ±6,3	24,4 ±5,9	27,3 ±5,4	26,8 ±6,1	24,8 ±5,9	30,4 ±5	24 ±4,8	0,110
LDL	8,2 ±2,2	7,8 ±1,8	9 ±2,3	9,9 ±2,7	9,7 ±1,4	10,2 ±1,8	7,7 ±1,4	0,138
AST	125,8 ±64,1b	445,4 ±217,3b	978,8 ±660,8a	344,4 ±316,8b	173,5 ±94,2b	485,0 ±312,6b	332,0 ±320,2b	0,001
ALT	36,7 ± 4,9c	164,8 ±98,4bc	375,9 ± 225,9a	148,4 ±138,5bc	58,3 ± 55,9c	237,8 ±129,1ab	140,1 ± 123,5bc	0,001
GGT	-,3 ±0,8c	6,5 ±5,8ab	8,4 ±5,8a	3,9 ±4,5abc	-,2 ±1,3c	8,0 ±5,1a	2,6 ±3,9bc	0,005
ALB	3 ±0,2	2,8 ±0,3	2,8 ±0,3	2,8 ±0,2	2,9 ±0,2	2,6 ±0,3	2,9 ±0,2	0,566
FT3	1,8 ±0,1a	1,3 ±0,1ab	1,4 ±0,1ab	1,8 ±0,6a	1,8 ±0,2a	1,2 ±0,3b	1,7 ± 0,3ab	0,035
FT4	1,3 ±0,2a	,8 ±0,1c	,9 ±0,1bc	1,1 ±0,3abc	1,3 ±0,2ab	,8 ±0,2c	1,1 ± 0,3abc	0,044
Ca	9,5 ±0,2bc	9,4 ±0,5c	9,9 ±0,5abc	10,1± 0,7ab	9,6± 0,1bc	10,3±0,5 a	9,8 ±0,5abc	0,036
Mg	1,9±0,2	4,6±7	2,5±0,5	2,4±0,6	2±0,1	2,6±0,3	2,1±0,3	0,001
Na	137,7± 1,5ab	135,3 ±5,1ab	142,4± 4,7a	141,0 ±4,3ab	136,7± 3,6ab	140,2 ±1,5ab	122,3± 39,5b	0,033
Cl	101,8 ±1,7a	97,1 ±6,6bc	99,2 ±2,5ab	97,8 ±3,8abc	97,2± 3,5bc	94,0 ±1,6c	94,7 ±3,4bc	0,010

Difference between the groups with different letters is statistically significant ($p<0.05$).

As to histological cross sections, kidney structure was detected normally with normal glomerules surrounded by Bowman's capsule, proximal and distal convoluted tubules, and collecting tubules were observed in normal morphological properties in the control group. In STZ group without any treatment, the degenerated glomerules that was infiltrated by the inflammatory cells were detected. In addition, the basement membrane of glomerule in STZ group was slightly thicker than in the control group. Edematous changes in the proximal convoluted tubules were also detected. The histological properties of kidney in group 3,4,5,6 and 7 revealed the features of healing such as normal structure of the glomerule, less inflammatory cell infiltration, and less edematous changes of the convoluted tubules. However, the absence of inflammatory cells, normal basement membrane thickness, and non-edematous structure of convoluted tubules were observed in group 5 that

Discussion

DM contains a group of metabolic diseases, and it is characterized by the increase of blood glucose as a result of the disorders in insulin secretion, insulin action, or both. The prolonged exposure of the organ systems to hyperglycemia is resulted with the damage, dysfunction, and failure of different organs, such as kidneys, blood vessels, and nerves [9] TVL and TSL are known with their antimicrobial and antiviral effects, thus the use is common among the population for the treatment of DM and various diseases. They are characterized by the large scale component of free radical scavenger essential oils such as thymol, carvacrol, 8-terpinene, p-ceymene and α-pinene. In addition, the chemical compositions of TVL and TSL essential oils varies in a wide range. Carvacrol and thymol are the main components of the mint family Lamiaceae plants. The rate of these components is

due to the environment and ecology [8] The aim of the present study was to analyze the impact of TVL and TSL in terms of blood glucose, vascular, renal and cognitive systems in diabetic rats. Streptozotocin is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medical research to produce an animal model for Type 1 DM in single large dose as well as Type 2 DM with multiple low doses. In a long period of time, diabetic rats are characterized by high blood glucose and body weight loss. In our study, we produced Type 1 DM by a single large dose of STZ administration (50 mg/kg) in the wistar albino rats. The diabetic rats were treated by glibenclamide, TVL and TSL aqueous extracts. It was seen that TVL was significantly effective in the avoidence of the increase in blood glucose, weight loss, the decline in cognitive functions, and also in the improvement of them.

In clinical practice, the more commonly used liver function tests (LFTs) including the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time are commonly used for screening for liver disease, monitoring the progression of the disease, and reflect the damaging effects of hepatotoxic drugs. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are called as aminotransferases, the level of them in the blood increase as a result of hepatocyte injury. Thus, they are commonly used as a marker reflecting hepatic disorder. The patients with type 2 DM have a higher incidence of the abnormalities in liver function tests than the normal one. Antidiabetic agents have generally been shown to decrease alanine aminotransferase levels as tighter blood glucose levels are achieved [10] In our study, the greatest improvement in hepatic enzymes was significantly seen in TVL group. These enzymes much more decreased in TVL group than in the other groups. Therefore, it was supposed that TVL might provide the protecting effect against the toxic effects of DM and DM related hepatic dysfunction by providing the regulation of blood glucose level.

In addition to the biochemical analysis, the histological sections of rat aorta were examined in terms of macrovascular complications. DM has multisystemic damaging effects, thus diabetic nepropathy (DN) is one of the most severe complications of DM and has become the most common major cause of end-stage renal disease (ESRD) in the United States and Europe [11] DN is commonly defined as an increase in urinary albumin excretion and detoriorated renal function as diagnosed by as abnormal plasma creatinine level [12] The pathogenesis of DN is complex and is not clearly defined yet. Thus, many researchers investigate the pathogenesis of DN and the prevention, as well as, the cure [13] Glucose and its metabolites activate protein kinase C, the polyol pathway and non-enzymatic glycation, and these pathways cause the occurrence of renal functional and structural changes [14] Therefore, hyperglycemia has the primary role in the cascade of the damage mediated by cytokines that produces oxidative stress, abnormal glycosylation, lipid peroxidation, and the production of further inflammatory elements [15] There were increasing findings stated that immunologic and inflammatory mechanisms play important roles in the development and progression of DN [16] Inflammatory cytokines including IL-1, IL-6, IL-18, TNF-α, TGF-β, and MCP-1 have been determined as a part of the pathophysiological processes of DN [17] Some alterations in blood pressure and hemodynamics are superimposed to these mechanisms [18] The hemodynamic changes including glomerular hyperperfusion and hyperfiltration become evident before the appearance of earliest measurable clinical signs of nephropathy [15].

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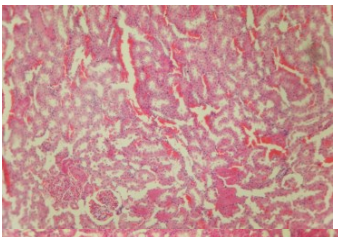
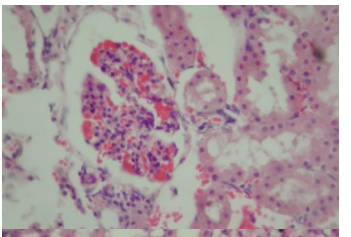
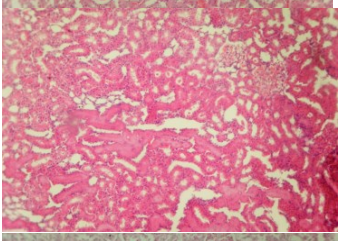
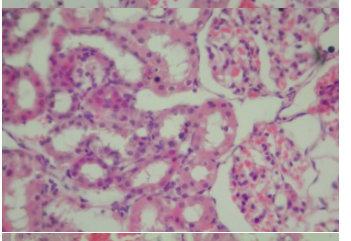
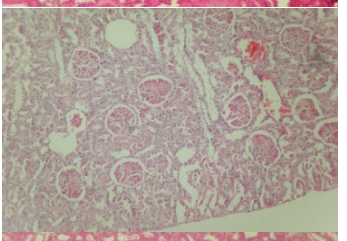
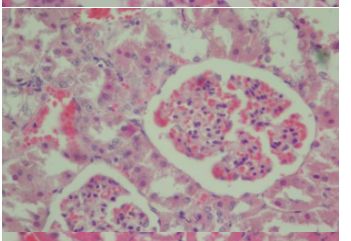
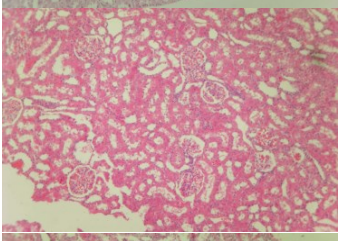
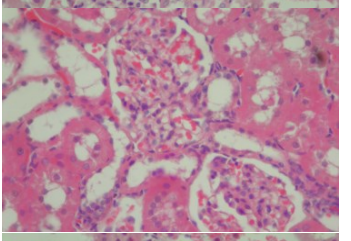
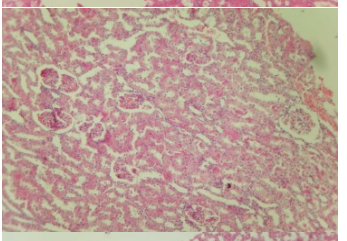
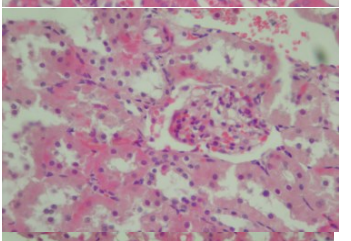
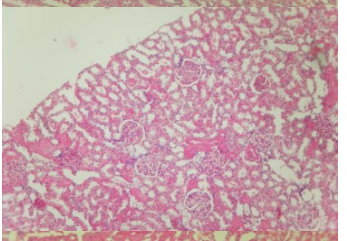
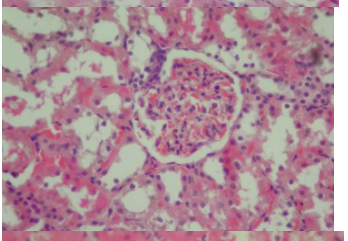
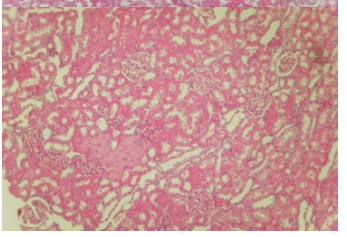
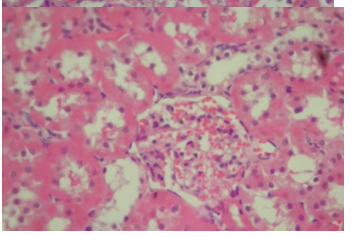
Table 4: Histological section of kidney tissue taken with microscope. Hematoxylin and Eosin (H&E) Stain. CRs: Control Rats DRs: Diabetic Rats, STZ 50 mg/kg, Glibenclamid 5mg/kg

Groups	Kidney x100	Kidney x400
1 CRs		
2 DRs		
3 DRs+Glibenclamid		
4 DRs+TVL 100mg/kg		
5 DRs+TVL 200mg/kg		
6 DRs+TSL 100mg/kg		
7 DRs+TSL 200mg/kg		

Increased blood glucose levels, deteriorated hemodynamics, immunological and inflammatory reactions, and injury of the glomerular basement membrane and podocytes create the pathophysiological basis of DN related renal dysfunction [19]

The earliest renal manifestation of DM is glomerular hyperfiltration that results from functional changes in the nephron at the level of the glomerulus [20] Subsequently, thickening of the glomerular basement membrane, glomerular hypertrophy and mesangial expansion is occurred [21] When we particularly look at the primary hystopathological alterations in kidney with DN, it is seen that DN related renal damage is characterized by some alterations in glomerular permeability and structure. Normally, the glomerular wall contains three layers: endothelial cells, basement membrane, and epithelial cells (podocytes). The selectivity of glomerular filtration is commonly provided by the basement membrane where the filtration barrier excludes proteins on the basis of their size and charge [22] However, the damage of the permeability barrier cause to proteinuria in DN [23] The structural damage of diabetic nephropathy in kidney include the accumulation of mesangial matrix and thickening of the basement membrane in the glomeruli, tubular hypertrophy and associated alterations in the tubulointerstitium with tubolointerstitial fibrosis [24] These abnormalities are associated with the renal overproduction of extracellular matrix proteins [25] In our study, we histologically analyzed the possible alterations associated with kidney in 7 groups to determine the possible impact of TVL treatment on the damaging effects of DN. The histological analysis of renal tissues revealed that kidney structures with normal morphological properties in group 1 replaced with degenerated glomerules that were infiltrated with inflammatory cells, slightly thickened glomerular basement membrane, and proximal convoluted tubules with edematous changes in group 2. It was seen that STZ created significant damages in the structure of kidney. Nevertheless, in the group of 3, 4, 5, 6, and 7, while different agents with various doses were applied to improve the damages of STZ exposure, the subsequent histological analyses revealed that the significant findings of healing including normal structure of glomerule, less inflammatory cells infiltration, and less edematous changes of convoluted tubules were determined. In addition, more improvements including the absence of inflammatory cells, normal basement membrane thickness, and non-edematous structure of convoluted tubule were particularly observed in group 5. Therefore, it was concluded that TVL 200 mg/kg provided more positive impacts on the damaging effects of DM on the structure of glomerules.

In conclusion, it was supposed that both TVL and TSL had protecting and healing effects against the damaging effects of DM in terms of blood glucose, renal and liver enzyme systems in diabetic rats. However, it was also determined that TVL 200 mg/kg could provide the greatest improvement in these terms, thus it should be preferred as an effective and alternative treatment modality in DM related disorders. These findings should be investigated by new comprehensive studies.

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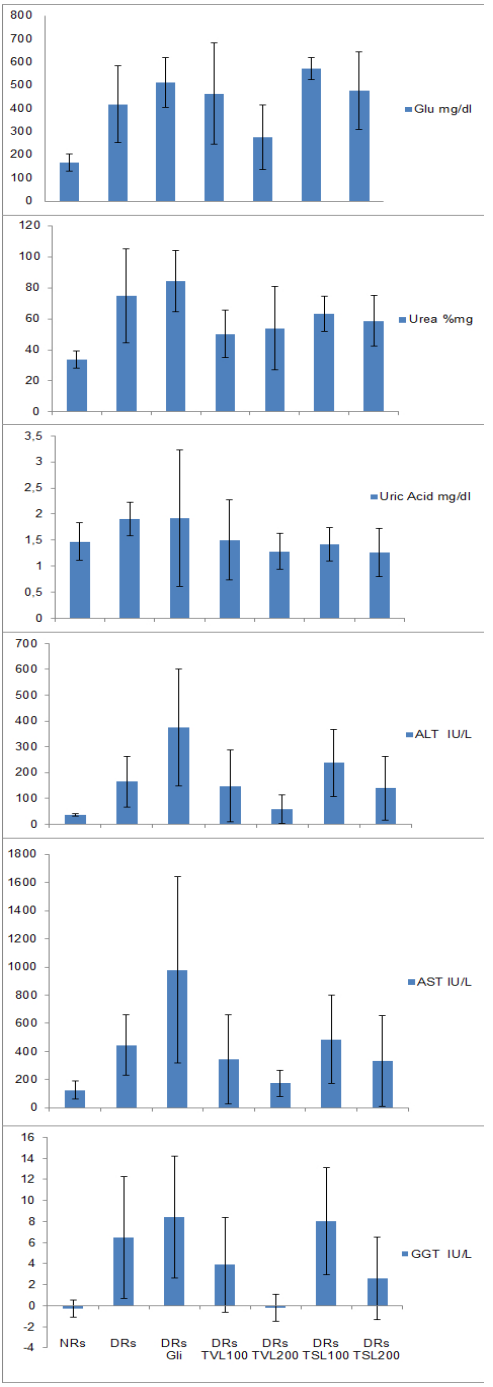
Conflict of Interest: The authors declared that they had no conflicts of interest.

Figure 1: The effect of TVL and TSL on important biochemical parameters of STZ induced diabetic rats. Each point represents data (mean ± SEM).



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A case of complex suicide: Self-electrocution

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Abstract

A 57-year-old man was found at his home by his neighbor. The body of the victim was lying on the right side of the door. A copper wire, whose plastic parts were untied, and surrounded the left and right hand palmar and dorsal faces. The wire was connected by an extension to an electric socket in the room. There was blood and toilet paper around the left hand wrist. Suicide note was found on the taboret

Keyword: Self Electrocution, Suicide

Introduction

Complex suicide is defined as using more than one suicide methods together [1, 2]. Using another method concurrently when the first one has the possibility of inefficiency is named as primary complex suicide, using another method to accelerate death when selected method becomes unsuccessful or goes slowly or gives much pain is named as secondary complex suicide [1-3].

Complex suicides account for 1.5-5.6% of all suicides in the forensic autopsy material [1,4]. Most of the suicides that killed themselves by self-incineration in combination with other methods were young to middle aged [4]. In this study, we aimed to discuss a case in which the death occurred as a result of complex suicide.

Case

A 57-year-old man was found at his home by his neighbor. The body of the victim was lying on the right side of the door. A copper wire, whose plastic parts were untied, and surrounded the left and right hand palmar and dorsal faces. The wire was connected by an extension to an electric socket in the room.

There was blood and toilet paper around the left hand wrist. Suicide note was found on the taboret. Autopsy was performed by the Division of Council of Forensic Medicine in Izmir. In the external examination; it was seen that there were bare plastic parts which were removed from the copper wires which were tied to and surrounded the hand totally on the left hand palmar and dorsal faces (Picture 1). On the skin parts that wires contact, typical electric burns; on the palmar and dorsal faces of right hand, electric burns which surrounded totally; on the inner face of left wrist, six superficial lacerations which varied between 1.5 and 4 cm. in length, involving skin-subcutaneous tissue and soft tissue that were smooth marginated were detected (Picture 2).

Specimens were taken for toxicological and pathologic analysis. In toxicological analysis, there was no toxic substance at blood and urine. In pathological analysis, there was no microscopic pathology on major organs. Lacerations on the left wrist were not fatal and it was understood that death was caused by cardiac arrest linked to the electric current.

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Discussion

The methods most frequently used in complex suicides are those used in so-called ‘‘classic’’ suicides: hanging, firearms, medication overdose, fall from a height, and drowning [5]. More rarely, cases combining simultaneous shots from two firearms [6-8], two shots followed by hanging [9] a shot followed by self-immolation [10], or self-immolation and fall from a height [4] have been described.

For differential diagnosis, cremation to cover up a homicide should always be taken into consideration when examining fire victims showing additional injuries, such as gunshot wounds or sharp force injuries [11, 4]. In the individual case, the differentiation between suicide and homicide may be difficult, especially if the body is found in the open with additional injuries being (co-) responsible for death and there are no independent witnesses who are not involved in the case [4].

Many questions may remain unanswered if the scene of death is not visited. The scene may reveal features of suicide-privacy, suicide note and so on. Relatives or friends of the decedent at the scene may provide background information such as history of depression and previous suicide attempts, marital, social or financial problems [12]. In such cases, a forensic pathologist should be called to the scene before the body is moved and s/he should carefully note the position of body, the state of clothing, the position of stains, the condition of the surroundings and the presence or absence of a suicide note [13]. On the other hand, the presence of a weapon beside the body is not necessarily an indicative of a suicide as a murderer may leave a weapon at the scene of the crime to simulate suicide. While investigating a scene of death, a forensic pathologist should ask himself/herself whether the death is a suicide or a homicide [13].

A study of planned complex suicides including the ingestion of toxic substances shows that a wide range of molecules are used [12, 13]. In our case on toxicological evaluation, no toxic substance detected in blood or urine. Electrocution is a relatively rare method of suicide and tends to be used by men more than by women [14]. In the present case, the precise cause of death is electrocution, the superficial nature of the cuts definitely excludes the hypothesis of wrist-cutting.

Conflict of Interest

The authors declared that they had no conflicts of interest.

Picture 1. Plastic parts removed from the copper wire surrounding the left hand

Picture 2. Superficial lacerations on the inner face of the left wrist

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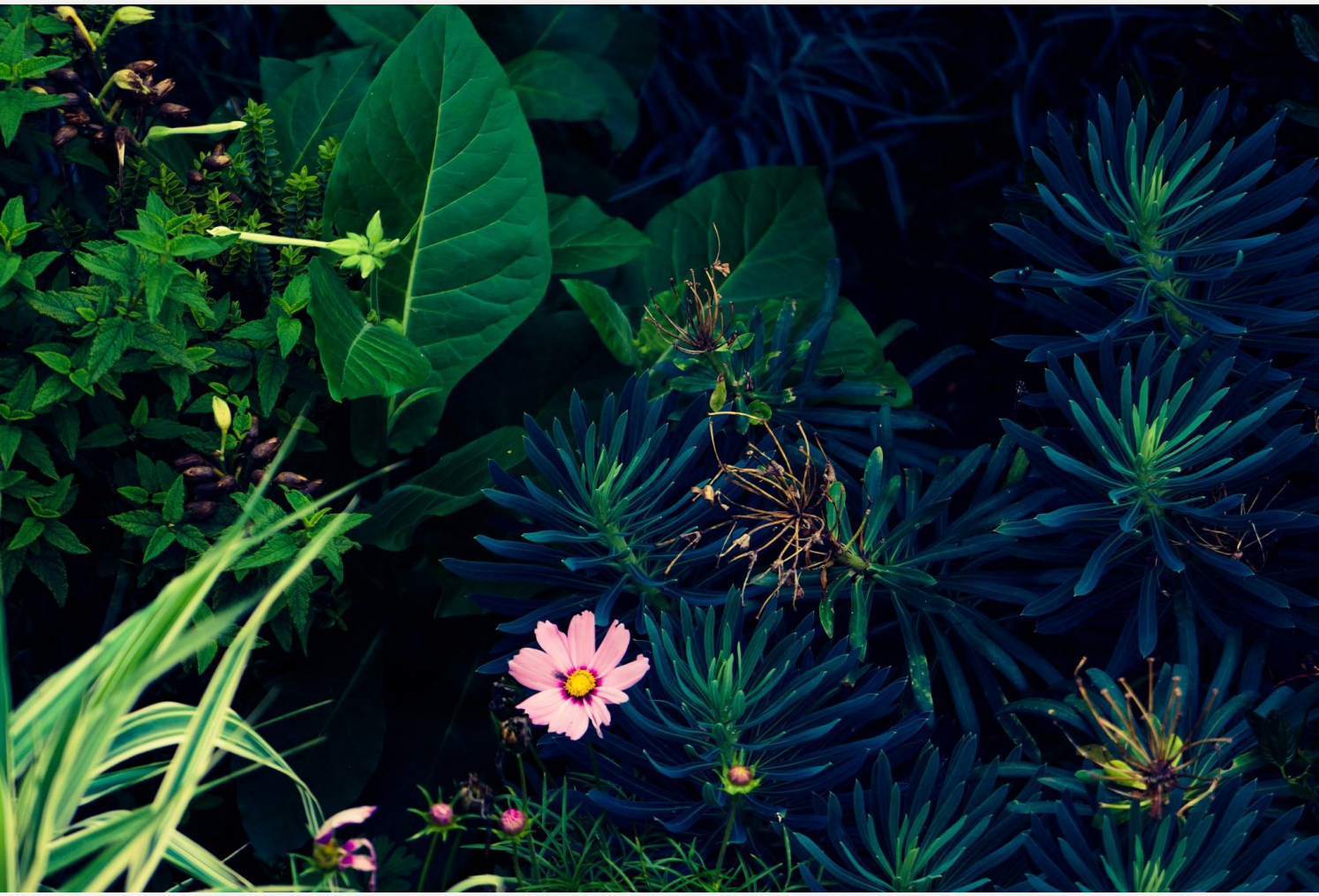

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