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Comparison of conventional Doppler imaging techniques and superb microvascular imaging in determination of vascularization in undescended testes

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Abstract

Aim: Our aim was to gain an idea about testicular injury by comparing the reduced volume, which is one of the indirect indicators of testicular damage in undescended testes, and by evaluating the reduced microvascular blood flow by superb microvascular imaging, and also to determine whether superb microvascular imaging modes could detect microvascular blood flow in more detail in the decreased volume of undescended testes. **Material and methods:** We compared testicular blood flow in undescended testes via conventional Doppler imaging, color superb microvascular imaging, and monochrome superb microvascular imaging techniques with contralateral normally located testis and normal control group. Each sample of testicular tissue was evaluated using a qualitative method. Spot color encoding and linear flow color encoding counts determined in testicular parenchyma were counted separately and expressed as numerical data. The localization of the examined testes in the grayscale was noted (proximal inguinal canal, medial inguinal canal, distal inguinal canal, and scrotal). The volume of undescended testes was calculated automatically via a formula for volume. **Results:** Monochrome superb microvascular imaging is significantly superior in visualizing the vascularity of undescended testes compared with color Doppler, power Doppler and color superb microvascular imaging ($p = 0.001$). Also, undescended testes have a significantly lower blood flow compared with contralateral normal testes ($p = 0.001$). The volume of undescended testes was significantly lower than the contralateral normal testes. **Conclusions:** The volume, structure and blood flow are indirect signs of testicular damage in undescended testes. Monochrome superb microvascular imaging can detect vascularity in undescended testes better than the conventional Doppler imaging technique and color superb microvascular imaging. Based on our findings, we can report that monochrome superb microvascular imaging can be used to evaluate testicular injury and vascularity of undescended testes.

Introduction

The testes consist of gonadal folds at the level of the kidneys and are displaced from the abdomen towards the scrotum in intrauterine life⁽¹⁾. Undescended testis (UDT) is the most common disease of the external genitalia in newborns. It is seen in 3% of full-term newborn babies, and in 70-75% of cases the testes descend to the scrotum within three months after birth. The rate decreases to 1% around the one-year-old age mark. The condition is more frequently observed in premature cases than those born at full-term⁽²⁾. UDT may be located between the kidney and the inguinal canal, but it is usually lo-

cated in the inguinal canal (80%). Approximately 70% of UDT cases are palpable. Of those that are not palpable, 30% are in the inguinal area, 55% are intra-abdominal, and 15% are absent or vanishing⁽³⁾.

In cases that do not complete the descent after one year, atrophy begins in the testes. Seminoma is the most common cancer in UDT. In addition, torsion is a common complication in UDT, and microli-thiasis may also be seen⁽²⁻⁴⁾. In the literature, the reported volume of UDT is significantly smaller than normally located testes in all age subgroups⁽⁵⁾. Progressive histopathological degeneration and atrophy delay germ cell maturation and reduce the number of germ cells

that may develop due to aberrant (ectopic) location. Early diagnosis is thus very important, and orchiopexy is recommended as early as possible to prevent tissue damage^(6,7). Therefore, children with UDT should be operated on between six and 18 months of age. If UDT is not treated, fibrosis will occur, leading to a decrease in testicular size and, consequently, loss of function^(8–10).

Ultrasound (US) is the first preferred imaging method for evaluating the scrotum and testes in patients with UDT because it is a readily available and easy-to-apply non-invasive imaging method. The testes are most commonly located at the level of the inguinal canal and can be easily assessed by US^(9,11). However, US may be insufficient to detect intra-abdominal or atrophic testes. Magnetic Resonance Imaging (MRI) does not contain ionizing radiation and is, therefore, a more attractive imaging method for localizing intra-abdominal testes in pediatric patients. However, MRI is expensive and hard to access. Also, MRI in children also requires anesthesia⁽¹¹⁾.

Testicular volume, structure, and blood flow (BF) of UDT are indirect signs of testicular damage in UDT^(12,13). The cryptorchid testis is usually smaller and isoechoic or hypoechoic in comparison to the normally located testis; in addition, testicular BF decreases compared to the contralateral normally located testis, as reported in the literature⁽¹⁴⁾. Vascularity and volume should definitely be evaluated in UDT, but there are some difficulties in the evaluation of BF in the testicles of small children. Various studies have shown that conventional Doppler imaging techniques (CDIT) may not be sufficient to make a diagnosis, especially in children^(9,14). CDIT can visualize BF at a higher resolution, but they cannot distinguish between the actual low-speed BF and motion artifacts. These techniques demonstrate high-speed and macrovascular BF better⁽⁹⁾.

Superb Microvascular Imaging (SMI) is a new Doppler imaging method developed to overcome the limitations of CDIT. SMI allows the visualization of microvascular structures with low velocity flow signals at a high image resolution and high frame rate (>50 fps), which is why it is very useful especially in inflammatory conditions in different body tissues^(15–18). SMI can be operated in two modes: color SMI (cSMI) and monochrome SMI (mSMI). The cSMI mode simultaneously displays the conventional grayscale US and color-coded Doppler signals. The mSMI mode increases the visibility of vascular structures by eliminating background signals through focusing only on the vascular systems⁽⁹⁾.

In this study, we compared testicular BF in UDT patients via color Doppler (CD), power Doppler (PD), cSMI, and mSMI techniques with contralateral normally located testes and normal control group. According to our knowledge, this is the first study in which vascularity was evaluated and compared with the use of CDIT and SMI in an isolated group of patients with UDT. Our aim was to gain an idea about testicular injury by comparing the reduced volume, which is one of the indirect indicators of testicular damage in UDT, and by evaluating the reduced microvascular BF by SMI. We also sought to determine whether SMI modes could detect microvascular BF in more detail in decreased volume of UDT.

Patients and methods

This prospective controlled study was conducted between June 2019 and June 2021. The study was approved by the local research ethics

committee. All patients were informed about the planned examinations and the procedure. Written consent was obtained before the US examination from each participant's parents. All study procedures involving human participants were performed in accordance with the ethical standards of the institution's research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The study enrolled pediatric patients with no scrotal pathology, who visited our radiology clinic for other reasons, and patients who visited our clinic with UDT, as well as pediatric patients who were followed up after an operation for UDT (operated undescended testes – OUT). The localization of the examined testes in the grayscale was noted (proximal inguinal canal, medial inguinal canal, distal inguinal canal, and scrotal). The length of the inguinal canal was measured in the grayscale, the total length was trisected, and the localization of the testes in the inguinal canal was determined by numerical measurement.

Patients whose testicular tissues could not be observed by US, patients with solid parenchymal lesions, active pediatric patients who could not undergo the examination, and patients with orchitis (too much BF that could not be counted) were excluded from the study. All procedures were carried out with the patient in the supine position. The probe was applied using an adequate amount of US gel. The operator adjusted the pulse repetition frequency, focal zone, gain, and wall filter as necessary to obtain optimal sonograms in each case. All Doppler US examinations were performed by a single radiologist (MSD) with 15 years of experience in Doppler ultrasound (US) and five years of experience in SMI. The images obtained were transferred to a US database and later to computer media. All sonographic images were stored on computer media (23-inch, full HD, 1920 × 1080 resolution, Lenovo/CHINA) at the same time.

Each sample of testicular tissue was evaluated using a qualitative method. Spot color encoding (SCE) and linear flow color encoding (LFCE) counts determined in testicular parenchyma were counted separately and recorded as numerical data (Fig. 1). SCE was defined as nodular BF, and BF with a long axis to short axis ratio of less than three. BF with a long axis to short axis ratio of more than three was defined as LFCE. The assessment was performed primarily visually, but undecidable BFs were scaled by measuring the long and short flow signal axis in a magnifying view and then classified depending on the determined ratio.

Canon Aplio 500 (Canon Medical System Corporation, Tokyo, Japan) with high-frequency (4–14 MHz) linear array transducers was used for the examination. In all the patients, testicular grayscale US, CD, and PD examinations were performed in the supine position. After the measurement of the three axes was completed [length (*L*) × width (*W*) × height (*H*)], the volume of the testis was automatically calculated by the built-in software of the US device using the formula $L \times W \times H \times 0.523$. In the next step, grayscale and CD, PD (frame rate 10–15 Hz), cSMI, and mSMI (frame rate >50 Hz) examinations were performed in the supine position (5 s for every examination). An additional 40 s period was allocated to each patient for vascular assessment. During the examination, by centering the focal zone on testicular parenchyma, the depth of testicular parenchyma was adjusted to the highest value that was possible within the imaging area, and it remained unchanged during the examination. Testicular Doppler examination was performed by observing the entire testicu-

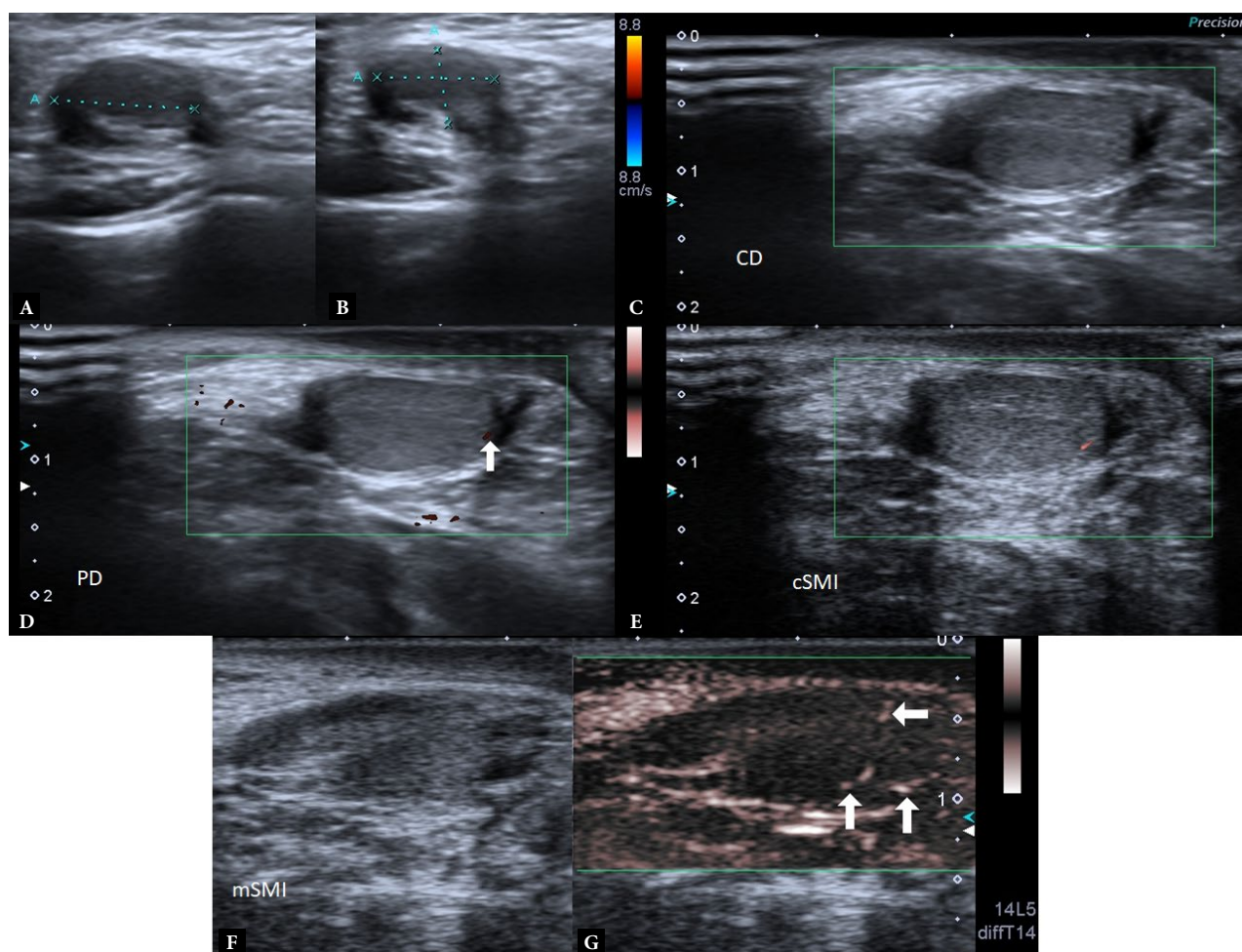


Fig. 1. Right testis in the medial section of inguinal channel was demonstrated by grayscale ultrasonography and testicular volume was calculated (A, B). No spot color encoding (SCE) and linear flow color encoding (LFCE) counts were observed in CD (C). In PD (D) and cSMI mode (E) one LFCE was observed. In mSMI; two LFCE and three SCE were observed, as shown with arrows (F, G). (CD: color Doppler; PD: power Doppler; cSMI: color superb microvascular imaging; mSMI: monochrome superb microvascular imaging; SCE: spot color encoding; LFCE: linear flow color encoding)

lar parenchyma in one maximum longitudinal plane within the examination area at the testicular hilus level from where the maximum vascular signal could be obtained for every four examinations. CD, PD, cSMI, and mSMI examinations were performed in all the patients at the constant parameters, as specified. The parameters were adjusted by an applicator before the study to obtain the most suitable images, and the parameters remained unchanged throughout the study. The average time spent on one patient's examination was approximately 10 minutes.

A total of 92 patients (181 testes, 90 left, 91 right), between 1 and 216 months old, were included in the study. The mean age of the patients was 73.8 ± 10.4 months. The testes of three patients who underwent unilateral orchiectomy (two left, one right orchiectomy) because of UDT were evaluated as unilateral testes; the other patients were evaluated as bilateral testes. The patients included in the study were recruited from a heterogeneous population.

A total of 40 patients (44 OUT) who had undergone surgery for UDT were included in our study. Four of these patients were operated for bilateral UDT (four OUT bilateral, 36 OUT unilateral, 36 normal scrotal placement since birth). A total of 44 testes which had an operation

history due to UDT [26 right (59.1%), 18 left (40.9%)] were classified as Group A. The patients' age at operation and period elapsed since surgery in Group A testes were noted and compared with the CDIT and SMI SCE and LFCE numerical values. A total of 39 testes were classified as Group B (six testes proximal of inguinal canal, 18 testes middle of inguinal canal, 15 testes distal of inguinal canal). Overall, 24 UDT were newly diagnosed and had inguinal canal placement (nine bilateral UDT, six unilateral UDT; five proximal of inguinal canal, 14 middle of inguinal canal, five distal inguinal canal). In total, 15 UDT were in follow-up (one right, six left: one middle of inguinal canal, six distal of inguinal canal). In total, 90 testes were classified as Group C; these were from 33 control group patients (66 testes) who agreed to participate in the study and were randomly selected from a group of patients who had undergone US for other reasons. We also used as part of the control group contralateral normally located testes of the patients who had unilateral OUT history (36 testes with normal placement) and contralateral normally located testes of patients who had UDT diagnosis (six testes). Group C testes had normal scrotal placement since birth, and their US findings were normal. Patients included in the study and groupings of testes are summarized in Tab. 1. The testes of patients included in Groups A, B, and C were statistically compared in terms of the CDIT, SMI SCE and LFCE numerical values.

Tab. 1. Testes included in the study according to localization, operation history, and grouping of testes. UDT was classified as inguinal canal proximal, inguinal canal medium, and inguinal canal distal. Control group including contralateral normally located of OUTs, follow-up UDT and control group of normally located bilateral testes

	Testicular localization	Group	Total n (%)
OUT	Scrotal	A	n = 44 (24.31%)
Newly diagnosed UDT (n = 24), (13.26%)	Inguinal canal proximal (n = 6) (15.39%)	B	n = 39 (21.55%)
	Inguinal canal medium (n = 18) (46.15%)		
Follow-up UDT (n = 15), (8.29%)	Inguinal canal distal (n = 15) (38.46%)		
Contralateral normally located of OUT (n = 27), (27.56%)	Scrotal	C	n = 98 (54.14%)
Contralateral normally located of UDT ((n = 5), (5.1%)			
Bilaterally normal testes (n = 66), (67.34%)			
Total (n = 173), (100%)	Scrotal or inguinal	A + B + C	n = 181 (100%)

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (IBM, Armonk, NY) software to evaluate the data. Descriptive statistics were expressed as mean, standard deviation, minimum-maximum values, frequency, and percentile. The Kolmogorov-Smirnov test was used to analyze the normal distribution of data. Kruskal-Wallis test, a non-parametrical test, and the Mann-Whitney U test were used to assess the SCE and LFCE numerical of the CDIT-SMI values and testicular volumes in Groups A, B, and C. The same statistical method was used to determine the relationship between SCE and LFCE numerical values and testicular localization. Spearman's correlation coefficient was used to assess the relationship with alterations of SCE and LFCE numerical values and testicular volume, age at operation, and period since the operation. P values of less than 0.05 were considered statistically significant with a 95% confidence level.

Results

A total of 88 patients [173 testes, 86 (49.7%) left, 87 (50.3%) right] between 1 and 216 months old were included in the study. The testes were evaluated bilaterally in 85 patients. The testes of three patients who underwent unilateral orchiectomy (two left, one right orchiectomy) because of UDT were evaluated unilaterally; testes in the other patients were evaluated as bilateral testes. The patients included in the study were recruited from a heterogeneous population. The median age of the patients was 48 months (interquartile range: 87); and the median testicular volume was 0.5 (interquartile range: 0.3).

Tab. 2. Testes of operated 44 patients (OUT, testes having contralateral inguinal canal distal placement under follow-up and contralateral normally located testes) in terms of age, volume, and SCE-LFCE numerical values. (SCE: spot color encoding, LFCE: linear flow color encoding, CD: color Doppler, PD: power Doppler)

	Operated UDT (44)	Inguinal canal distal under follow-up (7)	Normal contralateral testicle (27)
Age (months)	74.06 ± 56.17	54.57 ± 45.06	60.2 ± 42.3
Volume	1.31 ± 3.12	0.60 ± 0.14	0.74 ± 0.28
CD	-SCE	0.50 ± 1.62	0.001 ± 0.001
	-LFCE	0.16 ± 0.57	0.001 ± 0.001
PD	-SCE	1.09 ± 2.40	0.001 ± 0.001
	-LFCE	0.34 ± 0.80	0.14 ± 0.38
cSMI	-SCE	1.68 ± 4.25	0.001 ± 0.001
	-LFCE	0.47 ± 1.13	0.001 ± 0.001
mSMI	-SCE	6 ± 6.58	2.14 ± 1.46
	-LFCE	1.61 ± 1.82	0.43 ± 0.53

In the patients operated on for UDT (Group A), the age at operation and period since the operation differed in our study. The median age at operation was 28.5 (interquartile range: 30.5) months. The median post-operative period was 14 (interquartile range: 19.75) months. When SMI and CDIT numerical values were compared with the age, no significant correlation was found. When the testes were operated on (period when testis was passed in the abnormal localization), no significant correlation was identified ($p = 0.736$). In addition, when Doppler numerical values were compared with the post-operative time, no significant correlation was found, either ($p = 0.582$).

A total of 40 patients operated for UDT (44 testes, four of the patients were operated on for bilateral UDT) were evaluated in terms of age, volume, and CDIT and SCE-LFCE numerical values. OUT patient group was compared with inguinal canal distal placement under follow-up and contralateral normally located testes in terms of age, volume, and Doppler numerical values (Tab. 2). There was no statistically significant difference between the three groups in terms of age. When testicular volumes were compared, the testicular volume of OUT group was significantly higher with followed UDT ($p = 0.001$). Furthermore, the testicular volume of Group C was significantly higher compared to Group B and Group A.

In all groups (Groups A, B, C) in SCE; PD was significantly higher when compared to CD ($p = 0.001$). However, no significant difference was observed between cSMI and PD ($p = 0.369$). mSMI was significantly superior to cSMI and PD ($p = 0.001$). In LFCE, PD was significantly superior to CD ($p = 0.001$). Also, mSMI was significantly superior to cSMI ($p = 0.001$) and PD ($p = 0.001$) - CD ($p = 0.001$). There was no significant difference between cSMI linear encoding and PD linear encoding ($p = 0.420$).

Operation history, age, volume, and Doppler numerical values of a total of 173 testes included in the study are summarized in Tab. 3. The volumes in Group C were significantly higher when compared with Group A and Group B.

Tab. 3. Age, volume, and CDIT-SMI numerical values of testes according to the groups. (SCE: spot color encoding, LFCE: linear flow color encoding, CD: color Doppler, PD: power Doppler)

		Group A	Group B	Group C
Age (months)		74.06 ± 56.17	59.95 ± 40.93	83.48 ± 76.80
Volume		1.31 ± 3.12	0.56 ± 0.31	2.74 ± 5.15
CD	-SCE	0.50 ± 1.62	0.16 ± 0.48	1.62 ± 2.71
	-LFCE	0.16 ± 0.57	0.04 ± 0.20	0.24 ± 0.61
PD	-SCE	1.09 ± 2.40	0.54 ± 0.98	5.43 ± 7.84
	-LFCE	0.34 ± 0.80	0.25 ± 0.74	0.77 ± 1.01
cSMI	-SCE	1.68 ± 4.25	1.0 ± 2.21	5.92 ± 10.33
	-LFCE	0.47 ± 1.13	0.5 ± 0.98	0.68 ± 1.20
mSMI	-SCE	6 ± 6.58	3.54 ± 2.90	10.68 ± 14.28
	-LFCE	1.61 ± 1.82	1.75 ± 1.45	2.27 ± 2.22

Tab. 4. Age, volume, and Doppler numerical values of all undescended testes (newly diagnosed and follow-up undescended testes) (SCE: spot color encoding, LFCE: linear flow color encoding, CD: color Doppler, PD: power Doppler)

		Inguinal canal proximal (n = 6)	Inguinal canal medium (n = 18)	Inguinal canal distal (n = 15)	Scrotal (n = 98)
Age (months)		39.00 ± 22.36	65.16 ± 40.77	60.40 ± 42.64	78.03 ± 68.35
Volume		0.27 ± 0.08	0.56 ± 0.26	0.57 ± 0.27	2.74 ± 5.15
CD	-SCE	0.001 ± 0.001	0.22 ± 0.55	0.001 ± 0.001	1.10 ± 2.38
	-LFCE	0.001 ± 0.001	0.05 ± 0.24	0.001 ± 0.001	0.21 ± 0.68
PD	-SCE	0.001 ± 0.001	0.61 ± 1.09	0.20 ± 0.41	3.33 ± 6.13
	-LFCE	0.001 ± 0.001	0.39 ± 0.85	0.001 ± 0.001	0.68 ± 1.01
cSMI	-SCE	0.66 ± 1.63	0.94 ± 2.41	0.33 ± 0.72	4.14 ± 8.78
	-LFCE	0.50 ± 1.22	0.33 ± 0.68	0.20 ± 0.77	0.73 ± 1.45
mSMI	-SCE	2.66 ± 2.34	4.11 ± 2.95	3.80 ± 5.29	8.62 ± 12.45
	-LFCE	1.16 ± 1.47	1.66 ± 1.61	0.93 ± 0.96	2.31 ± 2.50

Tab. 5. UDT testicular volumes and mSMI numerical values were compared. When volume values increased, mSMI SCE and LFCE values were significantly increasing (correlation coefficient form SMI SCE: 0.312; correlation coefficient form SMI LFCE: 0.365). No significance was observed between mSMI numerical values of the post-operative period and age at operation

mSMI	Post-operative period	Age at operation	Volume
-SCE	$p = 0.914$	$p = 0.728$	$p < 0.001$
-LFCE	$p = 0.006$	$p = 0.698$	$p < 0.001$

Testes were compared by their placement levels without including those operated on (Tab. 4). Testicular volumes in the proximal inguinal canal were significantly lower when compared with the mid and distal inguinal canal ($p = 0.001$). In addition, the mean age of the proximal inguinal canal group was significantly lower when

compared with the mid and distal inguinal canal placements of UDT.

When volume values were increased, mSMI SCE and LFCE values were significantly increasing (correlation coefficient for SMI SCE: 0.312; correlation efficient for SMI LFCE: 0.365). No significance was observed between mSMI numerical values and post-operative period ($p = 0.914$ for SCE, 0.006 for LFCE) and the age at operation ($p = 0.728$ for SCE, $p = 0.698$ for LFCE) (Tab. 5).

Discussion

Early diagnosis and treatment are particularly important for preserving fertility and preventing possible future testicular malignancy in patients with UDT⁽²⁾. US is an ideal imaging modality for children with UDT. Widespread availability, absence of ionizing radiation, and relatively shorter operating time are well-known advantages of the modality. It is the most common diagnostic imaging tool used for the evaluation of cryptorchidism and the scrotum. On conventional grayscale US; UDT appears as a hypoechoic, round, or oval structure between six and 16 mm in the maximum diameter in the inguinal canal^(19,20). Higher temperature in the inguinal canal and abdomen, and higher pressure in the inguinal canal, which inhibits testicular circulation, are the main causes of progressive damage in UDT⁽²¹⁾. In our study, there was a significant difference in testicular volumes across Groups A, B, and C. The volume in Group C was significantly higher compared to Group A and Group B. We evaluated Group B in three stages as inguinal canal proximal, inguinal canal medium, and inguinal canal distal. The testicular volume in inguinal canal proximal was significantly lower in relation to inguinal canal medium and distal. These findings confirm that testicular volume tends to decrease in UDT.

Although US is usually the first preferred imaging modality used to detect cryptorchid testes in the inguinal canal in routine radiology clinic, it has a very low success rate in locating non-palpable abdominal testes. In patients with intra-abdominal testes which were not detected by US; CT or MRI should be chosen to determine testicular localization⁽²²⁾. In our study, we did not include intra-abdominal testes detected by CT or MRI. The patients in this group were operated on to prevent the risk of testicular malignancy in the abdomen after they were examined by cross-sectional imaging methods. We included UDT in the inguinal canal which was detected by sonographic examination.

In addition to the risk of infertility and testicular cancer, testicular torsion is a major complication of UDT. The incidence of torsion is reported to be 10 times higher in patients with UDT⁽²³⁾. Therefore, demonstrating vascularization in the testicular tissue in such patients is also important in terms of emergency treatment, since the testicular volume is smaller in patients with UDT and especially if torsion is suspected, it is very difficult to detect vascularization in atrophic testes by CDITs. It is extremely challenging to evaluate testicular BF in children because of the small testes, low velocity of blood flow in the testicles, and decreased testicular volume in UDT. SMI, especially mSMI, was quite successful in showing testicular BF in such cases, as in our study, compared to CDITs. There are several studies reporting significant limitations of CDITs in assessing testicular vascularity in young children^(24,25). When the difference between the mean values of CD, PD and SMI was compared in our study, it

was revealed that CD gave the least comprehensive data about testicular BF and PD, while SMI provided more data in terms of evaluating vascularity. Several studies reporting that PD provides more data on testicular BF than CD are available in the literature^(24,26,27). In our study, in consistency with the findings reported in the literature, PD was significantly superior to CD in demonstrating vascularization. However, PD is too sensitive to motion artifacts. On the other hand, SMI is superior to CD and PD in demonstrating vascularization by eliminating motion artifacts in detecting BF in testicles, in accordance with the literature⁽²⁵⁾. mSMI was significantly superior to other methods in visualizing vascularization. In this area, there have been studies conducted with SMI on various organs in the body^(5,16,28).

There are several limitations of this paper. We did not evaluate the duration of CD, PD and SMI techniques in the busy clinic conditions. However, in the study by Karaca *et al.*⁽²⁶⁾ it was reported that the SMI technique had the fastest evaluation in testicular BF. We did not have the opportunity to detect degeneration histopathologically in atrophic testicles and operation of intra-abdominal testicles. This is the subject of another study. One operator did all the examinations; therefore, we could not evaluate interobserver variability. In addition, we could not evaluate whether the participants of the study had premature or term births, since no sufficient clinical information was available.

Conclusion

UDT is the most common congenital abnormality of the urogenital system. Early detection and testicular tissue determination are espe-

cially important from the viewpoint of treatment. Determination of testicular BF in UDT is essential for detecting torsion and testicular degeneration. Our study reveals that SMI provides more extensive data than the CD and PD techniques for assessing BF of UDT in the testicles. It needs to be highlighted that mSMI was clearly superior in this regard. In the course of our study, it was determined that mSMI was clearly superior in visualizing testicular BF in patients with UDT. For this reason, we think that it can be used in routine clinical conditions to evaluate testicular blood flow.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Author contributions

Original concept of study: MSD. Writing of manuscript: FA. Analysis and interpretation of data: MSD. Final approval of manuscript: MSD. Collection, recording and/or compilation of data: MS, TS, MG, IC. Critical review of manuscript: FA.

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