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Superb Microvascular Imaging for Detecting Ovarian Vascularity in Precocious Puberty, Premature Thelarche, and Pubertal Girls

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Abstract

Objective: We aimed to evaluate ovarian vascularity with superb microvascular imaging (SMI) and to compare it with other conventional Doppler imaging methods in girls with premature thelarche, precocious puberty, and those at puberty.

Methods: A total of 133 ovaries from 69 patients were evaluated. Among the 69 subjects, 50 girls applied with the preliminary diagnosis of precocious puberty, and 19 of them were pubertal adolescent girls. The color Doppler imaging (CDI), power Doppler imaging (PDI), color SMI (cSMI), and monochrome SMI (mSMI) techniques were performed, and the images at the same site of the ovary were obtained. The images were evaluated by 2 pediatric radiologists using a 4-level grading system to evaluate the degree of vascularity.

Results: A total of 69 patients were evaluated, including 19 who were pubertal, 39 with premature thelarche (PT), and 11 with precocious puberty (PP) were evaluated. Among the 50 patients, 11 were diagnosed with precocious puberty, while 39 received a diagnosis of premature thelarce. The sensitivity of the techniques according to vascularity grading was interpreted as mSMI > cSMI > PDI > CDI. The interrater agreement of vascularity grading among the two observers according to their κ values was almost perfect in the left CDI, left PDI, and right PDI (κ > 0.92), strong in the right CDI, left and right cSMI, left and right mSMI (κ > 0.80), and moderate in the right CDI (κ > 0.60). There was a significant difference between the vascularity of pubertal and prepubertal ovaries, which correlated with their volumes (P < .001). Ovarian vascularity was similar in precocious puberty and premature thelarche groups.

Conclusion: The SMI is superior to other Doppler methods, such as PDI and CDI, in the evaluation of ovarian vascularity. It is useful in evaluating parenchymal vascularity, especially in pediatric patients.

Keywords: Doppler imaging, premature thelarche, puberty precoccious, puberty, superb microvascular imaging

Introduction

Ultrasonography is the first choice imaging modality for evaluating the female genital system. In addition to being easily accessible, it is a method that provides diagnostic accuracy in pediatric genital imaging. Doppler methods such as color and power Doppler imaging (CDI, PDI) have been used for years to detect tissue blood flow. Conventional Doppler imaging techniques are inadequate for detecting low-velocity blood flow in microvascular structures.

Superb microvascular imaging (SMI) is a novel ultrasound technique developed by Toshiba Medical Systems and is used to detect low-velocity blood flow and very small vessels. It can distinguish microflow signals from tissue motion artifacts by using a higher frame rate (> 50 frames per second) and lower pulse repetition frequencies (220-234 Hz).[1] Superb microvascular imaging can produce images using 2 modes: color SMI (cSMI) and monochrome SMI (mSMI). The cSMI displays conventional grayscale ultrasound and color signals, while mSMI displays vascular structures as subtraction with suppressed background images.[2]

Precocious puberty (PP) is the development of secondary sexual characteristics before 8 years of age in girls and 9 years in boys. The role of imaging is to determine the lesions responsible for abnormal sex hormone production and demonstrate the accelerated development of the reproductive organs.[3] Most girls who present with early pubertal development have common variants rather than pathologic disorders requiring treatment.[4] Premature thelarche (PT) is featured by an isolated appearance of breast development that is not progressive and does not require treatment.[5]

In the pediatric population, especially premenarcheal girls, it is difficult to demonstrate the vascularity of a normal ovary with conventional Doppler imaging.[6] To the best of our knowledge, there have only been a few studies about ovarian vascularity with SMI and there are no studies on ovarian vascularity in girls with the precocious puberty spectrum.[7,8] Thus, we aimed to evaluate and

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compare ovarian vascularity with CDI, PDI, and SMI in girls with premature thelarche, true precocious puberty, and those at puberty.

Methods

The prospective study was approved by the University of Health Sciences Ankara Child Health and Disease Hematology Oncology Training and Research Hospital Clinical Research Ethics Committee (Approval no: 2018023, Date: February 26, 2018). Written informed consent was obtained from the parents of all patients, and the study adhered to the Declaration of Helsinki.

Subjects

A total of 133 ovaries of 69 subjects were evaluated. In five patients, one of the ovaries could not be visualized due to inadequate bladder distention. The majority of patients were from our Endocrinology department, and the referral diagnosis was precocious puberty. Among the 69 subjects, 50 girls presented with a preliminary diagnosis of precocious puberty at an average age of 7.3 years. Nineteen of them were pubertal adolescent girls for whom pelvic ultrasound was requested for other reasons. All prepubertal cases were consistent with their karyotype. Patients with congenital adrenal hyperplasia and polycystic ovary were excluded because of the small number of patients with these conditions. Patients with adnexal masses or cysts were also excluded. All patients with precocious puberty had central precocious puberty. All patients with premature thelarche had neither central precocious puberty nor peripheral precocious puberty.

Imaging

All the ultrasonography (US) imaging was performed using a 6-MHz convex abdominal probe (Toshiba Aplio 500; Toshiba Medical System Corporation, Tokyo, Japan). Patients underwent examinations in supine positions with bladder distention. All examinations were performed by a 5-year experienced pediatric radiologist. In B-mode ultrasound, uterus and ovary measurements were assessed, and ovarian volumes were noted. The volume of ovaries (length × width × depth × 0.523) was calculated automatically. All subjects were evaluated with the same device without changing the imaging parameters of Doppler techniques (pulse repetition frequency set at between 220-240 and 880-960 Hz, frame rates were for SMI >50 Hz, for CDI and PDI 10-15 Hz). The CDI, PDI, cSMI, and mSMI images were obtained from the same site on each ovary. The richest vascular site of the ovary was recorded. Four Doppler images of each ovary were independently evaluated by two pediatric radiologists who were blinded to the patients. A four-level grading system was used to evaluate the degree of vascularity (Table 1).[7-10]

Clinical Assessment

Evaluation of patients included a physical examination, and laboratory tests for levels of basal follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (e2). Biometric data such as chronological age, bone age, weight, height, body mass index (BMI), and stage of thelarche were retrieved. The standard deviation score (SD) of height, body weight, and BMI for the same age and sex were calculated. FSH, LH, and e2 levels were measured using the chemiluminescence immunoassay (CLIA) technique. The assessment of bone age was performed using the Greulich-Pyle atlas,[11] and pubertal grading was done according to Marshall and Tanner.[12] None of the subjects had hirsutism, underlying endocrine diseases, or congenital anomalies. They did not have hormonal therapy or other drug therapies.

Table 1. The Vascularity Grading

Grade 0	No vascularity
Grade 1	1 or 2 punctate color coding
Grade 2	One linear or punctate vascularity more than 2
Grade 3	Multiple linear flows (±punctate colors)

Basal and stimulated LH levels were supporting tests to detect hypothalamus–pituitary–gonadal (HPG) axis activation in central PP. The diagnostic value of basal measurements is limited due to the pulsatile release of gonadotropins; a GnRH stimulation test is the gold standard test to distinguish central PP from PT. Luteinizing hormone and FSH responses to LHRH stimulation were determined for definitive diagnosis when necessary, and stimulated gonadotropin levels were interpreted according to the measurement method. Patients with a peak value greater than 5 IU/L in the GnRH test or a basal LH value greater than 1.1 IU/L were considered to have central PP.[13,14]

The patients with bone age to chronological age ratio > 1 and who had a pubertal response to the GnRH stimulation test were accepted as having central PP, and those who did not meet these criteria were considered to have PT.

Statistical Analysis

All the statistical analyses were performed with the Statistical Package for Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA). The Kolmogorov–Smirnov and Shapiro–Wilks normality tests were used to check whether the numerical variables obtained from the patients were compatible with the normal distribution. Summary statistics of variables conforming to the normal distribution were given as mean ± standard deviation, and when not normally distributed, they were presented as median (minimum–maximum). Descriptive statistics for categorical variables were given in terms of numbers and percentages. In group comparisons in terms of continuous variables, a parametric 2-sample independent *t*-test was used, and variance homogeneity was checked by the Levene test. In the case of comparisons of more than 2 groups, the analysis of variance test was used. In cases where there was a difference, the determination of which groups the difference was between was made using the Sidak post hoc multiple comparison test. A *P*-value of < .05 was considered statistically significant for the Sidak post hoc test. Spearman's correlation coefficient was used to evaluate whether there was a statistically significant relationship between ovarian dimensions and vascularity grades. Inter-rater agreement control between Doppler techniques was evaluated with Cohen's Kappa statistics. Agreement of diagnostic methods was evaluated using Cohen's Kappa for which a value < 0.2 is considered to indicate no agreement, 0.21-0.39 is minimal agreement, 0.40-0.59 is weak agreement, 0.60-0.79 is moderate agreement, 0.80-0.90 is strong agreement, and > 0.90 is almost a perfect agreement. The coefficients obtained were summarized with 95% confidence intervals. *P* < .05 was considered statistically significant.

Results

Based on the laboratory test results in prepubertal girls, among the 50 patients, 11 of them were diagnosed with precocious puberty, while 39 received a diagnosis of premature thelarche. In this study, out of a total of 69 patients, 19 were in the pubertal group, 39 were diagnosed with premature thelarche, and 11 were diagnosed with precocious puberty. The mean age was 8.89 (3-16

Table 2. Characteristics of Premature Thelarche and Precocious Puberty Groups

	PT (n = 39)	PP (n = 11)	*P*
Bone age (years)	7.48 ± 2.05	8.66 ± 1.82	.090
Chronological age (years)	7.33 ± 1.74	7.36 ± 1.61	.109
Weight-SDS	0.40 ± 0.97	0.57 ± 0.83	.608
Weight (kg)	27.4 ± 8.7	27.4 ±5.8	.989
Height-SDS	0.05 ± 1.10	0.60 ± 1.11	.148
Height (cm)	124.5 (71-151.5)	125.9 (107.1-145.0)	.542
BMI-SDS	0.59 ± 1.03	0.50 ± 0.47	.659
BMI (kg/m^2)	16.8 (13.5-23.1)	16.4 (15.4-19.8)	.381
Basal FSH (IU/L)	2.39 (0.27-6.20)	3.97 (2.18-9.52)	**.0010***
Basal LH (IU/L)	0.2 (0.1-1.3)	0.39 (0.20-1.00)	**.0004***
Basal e2 (pg/mL)	8 (5-41)	32 (18-64)	**<.0001***

Data are presented as mean ± SD or median (minimum–maximum); bold values indicate statistical significance.
BMI, body mass index; e2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SDS, standard deviation score.
*$P < .05$.

Table 3. Pairwise Comparisons of Groups were Obtained by the Analysis of Variance Test Followed by the Sidak Posthoc Test

Variable	Groups	Mean ± SD	*P*	PT, *P*	PP, *P*
Right ovary (cm^3)	Puberty	5.39 ± 2.90	**<.001***	**<.0001***	**.0002***
	PT	2.14 ± 1.45			>.9999
	PP	1.81 ± 1.12			
Left ovary (cm^3)	Puberty	5.16 ± 2.85	**<.001***	**<.0001***	**.0016***
	PT	2.02 ± 1.42			>.9999
	PP	1.98 ± 1.43			
Chronological age (years)	Puberty	13.06 ± 1.86	**<.001***	**<.0001***	**<.0001***
	PT	7.33 ± 1.74			>0.9999
	PP	7.36 ± 1.61			

Bold values indicate statistical significance.
PT, premature thelarche; PP, precocious puberty; SD, standard deviation.
*$P < .05$.

years old). The clinical and laboratory characteristics of a total of 50 PT and PP patients are described in Table 2. A statistically significant difference was found between the groups in terms of basal FSH ($P = .010$), basal LH ($P = .036$), and basal e2 ($P < .001$) values. Most of the patients in each group were in stage of 2 of thelarche. The GnRH stimulation test was performed on all patients in the PP group. Peak LH values were a minimum of 7 IU/L, a maximum of 29 IU/L, and a mean of 12.62 IU/L, while peak FSH values were a minimum of 5.2 IU/L, a maximum of 17 IU/L, and a mean of 10.19 IU/L in the PP group. There was a statistically significant difference in ovarian volumes. The patients in the pubertal group were statistically significantly different from those in both the PP and PT groups ($P < .001$) (Table 3). In our study, we demonstrated that ovarian vascularity increases with menarche, and we could easily reveal the degree of vascularity with SMI (Figures 1 and 2).

A total of 133 ovaries were evaluated. Observer 1 scored 128 (96.2%) of the ovaries as 0, 4 (3.0%) as 1, and 1 (0.75%) as 2, but none of them scored as 3 with the CDI. With PDI, 84 (63.2%) of ovaries scored as 0, 34 (25.5%) as 1, 11 (8.3%) as 2, and 4 (3.0%) as 3. Thirty-one (23.5%) of the ovaries scored as 0, 34 (25.5%) as 1, 34 (25.5%) as 2, and 34 (25.5%) as 3 with cSMI. And with mSMI, 26 (19.5%) of the ovaries scored as 0, 38 (28.6%) as 1, 33 (24.8%) as 2, and 36 (27.1%) as 3. Observer 2 scored 129 (97%) of the ovaries as 0, 3 (2.2%) as 1, and 1 (0.7%) as 2, but none of them scored as 3 with the CDI. With PDI, 84 (63.2%) of ovaries scored as 0, 35 (26.3%) as 1, 12 (9.0%) as 2, and 2 (1.5%) as 3. Thirty-two (16.6%) of the ovaries scored as 0, 37 (27.8%) as 1, 39 (29.3%) as 2, and 35 (26.3%) as 3 with cSMI. And with mSMI, 21 (15.8%) of the ovaries scored as 0, 36 (27.1%) as 1, 41 (30.8%) as 2, and 35 (26.3%) as 3. According to the data obtained from both observers, Doppler techniques were ranked in the following order according to vascularity grades: mSMI > cSMI > PDI > CDI (Figures 3 and 4). There was a statistically significant difference in vascularity grades between pre- and postpubertal ovaries with cSMI ($P < .0001$) and mSMI ($P = .0021$).

The inter-rater agreement was evaluated separately for each Doppler technique. The inter-rater agreement of vascularity grading among the two observers according to their κ values was almost perfect in the left CDI, left PDI, and right PDI ($\kappa > 0.92$), strong in the right CDI, left and right cSMI, left and right mSMI ($\kappa > 0.80$), and moderate in the right CDI ($\kappa > 0.60$) (Table 4).

The right and left ovary measurements were evaluated according to their respective sides. In the PP group, a moderate positive correlation was found between the right ovarian volume and mSMI grades ($r = 0.67$, $P = .022$), and a strong positive correlation was found between the left ovarian volume and cSMI ($r = 0.78$, $P = .004$) and mSMI ($r = 0.85$, $P = .008$) grades (Table 5). In the PT group, there was a statistically significant relationship between evaluations and right and left ovarian measurements; however, their correlations were low ($r < 0.5$).

Discussion

In this study, we demonstrated the utility of SMI for the evaluation of parenchymal perfusion in the ovaries of prepubertal girls. We observed that SMI showed better vascularity than the other Doppler techniques, such as CDI and PDI, with low interobserver variability. We also found that there is a significant difference between the vascularity of pubertal and prepubertal ovaries, which is correlated with their respective volumes. There have been few studies in the literature about ovarian vascularization with SMI, and this is the first study in this area with the population of precocious puberty and premature thelarche.

Precocious puberty is a condition in which sexual and physical development starts before the age of 8 years in girls and 9 years in boys.[15] Precocious puberty is classified into 2 major categories clinically: complete PP (true PP) and incomplete PP (refers to isolated premature thelarche, isolated premature pubarche, and isolated menarche). Etiologically, PP can be divided into central PP (GnRH dependent) and peripheral PP (GnRH independent).[16] Central precocious puberty (CPP) is caused by the premature activation of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator and is generally idiopathic.[17]

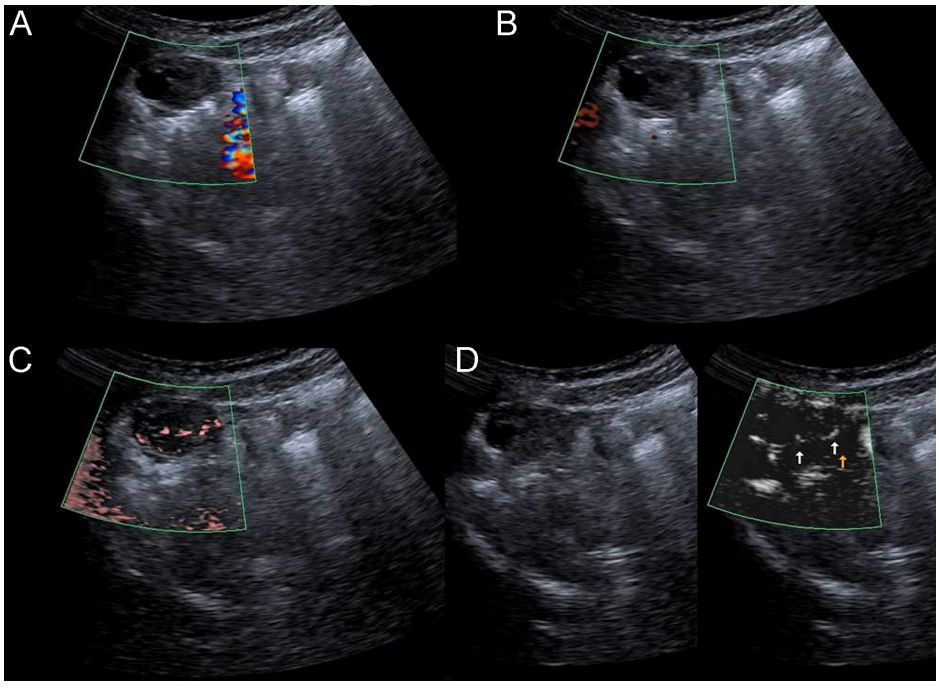

Figure 1. Doppler imaging of a 7-year-old girl with precocious puberty. There is no vascularity with color Doppler imaging (A) and power Doppler imaging (B). Both ovarian vascularity grades were 0. There are 2 linear and several punctate vascularities with cSMI (C) and mSMI (D), graded as 3.

Pelvic ultrasonography detects ovarian tumors or cysts in cases of peripheral PP and gives information about the development of internal genitalia in cases of central PP. The distinction between CPP and PT is confirmed by clinical, radiologic, and laboratory tests such as physical examination, evaluation of bone age, basal FSH and LH values, BMI index, and GnRH stimulation test.[18]

Prepubertal ovary sizes are small, and it is difficult to demonstrate vascularity with conventional Doppler methods due to their inability to detect low-flow microvessels. SMI is a modern technique that is sensitive to the visualization of microvascular structures. It effectively separates flow signals from tissue motion artifacts, which helps to demonstrate flow images with high accuracy.[19]

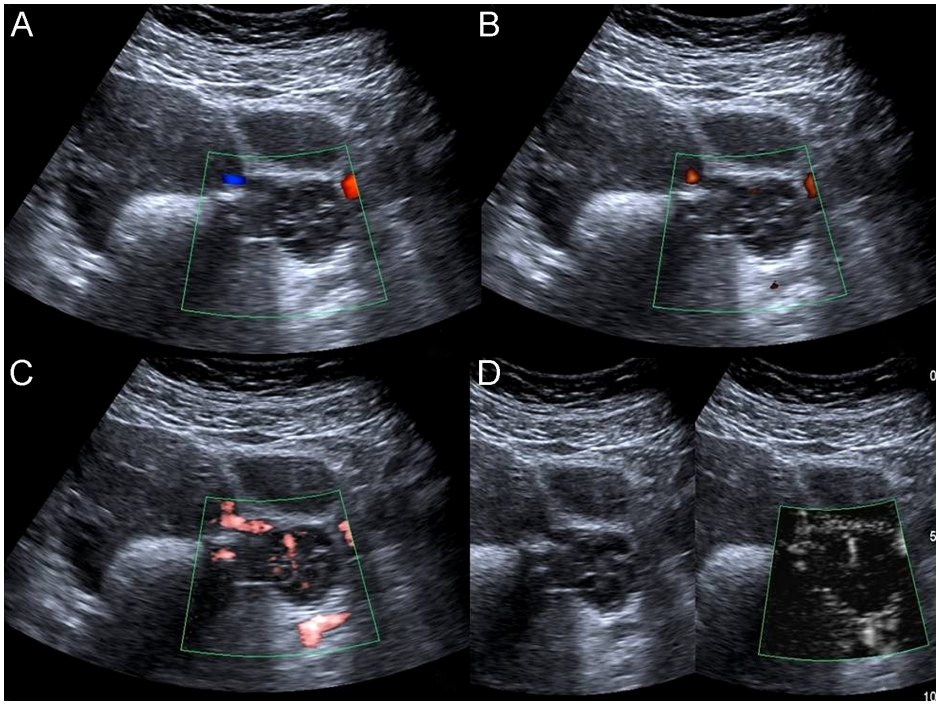

Figure 2. Imaging of a 12-year-old postpubertal girl. There is no vascularity with color Doppler imaging graded as 0 (A). There is one punctate vascularity with power Doppler imaging graded as 1 (B). There are one linear and several punctate vascularities with cSMI graded as 3 (C). There is one linear vascularity with mSMI graded as 3 (D).

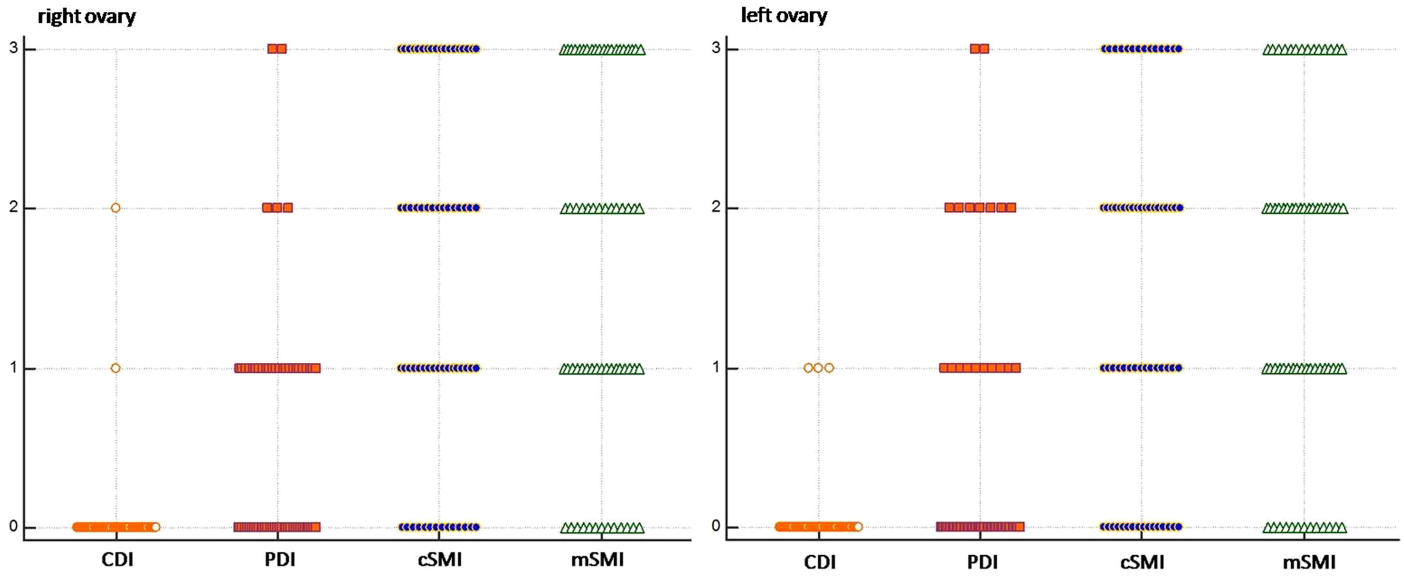

Figure 3. Differences between techniques (CDI, PDI, cSMI, mSMI) according to the vascularity grades (0, 1, 2, 3) in the left and right ovary are shown in graphics (observer 1).

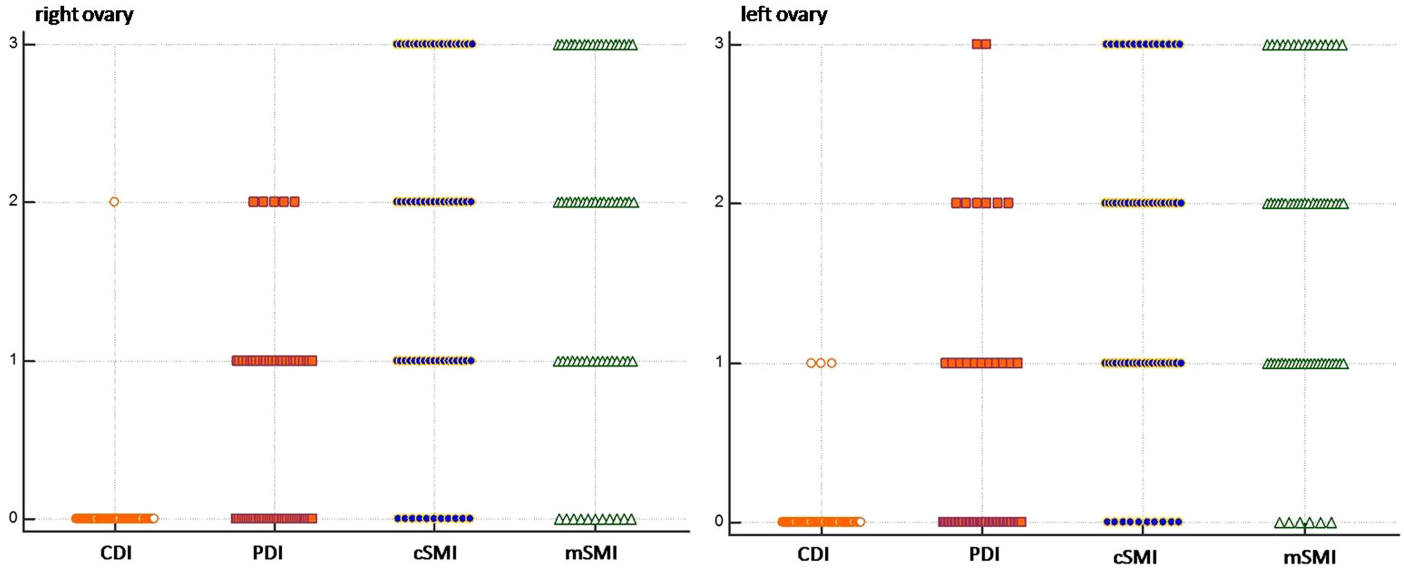

Figure 4. Differences between techniques (CDI, PDI, cSMI, mSMI) according to the vascularity grades (grade 0, 1, 2, 3) in the left and right ovary are shown in graphics (observer 2). CDI, color Doppler imaging; cSMI, color superb microvascular imaging; mSMI, monochrome superb microvascular imaging; PDI, power Doppler imaging.

Table 4. The Inter-Rater Agreement

	CDI		PDI		cSMI		mSMI	
Technique	**κ**	**95% CI**	**κ**	**95% CI**	**κ**	**95% CI**	**κ**	**95% CI**
Observer 1-2 (left ovary)	1.000	1.00-1.00	0.934	0.86-1.00	0.853	0.77-0.93	0.817	0.73-0.91
Observer 1-2 (right ovary)	0.796	0.35-1.00	0.953	0.90-1.00	0.885	0.81-0.96	0.885	0.81-0.96

CDI, color Doppler imaging; cSMI, color superb microvascular imaging; κ: Cohen's kappa value; mSMI, monochrome superb microvascular imaging; PDI, power Doppler imaging.

Table 5. Correlation Between Ovarian Volumes and Doppler Imaging Vascularity Grades

	PP (n = 11)				PT (n = 39)				Puberty (n = 19)			
	Right Ovary Volume	*P*	Left Ovary Volume	*P*	Right Ovary Volume	*P*	Left Ovary Volume	*P*	Right Ovary Volume	*P*	Left Ovary Volume	*P*
CDI	*r* = 0.00	> .05	*r* = 0.12	.723	*r* = 0.45	**.004***	*r* = −0.23	.8936	*r* = 0.42	.1952	*r* = −0.08	.738
PDI	*r* = 0.13	.692	*r* = 0.01	.096*	*r* = 0.42	**.007***	*r* = 0.36	**.0029***	*r* = 0.18	.458	*r* = −0.23	.337
cSMI	*r* = 0.58	**.057***	*r* = 0.78	**.004***	*r* = 0.34	**.031***	*r* = 0.53	**.0007***	*r* = 0.19	.412	*r* = 0.34	0.149
mSMI	*r* = 0.67	**.022***	*r* = 0.85	**.008***	*r* = 0.36	**.023***	*r* = 0.54	**.0006***	*r* = 0.21	.3836	*r* = 0.34	.149

CDI, color Doppler imaging; cSMI, color superb microvascular imaging; mSMI, monochrome superb microvascular imaging; PDI, power Doppler imaging; PT, premature thelarche; PP, precocious puberty; r: Spearman's correlation coefficient.
*$P < .05$.

Intraovarian arterial vessels enlarge or regress in relation to the focal follicular activity, paralleled by a venous system with a pampiniform plexus that directly connects to gonadal veins. Upon ovulation, a vascular ring is formed in the wall of the corpus luteum. Intraovarian vascularity varies with follicular development.[20]

There are few studies about ovarian vascularization with SMI. Ayaz et al. evaluated 146 healthy ovaries of different ages.[7] According to their findings, cSMI and mSMI techniques appear to be more effective in detecting vascularity in girls with healthy ovaries and may provide additional information compared to conventional techniques. They found that although the volume of the ovaries slightly increases after menarche, there was no statistically significant difference in vascularity between pre- and postmenarcheal girls of the same age. In another study, Arslan et al. found that as the ovarian volume increases in the age of healthy volunteers, an increase in vascularity was detected by Doppler methods.[8] In their study, ovarian vascularity grades were higher in PDI than in CDI. They also detected that while both PDI and CDI were insufficient in small ovaries, cSMI and mSMI detected better blood supply in all age groups and ovarian volumes. The findings of our study were consistent with these studies. We found a significant difference between the pre- and postpubertal groups in both ovary volumes and vascularity grades (Figure 5). In our study, while there was a moderate correlation between ovarian volume and vascularity in prepubertal cases (Table 5), in the pubertal group, statistically significant increased vascularity was observed with SMI independent of ovarian volume compared to the prepubertal group. Since intraovarian vessels enlarge with follicular activity,[20] the increased vascularity demonstrated by SMI can be considered a secondary indicator of increased follicular activity in the pubertal ovary.

In our patient population, according to the data obtained from both observers, Doppler techniques were ranked in the following order according to vascularity grades: mSMI > cSMI > PDI > CDI. Our findings were consistent with previous studies and emphasized that SMI has a better potential to demonstrate ovarian vascularity in our study groups. In clinical practice, increased vascularity confirmed with SMI may suggest increased follicular activity and is also useful for assessing the vascularity of ovarian lesions in cases of peripheral precocious puberty.

There are a few limitations to this study. First, the relatively low number of true precocious puberty cases compared to premature thelarche cases. Second, the absence of a healthy control group. Because our study population was composed of referral patients from the Endocrinology department, we could not establish a healthy control group.

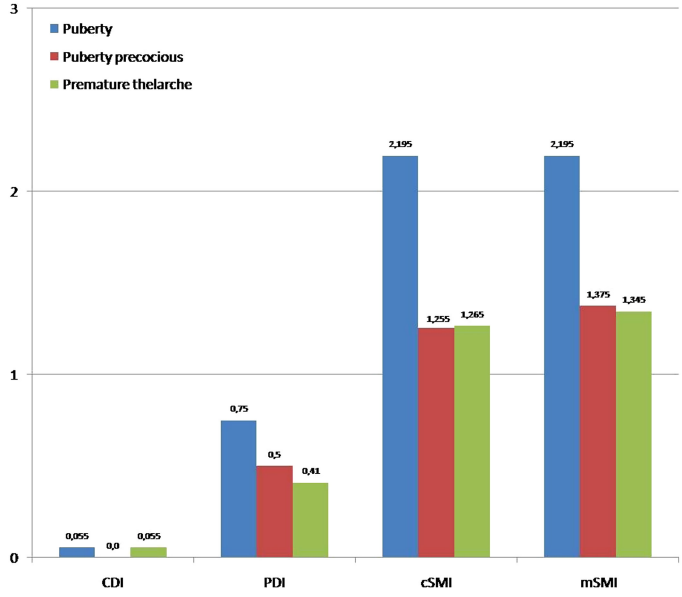

Figure 5. Comparison of the vascularity grading (grades 0, 1, 2, 3) according to techniques (CDI, PDI, cSMI, mSMI) in puberty, precocious puberty, and isolated premature thelarche groups. CDI, color Doppler imaging; cSMI, color superb microvascular imaging; mSMI, monochrome superb microvascular imaging; PDI, power Doppler imaging.

Conclusion

According to our findings, SMI is superior to other Doppler methods, such as PDI and CDI, in the evaluation of ovarian vascularity. SMI is a useful tool in evaluating parenchymal vascularity, especially in children, and can be used as part of imaging in routine practice. Further studies are needed to describe its usefulness and role in the peripubertal pediatric population.

Ethics Committee Approval: Ethics committee approval was received for this study from the University of Health Sciences Ankara Child Health and Disease Hematology Oncology Training and Research Hospital Clinical Research Ethics Committee (Approval no: 2018023, Date: February 26, 2018).

Informed Consent: Written informed consent was obtained from the parents of all patients, and the study adhered to the Declaration of Helsinki.

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Author Contributions: Concept – N.G.A.; Design – N.G.A.; Supervision – A.G.A.; Resources .; Materials – A.G.A., H.A.U.; Data Collection and/or Processing – N.G.A., E.M.U.; Analysis and/or Interpretation – N.G.A., M.S.; Literature Search – E.M.U., A.G.A., H.A.U.; Writing Manuscript – N.G.A., M.S.; Critical Review – S.A.U.

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