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Original article

Comparison of the planimetry and point-counting methods for estimating kidney volume using magnetic resonance imaging

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ABSTRACT

Introduction: Kidney volume (KV) is an important parameter for clinical assessment of patients with diabetes or renal artery stenosis and for assessment of kidney transplant candidates. The purpose of this study was to compare KV estimations obtained by using the Cavalieri principle combined with point-counting and planimetry techniques. In addition, we evaluated the results to construct a confidence interval value for KV according to a new approach.
Methods: The KV of 15 volunteers (30 kidneys) with no known history of renal diseases. Their age ranged from 18 to 25 years. A 3D- fast spoiled gradient-echo dual echo array spatial sensitivity encoding technique axial plan was performed using 1.5-T scanner. We used magnetic resonance (MR) images using the point-counting and planimetry methods to estimate KV.
Results: Kidney volumes obtained by the two different methods were not statistically different and correlated well with each other. The reference values of KV parameters with 95% confidence interval (CI) for lower and upper mean values were 121.50 cm^3 and 144.90 cm^3 respectively. The mean coefficient of error (CE) for KV estimates derived from the stereologic technique was between 0.5 and 1%.
Discussion: For accurate and precise estimation of KV, MR imaging with use of the two methods: should be preferred using our MR protocol. We also evaluated a satisfactory predicted CE values and this provided a relatively narrow confidence interval.

1. Introduction

Kidney volume (KV) estimates correlate with renal function and permits concurrent evaluation of differential renal function.[1] KV is an important parameter for clinical assessment of patients with diabetes or renal artery stenosis and for assessment of kidney transplant candidates. Computed tomography (CT), magnetic resonance imaging (MRI) and three-dimensional (3D) ultrasound can be used to estimate KV using different methods such as voxel-count method, segmentation, and planimetric method.[2,5] Several studies have validated the use of the voxel-count method in estimating kidney and liver volumes by prospectively comparing obtained volumes with water displacement of explanted kidneys and livers.[6,7] KV can be measured by ultrasound, but it needs calculation using ellipsoid formulae of 3D value of the kidney.[6] Moreover, the calculation using ellipsoid formulae tends to underestimate KV.[2] Using CT is time-consuming or require specialized 3D volumetric software for KV.[8,9] Bakker et al.[2] stated that KV calculations obtained by using ultrasound with ellipsoid formula resulted in a substantial systematic underestimation (25%) of the KV compared with those obtained by using MRI with the voxel-count method. In a recent *in vitro* study, the accuracy of MRI and US in measuring the volumes of porcine kidneys was evaluated.[10] The fluid displacement method was used as a gold standard. Volumes calculated with the voxel-count method applied to MRI resulted in no substantial deviation from the true renal volume. Cheong et al.[3] stated that volumes which were calculated by the ellipsoid formula were significantly smaller when they were compared with the MRI disc-summation method. The

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mean KV was approximately 18% less by the ellipsoid method in men and 15% less in women. KV can be estimated using the techniques of planimetry and point counting. Both techniques were used in combination with the Cavalieri method of modern design stereology. Our aim was to compare the efficiency of the volumetric techniques of point counting and planimetry in estimating KV using MRI.

2. Materials and method

2.1. Study population

A total of 15 volunteers, with 7 females and 8 males, with a mean age of 20 years (range 18 to 25 years) were studied. Patients who had no history of renal disease, hypertension, or other vascular disease were included in this study. The volunteers were students from the school of health sciences. All participants were informed about the study, and their written consents were obtained. The official permissions were taken from the university and state hospital administrators.

2.2. MRI data

Three-dimensional fast spoiled gradient-echo dual echo (3D-FSPGR-DE) 2 breath holds array spatial sensitivity encoding technique (ASSET) axial plan was obtained in a 1.5-T scanner (GE Signa Systems, Paris, France). The slice thickness was 5 mm with 1 mm interval. This is a 3D volume gradient echo pulse sequence spoiled with radiofrequency. The sequence was acquired over a period of 4 min. The MRI parameters used were a repetition time (TR) of 170 ms and an echo time (TE) of 15 ms; the ECHO was 1/1 with 16 kHz. The flip angle was 80°, the data set contained image matrix: Matrix of 256 × 256 pixels × 24 slices for an FOV of 42 cm, respectively. Thus, image voxels are 0.09375 × 0.09375 × 6 mm.

2.3. The Cavalieri estimator point-counting method

We use a series of systematic slices of thickness t, with a distance T > t between slice midplanes and with a random start between 0 and T. More precisely, the slices are {[z + kT, z + kT + t], k integer}, where z = U.T and U is uniform random in the interval (0.1). Cavalieri estimator of V is as follows:

$$V = \frac{T}{t}\sum_{i=1}^{n} V_i \quad (1)$$

V_i is the total volume of tissue of slice (which may comprise several slice profiles) in the slab.[11]

MRI series with 5 mm thicknesses (1 mm interval) were used to estimate KV. The transparent square grid test system with d = 0.40 cm between test points was superimposed, randomly covering the entire image frame. The points hitting the kidney sectioned surface area were counted for each section and the volume of the kidney was estimated using the modified formula for volume estimations of radiological images.[12,13]

$$V(PC) = \frac{T}{t}\left[\frac{SU \times d}{SL}\right]^2 x \sum P \quad (2)$$

T is the total section thickness, "t" is section interval, 'SU' the scale unit of the printed film, 'd' the distance between the test points of the grid, 'SL' the measured length of the scale printed on the film

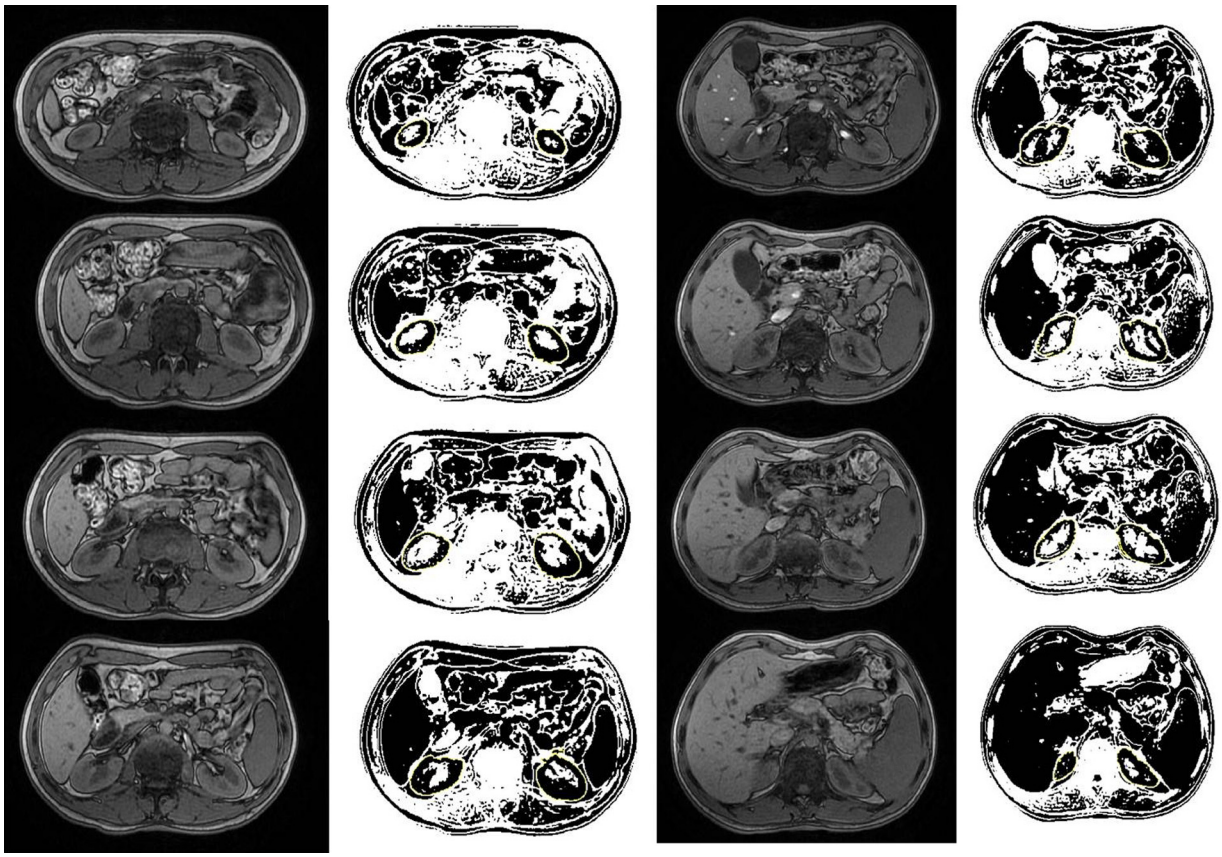

Fig. 1. Calculation of the KV using the ImageJ. Delineation the boundaries of the kidney. Threshold image for the measurement of kidney contour. (a) Original image, (b) Thresholded image.

and ΣP' is the total number of points hitting the sectioned cut surface areas of the kidney. According to this volumetric technique, a square grid of test points was positioned on each MR image, and all points hitting the kidney were counted.[14,15]

2.4. Planimetry method using ImageJ

3D-FSPGR-DE 2 breath holds ASSET axial plan acquisitions were transferred to a computer and further image processing was done using the in-house developed general purpose image analysis software as a plug-in to ImageJ. We used this software for morphometric measurements. It can be downloaded from website.[16] The images were displayed using consistent image and display levels on a monitor with fixed contrast settings. The same observer who carried out the stereological volume estimates performed the KV estimates using planimetry.[17]

The analysis included the following steps: The DICOM files were transformed into a "stack" using the function "Convert Images to Stack"in the submenu "Stacks". The region of interest (ROI) relevant for the present study is the kidney contour. Before outlining the ROI on each kidney border, the ROI manager in the pull-down menu "Analyse > Tools" was opened. The right and left kidney border were manually outlined using the "Polygon selection tool". This tool can create an irregularly shaped selection defined by a series of line segments.[18] The respective ROI of each slice was added to the ROI manager with the function "Add" in the ROI manager menu. To calculate the areas, all the ROIs must be selected in the ROI manager. The area of each ROI was calculated with the function "Measure" in the ROI manager menu. All images were created as masked images and image sequences saved in a BMP format. The outer boundaries of the kidney were delineated using threshold tool and then the wand tool was used to delineate the boundaries of the kidney. We opened threshold tool for true a threshold value. We selected dark background and gray-white value among 77-250.

The sectional cut surface of the structure of interest was measured by the software automatically (Fig. 1).

The KV was calculated according to Eq. (3).

$$V_{LV} = txPx\sum_{i=1}^{N} A_i \tag{3}$$

Here, t represents slice thickness, P denotes the pixel area, N denotes the number of images and Ai represents the number of pixels in the selected region of image i.

2.5. Calculation for confidence interval values

The error predictors given below come from the recent literature.[19–22] We make the calculation steps involved in the estimation of a lower and upper bound values for the KV by applying to the Cavalieri sample.

In particular, the estimation of volume, variance of the volume estimate and bounded intervals for the true volume are calculated as follows. An unbiased estimator of Q can be constructed from a sample of equidistant observations of f, with a distance T apart, as follows:

$$\hat{Q} = T\sum_{k\in z} f(x_0 + kT) = T(f_1 + f_2 + \ldots\ldots f_n) \tag{4}$$

where x0 is a uniform random variable in the interval (0,T) and {f1, f2, . . . , fn} is the set of equidistant observations of at the sampling points which lie in [a, b]. In many applications, Q represents the volume of a structure and f(x) is the area of the intersection between the structure and a plane that is perpendicular to a given sampling axis at the point of abscissa x.[20–22]

This data sample represents the area of kidney in cm^2 on sixteen MR sections a distance T = 0.5 cm, interval t = 0.1 mm apart (Table 1).

The Cavalieri volume estimate is, by applying Eq. (4):

$$Q_T = \frac{0.5}{0.1} \times (7.04 + 14.73 + 14.55 + \ldots\ldots\ldots + 6.73 + 3.15) = 105.72 cm^3$$

To estimate Var (Q̂T) *via* Eq. (5) we first have to calculate (q), C0, C1, C2 and C4. From Eq. (6), we have

$$Var(Q_T) = a(q)(3C_0 - 4C_1 + C_2)T^2 \quad q \in [0,1] \tag{5}$$

$$C_k = \sum_{i=1}^{n-k} f_i f_{i+k} \quad k = 1, 2, \ldots\ldots n-1 \tag{6}$$

Eq. (6) is an extended version of the variance estimator given in.[19,21,23] The quantities C0, C1 and C2 can be computed from the systematic data sample of as follows:

The smoothness constant can be estimated from Eq. (7) as given below.

$$q = \left\{0, \frac{1}{2\log 2}\log\left[\frac{3C_0 - 4C_2 + C_4}{3 \times C_0 - 4C_1 + C_2}\right] - \frac{1}{2}\right\} \tag{7}$$

$$q = \left\{0, \frac{1}{2\log 2}\log\left[\frac{3 \times 3123.94 - 4 \times 2859.74 + 2608.49}{3 \times 3123.94 - 4 \times 3038.69 + 2859.74}\right] - \frac{1}{2}\right\} = 0.53$$

We applied Eq. (8) with = 0.53.

The coefficient (q) has the following expression:

$$a(0.53) = \frac{\Gamma(2_q + 2)\zeta(2_q + 2)\cos(\pi q)}{(2\pi)^{2_q+2}(1 - 2^{2_q-1})} q \in [0,1] \tag{8}$$

where Γ and ζ denote the gamma function and the Riemann Zeta function, respectively.

$$a(0.53) = \frac{\Gamma(3.8)\zeta(3.8)\cos(0.9\tau)}{(2\tau)^{3.8}(1 - 2^{0.8})} = 0.019$$

Table 1
Calculation of the constants C_0, C_1, C_2, C_4 using Eq. (3).

Section, i	Pi	P2	Pi.Pi + 1	Pi.Pi + 2	Pi.Pi + 4
1	7.04	49.56	103.70	102.43	116.02
2	14.73	216.97	214.32	242.75	283.41
3	14.55	211.70	239.78	279.94	292.16
4	16.48	271.59	317.08	330.92	289.88
5	19.24	370.18	386.34	338.43	311.50
6	20.08	403.21	353.21	325.10	307.42
7	17.59	309.41	284.78	269.30	236.59
8	16.19	262.12	247.87	217.76	213.22
9	15.31	234.40	205.92	201.63	199.64
10	13.45	180.90	177.14	175.39	148.49
11	13.17	173.45	171.74	145.40	127.22
12	13.04	170.04	143.96	125.97	87.76
13	11.04	121.88	106.65	74.30	34.78
14	9.66	93.32	65.01	30.43	0.00
15	6.73	45.29	21.20	0.00	0.00
16	3.15	9.92	0.00	0.00	0.00
	211.45	3123.94	3038.69	2859.74	2648.09
		Co	**C1**	**C2**	**C4**

Therefore, the estimate of Var(QT) obtained *via* Eq. (5) is

$$Var(Q_T) = a(q)(3C_0 - 4C_1 + C_2)T^2$$
$$Var(Q_T) = 0.019(3 \times 3123.94 - 4 \times 3038.69 + 2859.74) \times (5)^2$$
$$Var(Q_T) = 38.02$$

The bounded interval for the volume of kidney is obtained by applying Eq. (9). We have:

$$\hat{Q}_T mT\lambda_q \sqrt{a(q)(3C_0 - 4C_1 + C_2} \quad (9)$$

$$\left(105.72 - 3.3 \times \sqrt{0.38}, 105.72 + 3.3 \times \sqrt{0.38}\right)$$
$$= (103.68 - 107.75)cm^3$$

We used the identity $\lambda_{0.53} = 3.3$ according to García-Fiñana.[20] We evaluated a satisfactory predicted interval and it provided a relatively narrow confidence interval. The upper and lower bound are located at approximately 2% the volume estimate for kidney.

We calculated in the estimation of a lower and upper confidence interval values for the KV by applying to the Cavalieri sample. In prior our study, we calculated the CE values.[24]

In this study, we calculated the CE values as predictive using the R program. First, by using the statistical package R, codes were developed to calculate the contribution to the predictive CE.[21]

2.6. Statistical analysis

The results were presented as mean ± standard deviation (SD). The differences of the estimated volumes obtained by two different approaches, namely point-counting and ImageJ planimetry, were compared using paired *t*-test to check the methodological differences. To assess the agreement between the volume measurements of the ImageJ planimetry method and the Cavalieri method, statistical agreement measurements including the concordance correlation coefficient (CCC), intraclass correlation coefficient (ICC), and Pearson correlation coefficient (PCC) were used. We considered ICC > 0.7 to be acceptable. A "p" value lower than 0.05 was considered to be statistically significant.

3. Results

The mean KV values for all subjects that we observed for point counting method and planimetric technique were 133.43 ± 22.08 cm^3 (range 93 to 182 cm^3) and 132.10 ± 22.81 cm^3 (range 89 to 181 cm^3), respectively (Table 2).

The reference values of KV parameters with 95% CI for lower and upper mean values were 121.50 cm^3 and 144.90 cm^3 respectively (Table 3).

An excellent agreement was observed between the two volumetric techniques with mean differences of 1.33 ± 5.10 cm^3. No statistically significant difference was observed between the values obtained from the both techniques (p > 0.001, Table 4).

Both techniques were highly reproducible. The scatter diagrams in Fig. 2 help to compare the performance of the two techniques (point counting and planimetry) at the individual specimen level.

Table 2
Mean KV measurement obtained with point counting and planimetric techniques.

	PC (n = 30)	PL (n = 30)	Difference
Min-Max	93.04–182.77	89.46–181.14	(−15.2)–(12.63)
Mean ± SD	133.43 ± 22.0	132.10 ± 22.7	1.33 ± 5.1

PC: Point-counting, PL: Planimetry.

Table 3
Mean and CI of KV parameters with 95% CI.

	Kidney volume (KV)		95% CI	
	Mean	SD	Upper value	Lower value
PL	132.10	22.7	122.80	143.20
PC	133.43	22.0	121.50	144.90

PC:Ponit-counting, PL:Planimetry.

Table 4
Statistical comparison of the stereological techniques in the whole study population.

	n	Min-Max	Mean	Std. Deviation	Std. Error	p
PC	30	93.04–182.77	133.43	22.0	3.90	0.164
PL	30	89.46–181.14	132.10	22.7	3.94	
PL-PC	30	(−15.2)–(12.6)	1.33	5.10	0.92	

PC: Point-counting, PL: Planimetry.

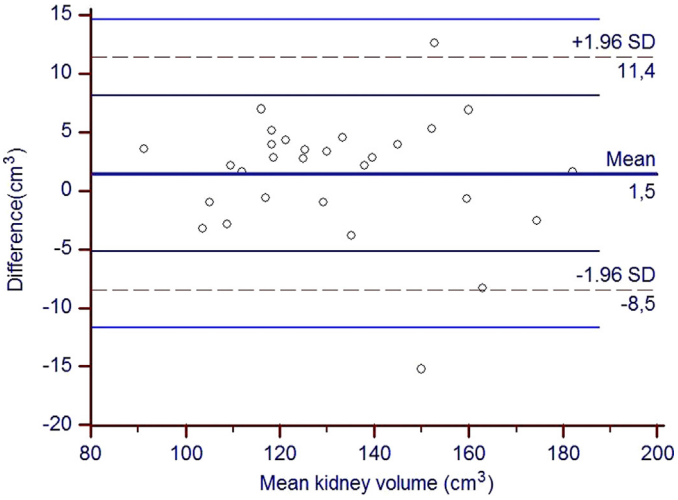

Fig. 2. Bland-Altman plot to demonstrate the agreement of planimetric and point counting methods.

Table 5
KV measurements and gender differences in the whole study population.

	Sex	N	Mean	Std. Deviation	Std. Error	p
PC	Male	16	142.37	22.14	5.53	0.015
	Female	14	123.21	17.66	4.72	
PL	Male	16	142.55	22.59	5.64	0.005
	Female	14	120.15	16.67	4.45	

PC: Point-counting, PL: Planimetry.

The mean KV obtained with planimetric method of the males (142.55 ± 22.59 cm^3) was larger than the KV of the females (120.15 ± 16.67 cm^3). The difference in KV between the genders was statistically significant in both stereological techniques (p < 0.001, Table 5).

The mean right KV obtained with planimetric method (128.47 ± 22.95 cm^3) was larger than the mean left KV (135.73 ± 24.60 cm^3). These differences were not statistically significant in the both stereological techniques (p > 0.001, Table 6).

The mean CE for the KV was for point-counting 2% and for planimetry 5%, respectively. The mean time for estimating the KV using the point counting technique was 4 ± 1.6 minutes (range 3

Table 6
KV measurements and side differences in the whole study population. .

		Mean	N	Std. Deviation	Std. Error Mean	p	Correlation (r)
PC	Right	130.45	15	22.01	5.68	0.223	0.670
	Left	136.40	15	22.51	5.81		
PL	Right	128.47	15	20.95	5.41	0.151	0.681
	Left	135.73	15	24.60	6.35		

PC: Point-counting, PL: Planimetry.

to7 minutes) and for planimetry was 8 ± 2.5 minutes (range 6 to_11 minutes).

Thus, we conclude that an absolute agreement was present between two methods. A perfect agreement, with 0.979 (0.963–0.986) ICC and 0.968 (0.936–0.981) CCC, was observed between ImageJ and point counting method.

The agreements between methods were subjected to Bland–Altman plots using volume differences of 95. This showed that the volumes estimated by Image J and point counting methods differed by 11.4 and $-8.5\,cm^3$ ($P > 0.001$) (Fig. 2).

4. Discussion

There are a lot of studies using the water filling method and stereological measurement for volume estimation in different organs. They use both water displacement and MRI or CT slices. Results of these studies showed a good correlation and there was no statistical difference between techniques.[13,25] Some studies have proven this estimator to be as accurate as digitization-based methods and to correlate closely with displacement volume measurements.[26,27] Sahin et al.[28] stated that there are no statistical differences between the performers and real liver volumes ($p > 0.05$). The mean of volumes determined by the Cavalieri estimator and the water displacement technique were highly correlated and the mean coefficient of correlation (r) was 0.993. Measurement of KV is clinically important because renal mass gives insight into renal function.[5] *In vivo* 3D measurements of renal volume using MRI can provide the most accurate estimate of kidney size. MRI can provide high resolution imaging of the kidneys and collecting system.[4,29] Several authors have reported estimates of normal KV using radiological methods such as MRI, CT, and ultrasound.[3,5,6,10,29]

Various methods of evaluating MRI for KV have been described in these studies. KV has also been assessed using MRI with the voxel-count method *in vitro*.[10] The repeatability of renal volumetry with the voxel-count method with MRI was excellent.[2] Currently the voxel-count method is considered the most accurate noninvasive method for estimating renal volume.[5,26] In contrast to previous reports, the results from our study suggest that there is no significant difference between the left and right sides but there is a significant difference between genders. A systematic slice sampling procedure was performed to estimate KV using both volumetric techniques. The agreement was found between the two techniques in our study. MRI may be uniquely suited for noninvasive evaluation of kidney pathology. Although CT also can provide noninvasive determination of KV, the technique entails substantial ionizing radiation that limits its use as a method of choice for routine noninvasive evaluation, particularly in patients with potential kidney pathology. MRI has the benefit of acquiring true tomographic data along any orientation, without the constraints of ionizing radiation and nephrotoxic contrast burden. Nevertheless, the literature contains few reports of renal dimensions determined by MRI.[1,2,10] In addition, compared to conventional MRI, Axial Dual echo FSPGR ASSET protocol more finer slices, which can minimize disparity between real size and measured size. Axial Dual echo FSPGR ASSET protocol is an established MRI technique that can provide clear images of the KV estimation. So, calculating KV for every kidney related patient is practical if Axial Dual echo FSPGR ASSET protocol is used. The routine MRI such as T1- and T2-weighted sequences are not suitable for KV estimation because of kidney contour is flue. The current clinical practice of using protocol in Axial Dual echo FSPGR ASSET can be improved on by the point counting and planimetric techniques *via* MRI, providing more accurate data for clinical decision- making. There is no study reporting the confidence interval values for KV estimation. This is the first study that applied the confidence interval calculation using stereology for KV. We evaluated a satisfactory predicted interval and it provided a relatively narrow confidence interval.

In this study, we report that the above MRI protocol could be used to measure KV in humans. By using MRI to estimate KV, we found that we were able to show good intra-observer reliability and performed well compared to two measurements.

Both techniques could be considered as a more efficient approach for estimating KV from MRI, due to its speed and simplicity. We think that our results will contribute to volumetric studies which evaluate the development, pathology, and abnormalities of KV.

Conflict of Interest

The authors declare that there is no conflict of interest.

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