Stereological Estimation of Bone Cyst Volume Using Computed Tomography Images: A Comparison with the Planimetry Technique

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ABSTRACT

Objective: The exact volume of the bone cyst is fundamental for evaluation, treatment, and surgical management of the pathology related to any bone. The aim of this study was to introduce the stereological point-counting technique in bone cyst volume measurements on computed tomography images and to compare it with the planimetry technique.

Methods: A total of 30 bone cyst volumes were estimated on CT images using stereological point-counting and the planimetry technique, which is based on the Cavalieri principle. The planimetric measurements were regarded as reference values. The stereological and planimetric volume estimations were performed by two independent observers twice. The estimation results of the two volumetric techniques were compared with the Wilcoxon signed-rank test. Intra-observer and inter-observer reliability of each volumetric technique was assessed.

Results: For each bone cyst, 7-11 systematically sampled CT slices enabled reliable cyst volume estimations with a low coefficient of error (0.39%-3.12%). There was no significant difference between point counting and planimetry methods regarding volume measurements in both sessions (p>0.05), and these methods correlated well with each other. There was a significant inter-and intra-observer agreement for each volumetric method (ICC=0.9984 to 0.9988). The stereological approach was observed to take less time than the planimetric approach (mean 01:43 \pm 0.44 vs. 03.33 \pm 1:47 minutes)

Conclusion: The stereological point-counting method can be well pertained to CT images for the reliable and reproducible assessment of bone cyst volume. Application of the point-counting method for volume estimation of bone cysts with different morphological features provides a great advantage in terms of both time-saving, applicability, and practicality in comparison with the planimetry technique.

Keywords: Bone cyst, computed tomography, planimetry, stereology, volume measurement

INTRODUCTION

Bone cysts are clear fluid-filled cystic lesions that tend to expand and weaken the bone. This benign lesion represents one of the most frequent osseous lesions. The cysts have a predilection to occur in males more frequently than in females. Bone cysts are one of the most frequently seen osseous lesions, and most commonly observed in the period from birth to 20 years of age (1,2). Common locations include the proximal humerus and femur, although any section of any long bone may be involved (3). Most bone cysts are asymptomatic and detected incidentally on imaging. However, cysts may also be diagnosed because of pain, which may reflect a microscopic pathological fracture as a result of a minor trauma (4). Treatment methods include intralesional injections, decompression, and combined surgical techniques (5).

The volume of benign bone lesions such as a bone cyst or enchondroma is fundamental for evaluation, treatment, and surgical management of the pathology related to any bone (6). As most benign lesions respond well to surgical removal, surgical treatment is one of the preferred options for the management of benign lesions. Precise information of cyst volume is often required to able to establish the healing process of the cyst as cyst volume is closely pertaining to pathological fracture risk (7). The exact volume of the cyst is also necessary for the planning of surgical treatment, especially to estimate the amount of graft required to fill the defect. Therefore, a technique for accurate and reliable estimation of the cyst volume has to be clinically oriented (6,7).

According to the Cavalieri principle, the two main volumetric methods for estimating the volume of anatomical structures are point-counting and planimetry techniques. In the Cavalieri principle, the volume of any structure can be estimated using 2-dimensional parallel sections separated by a definite distance (8).

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The point-counting technique of modern design stereology is based on surface area estimation of a region of interest on cross-sectional images via the simple method of point counting. The stereological technique is an accurate and effective approach, which can be utilized to safely assessing the volume of biological structures (9). Planimetry is an approach for estimating the sectional area particularly by tracing the boundaries of the object of interest and is regarded as a valid method for volume estimation (10).

The goals of this study were to introduce combining stereological point-counting technique with computed tomography (CT) image sections for bone cyst volume estimation, and compare the stereological measurements with those obtained with the planimetry technique.

METHODS

CT Scans

Before the study commenced, approval was obtained from the Institutional Ethics Review Board of our institution (Decision No: 611-2019). After obtaining Institutional Ethics Review Board approval for this study, we conducted a retrospective analysis of preoperative CT scans of 30 patients with varying sizes and shapes of the simple bone cysts, which were selected from the archives of the Department of Orthopedics and Traumatology, Faculty of Medicine, 19 Mayıs University, Samsun, Turkey. The 30 patients comprised 12 males and 18 females, with a mean age of 24.47±13.30 years. Axial plane CT scans of the bone cysts were obtained using a helical CT scanner (Toshiba Aquilion, Canon Medical Systems Corporation Toshiba, Dalian) with the following parameters; thickness: 1 mm, 120 kVp, 150-220 mAs. The axial image series were transferred to Digital Imaging and Communication in Medicine (DICOM) viewer software (Horos v.1.1.7, Purview, Annapolis, MD 21401, USA). In order to determine the superior and inferior margins of the cysts, CT scans were reconstructed in the coronal plane. Afterward, CT scans were divided into 1 mm sections without the slice gap in the coronal plane and recorded as separate DICOM files. Simple bone cysts with pathological fractures were not included in this study because the determination of the boundaries of the cysts was not possible on CT images. The localization of the cysts was 11 in the femur, 7 in the tibia, 4 in the radius, 3 in the humerus and fibula, and 1 in the metacarpal bone and calcaneus. Stereological and planimetric volume measurements were performed by utilizing ImageJ (ImageJ,1.37v: http://rsb.info.nih.gov/ij/) software, which is distributed free of charge by the National Institutes of Health, USA.

Main Points:

- Stereological point-counting technique is an efficient alternative approach for estimating bone cyst volume.
- Stereological bone cyst volume estimations from computed tomography images are practical, rapid, reproducible and accurate.
- The application of point-counting technique to measuring cyst volume reduces processing time.

Volume Estimation Techniques Planimetry Technique

The cyst boundaries were manually delineated on each CT image using the polygon selection tool of the software (Figure 1). The sectional surface area of the cyst was automatically calculated by the program for each slice. The total volume of the bone cyst was calculated by multiplying the obtained sectional surface areas by the slice thickness. The total volume of the bone cyst can be estimated using the following formula (10). Where T is the slice thickness of consecutive sections, a_i is the area of the cyst outlined in section i, and m is the total number of slices containing the region of interest.

In the planimetry technique, the coefficient of error (CE) was estimated with the formula described in previous studies (11). All calculations were recorded in a Microsoft Excel worksheet where the final data were automatically obtained by entering the section thickness and surface area of the structure into the worksheet. The mean time required to complete planimetric volume

Figure 1A. A coronal CT image showing a bone cyst in the proximal femur of patient no 28.



Figure 1B. Delineation of the borders of the bone cyst for planimetric volume measurement.



estimations was recorded. In the present study, planimetric volume estimates were regarded as reference values.

Stereological Technique

In the point-counting technique, instead of tracing the boundary of the region, area estimation was performed by superimposing a point-counting grid on the sections. By systematically sampling CT slices and regulating an optimum distance between test points of the grid, the point-counting technique may be optimized (12). This systematic slice sampling procedure is dependent on using a minimum number of systematically sampled slices to ensure precise and reproducible volume measurements with minimal user intervention (10). In the current study, the bone cyst was depicted on mean of 17.70±6.9 images (range 7-32). The sample types 1/2 and 1/3 were selected for stereological volume estimation. Finally, 7-11 CT sections including the cyst were obtained. Prior to the study, the point-counting grid with d= 0.7, 0.8, 0.9, and 1.0 cm between the test points, i.e. 0.49, 0.64, 0.81, and 1.00 cm² symbolizing area per point was tested on the first ten patients to determine proper point spacing. The point-counting grid with d=0.9 cm between the test points was selected as it enabled the determination of the bone cyst volume with CE<2%.

The grid was randomly overlaid on each CT image by the software and the number of test points accommodating within the cyst area was counted by the examiner on each section (Figure 2). According to the stereological technique, the bone cyst volume was assessed with the following formula (Figure 3) (13).

Where T is the section thickness (including interval) of consecutive sections, A represents the area associated with each test point, m is the number of sections depicting the cyst and Pi is the number of points lying within the cyst on section i.

The accuracy of volume assessment of any object using the stereological approach may be defined to obtain a CE<5% as described in previous studies (14). Calculation of the cyst volume, estimation of the CE value, and other associated data were implemented on MS Excel worksheets, where the results were automatically obtained by entering the point count, section thickness, and other related data into the worksheet. The mean time required to perform the stereological volume estimations was recorded.

The two observers independently estimated the bone cyst volume using both volumetric methods twice at an interval of one month to reduce recall bias. Each observer was blinded to the results of the other and to their own previous measurements of the same images for each measurement method. Prior to the study, 30 bone cysts were morphologically evaluated by an orthopedist (FS) in order to evaluate the efficacy and applicability of the volumetric techniques on cyst volume measurements.

Statistical Analysis

Version 22 of Statistical Package for Social Sciences for Windows software (SPSS, Chicago, IL, USA) was used for statistical analysis. Continuous variables were expressed as mean±standard deviation (SD), and min-max where appropriate. Conformity of the volumetric data to normal distribution was tested using the Shapiro-Wilk test.

Figure 2A. The point counting grid was superimposed to cover the entire cyst projection area randomly.



Figure 2B. The number of points within the area of the cyst were selected and counted for stereological volume estimation.



Figure 3. 3D-model of the segmented bone cyst for estimation of the total volume.



The Wilcoxon signed-rank test was conducted to detect statistical differences between the stereological and planimetric volume estimations obtained by the two observers. The intraclass correlation coefficient (ICC) (two-way mixed model) was calculated to define the intra- and interobserver reliability of each volumetric technique. The relationship between the estimation results of the two methods was examined using the Spearman correlation coefficient. Bland-Altman plots were generated to define 95% limits of agreement between the point-counting and planimetry methods. In addition, consistency between the two methods of measurement was assessed with Passing-Bablok regression analysis. A P value<0.05 was considered to be statistically significant for all tests.

RESULTS

There was no significant difference in age between male and female patients (P=0.638). The overall mean bone cyst volume estimated by the two observers using the optimized point-counting and planimetry methods was 20.71 ± 19.60 cm³ and 20.65 ± 19.60 cm³, respectively.

Evaluation of Stereological Volume Measurements

The mean cyst volume obtained using the stereological technique by the two observers in the first and second sessions was 20.67 ± 19.62 cm³ and 20.75 ± 19.74 cm³, respectively. According to the results of the Wilcoxon signed-rank test, there were no significant differences between each observer's estimation results in both sessions (p>0.05) (Table 1). There were no significant differences between the estimation results of the two observers in both the first and second sessions (p=0.651, p=0.829, respectively).

The ICC showed a high degree of intra-observer agreement in the stereological estimation for both the first and second observer (ICC=0.9986, p<0.001; ICC=0.9984, p<0.001, respectively). There was high inter-observer agreement in the stereological estimation for the first and second sessions (ICC=0.9988, p<0.001; ICC=0.9988, p<0.001; ICC=0.9988, p<0.001 respectively). The mean CE of the volume estimations obtained using stereology was 1.43±0.61%.

Evaluation of Planimetric Volume Measurements

The mean cyst volume obtained using the planimetry technique by the two observers in the first and second sessions was 20.67 ± 19.70 cm³ and 20.62 ± 19.67 cm³, respectively. Based on the

results of the Wilcoxon signed-rank test, no statistical difference was found between each observer's estimation results in both sessions (p>0.05) (Table 2). No statistically significant differences were determined between the planimetric volume estimates of the two observers in the first and second sessions (p=0.341, p=0.382, respectively).

The ICC showed a high degree of intra-observer agreement in the planimetric estimation for the first and second observer (ICC=0.9988, p=0.001; ICC=0.9988, p<0.001, respectively). Inter-observer agreement of the planimetric volume measurements was found to be almost perfect for the first and second sessions (ICC=0.9992, p<0.001; ICC=0.9991, p<0.001, respective-ly). The mean CE of the volume measurements estimated using the planimetry method was 1.36 ± 0.66 %.

Comparison of Two Volumetric Techniques

There was no statistical difference between the stereological and planimetric volume measurements obtained by each observer in the first and second sessions (p=0.711, p=0.658; p=0.339, p=0.666, respectively). Total cyst volume obtained using stereology by the first observer in both sessions was highly correlated with those obtained with the planimetry technique (r=0.997, p=0.001; r=0.996, p=0.001, respectively). The stereological estimation results of the second observer in both sessions showed a high correlation with those obtained from the planimetry method (r=0.995, p=0.001; r=0.996, p=0.001, respectively).

Bland-Altman Analysis revealed that both volumetric techniques were in good agreement for each observer and each session. The mean difference of cyst volume obtained using stereology and planimetry by the first observer in the first session was -0.07 cm³, and the limits of agreement were -1.60 and 1.47 cm³. The mean difference of cyst volume estimated using stereology and planimetry by the second observer in the first session was 0.16 cm³. The limits of agreement were -1.31 and 1.62 cm³. The mean difference of cyst volume evaluated with stereology and planimetry by the first observer in the second session was 0.07 cm³ and limits of agreement were found to be 1.41 and -1.55 cm³. The mean difference of the cyst volume estimated using stereology and planimetry by the second observer in the second session was 0.09 cm³ and limits of agreement were found to be -1.45 and 1.63 cm³ (Figure 4).

Table 1. The results of the bone cyst volume (cm3) estimations obtained using stereology in two sessions						
Observer	Mean ± SD	Min-max	Mean ± SD	Min - max	P value	
	First Session		Second Session			
Observer 1	20.66 ± 19.83	1.94 - 82.82	20.74 ± 19.90	2.08 - 83.60	0.551	
Observer 2	20.68 ± 19.74	2.18 - 82.62	20.76 ± 19.93	2.04 - 84.62	0.572	

Table 2. The results of the bone cyst volume (cm3) estimations obtained using planimetry in two session	ns
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Observer	Mean ± SD	Min-max	Mean ± SD	Min - max	P value
	First Session		Second S	Second Session	
Observer 1	20.72± 19.88	2.22 - 84.23	20.58 ± 19.74	1.79 - 82.60	0.162
Observer 2	20.61 ± 19.86	2.02 - 83.58	20.67 ± 19.94	1.88 - 83.32	0.517

Session	Observer	Intercept	95% CI	Slope	95% CI	Linearity
Session 1	Observer 1	- 0.31	- 0.51 to - 0.05	0.99	0.98 to 1.02	Yes
	Observer 2	0.37	- 0.17 to 0.61	1.00	0.99 to 1.02	Yes
Session 2	Observer 1	0.24	- 0.21 to 0.45	0.99	0.97 to 1.01	Yes
	Observer 2	0.23	0.08 to 0.47	0.99	0.97 to 1.01	Yes

Table 3. Passing–Bablok regression analysis results for measurement agreement between the stereology and planimetry techniques in the first and second sessions

Figure 4A. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the first observer in the first session. The dashed line represents the 95% limit of agreement.



Figure 4C. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the first observer in the second session. The dashed line represents the 95% limit of agreement.



Figure 4B. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the second observer in the first session. The dashed line represents the 95% limit of agreement.



Figure 4D. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the second observer in the second session. The dashed line represents the 95% limit of agreement.



The Passing-Bablok regression analysis indicated close agreement between the stereological and planimetric volume estimations for each observer in both sessions (Figure 5). The Cusum linearity test indicated a linear relationship between the volume estimates of the two measurement methods in all comparisons (p>0.05). Details of the Passing-Bablok regression analysis in the comparisons of the two techniques are given in Table 3. The mean time required to perform stereological volume analysis was 01:43±0:44 minutes (range, 0:41-03:10). The mean time needed to complete volumetric analysis of the cyst using planimetry was 03:33±1:47 minutes (range, 1:28-06:43).

Figure 5A. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the first observer in the first session. The solid blue line represents the regression line. Dashed brown lines indicate the confidence interval for the regression line. The dotted line shows the identity line. Orange dots represent measured samples.



Figure 5C. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the first observer in the second session.



Evaluation of the Bone Cysts in Respect of Morphological Characteristics

The 30 bone cysts were separated into 4 main groups according to the morphological characteristics. Group 1 included complete bone cysts with regular borders (example patient no 28-Figure 1). Group 2 included bone cysts that were complete but the borders were irregular (example patient no 18-Figure 6). Group 3 included bone cysts separated into two or more sections (example patient no 16-Figure 7). Group 4 included bone cysts which appeared as many separate independent cystic islets (example patient no 24-Figure 8).



Figure 5D. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the second observer in the second session.



Figure 6A. Application of the planimetry technique in volume estimation of the bone cyst, which has an irregular shape located in the distal femur of patient no 18.



Figure 7A. Application of the planimetry technique in the volume measurement of the bone cyst, which is divided into sections by two septum located in the distal femur in patient no 16.



Figure 8A. Application of the planimetry technique in the volume measurement of the bone cyst which consists of a large number of cystic islets in the distal fibula in patient no 24.



Figure 6B. Application of the point-counting technique in volume estimation of the bone cyst, which has an irregular shape located in the distal femur of patient no 18.



Figure 7B. Application of the point-counting technique in the volume measurement of the bone cyst, which is divided into sections by two septum located in the distal femur in patient no 16.



Figure 8B. Application of the point-counting technique in the volume measurement of the bone cyst which consists of a large number of cystic islets in the distal fibula in patient no 24.



DISCUSSION

The natural history of bone cysts has not been fully clarified. Active cysts are those which are closely adjacent to a growth plate and when localized at a distance from the growth plate, those are known as latent cysts. An active cyst in a child can be seen to grow in volume throughout the period of natural growth and development of the child. In the evaluation of radiographic healing following an intervention, knowledge of the cyst volume is crucial. Preoperative evaluation of cyst volume allows the preparation of a sufficient amount of therapeutic agent to fill the defect. Thus, in clinical practice, accurate and reliable estimation of the cyst volume is important (7).

Several studies have been conducted for volume estimation of similar pathological lesions using different imaging techniques. Gobel et al. (15) have calculated tumor volume in patients with Ewing sarcoma by assuming an ellipsoidal or cylindrical configuration on X-rays and CT scans. Similarly, Glowacki et al. (16) also utilized the above technique for the evaluation of solitary cyst volume using X-ray images. In another study, Docquier et al. (17) described a semi-automatic segmentation method for the measurement of bone cyst volume from magnetic resonance images. As the reliability and clinical applicability of the suggested methods have been shown to be limited, none of those approaches assessing bone cyst or lesion volume has been absolutely acknowledged.

The point-counting technique has been reported in the literature as a good alternative to the conventional planimetric technique for estimating the volume of various organs or structures (11,18,19). In those studies, the stereological technique has provided very similar results to those of the planimetry technique and it has shown a strong correlation with the planimetric approach.

In the current study, the stereological point-counting technique is introduced for the assessment of bone cyst volume on CT images. The study results showed that total cyst volume estimated by stereology in both sessions was not statistically different from the values obtained with planimetry (p>0.05) and stereological volume estimates displayed a high correlation with the planimetry values (ranging between 0.995 and 0.997). Bland-Altman plots indicated that the 95 % limits of agreement between the two volumetric methods were quite narrow and clinically acceptable. In addition, Passing-Bablok regression analysis showed close agreement between the stereological and planimetric volume estimations for each observer in both sessions. No significant deviation from linearity was observed (p>0.05). With respect to these results, the point-counting and planimetry techniques seemed to show sufficient agreement to be able to be used interchangeably for volume estimation of bone cysts.

Previous studies that have compared the two techniques have generally measured the volumes of structures and organs that have a holistic structure and specific shapes, such as the eye, brainstem, spleen, and liver (18,20,21). Unlike previous studies, in this study, the volume was measured of 30 bone cysts different from each other in size and structure. For example, as in the bone cyst situated at the proximal end of the femur of patient no 28, some of the bone cysts were whole and the borders were very regular. In such a case, both methods may be suitable for measuring cyst volume in respect of applicability and efficacy.

In other cases, although the bone cyst was complete, it may have quite irregular, indented, and protruding edges, as in patient no 18. Or the borders of the cyst may not be very obvious. In these cases, manually drawing the complex boundaries of the cyst on all CT sections using planimetry, is time-consuming and requires skilled users, while the stereological method has a great advantage in volume measurements as it uses the simple and fast process of point counting.

Some bone cysts may be divided into two or more sections by a septum located between the cysts as shown in patient no 16, or a cyst may consist of a large number of islets that appear to be independent of each other, as in patient no 24. If a cyst is divided into two or more cystic areas by a septum, the planimetric approach is more time-consuming as compared to the point-counting approach since the process of tracing the boundaries of the cystic areas is longer and laborious. If the bone cyst consists of a large number of lesions that seem to be independent of each other, it is necessary to trace the boundary of each cyst area individually. Application of the planimetry is both time-consuming and difficult in this condition. In both cases, the point-counting method offers a very practical, fast, and effective measurement opportunity as compared to the planimetry method.

Since the point-counting method relies on the simple and fast process of counting, the short processing time for volume estimation is its main benefit. Sahin and Ergur (21) reported that the point-counting method takes less time as compared to the planimetric method (mean 5:37 vs. 7:22 minutes) for the assessment of liver volume on MR images. Acer et al. (22) showed that the stereological technique is 30% faster than the planimetric technique for the assessment of total intracranial volume. In another study by Acer et al. (23), it was stated that the application of stereology to estimate cerebellum volume reduces processing time in comparison with the planimetry method (mean $8\pm3:6$ vs. $15\pm5:5$ minutes). In the current study, the time taken for stereological volume estimations was 48% shorter than planimetric volume estimations.

The stereological technique offers researchers the opportunity to make suitable changes in their sampling strategies and point density of the grids by assessment of the CE. A CE <5 % is in an acceptable range. Determination of the suitable grid size and the number of slices necessary for volume assessment of an object is essential at the beginning of the procedure as there is no need to calculate CE for repeated measurements. Mazonakis et al. (24) stated that bladder and rectum volume calculations can be performed on 5-7 CT image slices obtained by the adoption of the 1/3 systematic sampling procedure. In another study, Mazonakis et al. (18) also reported that 5-8 systematically sampled slices provided enlarged splenic volume measurements while 4-7 systematically selected slices enabled a precise normal splenic volume measurement. Manious et al. (10) suggested that abdominal fat volume may be estimated on only 6 CT slices by means of a systematic sampling process. In the present study, the bone cysts of patients were depicted on a varying number of CT images ranging from 7 to 32. Therefore, a sample type of 1/2 and 1/3 was performed to obtain the minimum number of slices containing the bone cyst. The results showed that 7-11 systematically sampled CT slices per bone cyst provided reliable cyst volume estimations with a low coefficient of error (0.39%-3.12%). In the point-counting technique, the slice sampling procedure allows the minimization of user interaction by providing a minimum number of systematically sampled CT sections for volume measurements. Stereological volume measurements are independent of cyst shape, which may vary widely among individuals and do not require user experience in outlining cyst contours.

This study was also designed to evaluate intra- and interobserver reliability and accuracy of the point-counting and planimetry methods for bone cyst volume measurement. There was high agreement in the intra- and interobserver results both in the planimetry and point-counting techniques (with all ICC values>0.998). Therefore, these techniques can be considered reliable techniques with low intra- and interobserver variation in cyst volume estimations.

Relatively higher doses of radiation exposure of the patients with CT imaging is a limitation of this technique. However, particularly for the bone cysts localized in the spine or pelvis as they cannot be easily identified on plain X-Ray images, CT scanning is recommended for initial evaluation. Another indication for CT is when there is a high index of suspicion regarding the structural integrity of a weight-bearing area (25,26). Nevertheless, it must be kept in mind that for pediatric patients, repeated CT evaluations are less desirable. Measurement and evaluation of only 30 CT images and differences between male and female patients in terms of the number are other limitations of the present study.

CONCLUSION

In conclusion, the stereological approach, based on point-counting and systematic sampling, may be efficaciously applied to CT images for identifying bone cyst volume. The estimated volumes of the point-counting technique were compatible with the results of the planimetry technique, which is a robust indicator of the accuracy of this technique. In addition to providing trustworthy and accurate estimations of bone cyst volume, the stereological point-counting technique provides a great advantage in terms of both time savings, applicability, and practicality for the volume estimation of bone cysts showing different morphological features.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University of School of Medicine (2019/611).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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