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Research article

Evaluation of the follicular space volume of lower third molars with different impaction positions and angulations: A cone-beam computed tomography and histopathological study

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ABSTRACT

Objective: To quantify the volume of the follicular spaces of impacted lower third molars (ILTMs) with different impaction positions and angulations using cone-beam computed tomography (CBCT) and to determine its association with the histopathological findings.
Study design: This study included 103 ILTMs of 33 men and 70 women aged 18–46 years (mean age, 29.18 years). The follicular space volumes were measured on CBCT by manual segmentation and correlated with the histopathological diagnosis of each ILTM having different impaction positions and angulations. Statistical Product and Service Solutions, version 24, was used for statistical analyses by applying the *t*-test and binary logistic regression and multiple linear regression tests ($p < 0.05$).
Results: Overall, 83 (80.6%) dental follicles presented a non-pathological diagnosis (mean follicular volume, 0.10 cm^3), whereas 20 (19.4%) presented a pathological diagnosis (mean follicular volume, 0.32 cm^3; $p = 0.001$). Similarly, the impaction depth in Position C cases was associated with a pathological diagnosis ($p = 0.010$).
Conclusion: The follicular volume of the ILTMs varied significantly in teeth with a histopathological diagnosis of a follicular cyst and was associated with the impaction depth, mainly in Position C cases, and its relationship with the mandibular ramus. A mean follicular volume of 0.32 cm^3 was associated with a greater probability of a pathological diagnosis.

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1. Introduction

The surgical removal of impacted lower third molars (ILTMs) is one of the most common procedures performed in oral and maxillofacial surgery [1]. The prevalence of ILTMs is approximately 24.40% [2], and the most frequently observed position is the mesioangular inclination, with an incidence of 41.17% [2]. Similarly, the dental follicle (DF) is a component of the tooth germ, which encompasses the crown of the impacted tooth and plays an essential role in tooth growth and eruption [3]. The DF space is seen as a pericoronal radiolucency with a width of 2–2.5 mm on radiography [4].

An impacted tooth maintains its DF, and its epithelium retains the ability to differentiate; this can progress toward odontogenic pathologies, such as the formation of dentigerous cysts, odontogenic keratocysts, and ameloblastomas. The prevalence of cystic and tumor pathologies is low, accounting for approximately 2% of all cases [5].

It is clinically accepted that follicular spaces (FS) measuring less than 2.5 mm are not considered pathological [6,7]. However, this is based on studies in which the FS size was measured on two-dimensional radiographs, mainly periapical or panoramic radiographs. Nevertheless, after excision, all DFs should be analyzed histologically for pathologies. Moreover, the amount of FS remains a matter of debate and research because numerous studies have found cystic changes in FS measuring less than 2.5 mm on radiographs. Haidry et al. [8] reported cystic changes in 24% of the DFs of ILTMs having normal dimensions. A study by Baykul et al. [9] showed cystic changes in 50% of the cases, which was very similar to the 46% reported by Saravana and Subhashraj [10]. Conversely, Güvenet et al. [11] described pathological changes in the DFs in 2.3% of cases, while Yadav et al. [12] reported changes in 4.44% cases. Thus, early diagnosis of small pericoronal radiolucent images of non-erupted teeth remains controversial, as does whether most asymptomatic ILTMs can be retained in the mouth [3,4]. FS measuring greater than 2.5 mm should be considered an indication of a pathological change [4]. Nonetheless, scientific evidence supporting this hypothesis is limited, and there is no internationally accepted consensus to differentiate between normal and pathological FS based on radiographic characteristics [8]. Moreover, no reference values are currently available for the volume of the FS in "cm^3" obtained using cone-beam computed tomography (CBCT) to support the advantages of this technique, such as gives undistorted three-dimensional images with a very good resolution that allows visualization of anatomical structures shape and their real size. Therefore, this study aimed to quantify the FS volume of ILTMs with different impaction positions and angulations using CBCT and to determine its association with the histopathological findings.

2. Materials and methods

Participants were informed about the objectives of the study and written informed consent was obtained. The study was approved by the Ethics Committee for Human Studies, Iberoamericana University (UNIBE), Dominican Republic (certificate number: CEI2022-19).

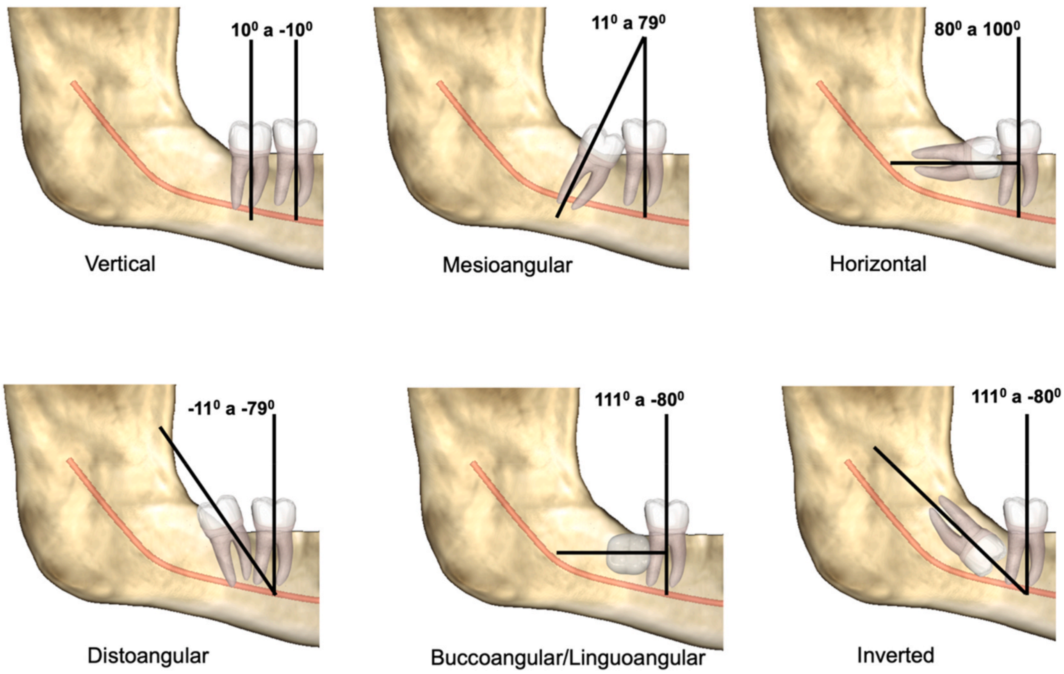

Fig. 1. Winter classification.

2.1. Characteristics of the sample

This prospective cross-sectional study included patients referred to the Dentomaxillofacial Imaging Center in Santo Domingo, the Dominican Republic for CBCT before third molar extraction.

The inclusion criteria were CBCT performed between 2021 and 2022 for participants over 18 years of age with ILTMs in the vertical, mesioangular, horizontal, and distoangular positions according to the Winter classification (Fig. 1), Position B or C according to the Pell & Gregory classification (Fig. 2), or Class I, II, or III according to the Pell & Gregory classification (Fig. 2). The sample consisted of all the patients who attended the imaging center with an indication for dental extraction of third molars and complied with the inclusion criteria.

The exclusion criteria were ILTMs in the vestibular-lingual or other positions (111° to −80°) according to the Winter classification (Fig. 1) and Position A according to the Pell & Gregory classification (Fig. 2). We evaluated DFs partially and wholly covered by the bone as well as ILTMs with macrodontia or microdontia alteration, those with obvious adjacent pathologies, and those with adjacent artifact areas on CBCT.

2.2. Histopathological study of dental follicles

After extraction, the ILTM follicles were immediately fixed in 10% formalin and sent to the Department of Oral Pathology for further processing and staining. Histological evaluations of all samples were performed using a research-grade Swift SW380T trinocular optical laboratory microscope (Swift Optical Instruments, Inc. Schertz, Texas, TX, USA) with a 10X/25X wide-field eyepiece, mechanical setting, ultra-precise focus, and 40–2500X magnification.

The characteristics of the histopathological evaluation are described below.

Type of epithelial tissue:

a. Reduced enamel epithelium
b. Stratified squamous epithelium

Type of connective tissue:

a. Loose fibrous tissue
b. Dense fibrous tissue

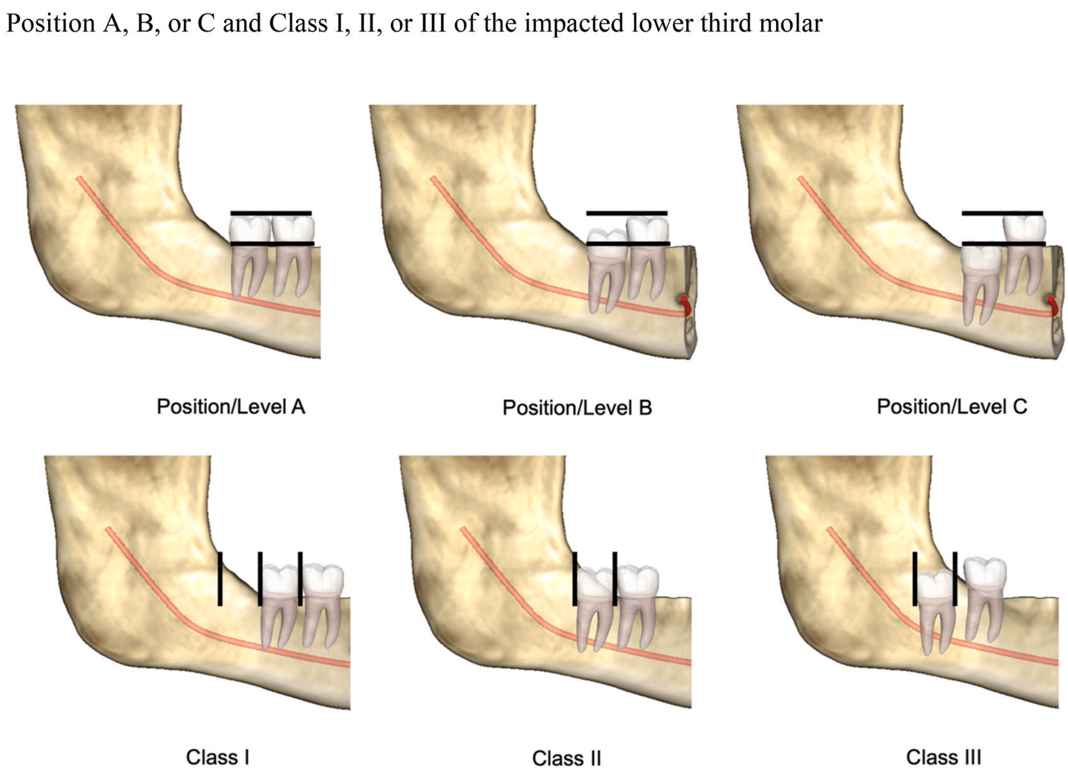

Fig. 2. Pell & Gregory classification.

The following histopathological criteria were used to differentiate between normal and pathological DFs based on the studies by Saravana and Subhashraj [10] and Adelsperger et al. [4]:

a. Normal DFs comprised reduced enamel epithelium and loose fibrous connective tissue (Fig. 4B).
b. Any DF that showed the presence of stratified squamous epithelium and dense connective tissue was considered pathological (Fig. 5B, D).

The samples were categorized as normal follicles or pathological follicles according to the histopathological results.

2.3. Image acquisition

CBCT images were taken with the Promax 3D MID equipment (Planmeca, Helsinki, FINLAND) using exposure values of 8 mA, 120 kV, field of view of 20 cm × 10 cm, voxel resolution of 0.2 mm, and exposure time of 12.076 s. Images were reviewed and analyzed on an iMac monitor (Retina 5K, 27-inch, N2020).

2.4. Measurement of the FS

The ILTMs were classified according to the Winter [13] and Pell & Gregory classifications [14] (Figs. 1 and 2). In the "explorer" window of the three-dimensional (3D) module of the Romexis 6.2 software, the sagittal plane was reoriented according to the major axis of the ILTM in the coronal and axial planes. The slice thickness was increased to 10 mm using the angle measurement tool. One line was drawn along the long axis of the ILTM and another along the length of the second molar, and the angle was calculated. The ILTM was classified according to the Winter classification based on this angle obtained [13].

Multiple sagittal slices were obtained by reorienting the long axis of the ILTM in the coronal and axial planes (Fig. 3A). The sagittal sections covered the entire FS, with approximately nine slices in the sagittal plane and a distance of 1 mm between the slices. The slices had a minimum integration/thickness of 0.2 mm. The FS were measured in the explorer tab of the 3D module of the Romexis 6.2 software; the free region grow tool was activated in annotations, and the FS was delimited by points throughout its periphery. This was performed for all the selected sagittal sections. At the end of the last slice, the computer mouse was double-clicked to manually generate the segmentation area (Fig. 3A and B). The measurements were expressed in "cm^3." The intra-examiner calibration procedure involved the primary investigator measuring five pairs of CBCT images twice at a 1-week interval. Intra-examiner reliability was assessed using the intraclass correlation coefficient (ICC). These measurements were performed before the surgical extraction and histopathological evaluation of the follicle. The Radiologist were blinded to the histopathological findings of the dental follicle.

2.5. Statistical analysis

The collected information was presented in two-dimensional frequency tables to quantify the research objectives. This made it possible to determine the behavior of the variables being investigated. Following this, inferential statistics, including the *t*-test hypothesis test, were used to determine the independence or dependence between the volume of the variables in "cm^3" according to the CBCT and histopathological diagnosis. The significance level was set at 95% ($p < 0.05$).

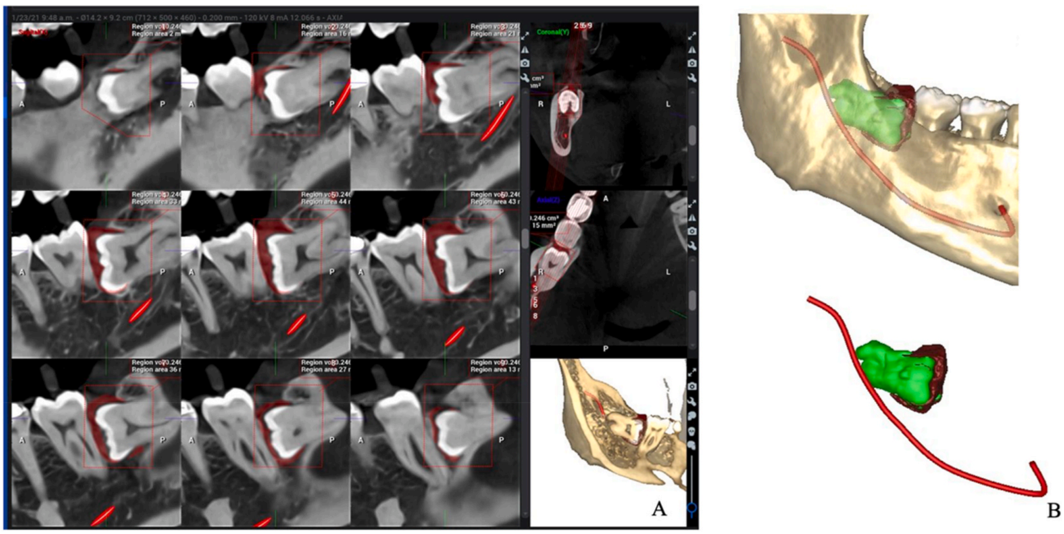

Fig. 3. A. Multiple sagittal slices reoriented on the long axis of the lower third molar in the coronal and axial planes: Measurement of the follicular space in the three-dimensional module of the Romexis software 6.2; **B.** Images of the renderings with the segmentation of the follicular space.

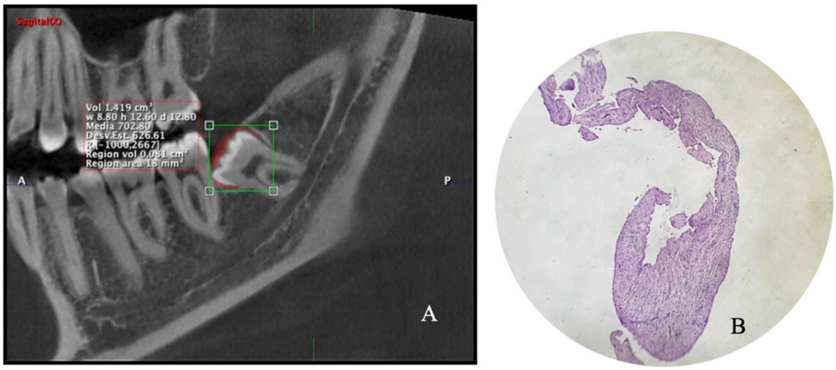

Fig. 4. A. Sagittal section of the lower left third molar shows the volume of the follicular space as 0.081 cm^3; **B.** Histopathological analysis shows the presence of reduced enamel epithelium and loose connective tissue, which indicates a normal follicle.

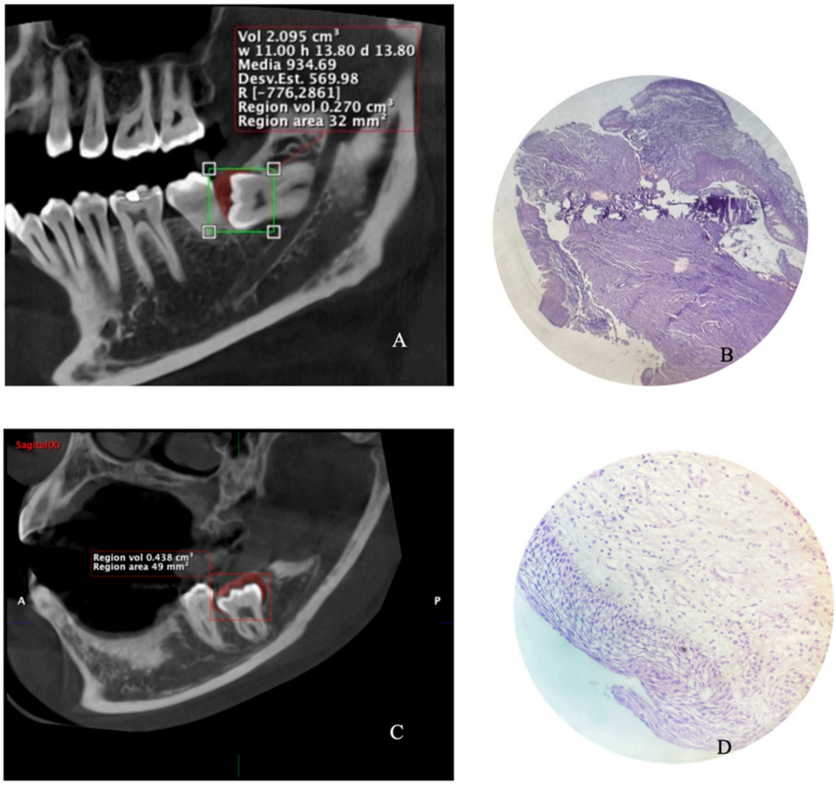

Fig. 5. A. Sagittal section of the lower left third molar shows the volume of the follicular space as 0.27 cm^3; **B.** Histopathological analysis shows the presence of stratified squamous epithelium and dense connective tissue, indicative of a pathological follicle (dentigerous cyst); **C.** Sagittal section of the lower left third molar shows the volume of the follicular space as 0.438 cm^3; **D.** Histopathological analysis reveals the presence of ameloblastic epithelium with a palisade of cuboidal cells with hyperchromatic nuclei with reverse polarity, indicative of a pathological follicle (unicystic ameloblastoma).

Binary logistic regression was used to evaluate the influence of the predictor variables on the histopathological diagnosis of a pathological follicle, and multiple linear regression was used to evaluate the influence of predictor variables on the follicular volume.

Statistical analyses were performed using the Office Excel 2016 program for Windows® and Statistical Product and Service Solutions version IBM® statistical software version 24.0 for Windows 10®.

3. Results

Intra-examiner reliability was evaluated using ICC, which revealed very good agreement for values greater than 0.90 for all measurements (confidence interval [CI], 0.900–0.998).

In total, 103 third molars were extracted, and the DFs were subjected to histopathological evaluation. The volume of the pericoronal radiolucency on CBCT for each ILTM was correlated with the histopathological diagnosis, and the data were analyzed for association with age, sex, angular position, and impaction depth. The age of the patients ranged from 18 to 46 years, with a mean age of 29.18 years (Table 1).

The follicular volumes of the groups with and without a histopathological diagnosis of a pathological follicle are shown in Table 2. The mean volume was 0.32 ± 0.17 cm^3 in the pathological group and 0.10 ± 0.06 cm^3 in the non-pathological group ($p < 0.001$).

The influence of the predictor variables on the histopathological diagnosis of a pathological follicle was evaluated (Table 3). The impaction depth of Position C cases was significantly associated with the diagnosis of a pathological follicle (odds ratio, 5.60; CI, 1.50–21.52; $p = 0.0109$). Similarly, there was an association between the impaction depth and volume (0.059 cm^3; $p = 0.041$) and between the ramus relationship and volume (0.045 cm^3; $p = 0.042$) (Table 4).

4. Discussion

This study showed that the mean follicular volume in the pathological and non-pathological groups was 0.32 cm^3 and 0.10 cm^3, respectively. To our knowledge, this is the first study to quantify the volume of the FS of ILTMs using CBCT and to determine its association with the histopathological findings.

Cyst formation associated with impacted third molars has been reported previously. The expanded radiolucent image in the pericoronal region of the ILTM may provide information regarding cystic changes [15]. Several studies evaluating the ILTM follicles using two-dimensional radiographic images have shown that follicles measuring greater than 2.5 mm could be indicative of subsequent pathological changes [6–12,16,17]. However, no study has evaluated the volume of the FS using CBCT. Therefore, there are no CBCT reference values for the FS volume of the ILTMs. Moreover, no data is available on the relationship between the FS volume and an increased probability of developing or evolving to a cystic or tumorous lesion on histological analysis, which is considered the gold standard.

Our results showed high intra-examiner reliability, which may be due to observer experience and prior calibrations. The images acquired were rendered from isotropic voxels (equal in length, height, and depth), which allowed geometrically precise measurements in any plane. The CBCT datasets used in this research were obtained using the same protocol for image acquisition and measurement of the FS, minimizing measurement errors. Several previous studies have validated CBCT as a measurement method [18–20].

This study included 103 ILTM follicles in various angles and positions and found that the mean volume of the normal follicle and pathological follicle was 0.10 cm^3 and 0.32 cm^3, respectively (Fig. 4A and 5A; Table 2). Therefore, the variables analyzed—follicular volume and histopathological diagnosis—were not independent and showed a dependence between their values ($p = 0.001$ and $p < 0.05$, respectively). Haidry et al. [8] reported cystic changes in 91.7% of the follicles showing radiolucency measuring 2.1–2.4 mm. However, these results were obtained using panoramic radiography; hence, they cannot be compared with our research directly.

In this study, 19.4% of the sample were pathological follicles. Our results are consistent with those of the study by Yildirim et al. [21], wherein the histopathological evaluation of the ILTM follicles was performed and pathological conditions were found in 23% of the cases. Similarly, Haidry et al. [8] reported pathological diagnoses in 24% of cases. While other studies have shown a higher incidence of cystic changes in ILTMs, such as 37% reported by Glosser and Campbell [22] and 46% by Saravana and Subhashraj [10], Baykul et al. [9] described cystic changes in 50% of the cases. On the other hand, some authors such as Güven et al. [11] described a low prevalence rate of 2.3% of cysts and neoplasia, and Yadav et al. [12] reported pathological changes in 4.44% of the follicular tissues.

Among all the pathologies that can develop from the pericoronal follicle of the ILTM, dentigerous cysts are the most frequent, accounting for approximately 18–20% of these developmental cysts [23]. Our results were similar with 19 (95%) cases of dentigerous cysts and 1 (5%) case of unicystic ameloblastoma (Fig. 5C and D). Although some studies reported the incidence of other pathological

Table 1
Initial sample characteristics.

Sex	n	Mean	SD
Male	24	30.00	7.99
Female	50	29.40	8.67
Third molar angulation	**n**	**%**	
Vertical	20	19.4	
Mesioangular	48	46.6	
Horizontal	26	25.2	
Distoangular	9	8.7	
Third molar impaction depth	**n**	**%**	
B position	67	65.0	
C position	36	35.0	
Third molar ramus relationship	**n**	**%**	
Class I	62	60.2	
Class II	34	33.0	
Class III	7	6.8	

SD: standard deviation.

Table 2
Comparison of follicular volume (cm3) between groups with and without a histopathological diagnosis of a pathological follicle.

Histopathological Diagnosis	N	Mean	SD	Mean differences	95% CI		p
					Lower limit	Upper limit	
Absent	83	0.10	0.06	−0.21	−0.26	−0.17	<0.001[a]
Present	20	0.32	0.17				

Independent student T-test.
[a] Significant; SD: standard deviation; CI: confidence interval.

Table 3
Binary logistic regression to evaluate the association of predictor variables with the histopathological diagnosis of a pathological follicle.

Predictor variable	p	Exp(B)	95% CI	
			Lower limit	Upper limit
Third molar angulatio				
Vertical	0.147	–	–	–
Mesioangular	0.162	0.345	0.078	1.535
Horizontal	0.449	1.831	0.383	8.756
Distoangular	0.733	1.548	0.125	19.097
Third molar impaction depth				
B position	–	–	–	–
C position	0.010[a]	5.690	1.504	21.527
Third molar ramus relationship				
Class I	0.972	–	–	–
Class II	0.817	1.154	0.344	3.868
Class III	0.894	1.176	0.107	12.919
Sex				
Male	–	–	–	–
Female	0.621	1.361	0.401	4.614
Age	0.579	0.978	0.904	1.058

CI: confidence interval.
[a] Significant.

Table 4
Multiple linear regression to evaluate the association of predictor variables with follicular volume (cm3).

Predictor variable	B	p	95% CI for B	
			Lower limit	Upper limit
Angulation	0.005	0.714	−0.024	0.035
Impaction depth	0.059	0.041[a]	0.002	0.115
Ramus relationship	0.045	0.042[a]	0.002	0.089
Sex	−0.003	0.901	−0.052	0.046
Age	−0.002	0.220	−0.004	0.001

CI: confidence interval.
[a] Significant

lesions such as odontogenic myxoma, Gorlin cyst, and more severe lesions such as malignant fibrosarcoma [24], there were no such findings in our sample.

Our study revealed a statistically significant relationship between cystic changes and impacted tooth depth. Pathological alterations in the DF were associated with the impaction depth of Position C third molars. In agreement with Haidry et al. [8] and Simşek-Kaya et al. [25], who reported a relationship between the impaction level and severe complications in ILTMs, our study found a strong correlation between the impaction depth and prevalence of pathological changes in the follicular tissue (Table 3).

Furthermore, the angular position of the ILTMs was evaluated, with pathological changes observed in 2 (10%) cases of distoangular angulation, 5 (25%) of vertical angulation, 6 (30%) of mesioangular angulation, and 7 (35%) of horizontal angulation. Haidry et al. and Yildirim et al. reported that vertical and mesioangular molars have a greater tendency to develop pathological changes [8,21]. In the study by Eliasson and Heimdahl, the incidence of cystic changes in horizontal ILTMs was higher than that in the others [16]. This study found no correlation between the angulation of impaction and prevalence of pathological changes. Patients with vestibular-lingual and inverted angulations were excluded from our study because of the low prevalence.

Furthermore, there was no association between sex and the presence or absence of normal DFs. Previous studies have reported different predispositions in men and women [4,21], although the reason for this discrepancy remains unclear [24].

Some studies have reported a relationship between age and the incidence of pericoronal pathosis, indicating that the probability of cystic changes in impacted teeth is higher in patients aged over 20 years than in the younger patients [4,21]. Our study did not find this association to be statistically significant.

One limitation of our research is the small sample size, as only those patients referred to the imaging center were included. Future research with a larger sample size is warranted. Moreover, only one observer measured the FS, and the results applied only to the lower third molars.

On the other hand, we didn't obtain a sample normal distribution in the groups; it is recommended that future research have a sample normal distribution in the groups to facilitate a better comparison of the volume of the follicular spaces of impacted lower third molars.

Based on the results of this study, we propose new FS reference values on CBCT, wherein a mean follicular volume of 0.32 cm^3 could be considered indicative of subsequent pathological changes. This association would allow the adoption of a suitably aggressive therapeutic approach in cases with a high likelihood of pathological development, and timely action would help avoid or reduce their adverse effects.

5. Conclusions

The follicular volume of the ILTMs varies significantly in teeth with a histopathological diagnosis of follicular cyst. The depth of impaction and relationship of the ramus are associated with average follicle measurements. A mean follicular volume of 0.32 cm^3 may be associated with a greater probability of pathological diagnosis. This information should be considered when planning third molar extractions.

Author contribution statement

Marlene Barroso: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Luis E. Arriola-Guillén: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Vinicius Dutra: Analyzed and interpreted the data; Wrote the paper.

Julio Escoto Rodríguez: conceived and designed the experiments; Wrote the paper.

Gerardo Ruales Suárez: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

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