Article

Cone Beam Computer Tomography and Histological Evaluation of Dental Follicle of Impacted Lower Third Molar Germs in Teens: A Histo-Radiographic Correlation Study in a Case Series

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Abstract: The dental follicle (DF) is the tissue that surrounds the crown of the developing tooth. In X-ray, images it appears as a radiolucent area. The removal of an impacted mandibular third molar is a common procedure in oral surgery. The radiographic evidence of pathology, commonly defined as a pericoronal radiolucency measuring at least mm 2.5 mm in any dimension, is accepted for the extraction of impacted mandibular third molars. Mesioangular impactions are usually more closely placed to the inferior alveolar canal, and the use of cone beam computer tomography (CBCT) before the removal of impacted mandibular third molars has been reported to be appropriate in these cases. The aim of this study was to evaluate the microscopic features of radiographically normal DFs associated with mesioangular impacted mandibular third molars examined through CBCT. Thirteen mesioangular impacted third molars from ten patients (5 males and 5 females, mean age $\pm$ SD: 15.1 ± 1.66) with a maximum width of the DF <2.5 mm, as digitally established by CBCT, were included in this study. All the DFs associated with the removed third molars were examined histologically through the analysis of different variables. The mean ($\pm$SD) and range of the maximum width of the DFs were 1.35 ($\pm$0.47) mm and 0.71–2.21 mm, respectively. Nine (69.23%) DFs showed odontogenic remnants, five (38.46%) showed focal squamous metaplasia and eight (61.53%) mild mesenchymal myxoid degeneration. The maximum width of the DF failed to show any significant correlation with all the histological variables considered in this study. Aware of the limited number of patients included in this study, the histo-radiographic correlation in our case series confirm data in the literature, according to which normal pericoronal imaging may be associated with DF tissue changes/variations that in turn are potentially associated with the development of pathologies including odontogenic cysts and tumors. Whether these changes/variations are enough to make prophylactic germectomy of impacted third molars the standard by themselves remains to be established. However, they require accurate correlations with the radiographic data for the appropriate histologic assessment of a DF.

Keywords: dental follicle; lower third molar germ; cone beam computed tomography; oral pathology; impacted tooth

1. Introduction

The dental follicle (DF) is the tissue that surrounds the crown of the developing tooth and remains until the tooth reaches its final position in the dental arch [1,2]. Radiographically, DF appears as a radiolucent area around the tooth; histologically, it consists of condensed ectomesenchyme that surrounds the enamel organ and the dental papilla in association to which forms the germ of the tooth [3–14]. In terms of function, DFs participate in tooth morphogenesis and directing tooth eruption through cascades of signaling pathways and different transcriptional factors [1,2,15].

In oral surgery, the removal of a symptomatic impacted third molar or its prophylactic removal is one of the most commonly performed surgical procedures [11]. Indeed, third molars are the most frequently impacted teeth, and the prevalence of third molar impaction has been reported to range from 16.7% to 73% [16–20]. Even though it is well known that DFs around impacted teeth have the potential to develop pathological conditions, there is no conclusive agreement among clinicians about their prophylactic removal [4–6,8–11,13,18,21]. Consequently, the pathological potential of impacted third molars is under continuous investigation.

The radiographic evaluation of the DF represents the main approach used to establish its physiologic development. Indeed, the DF is considered normal when its maximum width is lower than 2.5 mm [3–9,11–14,22,23]. At present, it is increasingly common to have a pre-surgical evaluation using tridimensional radiographic exams (e.g., cone beam computer tomography, CBCT) [22,24–34]. Because of the low dosage of X-rays associated with this method, it represents the foremost technique administered in pediatric patients [35,36]. In the specific context of impacted third molars, CBCT can be more accurate than conventional two-dimensional radiographic methods in displaying the morphology of the teeth and of their related anatomical structures, as well as in the measurement of DF width [14,22,24–34]. For these reasons, CBCT has been recommended for third molar surgical planning, especially when there is the potential to provide new information that can impact clinical management decisions not offered by conventional radiography.

Most dental practitioners discard extracted unerupted third molars rather than sending them for histopathological analysis. Consequently, there are not many studies focused on the morphology of radiographically normal DFs of impacted third molars [4–6,9,11,13,21,23,37–41]. In addition, the lack of radiographic evidence of pathology is not a reliable indicator of the absence of disease, and the prevalence of DF tissue pathosis has been estimated to be higher than generally assumed from radiographic evaluation alone [5,6,23,40]. For these reasons, we performed this case series study with the specific aim of histologically evaluating DFs from thirteen consecutive mesioangular impacted third molars that appeared normal through CBCT analysis.

2. Materials and Methods

2.1. Study Design and Case Selection

This histo-radiographic study was performed to correlate the DFs of the impacted third molars that were digitally evaluated through CBCT and the histological features of the DFs of the same excised teeth. All the impacted third molars were mesioangular and shared radiographically normal DFs. This is the position that is most frequently observed [14]. Since mesioangular impacted third molars are known to be more closely placed to the mandibular canal, in these cases CBCT represents a valid tool to assess the fine relationships between the impacted tooth and the mandibular canal [25,34,42]. DFs were considered normal when the maximum width of the DF was <2.5 mm, as reported previously [3–9,11–14,22,23]. This study was performed according to the guidelines recommended by the Consolidated Standard of Reporting Trials (CONSORT 2010) and was approved by the ethics review board of the Azienda Ospedaliero—Universitaria Policlinico Umberto I of Rome (Rif. 2947/24.10.2013). All patients and their parents/legal guardians were informed in advance that scans and histological samples might be anonymously used for research reasons later. All the extractions were performed at the Pediatric Dentistry

Unit of the Azienda Ospedaliero—Universitaria Policlinico Umberto I of Rome. Inclusion criteria included the following: patients aged between 13 and 17 years old, at least one germ of the mandibular third molar, mesioangular impaction according to Winter's classification, evidence of orthodontic indications for its extraction (e.g., the need to move the second molar distally), the absence of systemic disease, the availability of signed consent by the parents/legal guardians, and width of the DF at CBCT <2.5 mm [43]. Exclusion criteria included the following: patients aged outside the range 13–17 years, the absence of both germs of the mandibular third molar or indications of orthodontics, a lack of mesioangular impaction according to Winter's classification, the presence of systemic disease, the lack of signed consent by the parents/legal guardians, and width of the DF at CBCT >2.5 mm [43]. The excised DFs were examined histologically. Based on the above criteria, ten consecutive patients and thirteen impacted third molars were included in the study.

2.2. CBCT Evaluation of the DFs

CBCT was performed as described previously [36]. Briefly, imaging was executed with a Newtom 5G device (Cefla S.C., Verona, Italy). Device settings were set at 8 mA and 90 kV. Each field of view (FOV) mode was 5×5 cm, with an isotropic voxel size of 0.4 mm. The stage of development of the third impacted molar was established according to Nolla's classification [44]. The follicular spaces were digitally measured from the midpoint of the dental crown on axial, sagittal and coronal planes as described previously [14]. The maximum width for each impacted third molar was considered for the analysis. In each case, the impacted tooth was removed under local anesthesia. The DFs were carefully detached from the removed tooth. In all patients the sutures were removed ten days after surgery. Although studies in the literature describe better wound healing when a photobiomodulation was performed [45], no additional therapies were used in favor of healing in this study. Representative images of the impacted third molars as viewed in the different CBCT planes are illustrated in Figure 1.
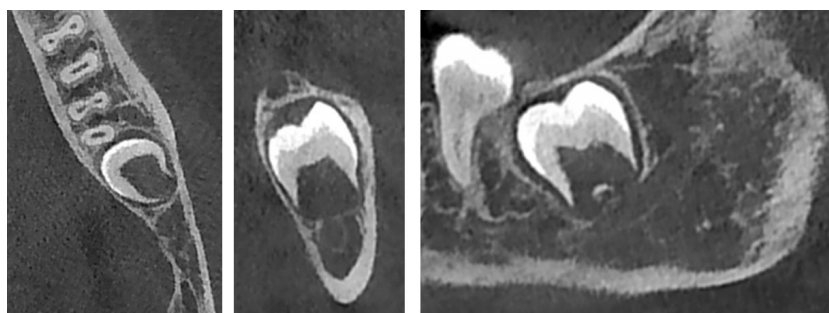

Figure 1. Representative CBCT views of impacted third molars on axial (**left panel**), sagittal (**middle panel**) and coronal (**right panel**) planes.

2.3. Histological Evaluation of the DFs

For histological analysis, DFs were routinely processed for paraffin embedding. Sections with 4 μm thickness were cut from the paraffin blocks and stained with hematoxylin-eosin and, to evaluate fibrosis more accurately, with Picrosirius red. As in the previous work of Esen et al. [13], the following parameters were considered: epithelial atrophy and spongiosis, type and severity of epithelial and mesenchymal inflammation, erosion/ulcer development, odontogenic remnants, fibrosis, granulation tissue, mesenchymal myxoid degeneration and squamous metaplasia. A semi-quantitative analysis of the DFs was performed as reported by Esen et al. [13]. Specifically, all the parameters were dichotomized as absent or present. In addition, when present, the epithelial and mesenchymal inflammation was defined as neutrophil predominant or mixed and lymphocyte predominant or mixed, respectively. They were graded as mild or moderate/severe in both cases, and the mesenchymal myxoid degeneration was also graded as either mild or moderate/severe.

Representative images of the epithelial lining of the DF and the histological parameters evaluated in this study are shown in Figure 2.
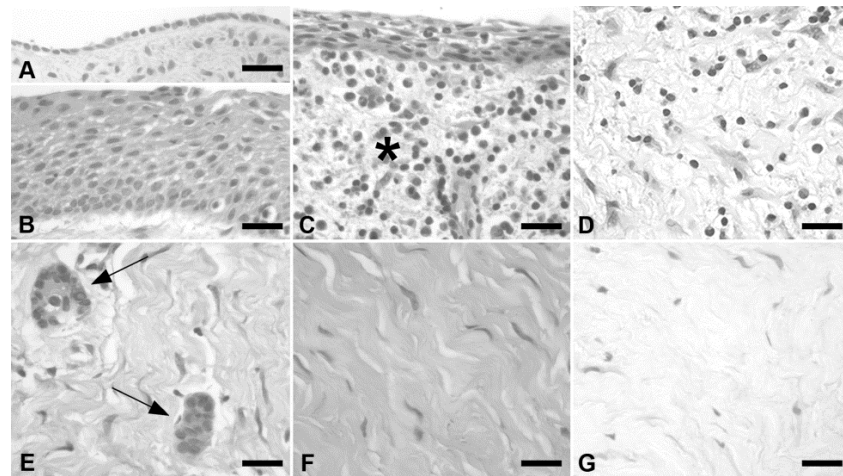

Figure 2. Representative histological images of the epithelial lining of the DF and of the parameters evaluated in this study. The epithelial lining of the DF appears cuboidal (**A**) or squamous (**B**). In (**C**), the squamous epithelial lining is atrophic, spongiotic and inflamed. Inflammatory infiltrates are also present in the superficial (asterisk in (**C**)) and in the deeper (**D**) portion of the DF connective tissue. Two odontogenic epithelial remnants are illustrated in (**E**) (arrows). The images in (**F**,**G**) show the dense (fibrosis) and loose (myxoid degeneration) appearance of the DF connective tissue, respectively. Hematoxylin-eosin. Bars: 50 μm.

2.4. Statistical Analysis

The data analysis was conducted with R and SAS Enterprise Guide (Version 7.15). Standard descriptive statistics were used to evaluate the distribution of each variable. Continuous variables were reported as means ± standard deviations and categorical variables as frequencies. The distribution of variables between male and females were compared with either a χ^2 test or Fisher's exact test and a Mann–Whitney U test, as appropriate. Correlations between variables were assessed by correlation matrices and the use of either Pearson (parametric) or Spearman (nonparametric) correlation methods where appropriate. A *p*-value < 0.05 was considered significant.

3. Results

3.1. Patients

The mean age (±SD) of the patients was 15.1 (±1.66) years (males 14.8 ± 1.09 and females 15.4 ± 2.19) and the age range 13–17 years (males 14–16 and females 13–17). Seven of the impacted third molars were from males (53.8%) and six from females (46.2%). All the impacted teeth were from the mandibular dental arcade (seven from the left and six from the right side). In three cases (two males and one female), the impacted third molar was bilateral.

3.2. CBCT and Histological Evaluation of the DFs

According to Nolla's classification, three of the impacted third molars (from two females) were stage 5, six (four from males and two from females) stage 6 and four (three from males and one from females) stage 7. The mean (±SD) and range of the maximum width of the DFs were mm 1.35 (±0.47) and 0.71–2.21 mm, respectively. Based on gender, the mean (±SD) and range of the maximum width of the DFs were mm 1.43 (±0.45) and mm 0.71–2.12 in males, and mm 1.26 (±0.52) and mm 0.72–2.21 in females, respectively. Statistical analysis failed to reveal significant differences between males and females, and no statistically significant correlation was demonstrated between the maximum width of the DF and patient gender ($\chi^2 = 0.929$; $p = 0.629$) or Nolla's stage ($\chi^2 = 0.3375$.; $p = 0.8447$).

At a histological level, all DFs showed some epithelial lining. As expected from the age of the patients [3,45], reduced enamel epithelium was predominant. Five DFs (38.46%) showed focal squamous metaplasia, two (15.38%) and three (23.07%) demonstrated some degree of epithelial atrophy and spongiosis, respectively, nine (69.23%) odontogenic remnants, four (30.76%) some degree of fibrosis, and eight (61.53%) mild mesenchymal myxoid degeneration. Odontogenic residues were occasionally centered by calcifications and detected in four of the DFs with squamous metaplasia, in two with some degree of fibrosis and in six with mild mesenchymal myxoid degeneration. These stromal changes were observed in four DFs with squamous metaplasia. Inflammation was detected in two DFs (15.39%) from the same patient (male, 16 years). In the right DF, it involved both the connective tissue and the metaplastic squamous epithelium, was graded as mild in the epithelium and moderate/severe in the connective tissue and included both neutrophil granulocytes (that were predominant within the epithelium) and lymphocytes. In the left DF, it was limited to the connective tissue, was graded as mild and included only mononucleated leucocytes. Focal erosion was detected in one DF (7.7%, the right DF described above) while ulcer and granulation tissue were consistently absent. Statistical analysis failed to reveal significant differences between males and females. A synopsis of the histological semi-quantitative analysis is reported in Table 1.

Table 1. Semi-quantitative analysis of the histological parameters evaluated in this study.

Histologic Parameter	Frequency (%)
-Squamous metaplasia	
No	8/13 (61.53%)
Yes	5/13 (38.46%)
-Epithelial atrophy	
No	11/13 (84.61%)
Yes	2/13 (15.38%)
-Epithelial spongiosis	
No	10/13 (76.92%)
Yes	3/13 (23.07%)
-Odontogenic remnants	
No	4/13 (30.76%)
Yes	9/13 (69.23%)
-Fibrosis	
No	9/13 (69.23%)
Yes	4/13 (30.76%)
-Mesenchymal myxoid degeneration	
No	5/13 (38.46%)
Mild	8/13 (61.53%)
Moderate/severe	0
-Epithelial inflammation	
No	12/13 (92.3%)
Mild (mixed inflammatory infiltrate)	1/13 (7.69%)
Moderate/severe	0
-Mesenchymal inflammation	
No	11/13 (84.61%)
Mild (lymphocyte predominant)	1/13 (7.69%)
Moderate/severe (mixed inflammatory infiltrate)	1/13 (7.69%)
-Erosion/ulcer	
No	12 (92.3%)
Erosion	1 (7.69%)
Ulcer	0
-Granulation tissue	
No	13 (100%)
Yes	0

A correlation matrix was used to summarize the data for statistical analysis. The coefficients between the different clinical-pathological variables are reported in Figure 3.

The maximum width of the DF failed to show significant correlation with all the histological variables considered in this study.
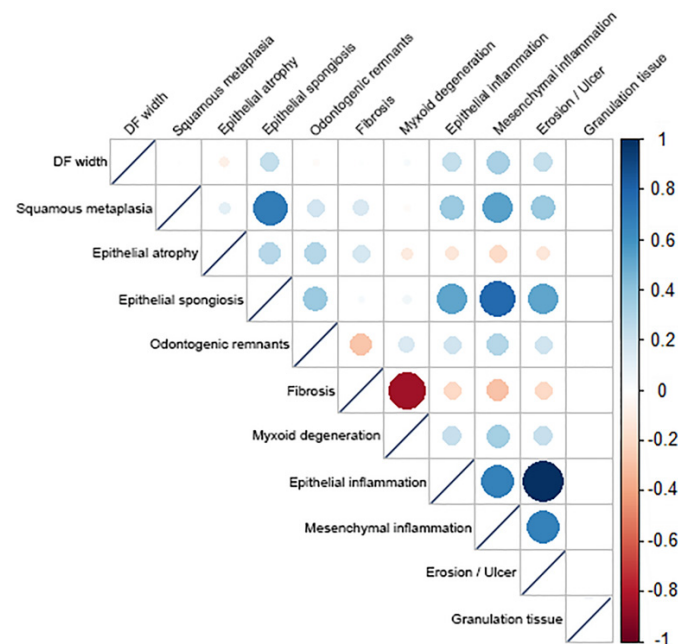

Figure 3. Correlation matrix between the maximum width of the DF and the diverse histological variables considered in this study. The different colors of the circles represent the correlation between the two variables. The diagonal indicates that each variable perfectly correlates with itself.

4. Discussion

DFs and the dental papillae are the immature tissues that compose the ectomesenchymal portion of tooth germ [1,2]. DFs develop into the periodontal ligaments and attachments, and dental papillae provide for the formation of dentin and mature into the pulp tissue [1,2]. They appear radiographically as semicircular radiolucencies around unerupted or impacted teeth and histologically consist of connective tissue with variable amounts of lining epithelium, epithelial odontogenic remnants, myxoid change and calcifications [4,13]. DF enlargement or asymmetry may occur, particularly in impacted third molars, and tissue components of the DF may be histologically misinterpreted with diverse pathological conditions [4]. Thus, accurate correlation of the clinical-radiographical and histological findings is mandatory to establish a proper diagnosis.

Since impaction of the third molar has been reported to occur in up to 73% [16–20], third molar removal is one of the most frequently performed operations in dentistry. However, the need for the removal of impacted third molars is still controversial. In symptomatic cases or when carious lesions, orthodontic problems, periodontal disease and cysts/tumors are present, the indication is clear. However, the rationale to remove them when asymptomatic (i.e., prophylactically) is not [4–6,8–11,13,18,21]. Prophylactic removal can be reasonably considered to preserve the results of orthodontic treatment, to avoid operative complications on the inferior alveolar nerve complicating avulsions in adulthood and, in principle, to prevent the development of inflammatory states (pericoronitis) or cysts/tumors (dentigerous cyst, odontogenic keratocyst, ameloblastoma). However, no more than 12% of impacted teeth have been estimated to be associated with pathological conditions, including cysts and damage to adjacent teeth [46,47].

According to the recommendations of the National Institutes of Health, impacted (and erupted) mandibular third molars with evidence of follicular enlargement should be considered for extraction, and the associated soft tissue always submitted for microscopic examination [48]. A pericoronal radiolucency of <2.5 mm on conventional two-dimensional radiographic methods is considered non-pathologic [3–9,11–14,22,23]. In this case series,

we investigated the tissue changes occurring in the DFs of thirteen mesioangular impacted third molars evaluated through CBCT in which the maximum width of the DF was <2.5 mm. CBCT is more accurate compared to conventional two-dimensional radiographic methods in the general evaluation of impacted third molars. Indeed, it makes it possible to define the relationship between bone structures and the impacted third molars with better spatial resolution. It also permits us to establish the type of impaction, the size of the follicle, the inclination of the long axis of the tooth, the 3D position of the tooth and its relationship with the medullary canal, as well as the amount of bone around the tooth [14,22,24–34]. In one study, Ghaeminia et al. evaluated the role of CBCT in the treatment of patients with impacted mandibular third molars at increased risk of inferior alveolar nerve injury [24]. Through CBCT analysis, the authors reclassified significantly more subjects to a lower risk for inferior alveolar nerve injury compared with panoramic radiography, with a consequent change in risk assessment and in the surgical approach.

In all cases included in this study, DFs were normal at CBCT evaluation, and third molar extraction was performed based on orthodontic indications. Pathologic analysis of the DFs included the evaluation of the same histological variables considered by Esen et al. in their series of patients [4]. Overall, our histological data indicate that tissue variations and changes may also occur in the DFs from impacted asymptomatic third molars when they appear normal through CBCT. However, of the considered histological variables, only odontogenic remnants and mild mesenchymal degeneration were appreciable in more than half of the samples. Of note, squamous metaplasia, the presence of which has been considered by some authors but not by others to be indicative of the development of a dentigerous cyst, was observed in 5 out of the 13 DFs (38.46%) [5,6,13,37,38,40]. Obviously, the evidence of odontogenic remnants, mesenchymal degeneration and squamous metaplasia has no prospective value per se, but their appropriate evaluation has important clinicopathologic implications for the correct assessment of a DF as normal. This is mandatory to avoid histological misinterpretation and, consequently, histological misdiagnosis that can be related to the lack of familiarity and experience with these tissue specimens [4].

The presence of odontogenic remnants within the connective tissue of DFs is a well-known phenomenon [4]. It is generally thought that odontogenic remnants are inactive and do not have clinical significance. Thus, their evidence is not synonymous of a pathologic DF per se. However, they represent the source of odontogenic lesions including cysts and tumors [38]. In addition, their frequency and number have been reported to decrease with the increase in patient age [45,49]. In the present series, odontogenic remnants were detected in 69.23% (9/13) of the samples. This value is remarkably like that previously reported by Kim and Ellis in their study on 847 DFs [4]. Features of ameloblastic differentiation including tall columnar cells with nuclear polarization and stellate reticulum type tissue, as well as nuclear and cytoplasmic pleomorphism and/or mitotic activity, amyloid deposition and Liesegang-ring-type calcifications have to be searched carefully to avoid misdiagnosis. Indeed, the absence of these features is the mainstay to differentiate normal DFs from diverse odontogenic tumors including ameloblastomas, ameloblastic fibromas and calcifying epithelial odontogenic tumor (Pindborg tumor). In the present study, these features were not detected in any of the DFs in which odontogenic remnants were found.

Myxoid changes were detected in 61.53% (8/13) of the samples included in this study. This value is also substantially like that previously reported by Kim and Ellis in their study [4]. To be aware of this change is important, because an absence of appropriate radiographic correlation may lead to the misinterpretation of a normal DF with myxoma [4]. Indeed, DFs appear radiographically as well-demarcated, thin, semicircular and usually symmetric radiolucencies around unerupted teeth; are virtually always less than mm 3 mm thick; and are never destructive. In contrast, myxomas appear as larger and typically poorly circumscribed, and are often expansile, destructive radiolucent lesions. When radiographic data are not available, the evidence of an epithelial lining, as in our samples, makes the recognition of DF possible because it always lacks in myxomas.

Squamous metaplasia has been considered a histopathologic feature indicative of the development of a dentigerous cyst in some studies [5,6,13,40]. Consequently, it is not a surprise that the diagnosis of dentigerous cyst is the most common misdiagnosis of DFs [4]. Of note, the evidence of squamous metaplasia (and the risk of dentigerous cyst development) is known to increase with age [5,6]. For example, the frequency of squamous epithelium was reported by Stanley et al. to be 16.7% in patients 13 to 21 years old and 73.9% in patients 22 to 69 years old [45]. Similarly, in the series of Adelsperger et al., squamous metaplasia was found in nearly one-fifth of specimens from patients under the age of eighteen and nearly half of specimens from patients aged 18 or older [6]. Thus, it is well established that squamous metaplasia may be observed, as in our series, in children and adolescents as well. In principle, removal of third molar teeth in these cases could result in the removal of DF tissues before they can progress to a radiographically detectable lesion. Since criteria for separating DF and dentigerous cyst have not been established, individual and differing perceptions of the requirements for the diagnosis of dentigerous cyst may determine which tissue changes are deemed cystic [23,37]. We completely agree with Tegginami and Prasad, according to which the evidence of squamous epithelium in place of reduced enamel epithelium reflects a squamous metaplasia rather than the evolution to a dentigerous cyst, at least when pericoronal radiolucency is, as in our cases, less than 2.5 mm [23].

Interestingly, in our series, in two DFs from the same patient (male, 17 years), squamous metaplasia was associated with florid DF connective tissue, as well as epithelial inflammatory infiltrate and changes (atrophy and spongiosis). This case may support the hypothesis of Esen et al., which claims an inflammatory state may contribute to enhance the development of the squamous metaplasia and that the delay in impacted third molar surgery can lead to an increase in the severity of inflammation. This in turn could contribute to further pathological changes including the development of a dentigerous cyst [13].

5. Conclusions

Aware of the small number of samples included in this study, making a general unequivocal conclusion impossible, our histo-radiographic study confirms that normal pericoronal imaging, as established using a highly sensitive technique, CBCT, may be associated with DF tissue variations and changes. It is currently not possible to predict which radiographically normal third molar impacted tooth will progress to clinically detectable pathologies. It remains to be established if these variations, as the presence of odontogenic remnants and changes (as the evidence of squamous metaplasia), are per se enough to make the prophylactic extraction of asymptomatic impacted third molars the standard. However, keeping in mind that the frequency of pathologies associated to impacted teeth is low, prophylactic third-molar extraction does not seem to meet the standard of evidence-based practice [50]. Without doubt, all DFs associated with the germ of an extracted impacted third molar need to be examined histologically, with clinical and radiographic features properly considered to avoid misinterpretation and misdiagnosis.

Data Availability Statement: Data are available from the authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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