

Original Article

Histopathological and Immunohistochemical Evaluation of Follicles of Asymptomatic Impacted Third Molars and their Pathological Risk Assessment

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ABSTRACT:

Introduction- Dental follicle and dentigerous cyst share common histopathological features. The present study was undertaken to evaluate and correlate the histopathological findings of the dental follicles with the dentigerous cyst and to compare the immunohistochemical expression of molecular markers bcl-2 and Ki-67 in dental follicles compared and dentigerous cyst. **Materials & Methods-** The study was carried out on 50 dental follicles obtained after surgical removal of impacted third molars and 10 archival tissue blocks of histopathologically confirmed cases of dentigerous cyst which were used as control. All the cases were examined histopathologically using routine haematoxylin and eosin staining (H & E) and special stains (PAS) for determining pathological changes in epithelial lining and connective tissue. **Results-** 14 out of 40 cases (35%) of dental follicle and 6 out of 10 cases (60%) of dentigerous cysts showed reduced enamel epithelial lining. 22 out of 40 cases of dental follicles and 4 out of 10 cases of dentigerous cysts showed squamous metaplasia. 8 out of 40 cases of dental follicles and 4 out of 10 cases of dentigerous cysts showed mucous prosoplasia. Squamous metaplasia was evident in 5 out of 14 cases of reduced enamel epithelium. Maximum number of cases showing dense collagenous type of connective tissue was present in generalized proliferating type of epithelium. 75% dental follicles had dense collagen connective tissue and 60% of dentigerous cysts had dental papillae type of connective tissue and plump shaped fibroblasts. The mean value (labeling index) of Ki-67 in positive cases of dental follicles (4.20 ± 4.043). Strong staining was seen in 2 cases of reduced enamel epithelium, 10 cases of generalized proliferating epithelium. **Conclusion-** A simultaneous need to conduct studies based on clinical, histopathological immune-histochemical and genetic factors to elucidate the actual inherent potential of the dental follicles to develop pathologies.

Key words- Dental follicle, Dentigerous cyst, Squamous metaplasia.

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INTRODUCTION

The development of a tooth from its initiation to its fully formed status requires principles of differentiation to be incorporated at varying stages. The same holds true in the disease process of lesions arising from the odontogenic apparatus where the disease formation is primarily mimicking the tooth ontogeny. Dental follicles physiologically develop into the cementum, periodontal ligament, alveolar bone, but occasionally remain adjacent to the crown of an impacted or unerupted tooth. Follicular tissues around impacted teeth have a

potential to develop into pathologic conditions. As suggested by Shear¹, dentigerous cysts may be of either extrafollicular or intrafollicular origin and that those of intrafollicular origin may develop by expansion of dental follicles resulting from accumulation of fluid either between the reduced enamel epithelium and the enamel, or within the enamel organ itself. Histologically, it consists of a fibrous 3 wall that may contain varying amounts of myxoid tissue and odontogenic rests.

Previous studies have reported squamous metaplasia to be a normal age related change in the pericoronal tissues of

impacted teeth; but some authors also stated that the metaplasia represents early pathosis. This metaplastic change from reduced enamel epithelium to this squamous epithelium marks an early stage of cystic evolution.² The transformation of normal follicle to pathosis along with the above histopathological changes has been studied by various molecular markers. The Ki-67, a non histone protein expressed by proliferative cells throughout the cell cycle including G1, S, G2 and M phase except the G0 phase. Ki-67 antigen is localized, during interphase, to the nuclear cortex and dense fibrillar components. It is suggested that the antigen is associated with a so called nuclear matrix intermediate filament scaffold. bcl-2, an anti-apoptotic protein, is believed to be localized in the outer mitochondrial membrane, endoplasmic reticulum, and nuclear envelope. bcl-2 has also been suggested as playing a role in the maintenance of mitochondrial membrane potential, indicating its possible association with the inner mitochondrial membrane.³

Edamatsu et al⁴ investigated the role of apoptosis related factors Fas, bcl-2, and ssDNA in epithelial components of dental follicles and dentigerous cysts associated with impacted third molars of the mandible, and the results were compared with immunoreactivity for Ki-67. They concluded that apoptosis related factors and proliferative markers differ between dental follicles and dentigerous cysts and play a major role in pathogenesis of dentigerous cysts and in dental follicles, expression of apoptosis-related factors and proliferative marker is most likely modulated by the morphologic characteristics of epithelial components as well as inflammatory changes. These molecular markers can be used for the risk assessment of these follicular tissues developing into pathological lesions. The present study was

undertaken to evaluate and correlate the histopathological findings of the dental follicles with the dentigerous cyst. It also analyzed the immunohistochemical expression of molecular markers bcl-2 and Ki-67 in dental follicles compared to dentigerous cyst.

MATERIALS & METHODS

This study was carried out in the Department of Oral and Maxillofacial Pathology and Microbiology, I.T.S Centre for Dental Studies and Research, Muradnagar, Ghaziabad. The study was carried out on 50 dental follicles obtained after surgical removal of impacted third molars and 10 archival tissue blocks of histopathologically confirmed cases of dentigerous cyst which were used as control. Out of 50 cases of dental follicles, 10 cases were excluded due to insufficient epithelium and inflammation for analysis.

All the cases were examined histopathologically using routine haematoxylin and eosin staining (H & E) and special stains (PAS) for determining pathological changes in epithelial lining and connective tissue. Tissue specimens were then assessed quantitatively and semiquantitatively by comparing the expression of both Ki-67 and bcl-2 in the dental follicular tissue and dentigerous cyst by observing the presence of chromogen within the nucleus for Ki-67(nuclear stain) and within the cytoplasm and nucleus of the individual cells due to the cellular localization of bcl-2 in the nuclear membrane, outer membrane of mitochondria as well as endoplasmic reticulum. Lymph node (bcl-2) and breast carcinoma (Ki-67) tissue sections were used as positive controls and lymphocytes were used as positive control for bcl-2. Results thus obtained were subjected to statistical analysis using chi-square test. P value < 0.05 was considered significant.

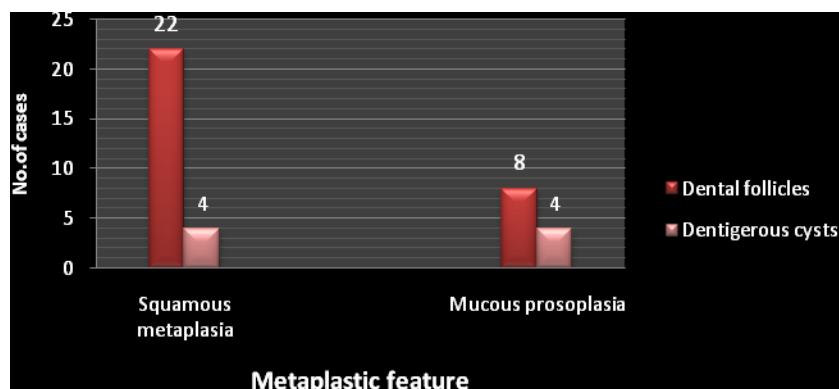
RESULTS

Table 1 Distribution of type of epithelium in dental follicles and dentigerous cysts

Type of epithelium	No. of cases		Percentage %		P value
	Dental follicle	Dentigerous cyst	Dental follicle	Dentigerous cyst	
Reduced enamel epithelium	14	6	35%	60%	0.732
Generalized proliferating epithelium	17	3	42.5%	30%	
Localized proliferating epithelium	9	1	22.5%	10%	
Total no. of cases	40	10	100%	100%	

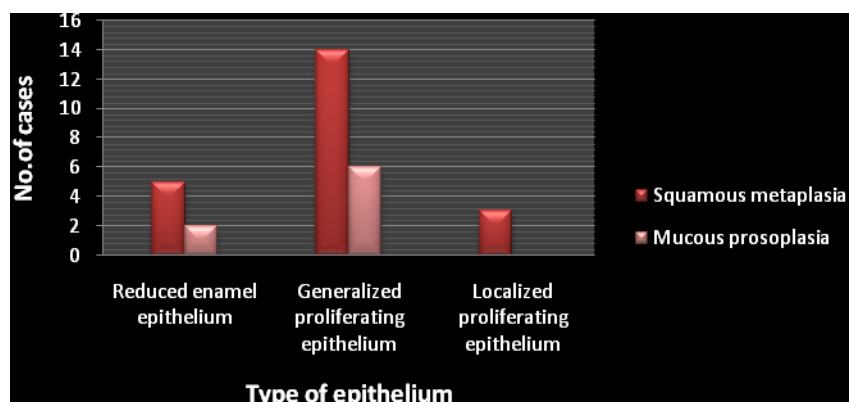
Table 1 shows that 14 out of 40 cases (35%) of dental follicle and 6 out of 10 cases (60%) of dentigerous cysts showed reduced enamel epithelial lining, 17 out of 40 cases (42.5%) of dental follicles. 3 out of 10 cases of dentigerous cysts (30%) showed generalized proliferating type of epithelium with minimal inflammation. 9 out of 40 cases (22.5%) of dental follicles. 1 out of 10 cases (10%) of dentigerous cysts showed localized proliferating type of epithelium. Comparison of distribution of type of epithelium between dental follicles and dentigerous cysts was done using Fisher's exact test and p value was found to be statistically non significant ($p > 0.05$).

Graph I Epithelial metaplastic features in dental follicles versus dentigerous cysts



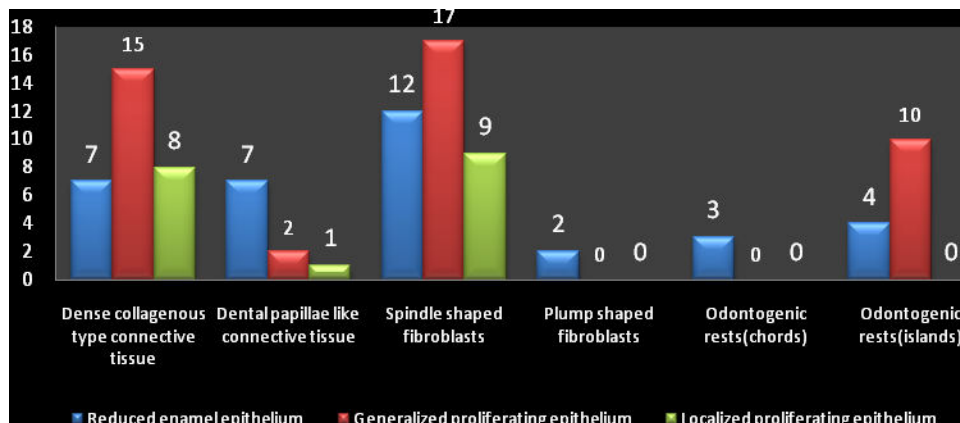
Graph I depicts that 22 out of 40 cases of dental follicles and 4 out of 10 cases of dentigerous cysts showed squamous metaplasia. 8 out of 40 cases of dental follicles and 4 out of 10 cases of dentigerous cysts showed mucous prosoplasia.

Graph II Epithelial metaplastic changes versus type of epithelium in dental follicles



Graph II shows that the distribution of squamous and mucous metaplastic changes in different types of epithelial lining the dental follicles. Squamous metaplasia was evident in 5 out of 14 cases of reduced enamel epithelium, 14 out of 17 cases of generalized proliferating epithelium and 3 out of 9 cases of localized proliferating epithelium of the follicular tissues. Mucous prosoplasia was seen in 2 out of 14 cases of reduced enamel epithelium, 6 out of 17 cases of generalized proliferating epithelium and none out of 9 cases of localized proliferating epithelium in follicular tissues.

Graph III Connective tissue histopathological features versus type of epithelium in dental follicles



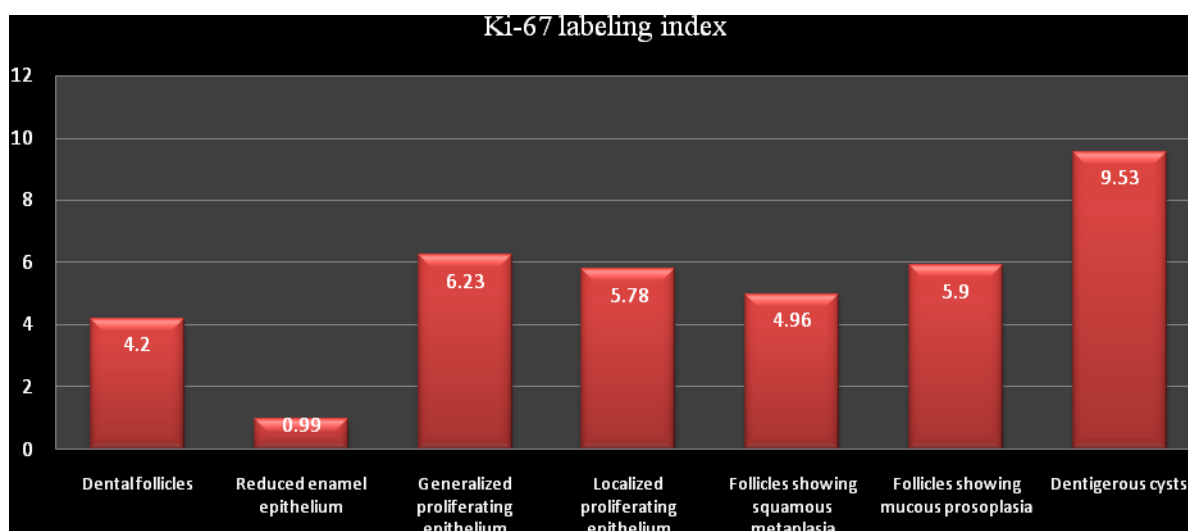
Graph III depicts that maximum number of cases showing dense collagenous type of connective tissue were present in generalized proliferating type of epithelium (15 out of 17), dental papillae type of connective tissue were present in reduced enamel type of epithelium (7 out of 14), spindle type fibroblasts were present in generalized proliferating type of epithelium (17 out of 17), plump type fibroblasts were present in reduced enamel type of epithelium (2 out of 14), chords of odontogenic rests were present in reduced enamel type of epithelium (3 out of 14) and chords of odontogenic rests were present in reduced enamel type of epithelium (4 out of 14).

Table II Connective tissue histopathological features in dental follicles versus dentigerous cysts

Connective tissue features	Dental follicles (n=40)	Dentigerous cysts (n=10)	P value
Dense collagen C.T	30 (75%)	4 (40%)	0.056
Dental papillae type of connective tissue	10 (25%)	6 (60%)	0.056
Spindle shaped fibroblasts	38 (95%)	4 (40%)	0.00
Plump shaped fibroblasts	2 (5%)	6 (60%)	0.00
Odontogenic rests (chords)	3 (7.5%)	2 (20%)	0.258
Odontogenic rests(islands)	14 (35%)	4 (40%)	1.00

Table II depicts that 75% dental follicles had dense collagen connective tissue and 60% of dentigerous cysts had dental papillae type of connective tissue and plump shaped fibroblasts.

Graph IV Quantitative assessment of Ki-67 expression in dental follicles and dentigerous cysts

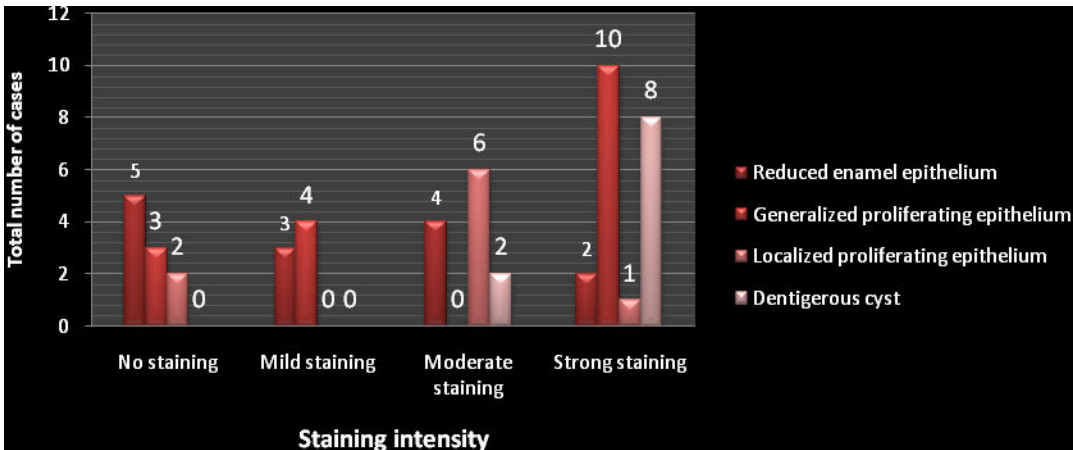


Graph IV depicts the mean value (labeling index) of Ki-67 in positive cases of dental follicles (4.20 ± 4.043). The mean value (labeling index) in reduced enamel epithelium (0.99 ± 2.00) and in localized proliferating epithelium (5.78 ± 4.20) is less as compared to mean labeling index of generalized proliferating epithelium (6.23 ± 3.14). Follicles showing metaplastic changes showed 4.96 ± 4.20 and 5.90 ± 4.20 labeling index for squamous metaplasia and mucous prosoplasia respectively. Dentigerous cyst showed 9.53 ± 1.81 labeling index.

Graph V depicts the number of cases showing immunopositivity for bcl-2 expression. Strong staining was seen in 2 cases of reduced enamel epithelium, 10 cases of generalized proliferating epithelium, 1 case of localized proliferating epithelium and 8 cases of dentigerous cysts. The difference was significant ($P < 0.05$).

Graph VI shows when no staining was seen in follicles with respect to bcl-2, 7 cases showed negative staining and 3 cases showed positive staining for Ki-67. When mild staining was seen with respect to bcl-2, 3 cases showed negative staining and 4 cases showed Ki-67 expression. When moderate staining was seen with respect to bcl-2, 3 cases showed negative staining and 7 cases showed Ki-67 expression. When strong staining was seen with respect to bcl-2, 3 cases showed negative staining and 10 cases showed Ki-67 expression.

Graph V Semiquantitative assessment of *bcl-2* expression in dental follicles and dentigerous cysts



Graph VI Assessment of *bcl-2* and Ki-67 expression in dental follicles

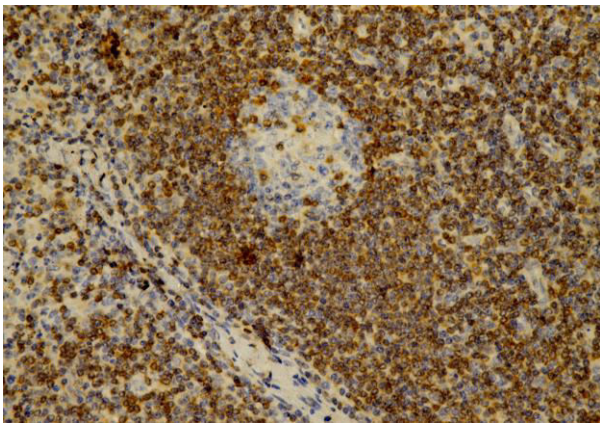
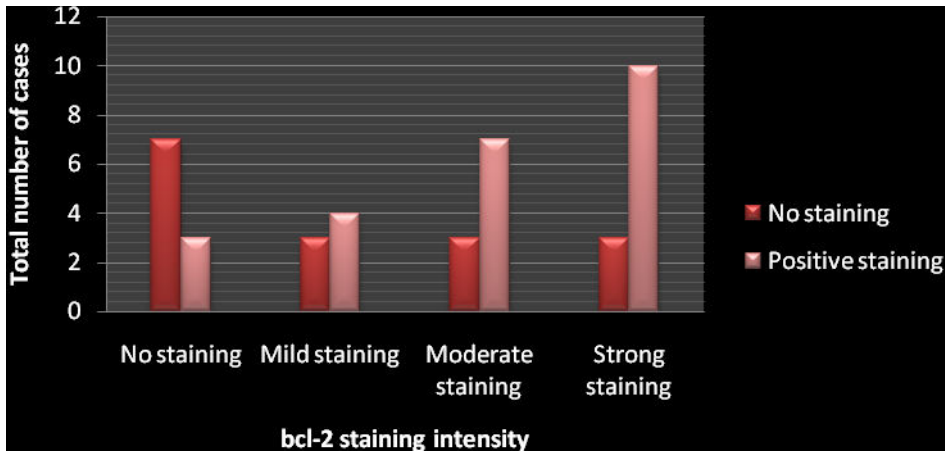


Figure 1: Positive control lymph node showing nuclear and cytoplasmic staining of *bcl-2* in mantle cell lymphocytes and scanty expression in germinal centre lymphocytes (40x)

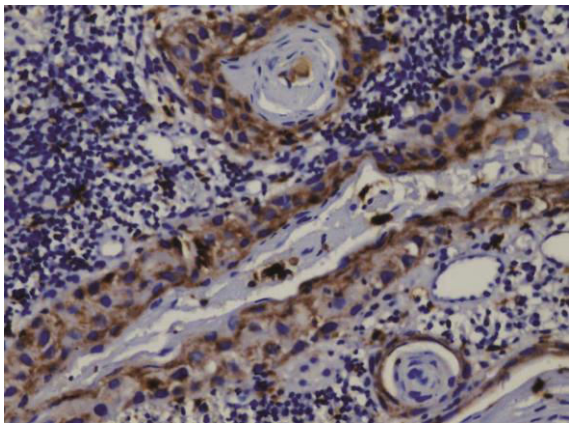


Figure 2: Well differentiated squamous cell carcinoma showing *bcl-2* positivity in nucleus and cytoplasm evident in peripheral cells of the entire island as well as in keratin pearl having diminished immunoreactivity in the centre (40x)

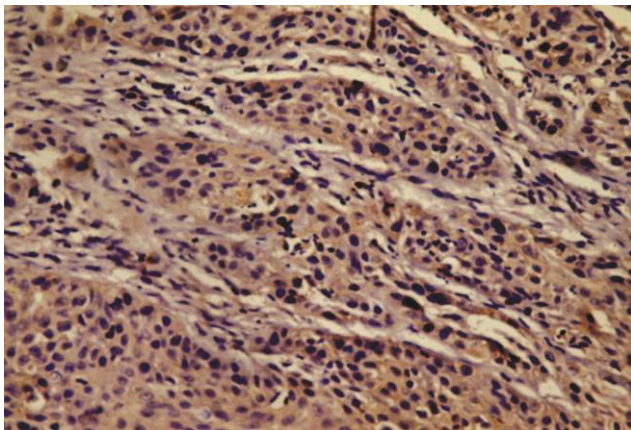


Figure 3: Moderately differentiated squamous cell carcinoma showing islands of tumor cells showing cytoplasmic and nuclear envelope expression of *bcl-2* (40x)

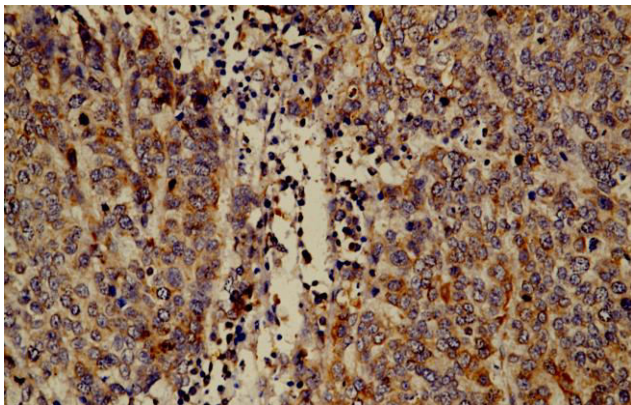


Figure 4: Poorly differentiated squamous cell carcinoma showing sheets of tumor cells having *bcl-2* positivity in both cytoplasm and nucleus (40x)

DISCUSSION

According to the Oral and Maxillofacial Surgery Clinics of North America the indications and contraindications for removal of impacted third molars have been put forth but the asymptomatic extraction of impacted third molar is still questionable. Histopathological analysis of our study revealed three different types of epithelium in dental follicles. The difference between the distribution of types of epithelium between dental follicles and dentigerous cysts was not found to be statistically significant ($p > 0.05$). Our results were in accordance with Kotrashetti et al.⁵

Squamous metaplasia was detected in 55% (22 out of 40 cases) of dental follicles and 40% (4 out of 10 cases) of dentigerous cysts showed in our study. Out of three types of epithelial lining in dental follicles, generalized proliferating epithelium showed maximum no. of cases with squamous metaplasia (14 out of 17 cases). Difference between three types of epithelium with respect to squamous metaplasia was statistically significant ($p < 0.05$).

Mucous prosoplasia was detected in 20% (8 out of 40 cases) in dental follicles and 40% (4 out of 10 cases) in dentigerous cysts in our study. Generalized proliferating epithelium in follicle showed maximum no. of cases (6 out of 17 cases) with mucous prosoplasia. Difference between three types of epithelium with respect to mucous prosoplasia was statistically non significant ($p > 0.05$). Our results were in concordance with Cabbar et al.⁶

In our study, while comparing the type of epithelium with connective tissue, 75% (30 out of 40 cases) of dental follicles showed dense collagenous type of connective tissue stroma and 25% (10 out of 40 cases) showed dental papillae type of connective tissue and generalized proliferating epithelial lining showed maximum number of cases with dense fibrous type of connective tissue (15 out of 17 cases). Tegginamani et al.⁷ showed 58% cases showing fibrous or myxomatous connective tissue 42% cases showed fibrocollagenous tissue in cases of dental follicles.

Results of our study revealed 95% (38 out of 40 cases) showed spindle shaped fibroblasts in dental follicles and 5% (2 out of 40 cases) showed plump type of fibroblasts and generalized proliferating epithelium showed maximum number of cases with spindle shaped fibroblasts (17 out of 17). The difference between dental follicles and dentigerous cysts was found to be statistically significant ($p < 0.05$).

With respect to the presence of odontogenic rests, 7.5% (3 out of 40 cases) showed odontogenic rests in the form of chords and 35% (14 out of 40 cases) showed islands of odontogenic rests. Difference between three types of epithelium was statistically significant ($p < 0.05$). Benn et al.⁸ reported the presence of these odontogenic rests in the connective tissue wall of the dentigerous cysts.

On comparing the histopathological features with the immunohistochemical results mean Ki-67 labeling index of cases in our study showing squamous metaplasia (4.96 ± 4.20) and mucous prosoplasia (5.90 ± 4.20) were more as compared to cases showing no metaplastic changes. Our results are in concordance with those observed by Guler et al.⁹

According to our data, it is highly likely that *bcl-2* and Ki-67 were expressed proportionally. The correlation of *bcl-2* and Ki-67 clearly indicates the interactive effect between these two oncoproteins to control growth and apoptotic indexes. Results of our study showed that the risk assessment of follicular tissues transforming to pathologies, in our study to dentigerous cyst was 33% when following parameters (Ki-67 immunopositivity, *bcl-2* immunopositivity and squamous metaplasia) were taken into account. When Ki-67 and *bcl-2* individually were compared with type of epithelium lining and metaplastic changes the risk was 60% and 75% respectively.^{10,11,12}

CONCLUSION

A simultaneous need to conduct studies based on clinical, histopathological immune-histochemical and genetic factors to elucidate the actual inherent potential of the dental follicles to develop pathologies. With studies conducted on large sample size a close approximation to the actual risk assessment could be inferred.

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