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Morphometric Study of Nuclear and Cellular Changes in Odontogenic Cysts and Tumor Using Image Analysis Software

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I. INTRODUCTION

Jaw lesions are frequently encountered in dental practice. Cysts and tumors form a major part of these lesions and hold great significance since they have a great potentiality to jeopardize health and longevity due to associated morbidity and mortality.

Maxillary odontogenic cysts are characterized by a pathological cavity either completely or partially covered with epithelium tissue. These lesions are considered as bone destructive lesions developed from the components of the odontogenic epithelium or their residual content that remains trapped inside the bone or its gingival tissue. Odontogenic keratocyst is the third most common type of cyst and it is found to arise from the remnants of the dental lamina and basal offshoots. It is well known for its unique aggressive clinical nature, increased mitotic rate, high tendency to reoccur and greater epithelial turnover rate. The Dentigerous cyst is a developmental cyst which encloses the crown portion of an unerupted tooth by expansion of its follical and is attached to its neck. It mainly develops in two different manners; firstly by the accumulation of fluid in between the reduced enamel epithelium and crown, or due to fluid accumulation between the layers of reduced enamel epithelium. Radicular cysts (also called as periapical cysts) are lesions that arise as a sequalae of pulpal necrosis and are considered being of an inflammatory origin.

The study of tumors of the oral cavity and adjacent structures holds importance in dentistry because of the important role which the dentist plays in the diagnosis and treatment of these lesions. Ameloblastoma is a true neoplasm of enamel organ type tissue which does not undergo differentiation to the point of enamel formation. It has been described very aptly by Robinson as being a tumor that is 'usually unicentric nonfunctional, intermittent in growth, anatomically benign and clinically persistent'.

Morphometric analysis is a method which provides early and quantitative factors to judge the behavioral pattern and aggressivety of the lesions for the diagnosis. Histomorphometric analysis involves the study of histological features of lesions, malignancies and cysts with the support of computer aided image analysis softwares. The nuclear cytoplasmic ratio increases at the expense of the cytoplasmic volume.

No study has been done yet to make a comparison between cysts and tumor using morphometry. In the current study we aim to analyse and compare the nuclear cytoplasmic ratio between cysts and tumors.

II. MATERIALS AND METHOD

Source of the Data

The material for the present study included a total no. of 100 Histopathological diagnosed cases of odontogenic cysts and tumor comprising of 25 cases each of odontogenic keratocyst, Radicular cyst, dentigerous cyst and Ameloblastoma. 10 cases of normal mucosa were selected as controls. Haematoxylin and eosin stained slides of the same were retrieved from archives of the department of Oral Pathology IDST, Modinagar and Department of Dermatology SMGS, Govt. Medical College Jammu.

Selection Criteria

Inclusion Criteria

The study samples included histopathologically diagnosed cases of odontogenic cysts and tumor.

Exclusion Criteria

Lesions associated with any other pathology.

ARMAMENTARIUM

The materials used in the study were as follows:

- One Haematoxylin and Eosin stained slide each of histopathologically diagnosed cases of Odontogenic keratocyst, Radicular cyst, Dentigerous cyst, Ameloblastoma and Normal mucosa.
- Computer aided image analysis software (IMAGEJ 4.1) for performing the morphometric analysis.

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- Olympus CX41RF microscope for analyzing the case slides.
- Digital camera (CANON IXUS 285) for capturing the photomicrographs of the all the case slides.

The selected case slides were imported one by one in the image analysis software. Areas of precession were enhanced automatically by software.
In each case slide 30 cells were manually selected and analyzed.

The cells were analyzed for cell area, nuclear area and nuclear cytoplasmic ratio in step ladder manner, moving the slide from left upper corner to right and then down in order to avoid measuring the same cell again.
The result table was automatically generated which was later saved in the form of excel sheets in the computer. The nuclear cytoplasmic ratio (N: C), was then calculated manually using the following formula .
Formula : N:C ratio = N area / C area- N area

Where, N: C ratio – Nuclear cytoplasmic ratio
N area – Nuclear area
C area – Cellular area

The data obtained was statistically analyzed.

III. RESULTS

A one way analysis of variance (ANOVA) is used to compare the mean cellular diameter, mean nuclear diameter and mean nuclear cytoplasmic ratio for each point of time followed by post hoc bonferroni to compare inter group variation, P value less than 0.05 was considered as significant.
Significance is assessed at 5% level of significance. ANOVA along with post hoc Bonferroni has been used to find the significance of study parameters on categorical scale and ordinal scale between two or more than two groups.

Mean

$$\mathbf{x} = \sum x / n$$

Where, $\sum$ x is the sum of measurements and n is the total subjects in the sample.

Standared deviation

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

σ = lower case sigma
$\sum$ = capital sigma
$\bar{x}$ = x bar

n = number of subjects examined

All the data compiled in master excel sheets was entered into SPSS software version 21.00. Data was presented in the form of mean and standared deviation. The nuclear cytoplasmic ratio was calculated by dividing nuclear area by the difference of cellular and nuclear area.

N/C = NA/NC-NA

Were, NA – nuclear area, NC- cellular area

The statistical analysis was divided into two parts. A one way analysis of variance (ANOVA) was used to compare the mean cellular diameter, mean nuclear diameter and mean nuclear cytoplasmic ratio of all the 25 cases of cysts and tumor along with 10 cases of normal mucosa. In **table 1**, the mean cellular area among the 5 study groups was calculated. **Graph 1** reveals the same depicting that the mean cellular area of the ameloblastoma was maximum followed by odontogenic kertocyst, dentigerous cyst, radicular cyst, being least in normal mucosa. In **table 2**, the mean nuclear area was calculated among the five study groups. **Graph 2** depicts the same and reveals that the mean nuclear area of ameloblastoma was maximum followed by odontogenic keratocyst, dentigerous cyst, radicular cyst, being least in normal mucosa. In **table 3**, the mean nuclear cytoplasmic ratio of all the five study groups was calculated individually. **Graph 3** illustrates the same in which the maximum nuclear cytoplasmic variation was seen in ameloblastoma, followed by odontogenic keratocyst, dentigerous cyst, radicular cyst and least value was shown in normal mucosa. By using ANOVA a statistically significant result was obtained where probability value (p value) 0.05 were considered as significant. It shows that variation in the nuclear cytoplasmic ratio occurs when the severity of the lesion increases. It shows that more variation in the nuclear cytoplasmic ratio is seen when the aggressiveness of the lesion increases.
The second part of the statistical analysis was done by using post hoc bonferroni test. It was used to do the inter-group comparison of the mean cellular

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area, mean nuclear area and the mean nuclear cytoplasmic ratio. All the groups are named as Group 1, Group 2, Group 3, Group 4 and Group 5.

- Group 1 - Odontogenic keratocyst.
- Group 2 - Dentigerous cyst.
- Group 3 - Radicular cyst.
- Group 4 – Ameloblastoma
- Group 5 - Normal oral mucosa

While doing the inter group comparison of mean cellular area between the groups **(table 4)** the p-value was found to be significant ($p \leq 0.05$)which reveals that there is a marked difference between the mean cellular areas of all cysts and tumor when compared with each other and with normal mucosa.

The inter group comparison of mean nuclear area was also done and significant difference was obtained among the group sets ($p \leq 0.05$).

The inter group comparison of mean nuclear cytoplasmic ratio was also done. On comparing the mean nuclear cytoplasmic ratio between the group sets a significant difference was obtained, but the comparison of group 4 with group 1 was not significant, signifying that both these groups (ameloblastoma and odontogenic keratocyst) are highly invasive in nature as compared to other study groups **(table 5).**

GROUP	Mean	S.D.	F-value	P value
OKC	163.645	24.251	103.248	0.0001
Dentigerous Cyst	159.813	28.219		
Radicular Cyst	144.958	23.491		
Ameloblastoma	167.488	26.356		
Normal Mucosa	144.949	25.352		

*One Way ANOVA test applied **TABLE 1: Mean cellular area among the five study groups**

GROUP	Mean	S.D.	F-value	P value
OKC	141.001	23.651	58.025	0.0001
Dentigerous Cyst	136.327	28.025		
Radicular Cyst	130.072	23.499		
Ameloblastoma	145.963	26.079		
Normal Mucosa	128.749	23.585		

*One Way ANOVA test applied **TABLE 2: Mean Nuclear Area among the five study groups**

GROUP	Mean	F-value	P value
OKC	37.997	6.043	0.0001
Dentigerous Cyst	37.271		
Radicular Cyst	29.197		
Ameloblastoma	74.451		
Normal Mucosa	15.103		

*One Way ANOVA test applied **TABLE 3: Mean Nuclear cytoplasmic ratio among the five study groups**

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Group (I)	Groups (J)	Mean Cellular Area	
		Mean Difference	p-value
Group 1 (OKC)	Group 3 (Radicular Cyst)	-3.832441	0.038*
	Group 5 (Normal Mucosa)	-7.674898	0.0001*
Group 2 (Dentigerous Cyst)	Group 1 (OKC)	-14.855179	0.0001*
	Group 3(Radicular Cyst)	-18.687620	0.0001*
	Group 5 (Normal Mucosa)	-22.530077	0.0001*
Group 3 (Radicular Cyst)	Group 5 (Normal Mucosa)	-3.842457	0.0001*
Group 4 (Ameloblastoma)	Group 1 (OKC)	-14.864372	0.045*
	Group 2 (Dentigerous Cyst)	-.009193	0.0001*
	Group 3 (Radicular Cyst)	-18.696813	0.0001*
	Group 5 (Normal Mucosa)	-22.539270	0.0001*

TABLE4: Intergroup comparison of mean cellular area using Post hoc Bonferroni

Group (I)	Groups (J)	Mean Nuclear Area	
		Mean Difference	p-value
Group 1 (OKC)	Group 3 (Radicular Cyst)	9.636309(*)	0.0001*
	Group 5 (Normal Mucosa)	4.961843(*)	0.040*
Group 2 (Dentigerous Cyst)	Group 1 (OKC)	-15.891064(*)	0.0001*
	Group 3 (Radicular Cyst)	-6.254755(*)	0.0001*
	Group 5 (Normal Mucosa)	-10.929221(*)	0.0001*
Group 3 (Radicular Cyst)	Group 5 (Normal Mucosa)	-4.674467	0.020*
Group 4 (Ameloblastoma)	Group 1 (OKC)	-17.214576(*)	0.044*
	Group 2 (Dentigerous Cyst)	-1.323512	0.0001*
	Group 3 (Radicular Cyst)	-7.578267(*)	0.0001*
	Group 5 (Normal Mucosa)	-12.252733(*)	0.0001*

The mean difference is significant at the .05 level

TABLE 5: Inter group comparison of mean nuclear area using Post hoc Bonferroni

The mean difference is significant at the .05 level.

Group (I)	Groups (J)	Mean NCR	
		Mean Difference	p-value
Group 1 (OKC)	Group 3 (Radicular Cyst)	59.348215	0.001*
	Group 5 (Normal Mucosa)	45.253871	0.001*
Group 2 (Dentigerous Cyst)	Group 1 (OKC)	-37.180185	0.028*
	Group 3 (Radicular Cyst)	22.168030	0.031*
	Group 5 (Normal Mucosa)	8.073686	0.001*
Group 3 (Radicular Cyst)	Group 5 (Normal Mucosa)	-14.094344	0.024*
Group 4 (Ameloblastoma)	Group 1 (OKC)	-36.454040	0.654
	Group 2 (Dentigerous Cyst)	0.726145	0.034*
	Group 3 (Radicular Cyst)	22.894175	0.021*
	Group 5 (Normal Mucosa)	8.799831	0.001*

TABLE 6: Inter group comparison of mean nuclear cytoplasmic ratio using Post hoc Bonferroni

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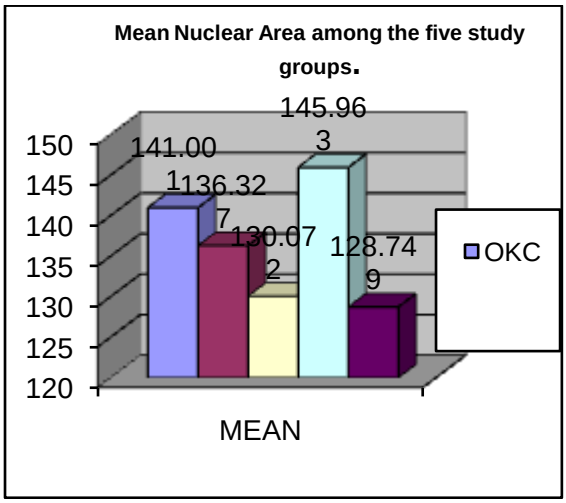
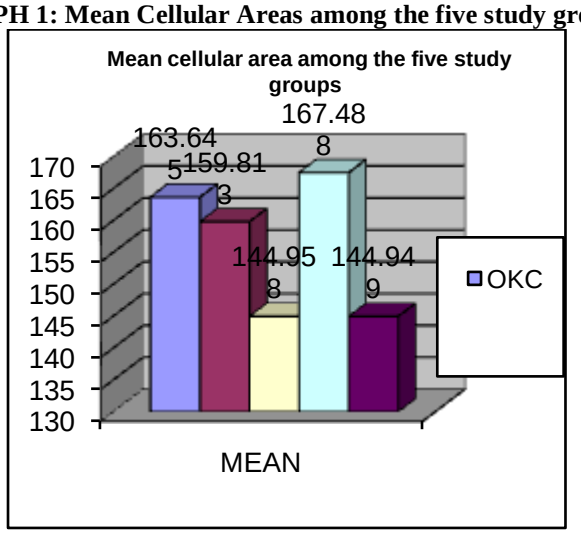
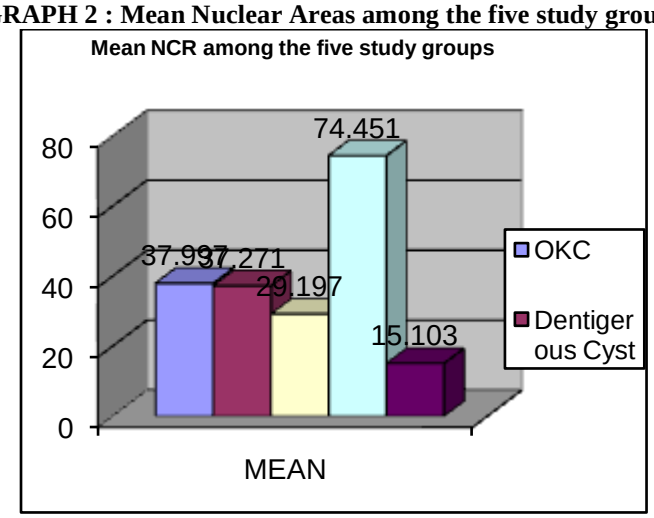


GRAPH 1: Mean Cellular Areas among the five study groups.



GRAPH 2 : Mean Nuclear Areas among the five study groups



GRAPH 3 : Mean NCR ratio among the five study groups

IV. DISCUSSION

It has been suggested that morphometry can be used as an emphasizing diagnostic and prognostic medium for various cellular and nuclear features. It has also been used as a parameter in predicting epithelial neoplastic transformation. It has been found that more prominent or severe the histological changes are, the more severe is the

grade of the lesion. Though, all the cases do not show these changes always as there is forever a particular subjectivity involved in the interpretation.

While performing the assessment of dysplastic or neoplastic lesions a great emphasis is laid on the changes in nuclear size and shape. Nuclear shape, nuclear size and their variability which is calculated as nuclear cytoplasmic ratio is considered of primary importance for assessing the severity of the lesion. Computerized nuclear morphometry has got various advantages such as objectivity, reproducibility and it can be quickly performed by means of conventional microscopic analysis and can be judged as a useful prognostic and diagnostic method.[54]

In the current study, for the first time we have evaluated the nuclear cytoplasmic ratio, (calculated by the mean of the nuclear and cellular areas) in the epithelium of three cysts and a tumor, compared them to each other and with the control specimens. Morphometric analysis was used for the comparison of the nuclear cytoplasmic ratio in 25 cases each of odontogenic keratocyst, dentigerous cyst, radicular cyst, amaeloblastoma and 10 cases of normal buccal mucosa. The mean nuclear cytoplasmic ratio was calculated from the mean of nuclear and cellular areas by using IMAGE J morphometric software. The inter group comparison among the five study groups was also done for the assessment of mean cellular area, nuclear area and nuclear cytoplasmic ratio as illustrated in **Table 4, 5 and 6** respectively. An inter group comparison of the mean cellular areas in all the five study groups revealed a highly significant p- value in all the comparative sets ($p < 0.05$). The comparison was assessed by statistical analysis using ANOVA and Post Hoc Bonferroni test.

The current study revealed that the mean of nuclear and cellular areas of cysts and tumors was significantly high as compared to that of normal mucosa. This indicates that in lesions the mean and the ratio of nuclear cytoplasmic values gets altered. Increase in the nuclear and cellular areas are two significant changes that occur in a proliferating cell as supported in literature.[56] Accordingly, it was observed that as we progress from normal to an aggressive lesion, the nuclear and cellular dimensions gradually increase.Hence, from the present study it can be concluded that morphometry could be used for the comparative assessment of nuclear cytoplasmic ratio between different lesions. A higher nuclear cytoplasmic ratio is indicative of a more aggressive lesion, which could aid the clinician in early prognostication.

V. CONCLUSION

The changes in the diameter of cell and nucleus bring out important information regarding the diagnosis and prognosis of various lesions. In this context, the enhancement of the proliferation may be one of the first indicators of malignant transformation. These changes are important to visualize and morphometric analysis is one method which can provide early and quantitative factors for the diagnosis of clinically relevant lesions. The current study reveals that on performing morphometric analysis, the mean cellular area, mean nuclear area and mean nuclear cytoplasmic ratio is maximum in ameloblastoma followed by odontogenic keratocyst, radicular cyst, dentigerous cyst, being the least in normal mucosa.

Thus it can be concluded that morphometry, when used with precision, could be efficient for quantifying the morphologic characteristics of cells and tissues. This can aid in assessing the severity of the case and also better prognostication.

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