Original Article

Analysis of Argyrophilic Nucleolar Organizer Region Score and Epithelial Dysplasia in Common Odontogenic Cysts Seen in a University Teaching Hospital

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Abstract

Aim: Carcinomatous transformation in odontogenic cysts (OC) lining may be more common than is generally appreciated. Thus, the aim of this study is to predict the potential for neoplastic transformation in the epithelial lining of OC using argyrophilic nucleolar organizer region (AgNOR) scores and degree of epithelial dysplasia. **Subjects and Methods:** A retrospective clinicopathological study was undertaken on OC histologically diagnosed over a 40-year period in a university teaching hospital in Southwestern Nigeria. Paraffin blocks of selected cases were retrieved and sectioned for hematoxylin and eosin and AgNOR stain. Evaluation of epithelial dysplasia was done using the WHO 2005 protocol. Estimation of atypical features and AgNOR scoring were done using modified and established protocols. Epi info and GraphPad InStat software packages were employed to manage the ensuing data. **Results:** The commonly observed OC was odontogenic keratocyst (OKC), which accounted for 44.5%, radicular cyst (RC) (24.2%), calcifying odontogenic cyst (COC) (12.7%), and dentigerous cyst (DC) (11.5%). Nuclear pleomorphism was the most common atypical feature and was present in 50% each of DC and COC, 42% of OKC, and 36% of RC. Drop-shaped rete pegs were rarely observed. A significant association was observed between the presence of moderate dysplasia and histologic type of OC ($P = 0.022$). Mean AgNOR scores per 100 cells was OKC (137.38 ± 35.82), RC (132.04 ± 34.22), COC and DC (119.00 ± 10.93) and (96.91 ± 38.88) respectively. This was not statistically significant ($P = 0.218$). **Conclusions:** As moderate epithelial dysplasia was more significantly associated with OKC than other cysts, it is concluded that the potential for malignant transformation is higher in OKC than other histologically categorized types of cysts.

Keywords: Argyrophilic nucleolar organizer regions, epithelial dysplasia, odontogenic cysts

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Introduction

Lining of odontogenic cysts (OC) is derived from odontogenic epithelium,[1] which can undergo carcinomatous transformation.[2,3] Primary intraosseous carcinoma (0.3%–2%) may originate from OC.[4-7] Epithelial dysplasia in cyst lining is the key to the diagnosis of carcinomatous transformation in OC.[8,9] Argyrophilic nucleolar organizer region (AgNOR), a cell proliferative marker, has been used in OC[10] and some other Nigerian studies.[11-13] The prevalence of odontogenic carcinoma in Nigeria varies from 4.9%[14] to 1.2%.[15] The proportion that originates from OC has, however, not been reported. This study investigated the potential for carcinomatous transformation of OC using the AgNOR technique and the degree of epithelial dysplasia.

Subjects and Methods

A retrospective clinicopathological study was undertaken on OC histologically diagnosed over a period of 40 years (1970–

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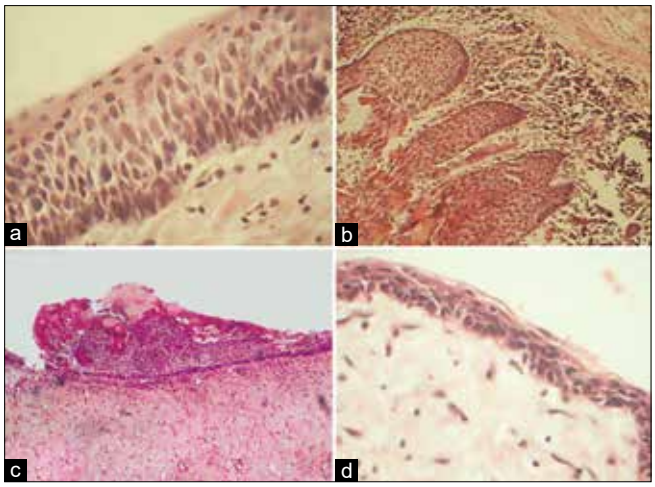

Figure 1: (a) Epithelial lining of odontogenic keratocyst showing nuclear hyperchromatism and cellular pleomorphism (H and E, ×400). (b) Epithelial lining of radicular cyst showing dropped shaped rete pegs and severe inflammation within the connective tissue (H and E, ×200). (c) Calcifying odontogenic cyst showing ghost cells and nuclear hyperchromatism in epithelial lining (H and E,) ×100. (d) Dentigerous cyst with thin flattened non-keratinizing epithelial lining showing nuclear hyperchromatism and pleomorphism (H and E,) ×400

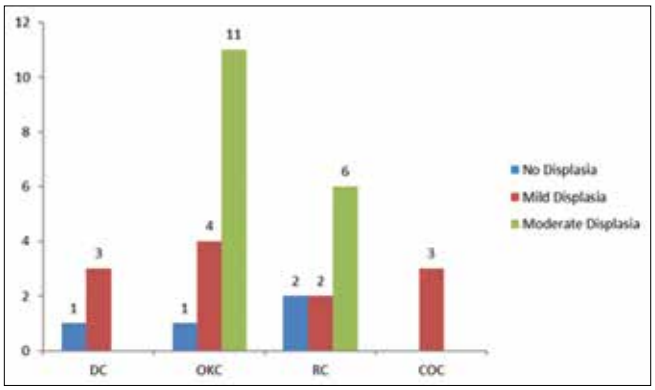

Figure 3: Distribution of epithelial dysplasia in common, categorized histologic types of odontogenic cysts

2010) in the oral biopsy records of the department of oral and maxillofacial pathology/biology, in a university teaching hospital in Southwestern Nigeria. The hematoxylin and eosin (H and E) slides were retrieved, diagnosis confirmed, and cases were grouped according to the Shear and Speight Classification.[16]

The categorized cases were further grouped into two: as cysts of low prevalence and cysts of high prevalence. The low prevalence OC were defined as cases whose proportion constituted <10% of classifiable cysts in the records, while high prevalence OC were defined as those cases whose proportion constituted 10% or more of classifiable cysts in the record. Cases within the high prevalence group that satisfied our inclusion criteria were selected for this study. To avoid the effect of inflammatory changes on the cystic lining, infected OC were excluded with the exception of a radicular cyst (RC) in which inflammation is part of the process of formation.

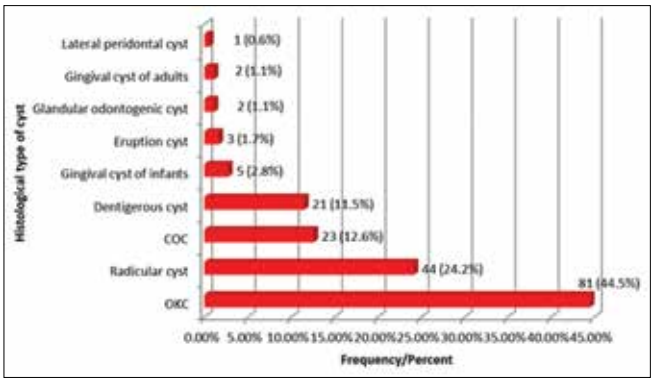

Figure 2: Prevalence of categorized histologic types of odontogenic cysts

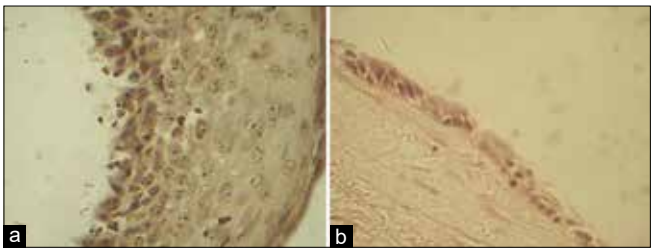

Figure 4: (a) Odontogenic keratocyst showing prominent Nucleolar Organizer Regions (argyrophilic nucleolar organizer regions stain, ×400). (b) Dentigerous cyst showing large Nucleolar Organizer Region dots (argyrophilic Nucleolar Organizer Regions stain, ×400)

Paraffin blocks of selected cases were retrieved, cut into 5 μm-thick duplicate sections, and stained with H and E and AgNOR [Appendix I]. Six slides from the same section were prepared, three of which were stained with H and E, while the remaining three were stained for AgNOR scoring.

Evaluation of epithelial dysplasia was done using the WHO 2005 protocol which entails a combination of architectural disturbance with atypical features. Estimation of atypical features was done by the protocol of Bánóczy and Csiba[17] modified by Odukoya *et al* [Appendix II].[18] Cases were evaluated as no dysplasia, mild, moderate, and severe dysplasia. No dysplasia was scored 0 and defined as cases with no atypical features present, with no evidence of disturbance in the entire epithelium. Mild dysplasia was scored 1 and defined as the presence of one to two atypical features, with disturbance in the epithelium limited to its lower third. Moderate dysplasia was scored 2 and defined as the presence of three to four atypical features, with disturbance in two-third of the epithelium from the basal layer. Severe dysplasia was scored 3 and defined as the presence of more than four atypical features and disturbance extending beyond two-third of the epithelium starting from the basal layer. For each case, three readings were taken by the authors, and the mean epithelial dysplasia score was computed. By consensus, reconciled observations were recorded for each parameter investigated.

Nucleolar organizer regions (NORs) for selected epithelial cells were evaluated using a modification of the counting protocol by Crocker *et al*.,[19] [Appendix III] and scores were recorded. From the three slides prepared for AgNOR

Table 1: Pattern of atypical features in common categorized histologic types of odontogenic cysts

Type of Cysts	Total	Increase nuclear cytoplasmic ratio	Cellular atypia				
			Nuclear pleomorphism	Nuclear Hyperchromatism	Enlarged Nucleoli	Hyperplasia of Basal Layer Cells	Drop Shaped rete ridges
		Freq (%)	Freq (%)	Freq (%)	Freq (%)	Freq (%)	Freq (%)
Radicular cyst	10	5 (20.0)	9 (36.0)	8(32.0)	2(8.0)	0 (0.0)	1(4.0)
Odontogenic keratocyst	16	2 (6.1)	14(42.4)	13(39.4)	3(9.1)	1 (3.0)	0(0.0)
Dentigerous	4	0 (0.0)	3 (50.0)	3(50.0)	0(0.0)	0 (0.0)	0(0.0)
COC	3	0 (0.0)	3 (50.0)	2(33.3)	1(16.7)	0 (0.0)	0(0.0)

Table 2: Proportion of categorized odontogenic cysts with different types of dysplasia

Histologic Type	No dysplasia	Mild dysplasia	Moderate dysplasia	Total dysplasia
Dentigerous	1(25.0)	3(75.0)	0(0.0)	4(100.0)
OKC	1(6.2)	4(25.0)	11(68.8)	16(100.0)
Radicular cyst	2(20.0)	2(20.0)	6(60.0)	10(100.0)
COC	0(0.0)	3(100.0)	0(0.0)	3(100.0)
Total	4(12.1)	12(36.4)	17(51.5)	33(100.0)

Table 3: Distribution of moderate epithelial in relatively common, categorized histologic types of odontogenic cysts (presence of moderate epithelial dysplasia)

Histologic Type	Yes Freq(%)	No Freq(%)	Total Freq(%)
Dentigerous	0(0.0)	4(100.0)	4(100.0)
OKC	11(68.8)	5(31.2)	16(100.0)
Radicular cyst	6(60.0)	4(40.0)	10(100.0)
COC	0(0.0)	3(100.0)	3(100.0)
Total	17(51.5)	16(48.5)	33(100.0)

Chi-square value=9.629 df=3, p=0.022 **. *p statistically significant.

stain, the mean NOR score was computed to obtain a final reading [Figure 1].

Data obtained were analyzed using the Epi info software version 3.3.2 and GraphPad InStat computer statistical software packages. Frequency distribution and Chi-square tests were used to compare differences between proportions, while ANOVA was used for comparison of means. The level of significance was set at $P \leq 0.05$.

Approval for this study was sought for and granted by the Ethics Committee of the university teaching hospital.

Results

From 361 cases of OC diagnosed during the period of study, 182 (50.4%) were categorized into various histologic types, while 179 (49.6%) were not categorized.

The most frequently observed categorized histologic type of OC in this study was odontogenic keratocyst (OKC) [Figure 1a], 81 (44.5%), followed by RC [Figure 1b], 44 (24.2%), calcifying odontogenic cyst (COC) [Figure 1c], 23 (12.6%), and dentigerous cyst (DC) [Figure 1d], 21 (11.5%). Other less commonly categorized histologic types of OC were gingival cyst of infants, 5 (2.8%), eruption cyst, 3 (1.7%), glandular odontogenic cyst, 2 (1.1%), gingival cysts of adult, 2 (1.1%), and lateral periodontal cyst, 1 (0.6%) [Figure 2].

Nuclear pleomorphism was the most commonly observed atypical cellular feature in each histologic type of cyst, with DC (50.0%) and COC (50.0%) having the highest, while OKC (42.4%) and RC (36%) had the lowest [Table 1].

Nuclear hyperchromatism was the second most commonly observed atypical cellular feature in each histologic type of cyst, with DC (50.0%), and OKC (39.4%) having the highest, while COC (33.3%), and RC (32.0%) had the lowest [Table 1].

Both nuclear pleomorphism and nuclear hyperchromatism were the most common atypical changes seen in each of the histologic types. Drop-shaped rete pegs were rarely observed, as these occurred at a frequency of only 4% in RC [Table 1].

The proportion/percentage of mild, moderate, and severe dysplasia in categorized OC is presented in Table 2. There were no cases with severe dysplasia.

Moderate dysplasia was most commonly observed in OKC (68.8%) and RC (60.0%) but was not observed at all in DC and COC [Table 3 and Figure 3]. Significant association was observed between the presence of moderate dysplasia and histologic type of OC ($\chi^2 = 9.629$, df = 3, $P = 0.022$**).

OKC cyst (137.38 ± 35.82) had the highest mean AgNOR score/100 cells [Figure 4a], followed by RC (132.04 ± 34.22), while COC and DC had mean AgNOR scores/100 cells of 119.00 ± 10.93 and 96.91 ± 38.88 [Figure 4b], respectively [Table 4]. However, the difference in mean AgNOR scores between the different histological types of OC was not statistically significant (F = 1.563, $P = 0.218$) [Table 4].

Table 4: Distribution of argyrophilic nucleolar organizer regions scores per 100 cells in relatively common histologic type of categorized odontogenic cysts

Histologic types of cyst	Range	Median	Mean+SD	Sample size
Radicular cysts	38.5 - 154	141.75	132.04+34.22	10
Dentigerous cysts	39.75 - 125.4	111.25	96.91+38.88	4
OKC	50.5 - 182.3	145.5	137.37+35.82	16
COC	109 - 131.4	118.0	119.72+10.93	3

F=1.563, p=0.218 ($p \geq 0.05$) not statistically significant

Discussion

This study which examined epithelial dysplasia and cell proliferation (using the AgNOR technique) in OC was undertaken on OC managed over a 40-year period in a university teaching hospital in Southwestern Nigeria.

In this study, it was observed that there was an association between the presence of moderate dysplasia and histologic type of OC. Furthermore, the proportion of cases of OKC with moderate epithelial dysplasia was found to be the highest, followed by RC. It could be argued that this observation corroborates the higher biologic and clinical aggression that is often associated with OKC.[20,21]

The association of moderate dysplasia with RC in this study may be attributed to the chronic inflammatory component of the cyst. Chronic inflammation has been documented to induce epithelial dysplasia in oral lesions.[22] Long-standing chronic inflammation has been suggested as the principal factor responsible for carcinomatous transformation in OC.[23] Furthermore, Gardner suggested that malignant transformation in the lining of OC is low when the cyst is not infiltrated with chronic inflammatory cells.[24] Schwimmer *et al*. have reported in their series that carcinomatous changes in cysts occurred mostly in a residual cyst, which is a form of RC.[25]

Although not statistically significant, observation of a higher mean AgNOR score in OKC than RC and any other OC in this study corroborates the earlier observation that OKC has a higher proliferative index than any other OC. This proliferative index contributes to its biologic aggression in respect of moderate epithelial dysplasia, as earlier mentioned. Proliferative markers such as p53 and ki67 have been used in a number of immunohistochemical studies to investigate cell proliferating activities in OC.[26-29] In one of such studies, p53 expression in OC showed that OKC had the highest expression,[26] thereby corroborating its relatively higher biologic behavior.

Conclusions

This study shows that moderate epithelial dysplasia was significantly associated with OKC compared to the other types of common OC. The mean AgNOR score was unable to significantly discriminate cell proliferative index between the histologic types of OC. It is concluded on the basis of epithelial dysplasia that OKC has a higher potential of carcinomatous transformation than other common OC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Appendices

Appendix I: Protocol for AgNOR Staining

Method:

1. Five micron paraffin section was de-waxed in xylene and hydrated through descending grades of alcohol
2. After thorough washing under running tap water, the slides were placed in coupling jar and rinsed in three changes of de-ionized water
3. 50% silver nitrate solution was prepared in de-ionized water warmed up to 45°C [1 volume of 2% gelatin in 1% aqueous formic acid (also at 45°C)] were added to 2 volume of the silver nitrate solution to make up the working solution
4. The slides (still in the coupling jar) were drained of the de-ionized water and treated with the working silver solution for 15 to 20 min at 45° in the dark
5. After rinsing in several changes of de-ionized water, the solutions were dehydrated through ascending grades of alcohol for about 2 min
6. The sections were then be cleared in xylene and mounted in disterene, tricresyl phosphate-xylene (DPX).

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Appendix II

List of architectural disturbances (A) and the features scored (B):

A. The Following Atypical Features were Considered as Features of Epithelial Dysplasia (Architectural Disturbances):

1. Irregular epithelial stratification
2. Hyperplasia of the basal layer
3. Drop-shaped rete ridges
4. Increased number of mitotic figures (at least two mitotic figures per high-power field, in a minimum of two different fields)
5. Loss of polarity of basal cells (this will be considered only in areas where the stratum basale is intact)
6. Increased nuclear-cytoplasmic ratio (at least five cells with this feature per high- power field, in a minimum of two different fields)
7. Nuclear polymorphism (at least ten abnormal cells per high-power field, in a minimum of two different fields)
8. Nuclear hyperchromatism (at least ten hyperchromatic nuclei per high-power field, in a minimum of two different fields)
9. Enlarged nucleoli (at least ten per high-power field, in a minimum of two different fields)
10. Keratinization of a single cell or cell group (at least two foci of keratinization per high-power field, in a minimum of two different fields)
11. Loss of intercellular adherence.

B. Enumeration of Epithelial Dysplasia (Features Scored).

1. No dysplasia: A case was diagnosed as no dysplasia and scored 0 if no feature of dysplasia was seen and there was no evidence of disturbance in the epithelium
2. Mild dysplasia: A case was evaluated as mild dysplasia and scored 1 if one or two features of dysplasia were observed and disturbance in epithelium was limited to lower-third of the epithelium
3. Moderate dysplasia: A case was evaluated as moderate dysplasia and scored 2 if three or four features of dysplasia were observed and disturbance in the epithelium extended up to two third of the epithelium starting from the basal layer
4. Severe dysplasia: A case was evaluated as severe dysplasia and scored 3 if features of dysplasia seen numbered more than 4 and disturbance in the epithelium extends beyond two-third of the epithelium starting from the basal layer.

Features of dysplasia or atypical features were evaluated by the protocol of Banoczy and Sciba[17] modified by Odukoya *et al*.[18]

Evaluation of epithelial dysplasia was done according to the protocol of WHO 2005 which combined architectural disturbance with features of dysplasia.

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Appendix III: Summary of the Counting Protocol of the Agnor Stain

1. Positive result showed brownish black intranuclear dots representing the nucleolar organizer regions (NORs)
2. Only the intranuclear dots in the epithelial cells of selected cases were assessed using NORs technique

3. Modified counting protocol of Crocker, 1989[19] was employed. Using this method, each slide was divided into four quadrants from each of which, five points were identified
4. From a circular view observed under ×20 objective lens, the identified points were labeled as North, South, East, West, and Center. In each of the identified points, 20 consecutive cells were evaluated for presence of NOR staining dots in epithelial cells at ×1000 magnification (×100 oil immersion objective lens). The total number of cells assessed per quadrant was 100 cells
5. The NOR scores per quadrant were scaled down to NOR dots per 25 cells, by dividing 100 by 4
6. A sum total for all the four quadrants gave the NORs score per 100 cells for each slide. Three slides were prepared per case. The mean NOR score for each case was computed from the averages of the three readings (slides) per case.

Diagram showing procedure for determining mean AgNOR score per 100 cell