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ORIGINAL ARTICLE

Actual Proliferating Index and p53 protein expression as prognostic marker in odontogenic cysts

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BACKGROUND: The purpose of this study was to evaluate the biological aggressiveness of odontogenic keratocyst/keratocystic odontogenic tumour (KCOT), radicular cyst (RC) and dentigerous cyst (DC) by observing the actual proliferative activity of epithelium, and p53 protein expression.
METHODS: The actual proliferative activity was measured by Ki-67 Labelling Index and argyrophilic nucleolar organizing regions (AgNOR) count per nucleus. The p53 protein expression was also evaluated.
RESULTS: Ki-67 positive cells were observed higher in suprabasal cell layers of KCOT with uniform distribution, a few of them were predominantly observed in basal cell layer in RC and DC. The AgNOR count was significantly higher in suprabasal cell layers of KCOT. The actual proliferative activity was noted to be higher in suprabasal cell layers of KCOT. The p53 immunolabelling was dense and scattered in basal and suprabasal cell layers in KCOT. The weakly stained p53 positive cells were observed diffusely distributed in KCOT, whereas they were mainly seen in basal cell layer of RC and DC.
CONCLUSION: The quantitative and qualitative differences of the proliferative activity and the p53 protein expression in sporadic KCOT may be associated with intrinsic growth potential that could play a role in its development and explain locally aggressive biological behaviour. AgNOR count and p53 protein detection in odontogenic lesions can be of great consequence to predict the biological behaviour and prognosis.
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Keywords: Actual Proliferating Index; odontogenic keratocysts/keratocystic odontogenic tumour; radicular cysts; dentigerous cyst; Ki-67 Labelling Index; p53 Labelling Index; argyrophilic nucleolar organizer regions

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Introduction

Odontogenic cysts are defined as those cysts, which arise from the enamel organ or their remnants. During and after odontogenesis, these cell remnants are common source of cystic change within the jawbones. Odontogenic keratocysts (OKC) represents 11.2% of all odontogenic developmental cysts, and are thought to arise from derivatives of embryologic dental lamina, or its remnants and extensions of basal cells from the overlying epithelium (Stenman *et al*, 1986; ; Stoelinga, 2003). The World Health Organization in 2005 (Barnes *et al*, 2005) based on behaviour, histology and genetics, reclassified the OKC as keratocystic odontogenic tumour (KCOT). Radicular cysts (RC) are the most common cysts of inflammatory origin (about 52.3% of all diagnosed jaw cysts). They arise caused by the effects of inflammation on epithelial residues (the Cell Rests of Malassez). Dentigerous cysts (DC) are the most common developmental odontogenic cysts making up to 16.6% of all jaw cysts (Shear, 1992). Earlier, it was thought that the epithelial lining of developmental odontogenic cyst has more proliferative potential than the epithelial lining of inflammatory cysts (Hume *et al*, 1990; Stenman *et al*, 1986).

Cell proliferation activity has been investigated in OKC/KCOT previously by various authors using Ki-67 Labelling Index (LI) (Kichi *et al*, 2005; Kim *et al*, 2003; Li *et al*, 1995; Mathews *et al*, 1988; Saracogly *et al*, 2005; Slootweg, 1995), argyrophilic nucleolar organizer regions (AgNOR) count (Allison and Spencer, 1993; Coleman *et al*, 1996; Eslami *et al*, 2003), autoradiography and DNA cytophotometry (Scharffetter *et al*, 1989), mitotic count (Stoelinga, 2003), proliferating cell nuclear antigen (PCNA) (Ogden *et al*, 1992; Li *et al*, 1994; El Murtadi *et al*, 1996; Piatelli *et al*, 1998), IPO-38 (Thosaporn *et al*, 2004), topoisomerase αII enzyme (Kimi *et al*, 2001) and enzyme histochemistry including oxidative enzyme and acid phosphatase (Magnusson, 1978). The actual proliferative activity can be assessed by measuring:

1. The proportion of cells committed to cell cycle that is 'growth fraction', so as to assess the intrinsic growth potential of the lesion, which is easily assessed by Ki-67 LI (Schluter *et al*, 1993). Ki-67 antigen is present in all the active parts of cell cycle – G1, S, G2, M phase and absent in G0 phase. Its expression increases with the cell cycle progression and reaches its peak during the G2 and M phases (Sawhney and Hall, 1992; Brown and Gatter, 2002).
2. The rapidity of the cell turnover is evaluated by speed of the cell cycle, so as to assess the growth rate of the lesion, which is easily assessed by AgNOR count per nucleus (Derenzini *et al*, 1995, 2004). The amount of AgNOR represents a cell kinetics parameter and can be used for prognostic purposes. Shorter the cell cycle, greater is the synthesis of rRNA for each time unit and thus, the total quantity of AgNOR present in the nucleolus (Derenzini *et al*, 1998, 2000).

Therefore the actual proliferative activity of lesion can simply be expressed by the equation [PA = Ki-67 or MIB-1 scores × AgNOR quantity] (Dong *et al*, 1997; Pich *et al*, 2004). In this study, for the first time, the actual proliferative activity of epithelium of KCOT, RC and DC was evaluated by using Ki-67 LI and AgNOR count per nucleus.

p53 protein is a product of the tumour suppressor p53 gene. The tumour suppressor gene functions in G1 arrest to allow the repair of DNA damage and to prevent the cell from entering the S phase of the cell cycle or alternatively to guide the damaged cells to apoptosis (Agarwal *et al*, 1998; Kumar *et al*, 2004). In this study, p53 expression was investigated to evaluate its role as proliferation marker in odontogenic cysts by using monoclonal DO-7 antibody, which detects both wild type and mutant p53 proteins. Thus, the AgNOR histochemistry and immunohistochemistry (IHC) for Ki-67 and p53 antigens were employed to evaluate the cell proliferation in epithelial lining of KCOT, DC and RC in this study.

Materials and methods

This study was carried out at the Department of Oral and Maxillofacial Pathology and Microbiology, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences, Sawangi (M), Wardha, Maharashtra, India. Samples were also collected from the Government Dental College and Hospital Nagpur, KLE's Institute of Dental Sciences Belgaum, Karnataka and VSPM Dental College and Hospital Nagpur. The study included 36 histopathologically diagnosed cases of KCOT, 30 cases of DC, 30 cases of RC and 12 normal oral mucosa (NOM). The study was performed on tissues fixed in 10% neutral buffered formalin, paraffin embedded tissue. The sections were cut serially to 4 um thickness for AgNOR staining and 5 um for IHC to evaluate expression of Ki-67 and p53 antigens.

Procedure

To enhance section adhesion and minimize section loss because of pretreatment, slides were precoated with silane (3-aminopropyl trimethoxy silane; Product code: A-3648, Sigma-Aldrich).

AgNOR staining method

Solution A: Two grams (2% w/v) of gelatin (Art. 3920, Loba Chemie) powder was added to 100 ml of distilled water with temperature maintained at 60̊–70°C over a water bath. Later 1 ml of formic acid (1%) (H.COOH = 46.03; Product code: 24205, Qualigens) was added and mixture was stirred thoroughly. **Solution B:** - 25 grams silver nitrate ($AgNO_3$ = 169.87; Product code: 27464, Qualigens) was added to 50 ml of distilled water. After the dissolution, the liquid was filtered with #1 Whatman filter paper. Working solution: 25 ml of solution A was mixed with 50 ml of solution B to get a resultant 75 ml of working solution. Staining was carried out according to 'International Committee on AgNOR Quantitation' (Derenzini *et al*, 2004). Slides were hydrated and heated in microwave oven at 750 watt for 10 min in 10mM sodium citrate buffer (pH 6.0). These sections were then washed with double distilled water three times for 5 min each and incubated in dark for 20 to 25 min in working solution at room temperature. The slides were washed in running double distilled water for 10 to 15 min.

Quantification of AgNOR

Two hundred nuclei were examined per slide in basal cell layer and suprabasal cell layers (4–5 cells above the basal cell layer) in five randomly selected areas among all the groups. The mean number of AgNOR per cell was calculated in basal cell layer and suprabasal cell layers and the mean was taken for complete epithelium. The counting was done by carefully focussing on the clearly defined dots of AgNOR within the nucleoli and small AgNOR outside nucleoli. The AgNOR appear as dark brown or black intranuclear bodies against a yellowish background. The modified counting protocol by Coleman HG and Altini M in 1996 was utilized.

Immunohistochemical method for detection Ki-67 and p-53 antigen

For IHC, Peroxidase Detection System (Streptavidin-Biotin Detection System HRP-DAB; Product Code: RE7110K, Novocastra Kit) was employed. The sections were deparaffinized with xylene and hydrated. The slides were heated in microwave oven for 10 min in 0.01 M sodium citrate buffer (pH 6.0) for antigen retrieval and bench-cooled for 20 min, and again the same cycle was repeated. Endogenous peroxidase activity was blocked by incubating the section with 3% H_2O_2 in methanol for 30 min. The sections were then washed three times with gentle shaking in PBS for 5 min each. To prevent non-specific reactions, sections were incubated with 10% serum for 10 min. Prediluted Ki-67 antibody (clone MIB-1; Product code: N1633; Dako, Denmark) and p53 antibody (clone DO-7; Product code: N1581, Dako, Denmark) were incubated at room temperature in humidifying chamber for 60 min, and then at 4°C, overnight. Known hyperplastic lymph node and squamous cell carcinoma samples showing good Ki-67 and

p-53 expression were used as a positive control respectively. One section from each positive control was used as the negative control by omitting the primary antibody and by incubating with PBS/serum. After the primary antibody and antigen reaction, the sections were rinsed in PBS three times for 10 min each. The secondary antibody (Biotinylated rabbit anti mouse IgG) was incubated at room temperature in humidifying chamber for 30 min. After the PBS washing three times for 10 min each, sections were incubated with Streptavidin-peroxidase reagent in a humidifying chamber at room temperature for 30 min. After the PBS washing three times for 10 min each, freshly prepared substrate/chromogen solution of 3,3′-Diaminobenzidine in provided buffer (mixing 5μl of concentrated DAB in 50ml of substrate buffer) was used to visualize the antigen-antibody reaction. Finally, the sections were counterstained in Mayer's haematoxylin. Sections were examined by conventional light microscopy (Leica microscope with image analyzer Leica Qwin standard software).

Assessment of immunohistochemically stained sections

The cells were considered positive for Ki-67 and p53 antigen if there was an intranuclear DAB staining (brown colour). All the stained nuclei were scored positive regardless of their intensity of staining. Cells that lacked a clear nucleus were excluded. Minimum of 1000 cells were counted in each section. Tissue sections were scanned at a 100 X magnification for most heavily labelled Ki-67 and p53 positive cells in the epithelial linings. Cell counts were made at 400 X magnification with conventional light microscope in 10 randomly selected fields. In KCOT and NOM, the constituent cells of the lining epithelium were divided into basal, suprabasal/intermediate and surface layers and the mean was taken for complete epithelium. The columnar/low-columnar cells located in one row at the basement membrane were considered as the basal cell layer. The superficial cell layers constitute flattened or polygonal cells consisting of 1–5 layers localized just underneath the surface of the lining epithelium. The suprabasal/intermediate cell layers were composed of relatively large round cells between the basal and the surface layers. In RC and DC, the counting was done in basal cell layer and suprabasal cell layers and, the mean was taken for complete epithelium.

The number of positively stained nuclei was expressed as a percentage of the total number counted for individual layer and in complete epithelium.

$$Ki-67/p53 labelling index(LI) = \frac{Number of IHC Positive Cells(Ki-67/p53) \times 100}{Total number of cells observed}$$

Actual proliferative activity was calculated by using the following equation (Dong *et al*, 1997; Pich *et al*, 2004).

'Actual Proliferative Index (PI) = Ki-67 or MIB-1 LI × AgNOR count'.

Statistical analysis

Group mean for Ki-67 LI, p53 LI, AgNOR count and API was derived for each group. The data were analysed statistically using (spss 14.0 version software) Paired Samples *t*-test, one-way ANOVA, Multiple Comparisons: Dunnett D (two-sided) test, multiple comparisons using Tukey-HSD test and Pearson's rank correlation analysis test.

Results and observation

Ki-67 LI

Ki-67 antigen was expressed in 97.23% of KCOT, 86.67% of RC, 93.34% of DC and 100.00% of NOM (Table 2). Ki-67 was expressed predominantly in suprabasal cell layers with uniform distribution in epithelial lining of KCOT. However, few Ki-67 positive cells in basal cell layers and very few focal areas of suprabasal Ki-67 positive cells were noted in RC in relation to elongated rete pegs and increased thickness of epithelial lining. In DC, Ki-67 positive cells were very few in basal cell layer (Figure 1).

Ki-67 LI was significantly higher in suprabasal cell layers than basal cell layer of KCOT, vice versa in RC, DC ($P < 0.001$) and NOM ($P = 0.001$). Ki-67 LI was significantly higher in KCOT than DC and RC with respect to basal cell layer ($P < 0.05$), supra basal cell layer and complete epithelium ($P < 0.001$). The higher Ki-67 LI was noted in KCOT than NOM in supra basal cell layer and complete epithelium ($P < 0.001$). Ki-67

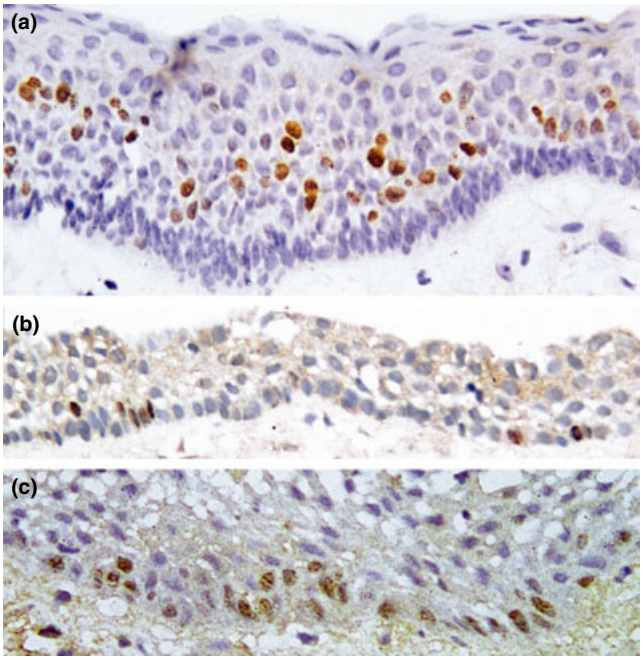

Figure 1 Photomicrograph of immunostaining for Ki-67. (**a**) In keratocystic odontogenic tumour, the Ki-67 immunopositive cells are predominantly noted in the suprabasal cell layers of lining epithelium. (**b**) In dentigerous cyst, the very few Ki-67 positive cells are found in basal cell layer. (**c**) In radicular cyst, the few Ki-67 immunopositive cells are found in basal cell layer (original magnification ×400)

Table 1 The mean Ki-67 Labelling Index, AgNOR count per nucleus and p53 Labelling Index in KCOT, RC, DC, and NOM

Cell layers	*Groups*	*n*	*Ki-67 Labelling Index*	*AgNOR count/nucleus*	*p53 Labelling Index*
Basal cell layer	KCOT	36	11.84 (±6.64)	2.28 (±0.35)	21.13 (±10.48)
	RC	30	7.13 (±4.50)	1.84 (±0.31)	5.32 (±6.40)
	DC	30	7.92 (±5.97)	1.64 (±0.32)	4.61 (±6.13)
	NOM	12	11.00 (±2.12)	1.88 (±0.21)	9.06 (±3.68)
Supra basal cell layer	KCOT	36	24.10 (±10.71)	2.65 (±0.47)	17.95 (±9.05)
	RC	30	2.87 (±2.31)	1.70 (±0.33)	1.97 (±2.37)
	DC	30	1.86 (±1.90)	1.39 (±0.24)	1.24 (±1.90)
	NOM	12	7.14 (±2.90)	2.06 (±0.29)	3.16 (±2.27)
Superficial cell layer	KCOT	36	0.66 (±0.93)	NA	0.70 (±0.98)
	NOM	12	0.00	NA	0.00
Complete epithelium*	KCOT	36	12.20 (±4.69)	2.47 (±0.39)	13.26 (±4.71)
	RC	30	5.00 (±3.29)	1.77 (±0.31)	3.64 (±4.28)
	DC	30	4.91 (±3.60)	1.51 (±0.27)	2.91 (±3.84)
	NOM	12	6.13 (±1.28)	1.97 (±0.21)	4.07 (±1.78)

KCOT, keratocystic odontogenic tumour; RC, radicular cyst; DC, dentigerous cyst; NOM, normal oral mucosa; NA not applicable [argyrophilic nucleolar organizing regions (AgNOR) not counted in superficial epithelium].
*Mean of basal cell layer, suprabasal cell layer and superficial layer was counted in complete epithelium.

LI was more in NOM than in DC in supra basal cell layer ($P < 0.05$) (Table 1).

The AgNOR count

There was a statistically significant difference for AgNOR count in all cysts ($P < 0.001$) and NOM ($P < 0.05$) between basal and suprabasal cell layers. Higher AgNOR count was noted in suprabasal cell layers than basal cell layer in KCOT. There were statistically significant variations of mean AgNOR count among all groups in each layer ($P < 0.001$) with descending order in KCOT, NOM, RC and DC. Significantly higher AgNOR count was observed in KCOT than DC, RC and NOM in all the cell layers ($P < 0.001$). AgNOR count was higher in NOM than RC and DC in supra basal cell layer. AgNOR count was higher in RC than DC in supra basal cell layer and complete epithelium ($P < 0.05$; Figure 2; (Table 1).

Actual Proliferating Index

Actual Proliferating Index (API) was calculated for the complete epithelium. It showed higher values for KCOT than for NOM, RC and DC ($P < 0.001$), but it was not statistically significant amongst RC, DC and NOM ($P > 0.05$; Table 2).

p53 LI

p53 antigen was expressed in 100.00% of KCOT, 63.34% of RC, 50.00% in DC and 100.00% in NOM (Table 2). p53 immunolabelling was dense and scattered in basal and suprabasal cell layers in KCOT, whereas very few densely stained cells were sporadically located in basal cell layer in RC, DC and NOM. The weakly stained cells were noted to be diffusely distributed in the complete epithelium of KCOT. While, in RC, DC and NOM, the weakly stained cells were noted mainly in basal cell layer and very few in the suprabasal cell layers (Figure 3).

There was a statistically nonsignificant difference ($P = 0.177$) for p53 LI in KCOT between basal and suprabasal cell layers. In contrast, there was a higher p53 LI in basal cell layer than suprabasal cell layers ($P < 0.001$) in RC, DC and NOM. Statistically

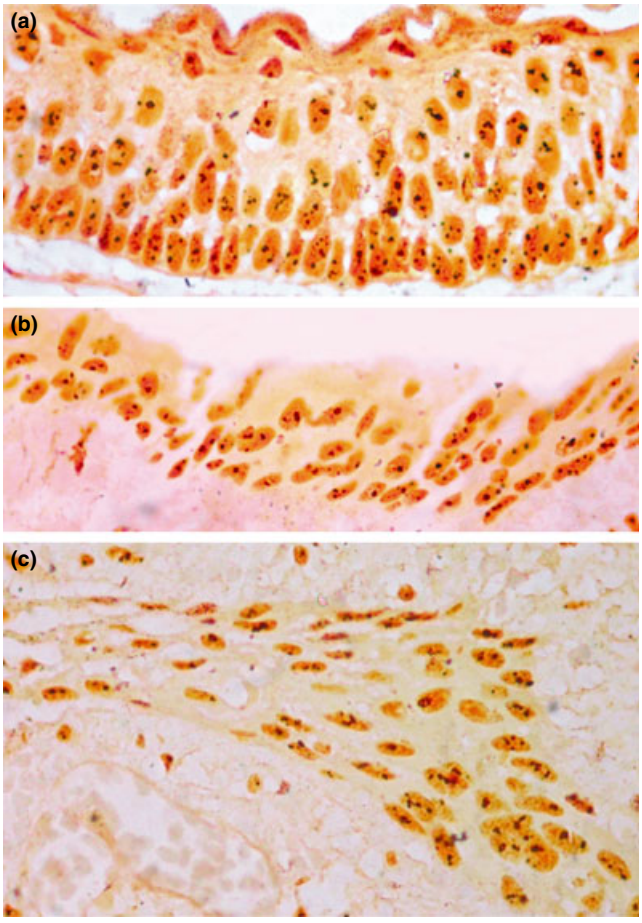

Figure 2 Photomicrograph of argyrophilic nucleolar organizing regions (AgNOR) staining. (**a**) In keratocystic odontogenic tumour, the AgNOR dots are more in the nucleus of suprabasal cells than basal cells. (**b**) In dentigerous cyst, the AgNOR dots are slightly more in the nucleus of basal cells than suprabasal cells. (**c**) In radicular cyst, the AgNOR dots are slightly more in the nucleus of basal cells than suprabasal cells (original magnification ×1000)

Table 2 The mean of Actual Proliferating Index in keratocyst/keratocystic odontogenic tumour (KCOT), RC, DC, and NOM. Positive cases for Ki-67 and p53 protein

Groups	*n*	*Actual proliferating index*	*Positive cases for Ki-67*	*Positive cases for p53 protein*
KCOT	36	30.87(±13.748)	35 (97.23%)	36 (100.00%)
RC	30	9.50(±7.683)	26 (86.67%)	19 (63.34%)
DC	30	8.20(±8.127)	28 (93.34%)	15 (50.00%)
NOM	12	12.09(±3.055)	12 (100.00%)	12 (100.00%)

Actual Proliferating Index was evaluated in the complete epithelium. 'API = Ki-67 or MIB-1 LI × AgNOR count'.
KCOT, keratocystic odontogenic tumour; RC, radicular cyst; DC, dentigerous cyst; NOM, normal oral mucosa.

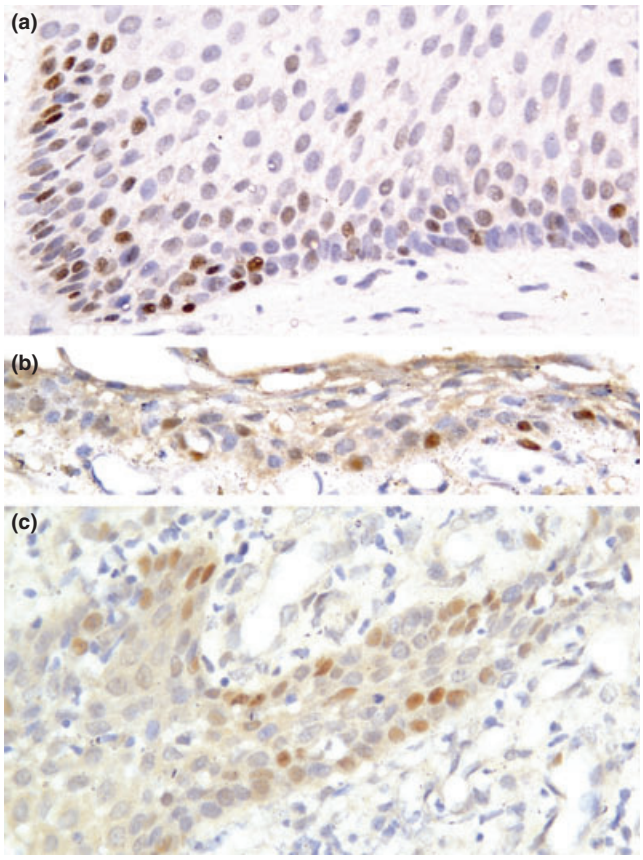

Figure 3 Photomicrograph of immunostaining for p53. (**a**) In keratocystic odontogenic tumour, the p53 immunopositive cells showing dense and week staining are observed in the suprabasal cell layers and the basal cell layer of lining epithelium. (**b**) In dentigerous cyst, the very few p53 positive cells showing weak staining are found in basal cell layer. (**c**) In radicular cyst, the few p53 immunopositive cells showing weak staining are found in the basal cell layer and very few in the suprabasal cell layers (original magnification ×400)

significant variation of mean p53 LI was noted amongst all the groups in basal cell layer, supra basal cell layer and complete epithelium ($P < 0.001$) which, was in descending order of KCOT, NOM, RC and DC. p53 LI was significantly higher in KCOT than NOM, RC and DC with respect to each cell layer ($P < 0.001$). In contrast, there was no significant difference of p53 LI amongst RC, DC and NOM in all layers (Table 1).

Correlation between AgNOR count and Ki-67 LI
There was a statistically significant positive correlation between AgNOR count and Ki-67 expression in KCOT in supra basal cell layer ($P = 0.002$), and complete epithelium ($P = 0.01$). Statistically significant positive correlation between AgNOR count and Ki-67 expression was noted in RC and DC in all the layers ($P \leq 0.001$).

Correlation between AgNOR count and p53 LI
There was a statistically significant positive correlation between AgNOR count and p53 expression in KCOT only in supra basal cell layer ($P < 0.05$), and in RC and DC in each layer. In contrast, no correlation was observed in NOM between AgNOR count and p53 expression.

Correlation between Ki-67 LI to p53 LI
There was statistically significant positive correlation between Ki-67 expression and p53 expression in KCOT in basal cell layer ($P = 0.009$), supra basal cell layer ($P = 0.009$), surface epithelium and complete epithelium ($P < 0.001$), in RC and DC in each layer ($P < 0.001$).

Discussion

In KCOT, the Ki-67 LI was significantly higher in suprabasal cell layers than in basal cell layer with uniform distribution. Similar findings were reported in previous studies that used Ki-67 antigen (Kichi *et al*, 2005; Kim *et al*, 2003; Li *et al*, 1995; Mathews *et al*, 1988; Saracogly *et al*, 2005; Slootweg, 1995) and other studies (Li *et al*, 1994; El Murtadi *et al*, 1996; Piatelli *et al*, 1998) that used PCNA as proliferation markers. This suggests that the highest proliferative activity is in the suprabasal cell layers. This unusual proliferation represents an epithelial disorganization and is perhaps; similar to dysplasia of oral squamous epithelium (Kurokava *et al*, 2003). This could also reflect a unique epithelial differentiation process, in which the basal cells assume some characteristics of preameloblasts (Li *et al*, 1994). In DC, Ki-67 positive cells were predominant in basal cell layer and very few in suprabasal cell layers (Tosios *et al*, 2000; Piatelli *et al*, 2002; Kim *et al*, 2003; Edamatsu *et al*, 2005). This suggests that the Ki-67 reactivity may be related to the regular maintenance of

2–3 cell layer thickness of epithelium. Thus, it confirms that the mechanism of expansion of the DC occurs passively by accumulation of fluid in the lumen rather than epithelial proliferation. In RC, Ki-67 positive cells suggest that the proliferative activity in lining epithelium of RC might be related to the grade of inflammatory reaction.

Argyrophilic nucleolar organizing regions count was significantly higher in suprabasal cell layers than basal cell layer of KCOT and vice versa in RC and DC. This suggests higher rate of proliferation in suprabasal cell layers of KCOT. AgNOR count was significantly higher in RC as compared with DC with respect to supra basal cell layer and complete epithelium. This could be because the suprabasal cells of DC may undergo apoptosis on a relatively rapid basis (Kichi *et al*, 2005), which seems to participate in maintaining regular thickness of cystic lining. These findings were not in accordance with previous study (Allison and Spencer, 1993; Coleman *et al*, 1996; Eslami *et al*, 2003). The inconsistency in results could be because of difference in staining method and counting protocol for AgNOR and because of the difference in the sample size. Thus, it is recommended that a universal protocol for AgNOR staining and counting must be developed and followed.

A significant positive correlation was found between the AgNOR count and Ki-67 LI in suprabasal cell layers and complete epithelium of KCOT. The API was significantly higher in KCOT than RC, DC, and NOM. The increased proliferative state and turnover rate of cells in suprabasal cell layers of KCOT suggest an abnormal control of the cell cycle resulting in higher intrinsic proliferative potential. Thus, the intrinsic growth potential could play a role in the development and locally aggressive behaviour of KCOT. AgNOR count and Ki-67 LI was significantly higher in suprabasal cell layers of NOM than DC and RC. Proliferative capacity of oral mucosal epithelium is dynamic in the sense that it regularly regenerates itself on relatively rapid basis.

In KCOT, p53 LI was significantly higher in all epithelial cell layers. There was no significant difference of p53 positive cells between basal and suprabasal cell layers of KCOT. These findings corroborated with the previous studies published by Slootweg, 1995 and Li *et al*, 1996; . This study results were not consistent with Ogden *et al*, 1992 and Lombardi *et al*, 1995;. The different findings might be because of variability of techniques. Notably; alteration of the detection threshold could be because of use of different antibodies, dilutions, and antigen retrieval (Battifora, 1994; Lambkin *et al*, 1994). The common findings between this study and previous ones (Kichi *et al*, 2005; Slootweg, 1995; Ogden *et al*, 1992; Li *et al*, 1996; Lombardi *et al*, 1995; Piatelli *et al*, 2001) was the predominance of p53 reactivity in basal cell layer and suprabasal cell layers in epithelium of KCOT than in RC, DC and NOM. The p53 LI was significantly higher in suprabasal cell layers of KCOT as compared with RC and DC, a finding in agreement with other investigators (Slootweg, 1995; Li *et al*, 1996; Lombardi *et al*, 1995; Piatelli *et al*, 2001). The high reactivity of p53 protein in KCOT can be explained by various factors peculiar to the KCOT such as: (i) locally aggressive behaviour, manifested by a large lesion extending along the cancellous bone without producing any noteworthy expansion of cortical plates [Ahlfors *et al*, 1984; Brannon, 1976; Partridge and Towers, 1987; Regezi, 2002; Shear, 2002a,b(Part I & II); Shear, 2003; (ii) high mitotic rate found in KCOT than other odontogenic cysts (Shear, 1992); (iii) tendency to recur, suggesting increased epithelial activity, although the rate of recurrence may depend on the method of treatment, (recurrence up to 62% was found) (Blanas *et al*, 2000; Myoung *et al*, 2001; Chirapathomsakul *et al*, 2006).

In this study, significant positive correlation was noted between Ki-67 and densely and weakly stained p53 expression in KCOT, which is in agreement with the previous reports (Kichi *et al*, 2005; Slootweg, 1995; Ogden *et al*, 1992; Li *et al*, 1996;). This could indicate that the negative growth regulation of normal p53 protein is suppressed at least to some extent, resulting in increased proliferation. The p53 detection by IHC in epithelium of KCOT is strongly related to the proliferative activity and an abnormal cell cycle control. The p53 positivity may result in increased cellular proliferation or the increase in p53 positivity may be because of the disturbance of growth regulation that results from increased cellular proliferation. However, the possibility that p53 stabilization is a result rather than the cause of rapid cell proliferation in KCOT cannot be excluded. The over expression of p53 protein in KCOT probably may not be solely attributable to p53 gene mutation, but is thought to be associated with overproduction and/or stabilization of normal/wild p53 protein product (Battifora, 1994; Hall and Lane, 1994; Lombardi *et al*, 1995; Dowell and Ogden, 1996; Chipuk and Green, 2006; Marine *et al*, 2006). Hence, we agree with other studies (Slootweg, 1995; Dowell and Ogden, 1996; Edamatsu *et al*, 2005) to hypothesize that a high proliferation activity may result in detectable concentrations of wild type p53 protein. Thus, p53 expression in the lining epithelium of sporadic KCOT does not necessarily imply an association with malignant disease but a tendency to be expressed in increasing quantities as the biological behaviour of KCOT becomes more aggressive (Lazzaro and Cleveland, 2000). Thus, p53 protein reactivity may explain its role in intrinsic growth potential of epithelium and locally aggressive biological behaviour of KCOT.

The immunohistochemical detection of p53 protein could be because of altered p53 protein metabolism, which is either caused by mutation or changed turnover of the wild p53 protein. However, the aggressive, locally invasive behaviour of KCOT may explain the presence of mutant or otherwise inactive p53 protein. In this study, densely positive p53 cells were noted in the basal and suprabasal cell layers of KCOT. Based on the similarity of staining pattern of p53 in ameloblastomas and KCOT, involvement of mutant p53 protein in lining epithelium of KCOT cannot be ruled out completely (Slootweg, 1995; Shibata *et al*,

2002; Agaram *et al*, 2004). Further study is required for detection of p53 mutation in KCOT by polymerase chain reaction.

In DC, the significant correlation between Ki-67 LI and weakly staining p53 protein in basal cell layer could indicate the normal wild type p53 protein expression; related to apoptosis and cell proliferation, thus maintaining the regular thickness of epithelium. In RC, the weakly stained p53 protein expression increased according to the degree of Ki-67 expression, which may indicate increased production and stabilization of normal wild type p53 protein, related to inflammatory stress (Dowell and Ogden, 1996; Marine *et al*, 2006).

In this study, significant correlation was found between AgNOR count and p53 protein expression in suprabasal cell layers of KCOT. AgNOR expression is directly related to the rate of ribosome biogenesis, which has been shown to be controlled by the tumour suppressor proteins pRb and p53 (Derenzini *et al*, 2004). From a clinical point of view, lesions with changes in pRb and p53 gene status are generally more aggressive than those with normally functioning pRb and p53 pathways. The higher AgNOR count and p53 expression in KCOT as compared with RC and DC are in favour of clinically aggressive behaviour and recurrence of KCOT. Thus, it can be stated that the AgNOR count and p53 protein detection by IHC are of great consequence to predict the biological behaviour and prognosis in odontogenic lesions.

In conclusion, our results may be supported by, (a) In KCOT the cells constituting suprabasal cell layers have prominent proliferative activity as well as unique epithelial differentiation process in which the basal cells assume some characteristics of preameloblasts; (b) Lateral spread of proliferating suprabasal cells along with abundant keratin produced by apoptosis (Kichi *et al*, 2005) and other enzymatic activity from cyst wall, may facilitate an expansion of the cyst cavity through cancellous bone; (c) p53 protein expression is probably a marker of cellular proliferation in KCOT; and (d) p53 protein expression and AgNOR count can be correlated with locally aggressive biological behaviour of KCOT. The intrinsic growth potential of epithelium and locally aggressive biological behaviour of KCOT further added evidence to hypothesize that OKC should be classified as KCOT (represent a locally aggressive benign low-grade cystic neoplasm rather than developmental cyst) (Barnes *et al*, 2005). Therefore, the treatment protocol for KCOT should be complete surgical excision with adequate normal margin rather than conservative approach.

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