Changes in cellular regulatory factors before and after decompression of odontogenic keratocyst

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Changes in cellular regulatory factors before and after decompression of odontogenic keratocyst

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This certifies that the Doctoral Dissertation of

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Decompression followed by enucleation, one treatment for odontogenic keratocyst (OKC), is frequently used in OKC lesions of large sizes. This method offers the advantage of minimizing the possibility of sensory impairment without creating a wide-range bone defect; moreover the recurrence rate can be significantly lower than following simple enucleation. This study aimed to assess the changes in histology and expression of proliferation markers in OKC before and after decompression treatment. A total thirty eight OKC tissue samples from nineteen patients who had undergone decompression therapy

were examined morphologically and immunohistochemically to observe changes in proliferative activity before and after decompression. The markers used for IHC staining were Bcl-2, EGFR, Ki-67, P53, PCNA, and SMO. The immunohistochemistry (IHC) positivity of the six markers was scored by using software ImageJ, version 1.49, by quantifying the intensity and internal density of IHC stained epithelium.

The results are as follows:

1. Progress after decompression was certain. The size of lesion had reduced, and the bone density on panorama x-ray was observed to be thickened. The decompression periods of the patients ranged between 4 months to 12 months, the average period being 7.3 months. After decompression periods, enucleation was performed.

2. The tissues of OKC showed differences before and after the decompression procedure. At the time of decompression, the cystic cavity wall was constructed of fibrous tissue covered by very thin regular parakeratinized stratified squamous epithelium which had five to eight layers. The basal cells consisted of columnar cells and palisading nuclei. Many epithelia were separated from the fibrous capsule. There was very loose connection between epithelium and connective tissue without rete pegs.

3. The tissues obtained at the time of enucleation had changed, the cyst wall epithelium having become hyperplastic stratified squamous epithelium and dense connective tissue with infiltration of inflammation cells.

4. Bcl-2 staining was expressed in the basal layer of the epithelium. However, in some samples, Bcl-2 positive cells were observed in all layers of the lining epithelium and connective tissue cells of OKCs. EGFR showed membranous and cytoplasmic staining of epithelial cells, progressively diminishing from the basal toward the superficial layers. Its expression was prominent in basal epithelial cells. Ki-67 positive cells were mainly distributed in the basal and parabasal layers of epithelium. PCNA showed an abundant expression in both basal and suprabasal areas. P53 positive cells were seen throughout the epithelium but mainly in the parabasal layers. Positive immunostaining of SMO was detected in the intermediate layer, but rarely in the superficial and basal layer.

5. Bcl-2, Ki-67, P53, and PCNA staining were confined to the epithelial cell nucleus of OKC. EGFR and SMO staining were confined to the cell membrane of epithelium. The positivity of the 6 markers after decompression showed similar patterns.

6. The quantified values of the six markers showed both increase and decrease without displaying a tendency before and after decompression.

7. The paired t-test was applied to numerical parameters of the 6 markers obtained by quantifying the IHC staining in terms of intensity. The values of EGFR before and after decompression resulted in a P-value of 0.040, which was considered significant. However, values of P53, SMO, Bcl-2, Ki-67, and PCNA before and after decompression yielded P-values of 0.370, 0.373, 0.785, 0.678, and 0.271 individually,

which were considered not significant. Statistics of EGFR values showed no significant difference between maxilla and mandible.

8. The other method for quantifying IHC staining was by using the internal density. Statistic evaluation of numerical parameters of P53, SMO, Bcl-2, Ki-67, and EGFR was performed using the Wilcoxon signed ranks test, yielding P-values of 0.968, 0.372, 0.147, 0.355, and 0.904, respectively. The paired t-test was used on numerical parameters of PCNA, yielding a P-value of 0.781. No P-values were considered significant.

Based on the above findings, the values of Bcl-2, Ki-67, P53, PCNA, and SMO in OKC before and after decompression showed no significant change. No correlation between clinical shrinkage and morphologic changes or expression of proliferation and growth markers could be found. There was no statistical evidence that decompression treatment reduces potentially aggressive behavior of OKC within the epithelial cyst lining itself. This might indicate that decompression does not change the biological behavior of the epithelial cyst lining, as well as the recurrence rate.

Key words: IHC staining, immunohistochemical staining, Bcl-2, EGFR, Ki-67, PCNA, P53, SMO, decompression, enucleation, odontogenic keratocyst

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Ⅰ. INTRODUCTION

The odontogenic keratocyst (OKC) is a distinctive form of developmental odontogenic cyst with potential for aggressive and infiltrative behavior that originates from the dental lamina remnants or from the basal cells of overlying epithelium (Ahlfors, et al., 1984; Neville, et al., 2015). OKC has a predilection for the posterior part of the mandible, a peak incidence in patients between second and fourth decades, and a slight male

predominant tendency. Radiographically, OKC is shown as a unilocular or multilocular lesion, most often surrounded by smooth or scalloped margins with sclerotic borders (Neville, et al., 2015).

The OKC shows aggressive clinical behavior with a high and extremely varied recurrence rate (Bataineh and Al Qudah, 1998; Kaczmarzyk, et al., 2012; Maurette, et al., 2006). The recurrence rate is reported to be 25~60%, varying according to the treatment method (Neville, et al., 2015). The OKC typically has a thin, friable wall, which is often difficult to enucleate from the bone in one piece. The epithelial lining is composed of a uniform layer of stratified squamous epithelium, usually six to eight cells in thickness. The luminal surface shows flattened parakeratotic epithelial cells, which exhibit a corrugated appearance. The basal epithelial layer is composed of a palisaded layer of cuboidal or columnar epithelial cells, which are often hyperchromatic (Neville, et al., 2015).

In 2005, the World Health Organization (WHO) renamed it “keratocystic odontogenic tumor” (KCOT), reclassifying it among the epithelial odontogenic tumors (Barnes, et al., 2005) due to studies which supported the hypothesis that OKC is a neoplastic condition (Gomes, et al., 2009; Shear, 2002a). This assumption was based on the fact that the lesion shows overexpression of proliferative markers and BCL-2, in addition to loss of heterozygosity or methylation of tumor suppressor genes and mutation of the *PTCH1* gene (Gomes, et al., 2009). However, in 2017, WHO moved ‘keratocystic odontogenic tumor’ from the neoplastic category (2005) back into the cyst category (2017), under the category of developmental odontogenic cysts. The 2017 classification reverted to the original and

well-accepted terminology of OKC because many papers showed that the *PTCH* gene mutation could be found in non-neoplastic lesions, including dentigerous cysts,; furthermore, many researchers suggested that resolution of the cyst after marsupialization was not compatible with a neoplastic process. It is, however, still important and clinically relevant to separate OKC from the other odontogenic cysts due to its diagnostic histologic features as well as distinctive clinical features (Soluk-Tekkeşin and Wright, 2018).

Its aggressive clinical behavior and frequent recurrence following curettage has been the focus of several studies, which indicate that the OKC epithelial lining may have some intrinsic growth potential (Li, et al., 1994, 1995; Ogden, et al., 1992; Shear, 2002a, 2002b, 2002c). The proliferative potential can be assessed by immunohistochemistry using monoclonal antibodies against specific cell cycle associated proteins (Brown and Gatter, 2002; Brøndum and Jensen, 1991; Rodu, et al., 1987).

Various treatment modalities are reported which can be generally classified as conservative or aggressive. Conservative treatment usually includes enucleation, and/or marsupialization, while aggressive treatment includes enucleation associated with adjunct therapies or resection (Boffano, et al., 2010). The reported frequency of recurrence in various studies ranges from 5% to 62%. This wide variation may be related to the total number of cases studied, the length of follow-up periods, and the inclusion or exclusion of orthokeratinized cysts in the study group (Neville, et al., 2015).

The recurrence rates of rather conventional therapies such as enucleation and

curettage are reported to be highest (Morgan, et al., 2005; Zhao, et al., 2002). In order to reduce the recurrence rates, mass excision including adjacent normal bone or use of Carnoy's solution should be considered, but such methods can lead to complications such as bone loss and sensory impairment (Frerich, et al., 1994; Stoelinga, 2005). It is known that in the case of OKC, mass excision or enucleation after decompression can minimize the possibility of sensory impairment without creating a wide-range bone defect; moreover, the recurrence rate can be significantly lower than that of simple enucleation (Brøndum and Jensen, 1991; Marker, et al., 1996). However, given recent published reports of rather contrary results, the recurrence rate is controversial.

The development of new surgical methods and drugs to reduce the recurrence rate is urgent but systemic research is difficult due to the low rate of morbidity. These treatments are still being reported only at the level of the presentation in case reports, with little research on changes in growth factors or cell apoptotic factors after decompression.

According to previous studies, proliferation factors of OKC before and after decompression has shown inconsistent and different results. Thus, present study hypothesized that expression of the proliferation factors would decrease. The surgeons of the Department of Oral and Maxillofacial Surgery had previously performed decompression and incisional biopsy on OKC lesions and later obtained tissue specimens when performing enucleation. Using these specimens, the present study aimed to investigate and identify in histology and expression of proliferation markers in OKC before and after decompression treatment through immunohistochemical staining methods.

Ⅱ. MATERIALS AND METHODS

1. Materials

Total 38 formalin-fixed paraffin-embedded tissue samples of OKC were retrieved from the files of the Department of Oral Pathology, College of Dentistry, Yonsei University. The samples met the inclusion criteria of histologically-confirmed OKC which had been subjected to decompression treatment, followed by enucleation on the same lesion at later on. Cases of OKC associated with basal cell nevus syndrome, orthokeratinized OKC were excluded. 9 patients had lesions on maxilla, and 10 patients had lesions on mandible, as summarized in Table1. All patients underwent the treatment at the Department of Oral and Maxillofacial Surgery, College of Dentistry, Yonsei University from year 2013 to year 2018. A polyethylene tube was inserted and fixed to the lesions at the time of primary incision biopsy. Patients were instructed to irrigate the cystic cavity with normal saline or clean water on a daily basis. The patients underwent periodic clinical and radiographic examination of the lesion to observe progression at an average interval of several months. Specimens for histology and pathology were taken during the decompression procedure for confirmation of diagnosis and subsequent enucleation. These surgical specimens constituted the materials upon which this investigation is based. The present research has passed deliberation of Institutional Review Board, which number was 2-2018-0050.

Table 1. Patient information

Patient number	Age	Gender	Area	Adjacent teeth	Decompression period (month)
1	38	Male	Maxilla Rt.	#18	5m
2	32	Female	Maxilla Rt., Sinus	#18	12m
3	36	Male	Maxilla Lt.		7m
4	23	Female	Maxilla Lt., Sinus	#28	4m2wk
5	24	Male	Maxilla Lt.	#28	5m
6	23	Male	Maxilla Lt.	#28	10m
7	20	Male	Maxilla Rt.	#18	11m2wk
8	44	Female	Maxilla Lt., Sinus	#23, 24, 25	4m
9	27	Female	Maxilla Lt.	#28	6m
10	40	Male	Mandible Rt.		7m3wk
11	59	Female	Mandible Rt.		8m
12	22	Male	Mandible Lt.		8m
13	68	Female	Mandible Rt., Ramus	#46, 47	6m3wk
14	61	Male	Mandible Rt.	#38	9m
15	61	Female	Mandible Rt.		8m2wk
16	81	Female	Mandible Rt., Condyle, Ramus		5m
17	19	Male	Mandible Lt.		7m
18	32	Female	Mandible Rt., Ramus, body	#38	10m
19	27	Male	Mandible Rt.,		4m

2. IHC Staining

2.1. Markers

A. Bcl-2

The *Bcl-2* gene has the ability to inhibit apoptosis without encouraging cell proliferation leading to cell cycle changes which facilitate cell survival independent of cell division. The expression of *Bcl-2* gene is connected with low-grade tumors and its inhibition of apoptosis is regarded as a common tumor genesis pathway (Cotter, 2009). Bcl-2 is located in the external membrane of mitochondria, endoplasmic reticulum and nuclear membrane. Overexpression of Bcl-2 in tumor cells causes apoptotic resistance and increased cell growth (Kumar and Cotran, 2003). Uncontrolled expression of Bcl-2 has been found before histopathologic changes in the early stage of neoplasm (Bronner, et al., 1995; Singh, et al., 1998).

B. EGFR

Epidermal growth factor receptor is the most important growth factor ligand on the cell surface. EGFR is involved in various cellular processes, including cell growth, motility, inhibition of apoptosis, and cell adhesion (Herbst, 2004; Laimer, et al., 2007). Lin et al. have demonstrated that the localization of EGFR is correlated with a highly proliferative status of tissues, and have provided evidence to support the potential roles of EGFR as a

transcription factor or coactivator, which might activate the genes required for its mitogenic effects. The location of this receptor within a cell may be related to its response to proliferative stimuli. Cells proliferating at a physiologic rate express this receptor in both membrane and cytoplasm (Lin, et al., 2001). The immunolocation of EGFR in odontogenic epithelium may therefore be associated with the origin of odontogenic cysts and tumors (da Silva Baumgart, et al., 2007). Overexpression of EGFR-related genes, seen in many neoplasms, causes the over sensitivity of cells to a normal level of growth factor. Nowadays, EGFR is known as an effective growth factor in many human cancers (Kumar and Cotran, 2003). EGFR signaling is associated with malignancy transformation, giving rise to specific phenotypes of cells which can affect the cellular reaction to the treatment (da Silva Baumgart, et al., 2007).

C. Ki-67

Ki-67 protein is a 319–358 kDa alternatively-spliced amphophilic non-histone nuclear protein that consists of multiple, unique Ki-67 motifs. Ki-67 is an absolute requirement for DNA synthesis (Sawhney and Hall, 1992). Ki-67 is known as a marker of cell proliferation because the Ki-67 antigen is preferentially expressed in proliferative cells during late G1, S, G2, and M phases, whereas resting, noncycling cells (G0 phase) lack Ki-67 expression. Ki-67 thus immunohistochemically provides a reliable index of cellular proliferation (Brown and Gatter, 1990). Although its level increases during the S-phase, MIB-1

recognizes Ki-67 antigen in the entire cell cycle (Li, et al., 1995; Thosaporn, et al., 2004). Because of its absence in quiescent cells (G0 phase), this protein has developed into a widely-used tumor marker in the fields of research and pathology (Oka, et al., 2011). Ki-67 is of prognostic value for many types of malignant tumors (Mateoiu, et al., 2011).

D. P53

The *p53* gene, located on chromosome 17q13, encodes a nuclear phosphoprotein which is thought to control cell growth at the G1/S checkpoint. P53 is produced by the tumor suppressor gene p53 effective at the G1 phase of the cell cycle, and participates in growth arrest, initiates repair, or induces apoptosis (Levine, et al., 1991). Detection of this marker immunologically may indicate stabilization of this protein and reflects cell cycle regulation in favor of proliferation (de Oliveira, et al., 2015).

E. PCNA

Proliferating cell nuclear antigen (PCNA) is a 36 kDa acidic nonhistone nuclear protein important for DNA synthesis and repair. In the presence of replication factor C, a multi-subunit complex, PCNA allows DNA polymerase to initiate leading-strand DNA synthesis (Mighell, et al., 1996). Immunostaining with a monoclonal antibody (PC10) against this antigen has been shown to demonstrate the proliferative compartment of

normal tissue (Yu, et al., 1992) and to correlate well with prognosis in some tumors.

F. SMO

The hedgehog (HH) signaling pathway is a key regulator of embryonic development, controlling both cellular proliferation and cell fate. Binding of sonic hedgehog (SHH) to its receptor, patched (PTCH1), is believed to relieve normal inhibition by PTCH1 of smoothened (SMO), a seven-span transmembrane protein with homology to a G-protein-coupled receptor r (Stone, et al., 1996). *smo* is a tumor-related gene located at 7q32.3, contains12 exons spanning approximately 24 kb, and encodes a 787-amino-acid transmembrane glycoprotein (Brastianos, et al., 2013). Its receptor is a G protein-coupled receptor that interacts with Patched, an important part of the HH signaling pathway during embryogenesis as well as adulthood (Ruat, et al., 2013; Wang, et al., 2013). The HH pathway has been demonstrated to play an important role in different development-related cancers (Amakye, et al., 2013; Jiang and Hui, 2008), but the exact mechanism of action has not yet been elucidated. The protein generated by SMO is downstream of PTCH1; that is, the expressionof PTCH1 restrains the activation of SMO, and thereby inhibits activation of the HH pathway (Shen, et al., 2013; Yang, et al., 2012).

2.2. Staining method

In order to identify the expression of cancer markers in tissues obtained at decompression and enucleation, immunohistochemistry staining using antibodies of Bcl-2 mouse mAb (Cell Signaling Technology Inc., #15071), EGFR rabbit mAb (Cell Signaling Technology Inc., #4267), P53 mouse mAb (Cell Signaling Technology Inc., #48818), Ki-67 rabbit mAb (Cell Signaling Technology Inc., #9027), PCNA rabbit mAb (Cell Signaling Technology Inc., #13110), and SMO mouse mAb (Santa Cruz Biotechnology Inc.,sc-166685). Formalin fixed tissue was made into paraffin block after the flush, dehydration, transparency, and paraffin penetration process. The paraffin fragments were made and dried by stripping the tissue to a constant thickness (4um). To conduct the immunohistochemical examination, the paraffin of each tissue section was removed using xylene and then hydrated, hydrogen peroxide (3% H_2O_2) then being added to inhibit intrinsic enzymes for 10 minutes at room temperature. In order to restore the cross-linking induced by formalin fixation to its original state, slices were put into the citrate buffer and boiled for 10 minutes in the microwave to perform heat antigen retrieval. The boiling slices were cooled at room temperature while immersed in a citrate buffer and treated at 5% BSA for an hour to suppress non-specific proteins. The diluted primary antibodies were then treated with incision and incubated at 4℃ for overnight. The dilution concentration of Bcl-2 and Ki-67 antibodies was 1/500, p53, EGFR, SMO antibodies 1/250, and PCNA antibodies 1/5000, achieved by diluting them in a 5% BSA solution. After primary antibody processing and washing, the secondary antibody with biotin and polymer-HRP were applied at room

temperature for 10 minutes following the protocol using the Polink-2HRP plus road 3, 3'-Diaminobenzidine (DAB) detection system (GBI Labs Inc. D41-18) and the color was manifested. Hematoxylin was used for contrast dyeing. After the primary antibody treatment, the phosphate-buffered solution with Tween was used to flush three times at room temperature for five minutes between each process. Dyeing-completed tissue slices were dehydrated, sealed into permounts, and observed under a microscope. The analysis was done by using the ImageJ program to measure the histogram and densities of the dyed parts of DAB.

3. Staining counting method

3.1. Measuring intensity

Using the ImageJ program, the original image of RGB (red, green, blue) was adjusted to 8-bit color, and the threshold then set. The histogram was generated in 3 fields to measure the intensity within a certain area (fig 1). This method was used to quantify the staining of several photos per sample.

3.2. Measuring internal density

Using the ImageJ program, the external part of epithelium was first removed from the original stained photo, after which, the color threshold was established. Statistics were analyzed measuring the internal density within a certain area in 3 fields (fig 2). In addition to the method using intensity, internal density was used to quantify the staining in several stained photos per sample.

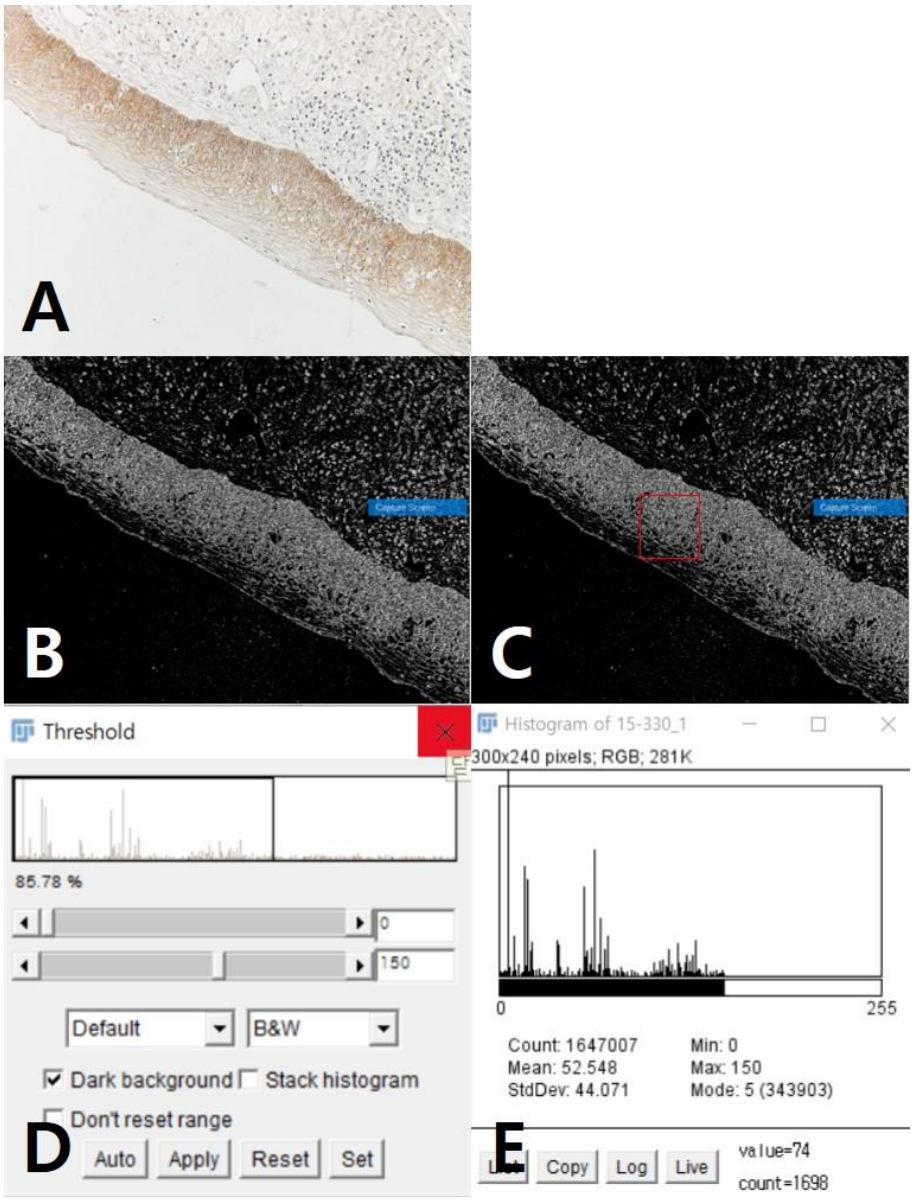

Figure 1. Measurement of IHC staining based on intensity

A: original stained photo with RGB adjusted to 8 bit color, **B**: setting threshold, **C**: measuring intensity within area, **D**: threshold control window, **E**: measured intensity in histogram form

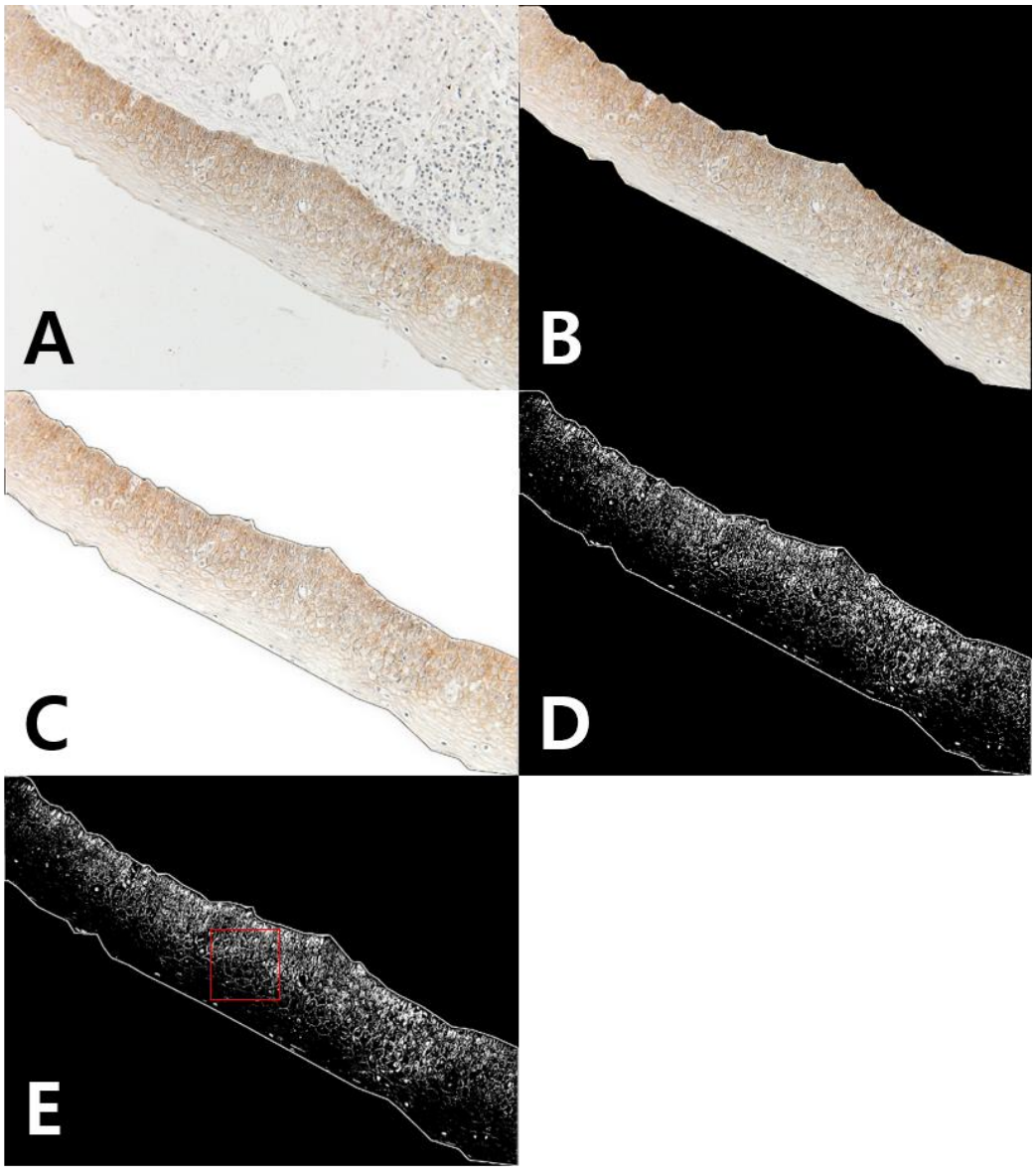

Figure 2. Measurement of IHC staining based on internal density

A: original stained photo, **B**: clearing outside, **C**: subtracting background, **D**: establishing color threshold, **E**: measuring internal density within area

4. Statistical analysis

Statistical analyses were performed to compare the staining values obtained from decompression specimens with those of enucleation tissues. Quantified values using intensity showed a Gaussian distribution. The paired t-test was used to compare value changes among the groups (SPSS® 25, IBM, USA). Most quantified values using internal density were considered to lack a Gaussian distribution. The Wilcoxon signed ranks test was used to compare value changes among the groups (SPSS® 25, IBM, USA). Statistical difference between the maxilla group and the mandible group was also determined. *P*-values less than 0.05 were considered significant differences.

Ⅲ. RESULTS

1. Decompression and follow-up

A total 19 patients, 9 female and 10 male, underwent the decompression procedure. The patients ranged between 19 to 81 years old, the average age being 38.8. 9 patients had OKCs on maxilla. 7 patients had 3rd molars with the lesion and 3 of them had a 3rd molar inside the sinus due to OKC enlargement. 3rd molars were removed during enucleation surgery. 10 patients had OKCs on mandible. 3 patients had OKC lesions on ramus, 1 of them with the lesion involved in condyle head to ramus. 2 patients had 3rd molars within the OKC lesion, which was also removed when enucleation was performed. The progress after decompression was certain. The size of lesion had reduced, and the bone density on panorama x-ray was observed to have thickened. The decompression periods of the patients ranged between 4 and 12 months, the average period being 7.3 months. After decompression, enucleation was performed. OKC recurred among 3 patients, all of whom had OKCs on maxilla. 2 patients experienced OKC recurrence 24 months after enucleation, and OKC recurred in 1 patient10 months after enucleation. Patients were all instructed to visit the Department of Oral and Maxillofacial Surgery for 10 years due to the characteristic high recurrence rate. However, 6 patients were lost in follow-up.

2. Immunohistochemical analysis

The OKC tissues showed differences before and after decompression. The cystic cavity wall was constructed of fibrous tissue covered by very thin regular parakeratinized stratified squamous epithelium consisting of five to eight layers. The basal cells consisted of columnar cells and palisading nuclei. Many epithelia were separated from the fibrous capsule. There was very loose connection between epithelium and connective tissue without rete pegs. The tissues obtained at the time of enucleation had changed. The epithelium of cyst wall had become hyperplastic stratified squamous epithelium and dense connective tissue with infiltration of inflammation cells. Bcl-2 staining was expressed in the basal layer of the epithelium. However, in some samples, Bcl-2 positive cells were observed in all layers of the lining epithelium and connective tissue cells of OKCs (fig 3). EGFR showed membranous and cytoplasmic staining of epithelial cells, progressively diminishing from the basal toward the superficial layers. Its expression was prominent in basal epithelial cells (fig 4). Ki-67 positive cells were mainly distributed in the basal and parabasal layers of epithelium. Photos showed a tendency toward less staining of Ki-67 in enucleation tissues. However, quantified values did not show the same tendency before and after decompression (fig 5). P53 showed abundant expression in both basal and suprabasal areas (fig 6). PCNA positive cells were seen throughout the epithelium but mainly in the parabasal layers (fig 7). Positive immunostaining of SMO was detected in the intermediate layer, but rarely in the superficial and basal layer (fig 8). Bcl-2, Ki-67, P53, and PCNA staining were confined to the epithelial cell nucleus of OKC. EGFR and SMO staining was

confined to the cell membrane of epithelium. The positive cells of 6 markers after decompression showed similar patterns.

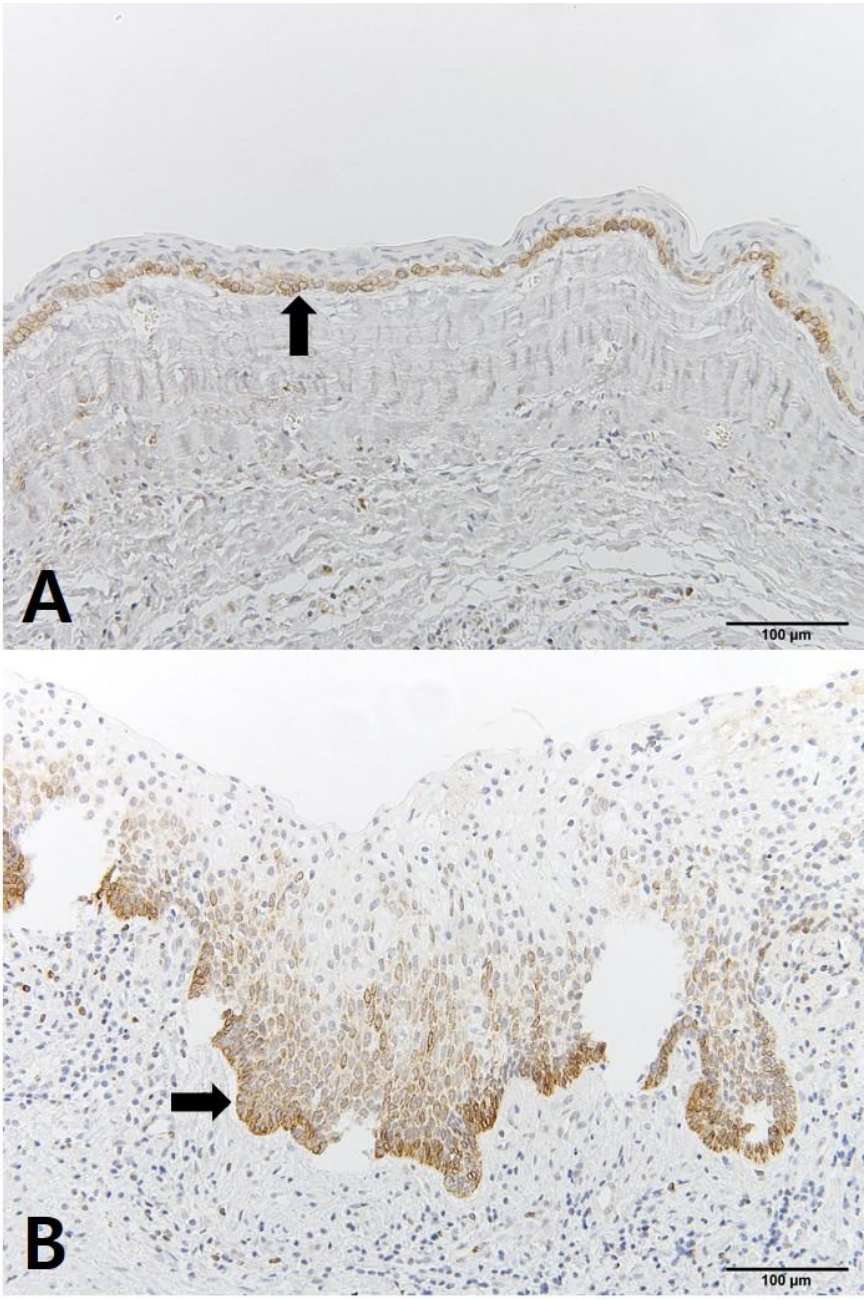

Fig 3. IHC staining of Bcl-2

A: tissue obtained at decompression, **B**: tissue obtained at enucleation

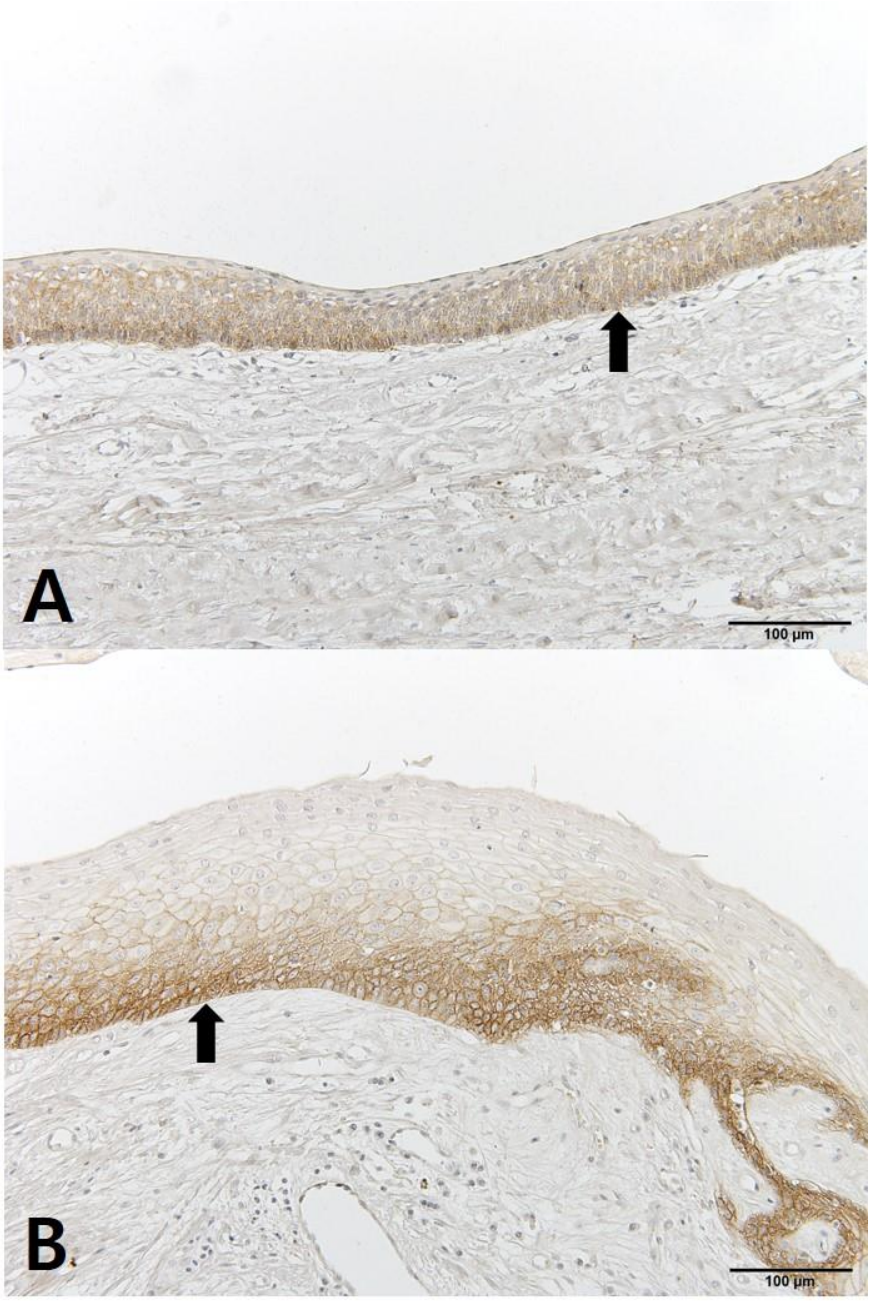

Fig 4. IHC staining of EGFR

A: tissue obtained at decompression, **B**: tissue obtained at enucleation

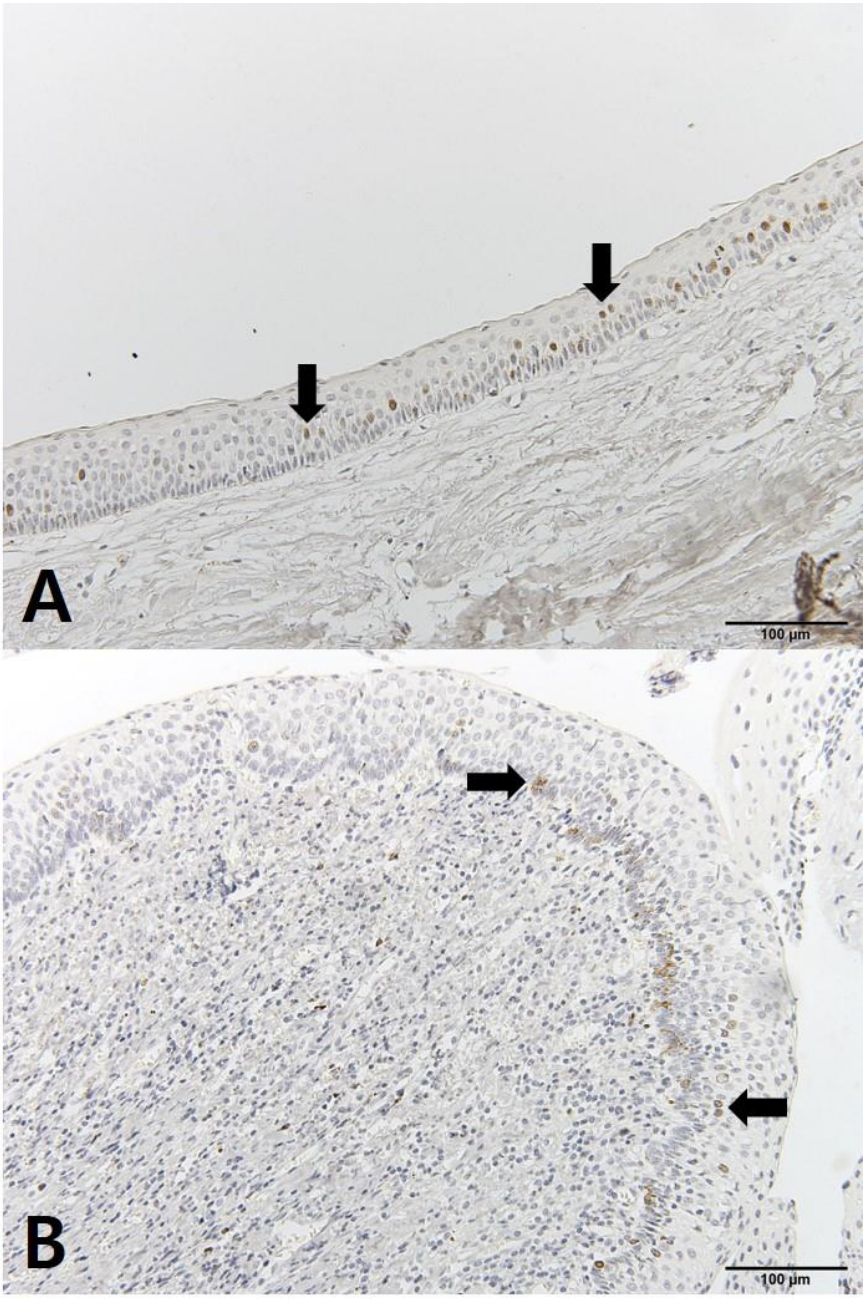

Fig 5. IHC staining of Ki-67

A: tissue obtained at decompression, **B**: tissue obtained at enucleation

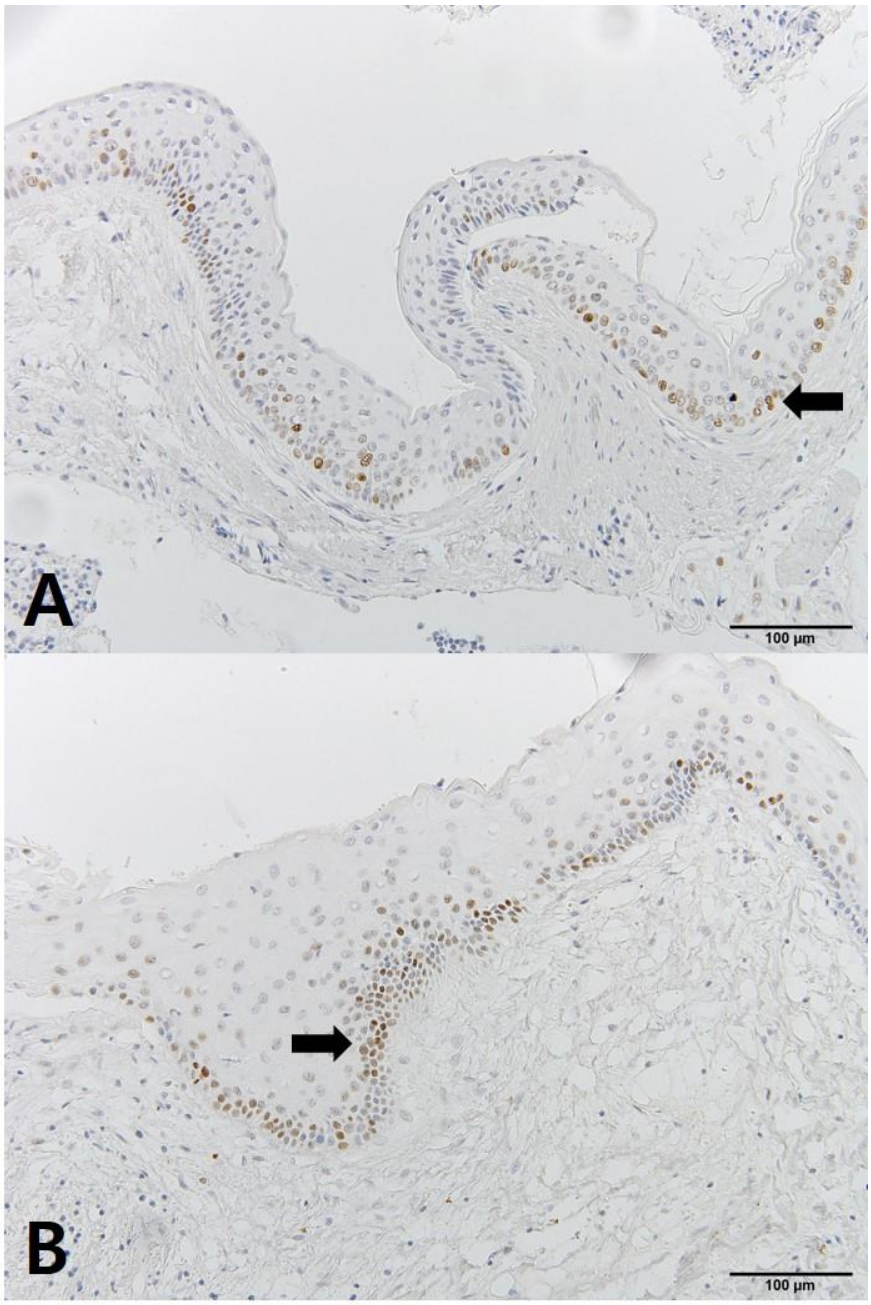

Fig 6. IHC staining of P53

A: tissue obtained at decompression, **B**: tissue obtained at enucleation

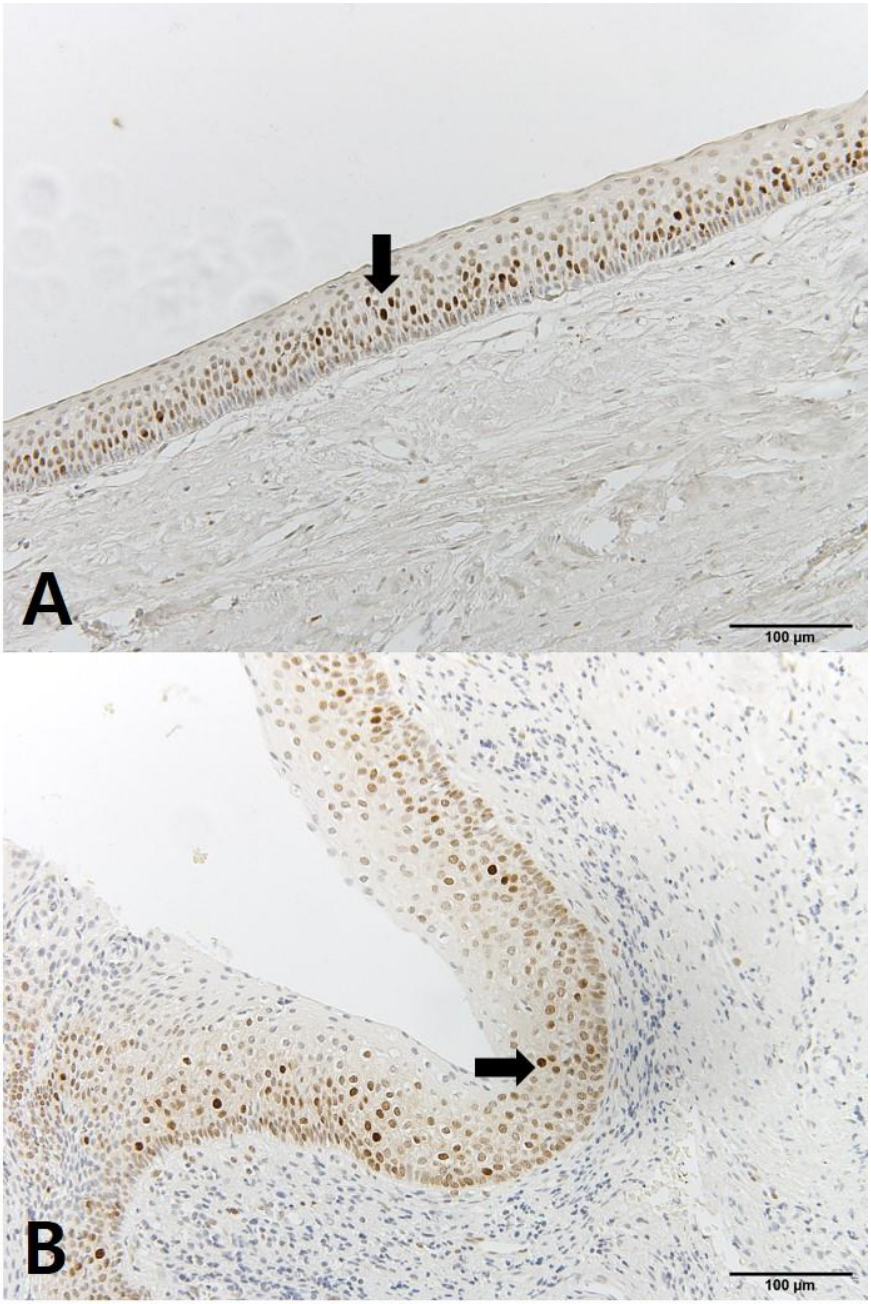

Fig 7. IHC staining of PCNA

A: tissue obtained at decompression, **B**: tissue obtained at enucleation

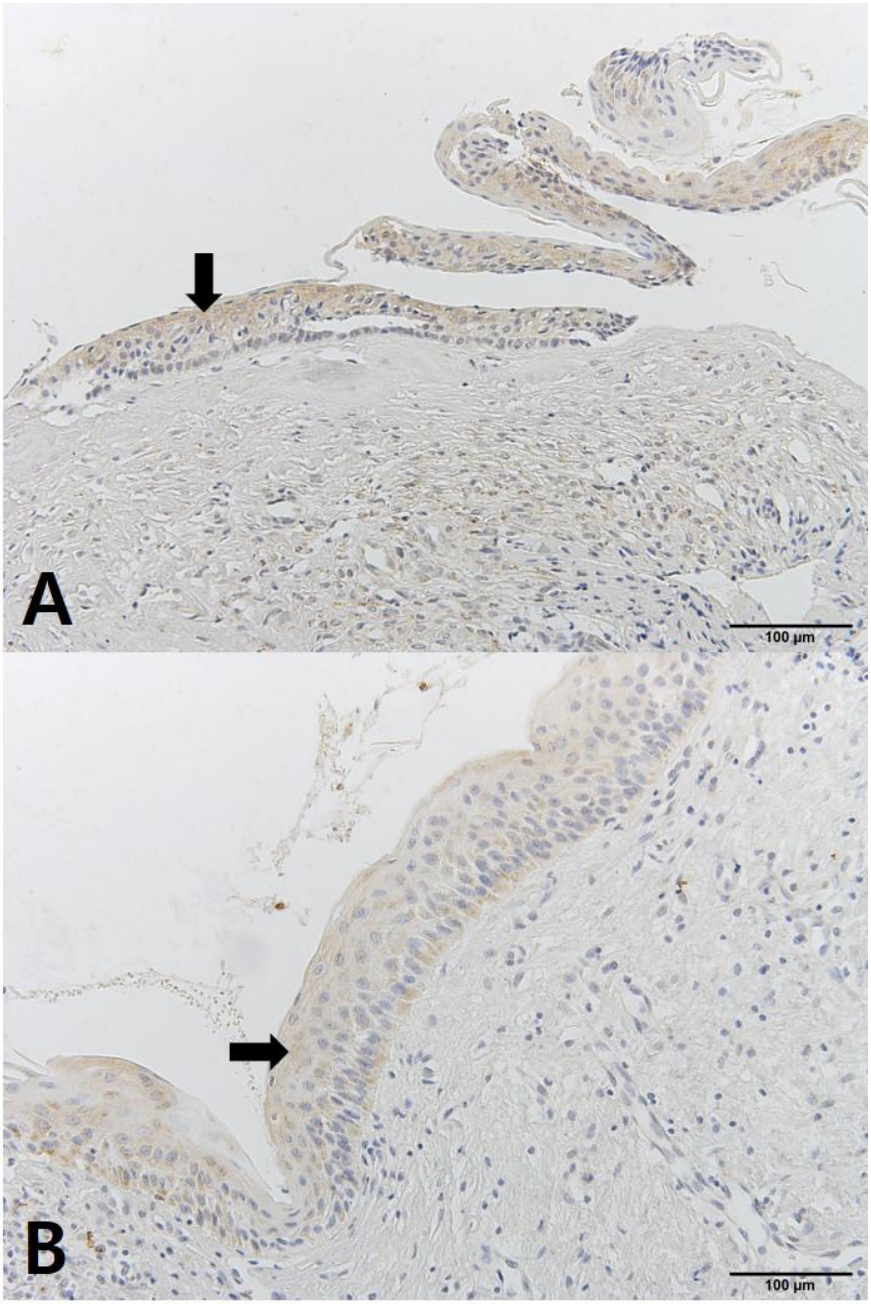

Fig 8. IHC staining of SMO

A: tissue obtained at decompression, **B**: tissue obtained at enucleation

3. Statistical results

The results for the investigated parameters are given in Table 2 and 3. The quantified values of six markers showed both increase and decrease without displaying a tendency before and after decompression. Statistic evaluation was performed to find significant differences in the values. The paired t-test was applied on numerical parameters of 6 markers obtained by quantifying the IHC staining intensity. The EGFR values before and after decompression yielded a P-value of 0.040, considered significant. However, values of P53, SMO, Bcl-2, Ki67, and PCNA before and after decompression resulted in P-values of 0.370, 0.373, 0.785, 0.678, and 0.271 respectively, which were considered not significant. Statistics of EGFR values showed no significant difference between maxilla and mandible.

Internal density was also used to quantify IHC staining. Numerical values obtained for the 5 markers P53, SMO, Bcl-2, Ki-67, and EGFR did not meet the test of normality. Statistics performed using the Wilcoxon signed ranks test yielded P-values of 0.968, 0.372, 0.147, 0.355, and 0.904 respectively. The paired t-test was used on numerical parameters of PCNA, yielding a P-value of 0.781. No P-values were considered significant.

Patient number	P53		SMO		EGFR		Bcl-2		Ki-67		PCNA	
	decom	enucleation	decom	enucleation	decom	enucleation	decom	enucleation	decom	enucleation	decom	enucleation
1	25.0124	24.5074	29.3349		50.4947	55.5087	35.0237	45.7029	37.1799	34.5308	33.57593	33.27127
2	38.4018	37.4386	41.787	35.1542	40.6627	50.8288	47.7429	47.3628	38.9512	44.3378	14.44733	7.633533
3	34.7583	34.0914	45.0117	46.8096	56.564	43.6838	51.0817	60.7123	41.452	50.3297	45.16333	24.75773
4	36.104	26.6477	44.3144	42.6455	70.39673	49.9735	59.3684	45.4353	47.0588	26.2504	52.38893	38.3601
5	36.4257	45.8278	38.9748	40.9927	51.3263	54.4129	50.5809	58.0132	40.8199	40.9262	16.5518	11.69493
6	31.0288	31.5607	40.6138	41.126	44.6381	81.0138	47.0884	24.7306	35.5879	40.6226	38.84955	50.4216
7	28.911	32.5193	32.4448	30.7163	73.402	62.3647	33.8703	47.2098	43.6486	48.6728	61.933	13.60327
8	26.1692	28.3475	21.5732	28.7682	86.7493	65.8529	33.8524	36.5697	38.5679	37.8657	12.1344	12.3017
9	29.1995	35.9683	30.553	39.049	48.7011	47.8829	48.32	45.1955	38.8456	30.1528	41.5466	35.36253
10	21.5059	18.3509	29.5511	34.3016	50.0194	41.0847	40.2528	42.218	18.1563	14.4132	52.4949	49.4736
11	15.3662	19.5092	39.655	26.2157	56.2871	31.2309	43.7492	46.3238	16.6931	22.0689	48.4937	31.7948
12	21.2601	22.7958	29.7912	29.2903	64.6252	43.2322	40.8435	50.2791	10.1073	15.5295	33.1182	39.593
13	26.8577	25.0546	45.0309	42.2114	61.2809	37.8622	54.291	41.6529	23.8276	13.4328	70.5684	61.9724
14	19.5709	32.6208	31.4108	33.287	53.5154	22.8134	41.5947	51.0266	10.8265	13.2331	38.9634	71.7943
15	21.6439	22.1587	36.9358	33.6182	46.9289	39.5982	43.8296	39.0106	16.2756	18.4216	66.6719	41.4219
16	21.886	14.8279	43.6597	27.3559	36.9253	27.3063	44.2893	45.5328	15.4373	13.5918	41.8062	48.9647
17	23.1867	27.7386	28.949	33.8088	45.5675	49.0716	57.4902	55.3479	17.8577	14.5702	29.3433	59.7442
18	23.5058	23.0818	42.8207	35.76	35.511	34.1092	36.5218	50.119	14.9716	18.0884	58.7303	51.3753
19	19.5944	18.3661	30.9006	32.2314	47.215	31.4734	52.731	42.1915	14.6933	10.9366	63.3259	44.4094
		(P = 0.370 Not significant)		(P = 0.373 Not significant)		(P = 0.040 significant)		(P = 0.785 Not significant)		(P = 0.678 Not significant)		(P = 0.271 Not significant)

Table 2. Quantified values of IHC staining using intensity

Decom, decompression- at time of biopsy; ecnucleation - at time of removal of cyst after decompression

Patient number	P53		SMO		EGFR		Bcl-2		Ki-67		PCNA	
	decom	enucleation	decom	enucleation	decom	enucleation	decom	enucleation	decom	enucleation	decom	enucleation
1	1221360	661886.3	159570		10946703.5	9401742	794427	2348836.6	3795541	702355	787070	1620213
2	1297895	1651919	169858	6020499	12978715	14244678.6	4005281	3720152	4196283	4852537	8250318	1362290
3	1210913	4326136	415264	460994.6	4768332.6	24469917.6	2989049.3	11101100	1086061	9664245	3435694	7642393
4	1100267	2584042	244947	3202104.6	13050229	6661497	3976664.6	16120202	2503290	4460643	5071506	3784806
5	358070	2934094	525009	1071066.6	7371294	9338641.3	4354102	3826833	9564397	3994580	2740623	3502349
6	2617972	581965.7	695948.5	2459057.5	8791084.3	25807126.6	12644365.6	37839358.3	4815109	3858343	8302469	12696463
7	572636	1131484	274488	344898	19323875.67	12277779	1384437	4464561.3	3411203	2593782	9922650	1898001
8	854203	456601.3	743487.5	319173	28428263	27539786	2592172.6	1606763	4662209	8662064	5050431	6075565
9	1173614	2684342	256765	246614.3	8365872	3050467.3	3403661	1648221.6	1808560	346986.7	2494948	505644
10	101443.3	43247	500141.3333	227394.6	4947151.3	1276840.6	1438076.3	3050363	50744.6	48613	2519860	3939132
11	160038.3	53904.67	2028081.5	385614.6	7852804	861543.6	3760483	8635895.5	54742.5	807558.7	3895514	1590900
12	118042	11022.67	206879.6	506003	12415767	647433.5	2717436	6914640.3	2799.3	972258.3	2675231	2298252
13	1591701	363906.7	933828.3333	913436	9281016.6	2761456.667	6234561.6	1021127.6	849262.3	2578.667	9026570	11690565
14	52882.33	134155.7	3352845.5	837549	4692212	837901	4230140.6	7423184.6	1971.5	2528	1602161	15459615
15	950395.7	628365.3	1098401.667	1743315	2395672	488422.5	2035971.6	1515056.3	200896.7	52865.33	9795084	2920485
16	122184.3	61751.33	876195.6667	390737	337179.3	136761.5	2840168.3	2485062.6	5011	8841.333	2720622	5728829
17	65406	42582.67	291139.6667	840242	615791.6	2634042.333	6948566.3	4895955	40814.67	17779.5	280163	4440092
18	240497.3	42703.67	1219010	1566785	514256	1697509.667	2719502.3	2100283.5	4135	230303.3	5986326	4843922
19	319510.7	155947.3	938439	589087.6	4711979.3	1424040.667	12456883.3	2305348.6	159179.5	918.3333	6558184	5188520
		(P = 0.968 Not significant)		(P = 0.372 Not significant)		(P = 0.147 Not significant)		(P = 0.355 Not significant)		(P = 0.904 Not significant)		(P = 0.781 Not significant)

Table 3. Quantified values of IHC staining using internal density

Decom, decompression- at time of biopsy; ecnucleation - at time of removal of cyst after decompression

Ⅳ. DISCUSSION

The object of this study was to assess the changes in histology and expression of proliferation markers in OKC before and after decompression treatment. Immunohistochemical analysis studies of OKC have demonstrated its aggressive character. Further, many studies have suggested that the epithelium adjacent to surrounding bone may be related to the aggressive character and proliferative activity of OKC. If the assumption is correct, rupture in the continuity of epithelium when performing decompression may decrease cyst size and mitigate proliferation. Interestingly, a study in which OKC walls were transplanted into athymic mice demonstrated that the features of the epithelial lining were only maintained in the presence of its cystic wall (Vedtofte, et al., 1982). There are markers relative to proliferation in the epithelium of OKC; if rupture of epithelium reduces proliferation of OKC, observation of associated markers before and after decompression may shed light on the relation.

Although intensity and internal density were utilized to quantify the positivity of IHC staining, the results showed no statistical significance. The values of EGFR before and after decompression were found to be significantly different using the intensity method. However, this method consistently reflected the background colors to the quantified values. Hence, the internal density method was assumed to be more precise. The values of the 6 markers before and after decompression obtained using the internal density method showed no significant differences. Other studies have calculated the number of positive cells per

unit length of epithelial basement membrane (BM) or the fraction of cycling cells within the investigated cell population at a given time (known as proliferation of labeling index). Studies using these methods usually involve two or more pathologists. Since it was not possible to apply those methods, expression of proliferation markers was analyzed with the aid of ImageJ software version 1.49.

The markers used in the present study have been used in several other studies comparing their expression in OKC with other lesions. The apoptosis-related factors P53 protein and Bcl-2 protein have been found in the lining epithelium of OKC (Kimi, et al., 2000; Li, et al., 1996; Piattelli, et al., 1998). Mendes RA et al observed mild to strong expression of COX-2 in all of 20 (100%) cases. Fifteen (75%) of OKC stained positive for P53 and 18 (90%) stained positive for Ki-67. There were no statistically relevant differences among the expressions of COX-2, Ki-67, and P53 (Mendes, et al., 2011). However, Slootweg has found the presence/absence of densely-stained P53 positive cells to be broadly related to Ki-67 cell numbers in highly proliferative areas as well as the converse (Slootweg, 1995). The present study did not find any relation between expression of P53 and Ki-67.

PCNA, Ki-67 and P53 protein were all expressed in actively proliferating cells, particularly in neoplasms. They were expressed more strongly in OKC than in other odontogenic cysts and more particularly so in the OKCs associated with nevoid basal cell carcinoma syndrome (NBCCS) (Shear, 2002b). Studies comparing OKC and dentigerous cyst have demonstrated a greater proliferative potential of the OKC epithelial lining

comparable to that of ameloblastoma (Li, et al., 1995; Thosaporn, et al., 2004). P53 was found in OKC more often than in other odontogenic cysts (Slootweg, 1995) or only in OKC(Ogden, et al., 1992), suggesting that increased epithelial activity explains the tendency to recur. In the present study, patients number 7 and 9 showed increased expression of P53 after decompression, but patient number 4 had decreased expression of P53.

Merva et al compared expressions of bax, Bcl-2 and Ki-67 in OKC, ameloblastoma, and radicular cyst. Ameloblastoma showed stronger Bcl-2 expression than OKC and radicular cyst. Bcl-2 expression in OKC was significantly higher than in radicular cyst. The lining epithelium of OKC showed stronger Ki-67 expression than that in ameloblastoma and radicular cysts. It was concluded that high expression of Bcl-2 and Ki-67 in OKC accords with its aggressive clinical behavior and high recurrence rate (Tekkesin, et al., 2012). Kichi et al also reported that Bcl-2 is seen only in OKC and not in dentigerous cyst (Kichi, et al., 2005). In a study done by Shear, expression of EGFR marker was reported in the epithelium of OKC, dentigerous and radicular cysts. The strongest reaction was related to OKCs, and the weakest was in the radicular cyst (Shear, 2002c). MG de Oliveira et al analyzed immunolabeling of Ki-67, EGFR and Survivin in the basal and suprabasal layers of OKC, dentigerous cyst and pericoronal follicles. OKC showed the highest proliferation rate among the three groups, with Ki-67 staining found mainly in suprabasal layers. EGFR immunolabeling was observed mainly in the cytoplasm in basal and suprabasal layers of OKCs (de Oliveira, et al., 2011). Razavi et al compared expression of Bcl-2 and EGFR in

OKC with that in dentigerous cyst and ameloblastoma. All cases of ameloblastoma and OKC, but no dentigerous cyst cases, were positively stained for Bcl-2. Expression of Bcl-2 was higher in the peripheral layer of ameloblastoma and the basal layer of OKC. Furthermore, all cases of ameloblastoma and dentigerous cysts, but no OKC samples, were positively stained for EGFR. Expression of EGFR was higher in the peripheral layer of ameloblastoma and basal layer of dentigerous cysts. It was concluded that the biological activity and growth mechanisms of OKC are different compared with those of other cystic lesions (Razavi, et al., 2015). In the present study, both Bcl-2 and EGFR were expressed in OKC tissues.

K. Ohki found immunoreactivity for SHH and GLI-1 was markedly higher in epithelial components than in subepithelial cells, while immunoreactivity for PTC and SMO was similar in epithelial components and subepithelial cells in OKC. The positive rate of PTC and SMO expression in subepithelial cells of OKC was significantly higher than that in gingiva. The positive rate of GLI-1 expression in subepithelial cells of BCNS-associated OKC was significantly higher than that in primary OKC. It was concluded that SHH signaling might be involved in the pathophysiologic nature of OKC (Ohki, et al., 2004). In the present study, SHH was tested for IHC staining with the tissue samples, however, staining with SHH did not work. SMO was thus used for the study instead of SHH.

T Yagyuu et al reported the immunoreactivity of proliferation-related SMO in OKC with recurrence was higher than that without recurrence, whereas the expressions of a ligand, SHH, and an inhibitory receptor, Patched, were not associated with OKC recurrence.

The expressions of SHH and SMO showed inverse correlation in whole OKC. It was concluded that recurrence of OKC is associated with multilocular large lesions and high SMO expression (Yagyuu, et al., 2008). In the present study, however, no relationship was found between high expression and recurrence in the values obtained from the three patients who experienced recurrence.

Odontogenic tumors were studied for the markers related to the SHH signal pathway. L Zhang et al suggested SHH, PTC, SMO and GLI1 proteins are predominantly epithelial expressions of the SHH signaling pathway in odontogenic tumors. Immunoreactivity for SHH, PTC, SMO and GLI1 was detected in both epithelial-derived odontogenic tumors and epithelial-mesenchymal derived odontogenic tumors with or without dental hard tissue formation. Mesenchymal-derived odontogenic tumors showed no positive staining except for the focal epithelial cells in island or cord forms within the central portion of the tumor. SHH, PTC, SMO and GLI1 were detected more in the cytoplasm of the epithelial cells than in stromal cells. Immunoreactivity for GLI1 was also detected in the base membrane of the tumor cells (Zhang, et al., 2006).

There are controversies over some staining factors in the context of decompression treatment. Ninomiya et al investigated Ki-67 labeling index and expression of IL-1 alpha mRNA following decompression treatment in OKC. Their results showed a decrease in Ki-67 labeling after decompression. They concluded that proliferation activity in the lining could also be affected by changes in the intra-luminal pressure and cytokine concentrations (Ninomiya, et al., 2002). Nakamura et al found that the average Ki-67 labeling index after

marsupialization was slightly lower than that before marsupialization, though, these results were statistically insignificant (Nakamura, et al., 2002). S Awni et al found no statistical changes in Ki-67 expression before and after the decompression procedure or with inflammation (Awni and Conn, 2017). The values of Ki-67 expression in the present study showed both increase and decrease after decompression, the results being statistically insignificant.

In an immunohistochemistry study, S Awni et al observed an increase in P53 expression with prolonged duration of treatment and in cases showing more inflammation. The same study found no statistical difference in expression of anti-apoptotic protein Bcl-2 after treatment (Awni and Conn, 2017). In the present study, inflammation cells were observed to increase in H&E stained photos of some cases after decompression, but quantification or statistic evaluation were not performed.

Pogrel et al reported 10 OKC resolved completely with marsupialization therapy alone, Bcl-2 staining being negative in all the samples taken of resolved OKC compared to the high expression observed in the pre-treatment biopsy (Pogrel and Jordan, 2004). Bcl-2 staining was positive in the tissues after decompression in the present study. However, this discrepancy may be due to differences in decompression and marsupialization, similarities notwithstanding.

Pia Clark et al examined expression of P53, Ki-67, and EGFR in OKC before and after decompression. They observed no significant change in expression values nor correlation

between the expression of Ki-67 and P53. No correlation between clinical shrinkage and morphologic change or between expression of proliferation and growth markers was found. Expression of EGFR was large and had a tendency to increase after decompression. OKC showed a high degree of EGFR expression that could indicate considerable growth potential of these. Thirteen of 16 (81.3%) of the cysts showed expression before decompression, the number increasing to 15 of 16 (93.8%) after decompression (Clark, et al., 2006). Expression of EGFR in the present study showed significant difference after decompression based on the values obtained by quantifying the intensity. However, no statistical difference was found on the other values, obtained by quantifying the internal density.

Studies regarding various treatments of OKC have been conducted. Resection was found to have the lowest recurrence rate (0%) but the highest morbidity rate. Simple enucleation was reported to have a recurrence rate of 17% to 56%. Simple enucleation combined with adjunctive therapy, such as the application of Carnoy's solution or decompression before enucleation, was reported to have a recurrence rate of 1% to 8.7%. Resection or enucleation with adjunctive therapy was associated with recurrence rates that were lower than those associated with enucleation alone (Blanas, et al., 2000). Other studies showed additional adjunctive therapies including liquid nitrogen and peripheral ostectomy. These adjuncts all described above, were supposed to eliminate epithelial islands and microcysts in the peripheral bone and decrease the recurrence rates (Tolstunov and Treasure, 2008), (Stoelinga, 2005).

Some studies have suggested that pathological changes after decompression are caused by the introduction of inflammation into OKC via decompression opening (Paula, et al., 2000), (Li, et al., 1996). In addition, some have suggested decompression therapy reduces the intra-lesional pressure. Other studies have found certain changes after treatment with decompression, including thickening of the cystic wall (Marker, et al., 1996), inhibition of IL-1α (Ninomiya, et al., 2002), epithelial dedifferentiation and loss of cytokeratin-10 production (August, et al., 2003), changes for Forsell and Sainio group Ia (parakeratotic type) to group II and III (orthokeratotic type) (Brøndum and Jensen, 1991), and Bcl-2 negativity (Pogrel and Jordan, 2004). It has been suggested that those changes might account for less aggressive behavior with decreased cyst recurrence after decompression (Brøndum and Jensen, 1991; Marker, et al., 1996).

It seemed that the same treatment yielded different immunohistochemical expressions of Bcl-2, EGFR, Ki-67, P53, PCNA, and SMO. Although IHC staining is possible in OKC, quantification of staining results seems uncertain. Clinically, shrinkage of the cysts after decompression could be observed, no matter what the histologic picture showed.

There were studies that supported the reliability of numericalization of IHC using software such as ImageJ. Fuhrich D. G. et al compared intraobserver and interobserver variation between traditional histological score (HSCORE) and digital HSCORE (D-HSCORE) performed by expert and naive researchers. Immunohistochemical analysis of β3 integrin subunit of 100 endometrial biopsies obtained from the midluteal phase of the menstrual cycle were reanalyzed using ImageJ software (D-HSCORE). The study

concluded the D-HSCORE performed by an inexperienced researcher has high correlation to traditional HSCORE performed by an expert (Lessey and Savaris, 2013). Similar results were yielded in other studies that compared manual counting with quantification using computer programs (Miksch, et al., 2017; Rizzardi, et al., 2012; Rizzardi, et al., 2016).

The results of the present study may be due to small sample size, tissues being harvested 4 to 5 years ago, and uncontrolled variation in staining conditions, as well as the smaller tissue sizes harvested in decompression relative to mass excisions. There was considerable difference in the proliferation rate even in epithelium of the same appearance. Quantifying IHC staining from another area of the cyst may have yielded a totally different result. The decompression time was determined by the clinical response, which varied and was relatively short in some cases.

Ⅴ. CONCLUSION

The values of Bcl-2, Ki-67, P53, PCNA, and SMO in OKC before and after decompression showed no significant change. Expression of EGFR values changed significantly after decompression. However, the results are uncertain because statistic confirmation was only done with the values obtained from quantification based on intensity. The values of EGFR obtained based on internal density showed no significant change. No correlation between clinical shrinkage and morphologic changes nor between expression of proliferation and growth markers could be found. According to the above results, the original hypothesis was rejected. There was no statistical evidence that decompression reduces the aggressive behavior of OKC within the epithelial cyst lining itself. This might indicate that decompression does not change the biological behavior of the epithelial cyst lining, as well as the recurrence rates.

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ABSTRACT (in Korean)

감압술 후 낭종적출술을 시행한 치성 각화낭에서 세포조절인자 변화

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감압술 후 낭종적출술은 치성 각화낭의 치료방법 중 하나이다. 이 방법은 크기가 비교적 큰 치성 각화낭 병소에 주로 적용된다. 이 방법의 장점은 넓은 골결손부를 형성하지 않고 감각 저하 가능성을 최소화 할 수 있으며 단순 낭종적출술에 비하여 재발률을 줄일 수 있다. 본 연구의 목적은 치성 각화낭에 감압술 시행 전과 후의 세포 조절 인자의 변화를 관찰하여 치성 각화낭의 증식 활성의 변

화를 규명하고자 하였다. 19명의 환자가 감압술 후 낭종적출술을 시행할 때 각 단계에서 조직검사를 위해 조직을 채취하였다. 총 38개의 치성 각화낭 조직에 형태학적인 검사와 면역조직화학 검사를 시행하여서 감압술 전과 후의 증식 활성의 변화를 관찰하였다. 면역화학조직 염색을 위해서 사용된 마커는 Bcl-2, EGFR, Ki-67, P53, PCNA 과 SMO 였다. 표피 염색 발현의 강도와 내적 밀도를 각각 수치화하기 위하여 ImageJ 라는 컴퓨터 프로그램을 사용하였다.

상기 연구 과정을 통하여 아래와 같은 결과를 얻었다.

1. 감압술 시행 후의 변화 과정은 분명했다. 병변의 크기는 감소하였으며, 방사선학적으로 골밀도가 두꺼워지는 것이 관찰되었다. 환자들의 감압술 시행기간은 4개월에서 12개월 사이였으며, 평균 7.3 개월이었다. 이 후에 낭종적출술을 시행하였다.

2. 치성 각화낭의 조직은 감압술 시행 전과 후의 차이를 보였다. 감압술을 시행하였을 때 채취한 조직에서, 낭 내강의 벽은 섬유성 조직이 매우 얇은 5~8 층 정도의 세포 두께를 가지는 부전 각화 중층편평상피로 덮혀 있었다. 기저세포는 입방형 세포와 책상배열을 한 핵으로 구성되어 있었다. 흔히 섬유피막으로부터 피복상피가 박리되어 있었다. 결합조직과 상피 사이의 결합은 매우 약하게 이루어져 있었으며 상피돌기의 소실을 보였다.

3. 낭종적출술을 시행할 때 얻은 조직에서 변화된 모습이 관찰되었다. 낭종

벽의 상피는 과증식된 중층편평상피와 염증세포의 침윤이 일어난 촘촘한 결합 조직으로 변화하였다.

4. Bcl-2 염색은 상피의 기저층에 발현하였다. 하지만 일부 조직에서는 상피 표면과 결합조직의 전 층에서 발현을 관찰할 수 있었다. EGFR 염색은 상피세포의 막과 세포질에서 발현이 되었고 기저층에서 표층을 향하여 점차적으로 감소하는 모습을 보였다. Ki-67 염색은 상피의 기저층과 부기저층에서 주로 발현되었다. PCNA 은 기저층과 부기저층에서 모두 풍부한 발현을 보였다. P53 염색은 상피에서 전반적으로 관찰되었으며 주로 부기저층에서 발현되었다. SMO 염색은 주로 중간층에서 발현되었다.

5. Bcl-2, Ki-67, P53 과 PCNA 염색은 치성 각화낭의 상피세포 핵에 국한하여 발현이 관찰되었다. EGFR 과 SMO 염색은 상피의 세포막에서 발현이 관찰되었다. 낭종적출술 시행시 채취한 조직에서도 6 가지 마커는 비슷한 양상의 염색 발현을 보였다.

6. 6 가지 마커의 염색 발현을 수치화한 값은 감압술 시행 전과 후에 특정한 경향을 보이지 않고 증가와 감소를 모두 보였다.

7. 6 가지 마커의 염색조직화학검사를 염색강도로 수치화한 값을 사용하여 대응표본 t 검정을 시행하였다. 감압술 시행 전과 후의 EGFR 수치는 유의확률수치가 0.040으로 통계적인 유의성을 보였다. 하지만 감압술 시행 전

과 후의 P53, SMO, Bcl-2, Ki-67, PCNA 는 유의확률수치가 각각 0.370, 0.373, 0.785, 0.678, 0.271 로 통계적인 유의성은 관찰되지 않았다. EGFR 수치에서 상악군과 하악군 사이에 통계적인 유의성은 관찰되지 않았다.

8. 다른 방법으로 염색조직화학검사를 내부 밀도를 사용하여 수치화하였다. P53, SMO, Bcl-2, Ki-67, EGFR 수치에 윌콕슨 부호 순위 검정을 시행하였으며 유의확률수치는 각각 0.968, 0.372, 0.147, 0.355, 0.904 로 통계적 유의성은 관찰되지 않았다. PCNA 수치에 대응표본 t 검정을 시행하였으며 유의확률수치는 0.781로 통계적 유의성은 모두 관찰되지 않았다.

이상의 결과를 바탕으로, 감압술 전과 후의 Bcl-2, EGFR, Ki-67, P53, PCNA 과 SMO 의 염색 발현을 수치화한 값에서 통계적 유의성은 관찰되지 않음을 확인할 수 있었다. 임상적인 낭의 수축과 형태학적인 변화나 증식 마커 또는 성장 마커 발현 간의 연관성은 관찰할 수 없었다. 감압술을 시행할 경우에 치성 각화낭 상피 표면 자체의 잠재적인 공격성이 감소한다는 통계적인 증거는 찾을 수 없었다. 이는 감압술이 치성 각화낭 상피 표면의 생물학적인 행동을 변화시키지 않을 수도 있다는 점을 나타내며, 또한 치성 각화낭에서 감압술을 시행하여도 재발률을 감소시키지 않는다고 생각해 볼 수 있다.

핵심되는말: 면역조직화학염색, Bcl-2, EGFR, Ki-67, PCNA, P53, SMO, 감압술, 낭종적출술, 치성 각화낭