Original Article

Histopathologic and immunohistochemical findings of odontogenic jaw cysts treated by decompression technique

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Abstract

Context: Odontogenic cysts are among the most common lesions to affect the oral and maxillofacial region. Cysts are capable of causing significant bony disfigurement, tooth displacement and pathological fractures. Several surgical approaches exist for the management of larger cysts of the jaws. These include enucleation, marsupialization and decompression.

Aims:

1. Analysis of histopathologic findings in odontogenic cysts before and after decompression
2. Analysis of Ki-67 expression in odontogenic jaw cysts before and after decompression.

Settings and Design: Decompression technique was used for the treatment of 10 cases of odontogenic cysts in the study. Incisional biopsies of cystic lining (pretreatment) and corresponding excisional biopsies (posttreatment) were received for histopathologic and immunohistochemical examination.

Subjects and Methods: Hematoxylin and eosin stain was used for histopathologic findings, and Ki-67 was used for immunohistochemical findings using antibody Ki-67 in fresh tissue samples.

Results: Overall, radicular cysts, dentigerous cysts, and sialo-odontogenic cyst contained fewer Ki-67 $^{+}$ cells than odontogenic keratocysts. The average scores were found to be 2.2 and 1 for before and after decompression, respectively. A statistically significant difference was observed between the two groups. The two-tailed *P* value was found to be <0.0001. The confidence interval was found to be 95%.

Conclusions: The proliferative activity evaluated by Ki-67 marker was greater in predecompression epithelial lining compared to postdecompression. Our study infers that proliferative rate of the cystic epithelial lining is significantly diminished after decompression.

Keywords: Decompression, Ki-67, odontogenic cysts

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INTRODUCTION

Odontogenic cysts are unique and affect the oral and maxillofacial region. They develop from components of odontogenic epithelium or its residuals entrapped in gingiva or bone.[1] A variety of cysts frequently occur in

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the jaws. Over 90% of cysts of the oral and maxillofacial region are of odontogenic in origin. Odontogenic cysts are the second most common oral and maxillofacial lesions in adults after mucosal pathologies. They account for 14%–15% of specimens. In terms of bone pathology, odontogenic cysts are by far the most common cause of bony swellings of the jaws. Cysts are capable of causing significant bony disfigurement, tooth displacement and pathological fractures. They are often asymptomatic and are frequently discovered incidentally, or when inflammation or infection develops. As cysts enlarge, they resorb bone and expand into the surrounding tissues and may also displace neighboring teeth. The surrounding structures may, therefore, suffer some damage before the cystic lesion is identified and managed appropriately. Several surgical approaches exist for the management of larger cysts of the jaws. These include enucleation, marsupialization and decompression.[2]

Decompression and marsupialization of cysts are probably the earliest advocated treatment and were first suggested by Partsch in the German literature in the late 19th century.[3] Decompression involves the creation of a small window/fenestration in the cystic wall which allows the lining of the cystic lumen to become confluent with that of the oral cavity. The insertion of a decompression stent/drainage tube is required so that the continuity between the cystic lumen and the oral cavity is maintained. Such continuity establishes free draining of cystic contents and equalization of the intra- and extracystic pressure.[4,5]

Cystic decompression is a conservative approach in the management of large cystic lesions that may significantly reduce the associated morbidity and cost.[6] After decompression treatment, the phenotypic look is changed considerably, and the changes indicate growth and proliferative activity. It is therefore interesting to study whether the pronounced clinical shrinkage which occurs in odontogenic cysts after decompression, is visible biologically/histologically.[7] Cell proliferation plays an important role in several biological and pathological events such as cysts and tumors. Assessing the proliferative capacity of epithelial cells involved in odontogenic cysts may be useful in determining cysts progression/recurrence and presumably prognosis. Ki-67 is a proliferative marker which is a nuclear nonhistone protein expressed maximally in cells in the G2 and M phases of the cell cycle but is absent in resting cells.[8]

The Ki-67 antigen was originally identified by German group in the early 1980s, by the use of a mouse mAb against a nuclear antigen from a Hodgkin's lymphoma derived cell line. This nonhistone protein was named after the researcher's location, Ki for Kiel University, Germany, with the 67 label referring to the clone number on the 96-well plate. Mostly, Ki-67 is measured on paraffin section by an immunohistochemical method, using MIB-1 antibody. In general, Ki-67 score is defined as the percentage of total number of tumor cells with nuclear staining. Hence, Ki-67 can be employed to measure the growth fraction of small tissues, as well as premalignant, malignant and cystic lesions.[9-12] The purpose of this study was to evaluate if the histologic changes that occur in odontogenic cysts after decompression can be detected biologically as a difference in growth and proliferation activity, before and after decompression, using the immunohistochemical expression of Ki-67 as marker. Histopathological study will also help to check the efficacy of decompression procedure in the treatment of cysts during decompression and after completion of decompression procedure.

SUBJECTS AND METHODS

Our study was carried out in the Department of Oral and Maxillofacial Pathology and Microbiology and Department of Oral and Maxillofacial Surgery. Decompression technique was done for all the ten cases. Ten fresh incisional biopsies of cystic lining (predecompression) and ten biopsies (postdecompression) were received in 10% buffered formalin solution. Two sections, each of 4 μ thickness, from paraffin-embedded tissues of Groups 1 and 2 were obtained. One section was stained with hematoxylin and eosin and another was immunostained with Ki-67 monoclonal antibody.

Antibodies

Primary antibody Anti-Ki-67 Antigen, Clone MIB-1 and Secondary antibody containing Super Sensitive Polymer DAB detection kit was acquired from Biogenex (Sikandrabad, India).

Immunohistochemistry

For immunohistochemical detection of Ki-67, 4 μ sections were cut from formalin-fixed paraffin-embedded tissue blocks. These sections were de-paraffinized by xylene and dehydrated in 96% ethanol. For antigen retrieval, the deparaffinized sections were kept in staining trough filled with citric acid (2.94 g/L sodium citrate, pH 6.0) and were boiled in pressure cooker for 15 min followed by gradual cooling to 30°C.[13] The sections were rinsed in 0.01 M phosphate-buffered saline (PBS; 7.4). After that, sections were introduced to peroxide block for 10 min, followed by power block for 10 min, at room temperature in a humidifying chamber. Sections were not

washed with PBS after exposing them with power block. Subsequently, slides were incubated for 60 min with Ki-67 primary antibody. After rinsing with two changes of PBS, sections were subjected to super enhancer. Then, they were incubated for 30 min with secondary antibodies, which conjugated with peroxidase-labeled dextran polymers. After rinsing those with PBS, sections were treated with 3,3'-diaminobenzidine for 20 min. Sections were counterstained with Mayer's hematoxylin for 3 min and then the expression of Ki-67 antigen was evaluated. For control study on antibodies, the primary antibodies were replaced with preimmune rabbit immunoglobulin (Ig) G or mouse IgG subclasses (Biogenex). The average intensity of Ki-67 expression before and after decompression was scored as +++, ++ and +; "+++" being scored for intense expression, "++" for moderate expression and "+" for mild expression. These symbols were allotted a numerical value of 3, 2 and 1, respectively. The average score was calculated for both, before decompression and after decompression. Chi-square test was applied to evaluate the results statistically.

RESULTS

The sex distribution of the studied cases was 6 males and 4 females. The male-to-female ratio was 3:2. The average age observed for the patients in the study was 26 ± 9.2 years. The most frequent site was the angle or ramus of the mandible. The average time of decompression was found to be 7.3 ± 2 months [Graph 1]. On radiographic evaluation, there was a complete resolution of the bone defect caused by the cyst and the lesion showed an average reduction of >80% [Table 1]. Size comparison of predecompression and postdecompression (radiographic evaluation) showed complete resolution as measured across the cavity of the panoramic X-ray [Figure 1 and Graph 2].

Histological examination of tissue removed at the initial surgery or predecompression showed that none of the cysts showed signs of inflammation or very mild in few cases in the underlying tissue, whereas after decompression, all the cysts had signs of subepithelial inflammation although to varying degrees [Figures 2 and 3]. The histologic appearance of the epithelium in the biopsies obtained after decompression was that of hyperplastic stratified nonkeratinizing squamous epithelium [Table 2]. Overall radicular cysts (RCs), dentigerous cysts (DCs) [Figure 4] and sialo-odontogenic cyst contained fewer cells with Ki-67 reactivity compared to odontogenic keratocysts (OKCs). This was confirmed by counts of single high-power fields in areas of greatest labeling within individual specimens. In OKC, the distribution of Ki-67 $^{+}$ cells was uniform, whereas in RCs, DCs and sialo-odontogenic cyst areas showing few or no labeled nuclei alternated with parts containing large numbers of positive nuclei. The position of Ki-67 $^{+}$ cells within the epithelium differed between lesions. In OKC, they were mainly confined to suprabasal and basal layer [Figure 5]. In DC, RC and sialo-odontogenic cyst positive nuclei were mainly confined to basal cell

Table 1: On radiographic evaluation there was a complete resolution of the bone defect caused by the cyst and the lesion showed an average reduction of >80%

Case number	Predecompression (cm's approximate)	Postdecompression
1	5×1.5	Resolved
2	6×2.5	Resolved
3	4×1.5	Resolved
4	3.5×1.5	Resolved
5	4×1.5	Resolved
6	4×1.0	Resolved
7	3.5×1.5	Resolved
8	4×1.5	Resolved
9	3.5×1.5	Resolved
10	4.5×3	Resolved

Table 2: Histopathological evaluation of biopsies obtained pre- and post-decompression

Case number	Histopathological diagnosis	Epithelium predecompression	Epithelium postdecompression
1	Sialio-dontogenic cyst	Proliferative	Normal hyperplastic
2	OKC	Proliferative	Normal hyperplastic
3	RC	Proliferative	Normal hyperplastic
4	RC	Proliferative	Normal hyperplastic
5	DC	Proliferative	Normal hyperplastic
6	OKC	Proliferative	Normal hyperplastic
7	RC	Proliferative	Normal hyperplastic
8	DC	Proliferative	Normal hyperplastic
9	RC	Proliferative	Normal hyperplastic
10	DC	Proliferative	Normal hyperplastic

OKC: Odontogenic kerato cyst, RC: Radicular cyst, DC: Dentigerous cyst

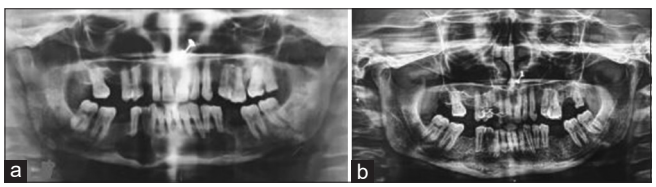


Figure 1: Radiographic evaluation of odontogenic keratocyst predecompression (a) postdecompression (b)

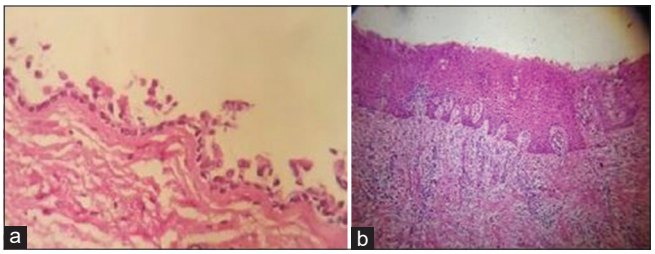


Figure 2: H&E-stained sections dentigerous cyst (a) predecompression (b) postdecompression ×100

layer except in inflamed areas where they were present throughout the entire epithelial thickness.

The average score was calculated for both, before decompression and after decompression. The average scores were found to be 2.2 and 1 for before and after decompression, respectively [Graph 3]. The difference in observation when numerically represented was 1.2. The grading was carried out subjectively depending on intensity of Ki-67 expression and areas involved [Table 3]. Numerical representation of expression of Ki-67 in cystic epithelium in predecompression was 2–3, whereas postdecompression, the value was 1 [Table 4].

A statistically significant difference was observed between the two groups [Table 5]. The two-tailed *P* value was found to be <0.0001. By conventional criteria, this difference is considered to be extremely statistically significant. The confidence interval was found to be 95%. The intermediate values observed were $t = 9.0000$ (distribution of data) and df = 18 (degree of freedom).

DISCUSSION

The average time of decompression was found to be 7.3 ± 2 months. In this study, we used I-CAT CBCT software (Carestream Dental, Rochester, New York, U.S.A.) to measure the bone density of cyst and adjacent normal bone before and after decompression. In most of the patients, lesions completely disappeared after completion of decompression.

Result of the present study is comparable to some extent with a study of August *et al*. who studied 14 cysts, out of which were six males and eight females with an average age of 32 years, whereas in our study, average age is 26 ± 9.2 years.[14] Ten cysts were mandibular, and four were maxillary, with an average duration of 8.4 months of decompression which is similar to our study 7.3 ± 2 months.

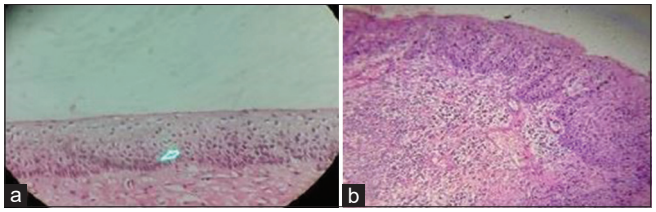


Figure 3: H&E-stained sections odontogenic keratocyst (a) predecompression (b) postdecompression ×100

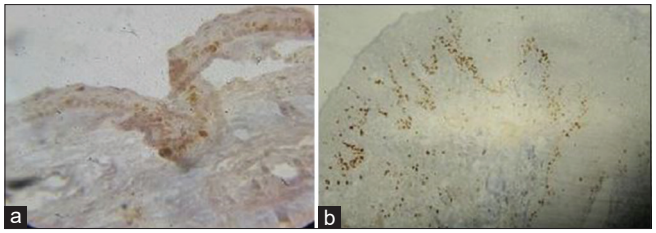
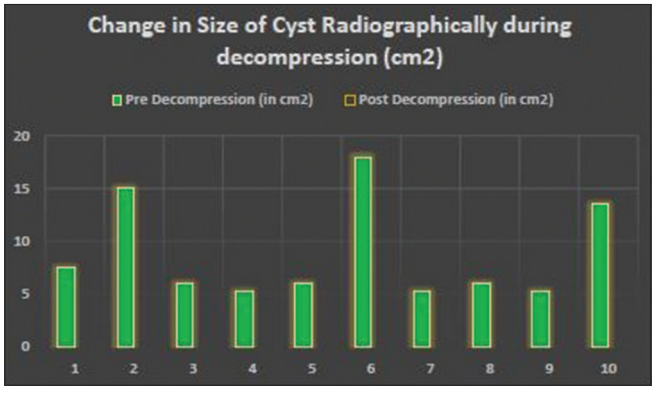


Figure 5: Immunoexpression of Ki-67 odontogenic keratocyst (a) predecompression (b) postdecompression ×400



Graph 2: Size comparison predecompression and postdecompression (radiographic evaluation)

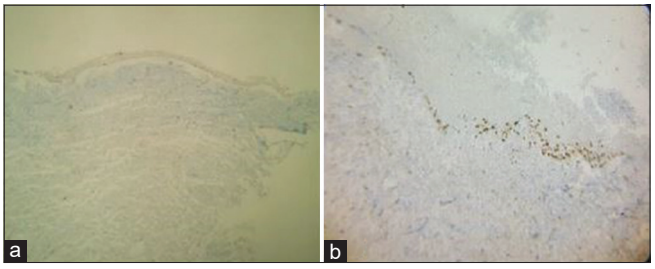
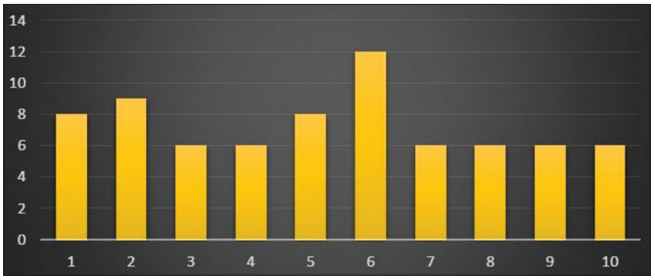
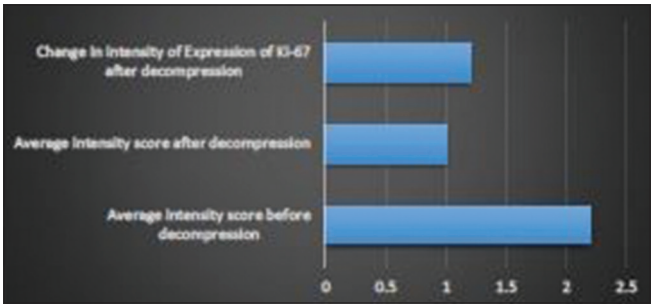


Figure 4: Immunoexpression of Ki-67 dentigerous cyst (a) predecompression (b) postdecompression ×100



Graph 1: Time taken for resolution of cyst (in months)



Graph 3: Average intensity of Ki-67 expression

Table 3: Immunoexpression of Ki-67

Case number	Type of Cysts	Ki-67 predecompression		Ki-67 postdecompression	
		Area	Intensity	Area	Intensity
1	Sialo-odontogenic cyst	Basal layer	++	Epithelium	+
2	OKC	Basal layer suprabasal layer	+++	Epithelium	+
3	RC	Epithelium	++	Epithelium	+
4	RC	Epithelium	++	Epithelium	+
5	DC	Epithelium	++	Epithelium	+
6	OKC	Basal layer	+++	Epithelium	+
7	RC	Suprabasal layer	++	Epithelium	+
8	DC	Epithelium	++	Epithelium	+
9	RC	Epithelium	++	Epithelium	+
10	DC	Epithelium	++	Epithelium	+

OKC: Odontogenic kerato cyst, RC: Radicular cyst, DC: Dentigerous cyst, +: Means mild expression, ++: Means moderate expression, +++: Means intense expression

Table 4: Numerical representation of expression of Ki-67

		Immunoexpression of Ki-67			
Case number	Type of Cysts	Ki-67 predecompression		Ki-67 postdecompression	
		Area	Intensity	Area	Intensity
1	Sialo-odontogenic cyst	Basal layer	2	Epithelium	1
2	OKC	Basal layer suprabasal layer	3	Epithelium	1
3	RC	Epithelium	2	Epithelium	1
4	RC	Epithelium	2	Epithelium	1
5	DC	Epithelium	2	Epithelium	1
6	OKC	Basal layer	3	Epithelium	1
7	RC	Suprabasal layer	2	Epithelium	1
8	DC	Epithelium	2	Epithelium	1
9	RC	Epithelium	2	Epithelium	1
10	DC	epithelium	2	Epithelium	1

OKC: Odontogenic kerato cyst, RC: Radicular cyst, DC: Dentigerous cyst

Table 5: Statistical analysis

Group	Predecompression	Postdecompression	Significance between two groups
Mean	2.2	1	$P<0.0001$ (statistically, extremely significant)
SD	0.42	0	
SEM	0.13	0	
n	10	10	

SD: Standard deviation, SEM: Standard error of mean

Schlieve *et al*. in their study of 25 cysts treated with decompression the mean age was 34 years and 14 were male and 11 were female. Most lesions were located in the mandible (angle and ramus); the remaining lesions were located in the posterior maxilla.[15] The average time of decompression; was 9–12 months which is somewhat similar to our study.

Enislidis *et al*. evaluated prospectively the effect of decompression as the primary treatment of large mandibular cysts in 24 patients, out of which four patients did not turn up for follow-up. Fourteen were male and 6 were female with an average age of 40 years. Eleven cysts were in mandibular angle and 9 in the mandibular symphysis. Their study group consisted of 8 keratocysts, 5 RCs, 6 DCs, and one 1 epithelial cyst. After a mean duration of decompression of 446 days, cysts had shrunk by a mean of 81%.[16] This study is in accordance with the present study.

Similar results were demonstrated by Anavi *et al*. in a study group of 67 patients with male-to-female ratio of 1.4:1, mean decompression time of 9.2 ± 5.2 months; it was 7.6 months in patients ≤18 years old and 10.2 months in older patients.[17]

Our results were compatible with other study done by Marker *et al*. where the sex distribution of the initial group was 8 men and 4 women. In the new group, there were six men and five women. The male/female ratio of the two combined groups was 14/9. The average age distribution was 47 years in the original group and 25 years in the new group. The most frequent site was at the angle or ramus of the mandible. The average time of decompression was 12 months for original group and 9.5 months for the new group.[18]

In the present study, the radiographic evaluation of predecompression and postdecompression showed evidence of bony infill and complete resolution of the radiolucent area, which is in accordance with the study conducted by Sammut *et al*. who presented a series of clinical cases of 14 large cystic lesions of the jaw treated with decompression, out of which 11 were male and three female patients. Patient's age range was from 13 to 78 years. Eight of the cysts were located in the maxilla

and six in the mandible. In all cases, a postoperative radiograph confirmed cavity shrinkage and bony infill.[4] These radiographic signs were noted as early as 2 months postoperatively in the younger members of the series which is somewhat same as seen in our study.

Lizio *et al*. in their study of twenty cases evaluated the three-dimensional radiographic variation in mandibular odontogenic cystic lesions after decompression.[19] The average decompression time was 5.7 months (3–12 months) which is in accordance with our recent study.

To evaluate the effectiveness of decompression as the primary treatment of odontogenic cystic lesion, Gao *et al*. analyzed predecompression and postdecompression panoramic radiographs in a total of 32 odontogenic cysts. Radiographic evaluation at 1–24 months after decompression showed gradual decrease in all the cysts, which suggested that bone regeneration occurred during decompression.[20] The result of this study was similar to the results of our study.

However, a study was conducted by Park *et al*. to verify the clinical effectiveness of decompression in decreasing the size of a cyst. Thirteen DCs, 14 keratocystic odontogenic tumors and 5 unicystic ameloblastoma cases were treated by decompression. The authors concluded that there was no difference in size due to decompression among different types of cysts.[21] The result was not in accordance with our study. They could not evaluate the cause, why there was no decrease in size after decompression.

On histological examination, the change in cystic epithelium appeared to be a gradual process. The biological mechanism for this phenomenon is unclear. Careful histologic evaluation of residual cystic lining showed transformation in some regions and not in others. The epithelium was normal and hyperplastic when compared to epithelium seen in predecompression sections where it was much proliferative in nature. Histologic examination of tissue removed at the initial surgery showed that none of the cysts show signs of inflammation in the underlying tissue, whereas after decompression, all the cysts had signs of subepithelial inflammation although to varying degrees. The histological examination in the present study showed that the epithelium of the cysts had changed in all the ten cases after decompression, which is somewhat similar to the findings of Marker *et al*.[18] who found that epithelium showed change in 83% of cases of large OKCs treated by decompression and later cystectomy.

Immunohistochemically, in the present study, the changes in proliferative activity before and after decompression were detected by immunoexpression of Ki-67 which is a proliferative marker. Overall, RCs, DC and sialo-odontogenic cyst contained fewer Ki-67 $^{+}$ cells than OKC. This was confirmed by counts of single high power fields in areas of greatest labeling within individual specimens. In OKC, the distribution of Ki-67 $^{+}$ cells was uniform, whereas in RC and DC areas showing few or no labeled nuclei alternated with parts containing large numbers of positive nuclei. The expression of Ki-67+ cells within the epithelium differed between the lesions. In OKC, the Ki-67 cells were confined immediately above the basal columnar layer with few positive basal layers. In DC, RC and sialo-odontogenic cyst-positive nuclei were mainly confined to the basal cell layer except in inflamed areas where they were present throughout the epithelial thickness. Similar results were obtained by Slootweg where OKC showed highest proliferative activity which was confirmed using Ki-67.[22] The distribution pattern of Ki-67+ cells was also somewhat similar to the present study.

There are few published case series describing the effects of decompression on a variety of odontogenic cyst-like lesions such as the glandular odontogenic cyst, the mural or intraluminal cystic ameloblastoma, or the DC. Few reports have commented on the final histologic diagnosis of the lesion after postdecompression definitive surgery, with only a single article by Anavi *et al*. discussing the final pathology of a wide range of odontogenic cyst-like lesions.

CONCLUSIONS

The proliferative activity evaluated by Ki-67 marker was greater in predecompression epithelial lining compared to postdecompression. There was a statistically significant difference in proliferation of epithelial lining before and after decompression.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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