Original Article

COMPARATIVE EVALUATION OF TOPICAL APPLICATION OF 5-FLUOROURACIL AND MODIFIED CARNOY'S SOLUTION IN MANAGEMENT OF ODONTOGENIC KERATOCYST

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ABSTRACT

The study was designed to assess the efficacy of topical application of 5-fluorouracil (5-FU) in the management of odontogenic keratocysts (OKC). The prospective, comparative, clinical study was conducted on seventeen patients with non-recurrent, non-syndromic OKC of the mandible who underwent cyst enucleation and peripheral osteotomy, followed by the application of 5-FU (group A, n = 9) and modified Carnoy's solution (MC) (group B, n = 8). Patients were assessed for nerve paresthesia, recurrence, and bone healing. Intergroup comparison of age, gender, location, and size was done by analysis of variance and Chi-square test. Recurrence was studied by Kaplan-Meier analysis. Bone healing was compared between the groups using an unpaired t-test.

Age of patients ranged from 26-61 years, with male-to-female ratio of 1.8: 1. Patients were followed-up for 43.7 and 47.5 months in groups A & B, respectively. They showed no recurrence in the 5-FU group and 25% (n=2) recurrence in the MC group. Kaplan-Meier analysis showed a disease-free survival probability of 0.9 at 30 months, which reduced to 0.7 at 60 months in group B. Although temporary paresthesia was seen in both group A (28.5%, n=2) and group B (42.8%, n-3), complete recovery was seen in all the patients. Bone healing assessment showed a mean increase in grey-level score by 36.52 ± 2.96 and 34.064 ± 6.52 in groups A and B respectively, which was statistically insignificant (p-value 0.225184). 5-FU is a novel option for the management of OKC. Its use resulted in lower post-operative paresthesia with no recurrence.

Key words: Keratocystic odontogenic tumor, Odontogenic cyst, Enucleation, 5-Fluorouracil.

Introduction

Odontogenic keratocyst (OKC) is a common jaw cyst, which is characterized by a high recurrence rate. Conventionally the management of OKC varies from conservative enucleation of the cyst lining to more aggressive jaw resection. Although enucleation results in low postoperative morbidity, it is associated with a recurrence rate of 56% [1, 2]. Resection of the jaw with safe bone margins reduces the risk of recurrence to almost 0%. However, jaw resection is associated with increased morbidity and the need for extensive reconstruction surgery. Adjunctive measures like the use of chemical cauterization with Carnoy's solution, peripheral ostectomy, and cryosurgery have been used with enucleation to reduce the chances of OKC recurrence [3]. Use of Carnoy's solution (CS) (a tissue fixative composed of absolute alcohol, glacial acetic acid, chloroform, and ferric chloride) after cyst enucleation has traditionally become popular, as it significantly reduces the recurrence of OKC [4]. However, the application of CS near the nerve carries the risk of peripheral nerve injury. 18% of patients reported inferior alveolar nerve paresthesia after the application of CS for treatment of OKC of the mandible [5]. In 1992, the Food and Drug Administration of the United States of America labeled chloroform, a carcinogenic agent and placed a ban on the use of therapeutic agents containing it [6]. This resulted in the removal of chloroform from CS which led to the use of modified CS. Dashow J. *et al*. showed that the application of MC resulted in a higher recurrence rate (35%) compared to the original CS (10%) [7]. Limitations of CS and MC and the morbidity associated with extensive resection, have necessitated the search for other treatment adjuvants to reduce the recurrence rate associated with conservative treatment of OKC with enucleation. The genetic pathogenesis of OKC and basal cell carcinoma appear to be similar, in the fact that both develop following mutation in the protein patched homolog (PTCH) gene [8]. PTCH gene mutation in cancer causes activation of smoothened and sonic hedgehog signaling, leading to neoplastic growth [9]. Smoothened and sonic hedgehog gene alterations have also been documented to play an important role in the development of OKC [10]. 5-fluorouracil (5-FU) is an antimetabolite drug that causes cell death through inhibition of the sonic hedgehog gene and has been used topically for the treatment of superficial basal cell carcinoma and other carcinomas including hepatocellular carcinoma and colorectal cancer [11, 12]. Although the application of 5-FU has not been studied extensively, recent results

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reported by Ledderhof *et al*. and Lone *et al*. has shown promising result, with reduced recurrence rate and lesser risk of nerve damage [13, 14]. This prospective clinical study was designed to evaluate the efficacy of 5-FU as an adjuvant to cyst enucleation and compare it with conventionally used modified Carnoy's solution in the management of non-syndromic and non-recurrent OKC of the mandible. The hypothesis to be tested was; topical application of 5-FU after enucleation of OKC involving the mandible, would yield results, similar to or better than the conventional used MC solution, in terms of recurrence rate and nerve paresthesia.

Materials and Methods

A prospective comparative randomized clinical study was conducted in the Department of Oral and Maxillofacial Surgery, Krishna Hospital, Karad, India after due approval of the Institutional ethics committee. Patients with histologically proven OKC (with incision biopsy report) involving the mandible and willing to participate in the study and comply with follow-up visits were enrolled for the study. Patients were enrolled from August 2012 to July 2018. Exclusion criteria included; recurrent OKC, multiple OKC as a part of Gorlin-Goltz syndrome, lesion involving the maxilla, lesion with significant cortical perforation(s), or involvement of overlying mucosa and patients with a pre-existing neurological disorder or nerve paresthesia.

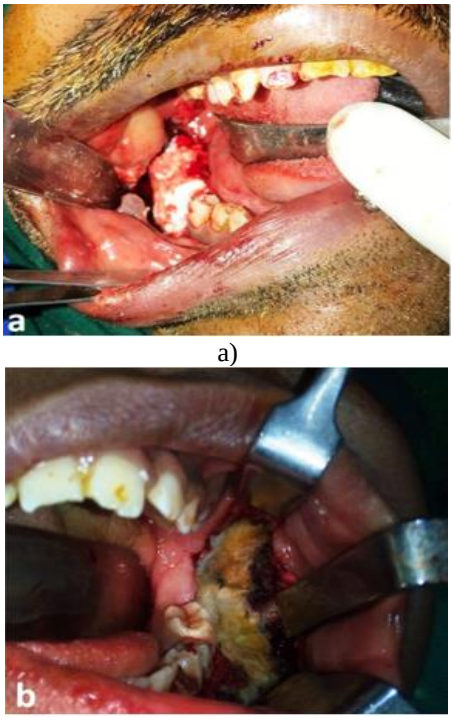
Patients fulfilling the above criteria and completing a minimum follow-up of 2 years were included in the study. Simple randomization was done to allocate the patients into group A (receiving 5-FU) or group B (receiving MC), using computer-based software (Clinstat, MS-DOS program, Bland M). The independent variables between the 2 groups included the application of 5-FU versus MC after enucleation of OKC, while the primary outcome variables included; presence or absence or recurrence, time of recurrence, the incidence of inferior alveolar nerve paresthesia and bone healing at 2 years postoperative period. Other variables evaluated were; age, gender, location (anterior mandible, body, body-ramus, ramus), and size of the tumor (measured as the largest dimension on computed tomography). Intra-oral biopsy report was reviewed for confirmation of the diagnosis of OKC to enroll the patients for the study. All the patients included in the study were subjected to screening radiographs (orthopantomogram), followed by detailed imaging using computed tomography (with contrast when indicated). Patients with multiple and/or large cortical perforations, significant involvement of the lower border of the mandible, and oral mucosal involvement were eliminated from the study. The surgical procedure, potential risks, and benefits of treatment with 5-FU and MC were explained to the patient and informed consent was obtained before taking up for enucleation of the lesion under general anesthesia.

Protocol for enucleation and topical 5-FU application (group A)

All the lesions were approached through an intraoral incision. A bone window was created over the buccal cortex and the cyst lining was enucleated. Peripheral ostectomy was done using round motor-driven bur. Care was taken to avoid ostectomy over the region of the inferior canal when the lesion was close to it. Subsequently, the bone cavity was prepared to be treated with 5-FU (Fluorouracil 5% w/w cream). A sterile ribbon gauge coated with 5-FU was packed in the bone cavity. **(Figure 1a)** Primary closure of the overlying oral mucosa was achieved and the distal end of the ribbon gauze was kept exposed into the oral cavity and removed on the first postoperative day (after 24 hours).

Protocol for enucleation and topical MC application (group B)

The cyst was approached and enucleated with the same protocol as in group A. After the completion of peripheral ostectomy, the cyst cavity was prepared for the application of MC. When the inferior alveolar nerve was exposed in the surgical defect, the application of MC was done after isolating the nerve with paraffin-impregnated gauze (Bactigras dressing, Smith & Nephew Pvt Ltd). Cotton applicators soaked in MC (60% ethanol, 10% glacial acetic acid, and 1 g of ferric chloride) were packed carefully into the bone cavity and maintained for 3 minutes as per the protocol described by Frerich *et al*. [15] **(Figure 1b)** After removal of cotton soaked in MC, the surgical defect was closed primarily by suturing of the overlying mucosa.



a)

b)

Figure 1. Intraoperative photographs showing the application of 5-FU in group A patient (a) and MC group B patient (b)

Patients in both the groups received same postoperative care. All the patients were kept on regular long-term follow-ups. Evaluation of Inferior alveolar nerve paresthesia was done for patients with lesions in the body and ramus region. Patients with OKC of the anterior mandible and where the nerve was directly exposed in the surgical defect were excluded from the evaluation (to eliminate bias due to direct manipulation of the nerve). All the patients underwent 6 monthly clinical assessments along with a screening radiograph (orthopantomogram) to study bone healing and the possibility of recurrence.

Bone healing of the surgical defect was studied on an orthopantomogram using a grey-level histogram score. Preoperative (baseline) and 2 years follow-up radiograph images were exported to Adobe Photoshop software (version 7). The area of the cyst on the pre-operative orthopantomogram was outlined using a selection tool and the baseline grey-level value was recorded. In case of the presence of an impacted tooth within the lesion, the area was removed from the selection. Similarly, the selection area was used over the 2 years postoperative orthopantomogram, and the grey-level value was recorded. **(Figure 2)** The difference in mean grey-level scores between the two groups was tabulated and compared.

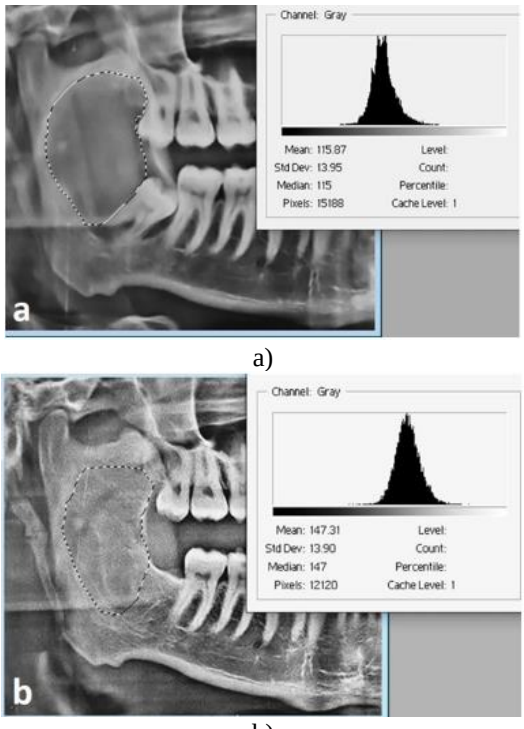


Figure 2. Evaluation of bone healing using gray-level histogram score at baseline (a) and at 2 years follow-up (b).

Statistical analysis

All the data was entered into Microsoft Excel 2010. Descriptive statistics for age, gender, location, and size were expressed as mean ± standard deviation (SD) for each group. Frequency distribution and percentage were used to elaborate on the result of the demographic details. The intergroup comparison of age and size of the lesion was done by the analysis of variance (ANOVA), while gender and location association were checked with the Chi-square test. The recurrence of the disease in both groups was assessed and disease-free survival analysis was done using Kaplan-Meier analysis. The bone healing between the group was compared using an unpaired t-test. For all the above tests 'the p' value < 0.05 was considered statistically significant. The software used for statistical analysis was Statistical Package for Social Sciences (SPSS version 19).

Results and Discussion

A total of 17 patients fulfilling the inclusion criteria were randomly divided into group A (receiving 5-FU; n=9) and group B (receiving MC; n=8). The age of patients ranged from 28-56 years (mean 36.88, SD 8.66) in group A and 26-61 years (mean 37.75, SD 11.58) in group B. The age difference between the two groups was statistically insignificant (p-value 0.863515). Group A comprised 6 males and 3 females, while group B included 5 males and 3 female patients, with an overall male-to-female ratio of 1.8: 1. There was no statistically significant difference in gender distribution between the two groups (p-value 0. 857596). While 2 lesions were localized over the anterior mandible (group A n=1, group B n=1), 7 OKCs were present in the body-ramus region (group A n=4, group B n=3) and 8 in the ramus of the mandible (group A n=4, group B n=4). The size of the pathology ranged from 32-56 mm in group A (mean 43.66, SD 7.71) and 35-61 mm in group B (mean 47.27, SD 10.43). There was no statistically significant difference in the location and size of OKC between the two groups (p values of 0.429546 and 0.958714 respectively).

Patients in group A were followed-up for 24-64 months (mean 43.7 months) and none of the patients showed evidence of recurrence on clinical and radiological examination. Whereas, two patients (25%) in group B showed recurrence when followed up for a period of 26-68 months (mean 47.5 months). The mean time of recurrence in group B was 31 months. Kaplan-Meier analysis showed a disease-free survival probability of 0.9 at 30 months, which reduced to 0.7 at 60 months of follow-up in patients treated with MC (group B) **(Figure 3)**.

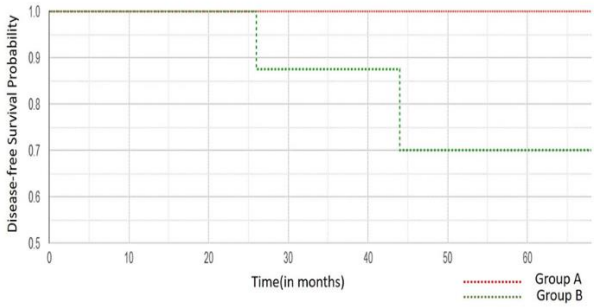


Figure 3. Disease-free survival probability assessment by Kaplan-Meier analysis.

Inferior alveolar nerve paresthesia was assessed in all the patients with OKC located in the body and ramus region. One patient in group A, having direct exposure of the nerve within the surgical cavity was excluded from the assessment (group A & B n= 7 each). Two patients in group A (28.5%) and 3 patients in group B (42.8%) showed temporary paresthesia, which completely recovered at a mean follow-up period of 6.5 and 11 months respectively. None of the patients in either group showed permanent Inferior alveolar nerve paresthesia. Bone healing assessment at two years follow-ups showed a mean increase in grey-level score by 36.52 ± 2.96 (range of 32.37- 39.72) in group A and 34.064 ± 6.52 (range of 23.63 - 41.22) in group B. The statistical difference in bone healing between the two groups was insignificant (p-value 0.225184).

OKC is an odontogenic cyst arising from the dental lamina of a tooth bud. The local aggressive nature of the cyst and its high rate of recurrence after enucleation led to its classification as an odontogenic keratocystic tumor by WHO in 2005. However, in 2007 nomenclature was reverted to OKC [16]. Traditionally the treatment of OKC has varied from aggressive resection to conservative enucleation. Although resection minimizes the chance of recurrence to almost zero, it results in high morbidity, damage to vital structures like the inferior alveolar nerve, the need for complex jaw reconstruction surgery, and increased hospitalization time and cost of treatment. Although enucleation has low morbidity, when performed alone, it results in a high risk of recurrence, ranging from 23-56% [1, 17]. Many adjunctive procedures like peripheral ostectomy, chemical cauterization, and cryotherapy have been conventionally combined with enucleation to reduce the risk of recurrence [17].

CS is a tissue fixative, containing chloroform, glacial acetic acid, absolute alcohol, and ferric acid. When used with enucleation, it lowers the reported recurrence rate <10% [7]. CS is applied over the bone lining the cyst cavity, as per the protocol described by Frerich *et al*. [15]. This causes chemical necrosis up to 1.5 mm of cancellous bone, thus eliminating the remnants of cyst lining and microcysts, which when left untreated would result in recurrence [18]. Conventionally enucleation has also been combined with peripheral ostectomy along with the application of CS to achieve good clearance and minimize the recurrence rate. Removal of chloroform from CS was necessitated due to its carcinogenic potential. The modified Carnoy's solution without chloroform has relatively low potency and a higher reported recurrence rate of 35% [7]. Use of CS and MC also results in a greater risk of nerve damage, resulting in temporary to permanent paresthesia of inferior alveolar nerve, when treating mandible lesions. With these limitations of CS and MC, the present study was designed to evaluate the application of alternative chemical agent 5-FU, as an adjunct to enucleation and peripheral ostectomy in the management of OKC.

The use of 5-FU for the treatment of OKC is novel and has not been widely studied. The first reported application by Ledderhof *et al*. in the year 2017 showed promising results with no recurrence and lower incidence of nerve damage in the management of OKC involving both jaws [13]. To standardize the selected sample and eliminate treatment bias, the present study included only mandible OK, which were well-localized without significant cortical perforation or involvement of overlying oral mucosa. Cases, where IAN was exposed within the surgical defect, were eliminated from statistical assessment for assessment of nerve paresthesia.

The present study included 17 mandibles OKC with male to female ratio of 1.8:1 and an age range of 26-61 years. The 5-FU group showed no recurrence (with a mean follow-up period of 43.7 months), as compared to the 25% recurrence seen in the MC group (with a mean follow-up period of 47.5 months). A similar risk of recurrence of 19% was reported by Ledderhof *et al*. after the use of MC [13]. Lone *et al*. reported a higher recurrence rate of 66.6% in patients treated with MC while those managed by 5-FU and resection showed no recurrence [14]. The possible reason for the lower percentage of recurrence in the MC arm of the present study can be due to the inclusion criteria of the localized corticated lesion and the addition of peripheral ostectomy with enucleation.

The procedure of application of 5-FU was relatively simple and easy to execute. A ribbon gauze coated with 5-FU was applied to the bone lining the surgical site after the enucleation of the cyst and was maintained for 24 hours. On the contrary, the application of CS/MC required utmost care to avoid any spill of the solution on the oral tissue, which can result in a chemical burn and tissue damage. Exposure of CS/MC to nerve results in nerve injury. Frerich B *et al*. reported axonal degeneration when Carnoy's solution was applied over the nerve for more than 3 minutes [15]. In the present study, temporary IAN paresthesia was higher in the MC group as compared to the 5-FU group (MC-42.8%, 5-FU-28.5%). However, complete recovery of nerve sensation was observed in both groups with a mean recovery period of 11 and 6.5 months in the MC and 5-FU group respectively. The reason for the lower risk of nerve damage observed in the study can be due to the inclusion of only well-localized lesions and the use of protective paraffin gauze dressing over the region of the inferior alveolar canal before the application of the topical agent. Also, the elimination of lesions where the IAN was exposed in the surgical defect (to eliminate bias of injury due to direct nerve handling), could have contributed to a lower incidence of IAN paresthesia in the studied patients.

Topical application of 5-FU in periorbital areas for the treatment of ocular surface squamous neoplasms, and sinus

region after maxillectomy and sphenoid-ethmoidectomy has shown no adverse effect on nerve or surrounding soft tissues [19, 20]. This makes the application of 5-FU safe, not only for localized mandibular lesions but also for lesions with cortical perforation(s), maxillary lesions with extension into the maxillary sinus, and OKC close to orbit, where CS/MC is contraindicated [14]. Bone healing evaluation after the application of 5-FU has not been studied previously [13, 14]. Grey level histogram scores evaluating bone fill at postoperative 2 years follow-up showed good bone fill after treatment with 5-FU. The result was comparable with healing in patients receiving MC.

Conclusion

5-FU is a novel alternative to MC in the treatment of OKC by enucleation and peripheral ostectomy. 5-FU resulted in no recurrence and a lower risk of postoperative IAN paresthesia. Ease of application, affordability, and easy availability make it a viable option. The limited sample size is a drawback of the present study, warranting future studies (possibly multicentric), and recruiting a greater number of patients to further evaluate the utility of 5-FU in the management of OKC.

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Conflict of interest: None

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Ethics statement: None

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