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Chemical adjuncts and cryotherapy in the management Of odontogenic keratocysts: A systematic review

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ABSTRACT

Introduction: Odontogenic keratocysts are benign lesions but are locally aggressive, forming multiple daughter cysts which have a tendency to recur. Practitioners who advocate a conservative approach suggest enucleating the lesion and then treating the residual bony cavity with an adjunctive therapy in order to minimise the possibility of recurrence. Many forms of adjunctive therapy have been described such as cryotherapy, chemical cauterisation with Carnoy's or Modified Carnoy's solution and the use of topical 5-fluorouracil (5-FU) but there has not been a systematic review to compare these various adjunctive treatments in the management of odontogenic keratocysts.

Method: PRISMA guidelines for systematic reviews were followed. An electronic literature search from the databases Medline, PubMed, the Cochrane database and the Web of Sciences were performed, and relevant articles were selected based on the inclusion criteria. Disputes were resolved by an independent third party. Statistical analysis was performed to identify trends of recurrence for each treatment arm.

Results: A total of 28 studies managing a total of 1430 odontogenic keratocysts were included in the review. It was observed that only Carnoy's solution resulted in statistically different outcomes compared to no adjunct therapy. There was weak evidence on the efficacy of modified Carnoy's solution but no evidence that cryotherapy or 5-FU resulted in statistically different outcome compared to no adjunct therapy.

Conclusion: Carnoy's solution appears to be the best adjunct therapy for now but further studies are needed to establish the roles of modified Carnoy's solution, cryotherapy and 5-FU in the management of odontogenic keratocyst.

1. Introduction

Odontogenic keratocyst (OKC), formerly known as keratocystic odontogenic tumour (KCOT) is a benign intraosseous cyst of the jaws which arises from the dental lamina. It was first described by Philipsen in 1956 [1]. It is the third most commonly occurring odontogenic cyst comprising of approximately 20% of all odontogenic cysts [2,3]. In 2005 it was classified by the World Health Organisation (WHO) as a keratocystic odontogenic tumour, a cystic neoplasm, because of its aggressive clinical traits, high recurrence rates as well as emerging evidence, at that time, of genetic mutations linked to its pathogenesis [4]. However, in 2017 the WHO reclassified the KCOT back to the cyst category as they found that there was insufficient evidence to demonstrate the genetic aetiology as well as more recent reports of KCOT responding positively to conservative treatments such as marsupialisation and decompression which is not associated with neoplasia [5].

Clinically, OKC may occur in any jaw but it has a marked tendency to involve the posterior body and ascending ramus of the mandible. It has a slight gender predilection of males to females at a ratio of 1.1–2:1 [2,3,6]. OKC tends to grow in an antero-posterior direction within the medullary cavity of the bone without causing significant expansion therefore developing to a significant size before causing any symptoms. A reported 50% of OKCs are asymptomatic and diagnosed incidentally during routine dental examinations [7,8]. Aspiration results in an aspirate which is a dirty white colour with viscoid keratin suspensions and a protein content of less than 4.8 g/dl [9]. Multiple OKC in a single patient are usually associated with Nevoid Basal Cell Carcinoma Syndrome (NBCCS) which is an autosomal dominant disorder characterised by multiple basal cell carcinomas and skeletal anomalies in addition to multiple OKC of the jaws [10].

Radiographically, the majority (approximately 70–80%) of OKC present as a unilocular radiolucency with well corticated border [7,8]

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but may also present as a multilocular radiolucency as well. They may be associated with an impacted or unerupted tooth which may mimic the radiographic appearance of a dentigerous cyst. Due to the large variability of clinical and radiographic appearance, diagnosis through purely clinical radiographic means is difficult and a biopsy is required to confirm the diagnosis.

The microscopic features of OKC are distinctive which enables accurate histopathological diagnosis. The epithelial lining of OKC is parakeratinised, uniform without rete ridges and about 6–8 cells thick. The basal cell layer is well defined, consists of columnar or cuboidal cells and often palisaded. The nuclei of the basal cells are oriented away from the basal membrane and are often intensely basophilic. The parakeratotic layers often have a corrugated surface and there is a frequent observation of satellite cysts or islands of odontogenic epithelium in the fibrous capsule [4]. These features however may be lost or complicated in the presence of infection or inflammation which may complicate the diagnosis of OKC.

The treatment goals for the management of OKC is the complete removal of the pathology as well as minimising the possibility of recurrence. However, the high reported recurrence rate of OKC has led to a wide range of philosophies in its management. Broadly speaking, surgical management can be classified into radical and conservative approaches. The radical approach utilises resection to manage OKC whereby the pathology is removed intact with a margin of uninvolved bone. Depending on the size of the pathology, the continuity of the mandible may be preserved by performing only a marginal resection or lost if a segmental resection is required. The en bloc removal of the pathology with a margin has resulted in extremely low recurrence rates (0% - 8.4%) but also greater patient morbidity [11–15]. The mutilating effects of radical treatment has resulted in many surgeons questioning the pragmatism of radical surgery as opposed to more conservative approaches [16,17].

Conservative surgical management utilises decompression, marsupialisation, and enucleation either exclusively or in conjunction with one another to treat OKC. However, this approach has reported higher recurrence rates compared to radical treatment. In order to reduce the recurrence rates, adjunct therapies such as chemical and thermal cauterisation with various compounds have been used in conjunction with conservative surgical treatment with varying success [13,14].

While disagreements still exist concerning the best management of OKC, the various surgical approaches have been discussed and reported on extensively. However, little data exists on the various adjunct therapies utilised in the management of OKC. This manuscript aims to present a systematic review of the treatment outcomes by various adjunct therapies when used in conjunction with conservative surgical management of OKC.

2. Materials and methods

2.1. Search strategy

This review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [18] to ensure a systematic approach and reliable result. An electronic search was carried out in the following databases: Embase, Medline, Cochrane and Web of Science. The keywords “odontogenic keratocyst”, “keratocystic odontogenic tumour”, “Carnoy’s solution”, “cryotherapy” and “5-fluorouracil” were used. The full search strategy is shown in Appendix 1. The reference lists for identified articles and relevant reviews performed previously were also hand searched to identify any additional studies.

2.2. Inclusion criteria

The inclusion criteria for this review was set according to the PICOS components. Population (P) – Patients with biopsy proven OKC. Intervention (I) – Studies that reported on the use of one or more adjunctive therapies in the management of OKC. Comparison (C) – Patients that were managed without the use of adjunctive therapies. Outcome (O) – Recurrence rates for the different adjunctive therapies and without adjunct therapies with adequate follow up time for at least 12 months. Study design (S) – Prospective randomised controlled trials, cohort studies, cross-sectional studies and case series. In addition, studies should be published in English.

2.3. Exclusion criteria

The exclusion criteria for studies were: Non – human studies, case reports, review papers, letters to the editor, studies without adequate description of adjunct therapies used, adequate details of follow – up periods or recurrence, and studies not published in English.

2.4. Study selection

The titles and abstracts for all reports identified by the electronic and hand search were reviewed independently by the reviewers (ZWT and WLS). For studies that did not have adequate information contained in the abstract or where the abstract was not available, the full report was obtained to assess eligibility. Eligible studies that were agreed on unanimously were included. Disagreements were resolved by discussion and if both reviewers were unable to come to an agreement, an independent third party (RML) was invited to mediate.

2.5. Data extraction

The data extracted from eligible articles were: authors, date of publication, study design, number of patients, number of OKCs in total, number of recurrences, range of follow up or mean follow up period, post-operative complications and number of patients with NBCCS.

2.6. Critical appraisal of studies

Two reviewers (ZWT and WLS) independently assessed the risk of bias of each of the selected studies using the Newcastle-Ottawa Scale [19] which is shown in Appendix 2. Disagreements were resolved by discussion or by an independent third party (RML). According to the guidelines, a study can be awarded one point for each of the categories and a maximum of two points in the comparability category. The greatest score that can be given is a total of 9 points which represents a low risk of bias. In this review, any study with a score of 7 or more points are classified as being high quality.

2.7. Summary measures and synthesis of results

Descriptive statistics were used to report the data. A funnel plot was used to identify publication bias within the selected studies. Recurrence rates for each included study were reported noting the adjunct treatment used as well as the follow up period. Regression analysis was then performed to identify any observable trends between the use of each type of adjunct therapy and recurrence rates over time.

In addition, a cohort of 159 patients obtained from the data published in 8 articles was assembled and Kaplan – Meier survival analysis as well as logistic regression analysis with generalised linear modelling was performed in this cohort.

Meta-analysis was not performed as no study had included hazard ratio analysis in their article. Furthermore, variations in data and follow up periods within each study was too great resulting in a high degree of heterogeneity.

3. Results

3.1. Results of literature search

The study selection process is shown in Fig. 1. The search strategy resulted in 528 articles from all databases. And additional 6 articles were identified by hand searches. After screening for duplication, 181 articles were eliminated resulting in 347 articles that were screened for eligibility.

After eligibility screening from reading the titles and abstracts, 54 articles were selected for full text reviews. 26 studies were excluded as they did not meet the inclusion criteria. The remaining 28 studies were included in the review [20–47].

3.2. Description of included studies

The full description of the selected articles is detailed in Table 1. Of the 28 selected studies, 4 were prospective studies [24,26,36,42] and 1 was ambispective [22]. The rest were retrospective studies [20,21,23,25,27–35,37–41,43–47]. 28 articles managed a total of 1494 patients with a total of 1524 OKCs. Of these, 1430 OKCs were managed conservatively with or without chemical adjunct therapies or cryotherapy with 212 reported recurrences.

Of 28 articles, 18 articles managed a total of 568 OKCs with Carnoy's solution. Follow up periods ranged from 12 to 313 months [21,25–29,31–35,37–39,41,42,44,47].

Four articles managed a total of 101 OKCs with Modified Carnoy's solution. Follow up periods ranged from 12 to 288 months [22,24,28,40].

Nine articles managed a total of 119 OKCs with cryotherapy. Follow up periods ranged from 6 to 120 months [20,23,30,36,37,43–46].

One article managed 11 patients with 5-FU with follow up period of 8–63 months, mean 35 months [22].

Sixteen articles managed a total of 631 OKCs without any adjuvant therapy with follow up periods ranging from 12 to 288 months [20,21,26,27,32,33,35,37–42,44,46,47].

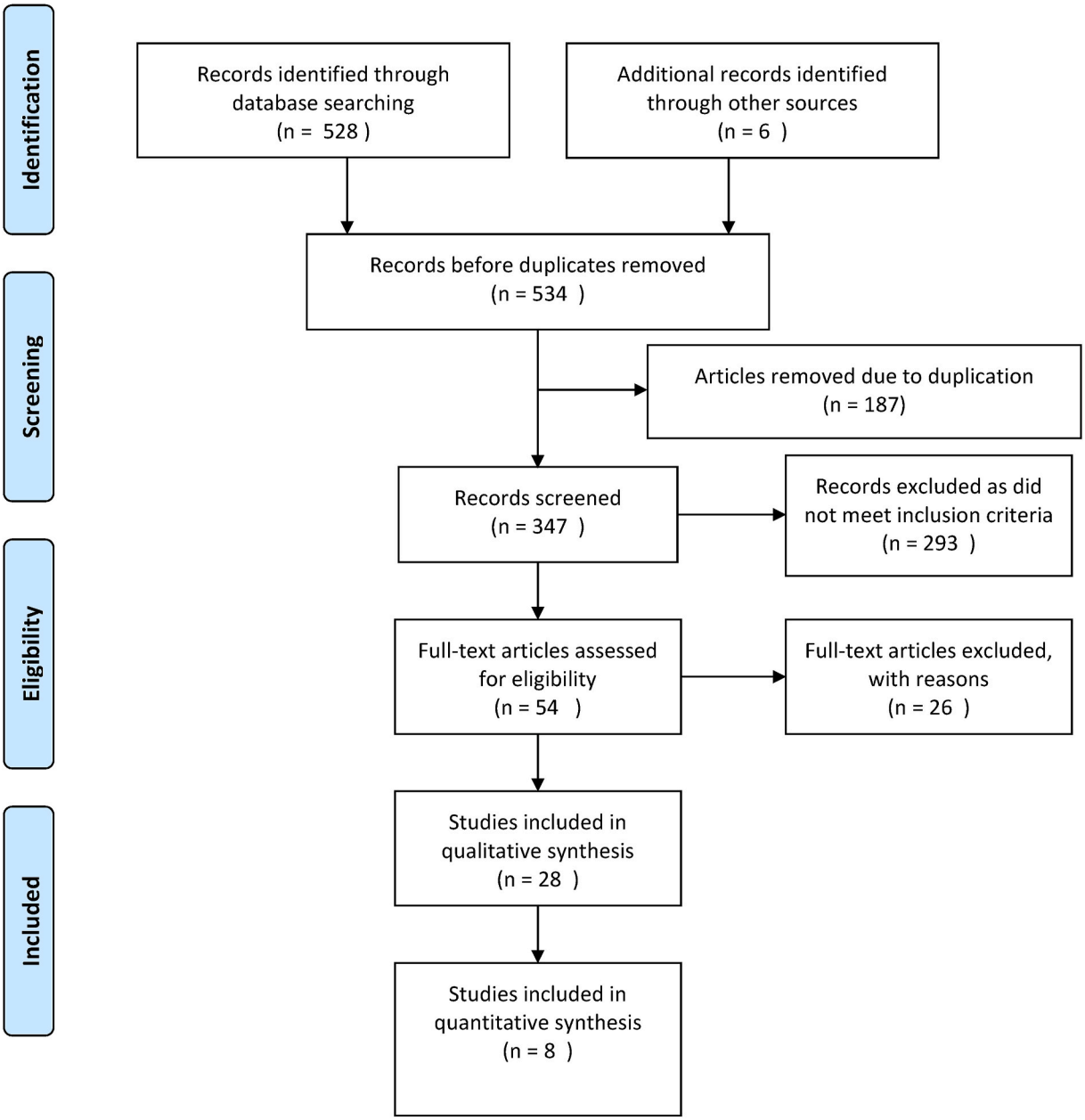

Fig. 1. Study selection process.

Table 1
Description of included studies.

Author	Year	Study design	No. of patients	No. of OKC Total	adjunct therapy	n Treated	Recurrence	Recurrence percentage	Mean Length of follow up	NBCCS
Daroit et al.	2018	retrospective	32	32	None	10	6	60.00%	21.6 months	11
					Cryotherapy	22	3	13.64%		
Alchalabi et al.	2017	prospective	29	29	Modified Carnoy's	29	0	0.00%	36–72 months	0
de Souza Cruz	2017	retrospective	10	10	cryotherapy	10	2	20.00%	64.3 months	nm
Ledderhof et al.	2017	ambispective	32	32	5-FU	11	0	0.00%	35 months	0
					Modified Carnoy's	21	4	19.05%	41.3 months	0
Ribeiro et al.	2017	retrospective	31	38	Carnoy's	28	4	14.29%	43.5 months	13
					None	10	1	10.00%		
Gupta et al.	2016	prospective	12	12	Carnoy's	3	1	33.33%	12–18 months	0
					None	9	5	55.56%		
Leung et al.	2016	retrospective	105	105	Carnoy's	105	12	11.43%	86.6 months	0
Dashow et al.	2015	retrospective	80	80	Carnoy's	44	4	10.00%	44.75 months	0
					Modified Carnoy's	36	13	35.00%	27.23 months	0
Levorova et al.	2015	retrospective	22	22	Carnoy's	11	5	45.45%	36 months	0
					None	11	5	45.45%		
Carneiro et al.	2014	retrospective	9	9	Cryotherapy	9	2	22.22%	68.78 months	nm
Rao et al.	2014	retrospective	32	34	Carnoy's	34	2	5.88%	33.6 months	0
Rajeshkumar et al.	2013	retrospective	7	7	Carnoy's	7	0	0.00%	6–18 months	
Guler et al.	2012	retrospective	39	43	Carnoy's	25	0	0.00%	40.54 months	0
					None	18	0	0.00%		
Ribeiro et al.	2012	retrospective	14	22	Carnoy's	22	1	4.55%	42.9 months	3
Titinchi et al.	2012	retrospective	65	65	Carnoy's	9	1	11.11%	12.6 months	15
					None	50	15	30.00%	23.5 months	
Zhao et al.	2012	retrospective	257	257	Carnoy's	124	7	5.65%	12–180 months	0
					None	133	12	9.02%		
Schussel et al.	2011	retrospective	24	24	Carnoy's	2	0	0.00%	46.09 months	2
					Cryotherapy	4	2	50.00%		
					None	18	11	61.11%		
Tonietto et al.	2011	prospective	8	9	Cryotherapy	9	0	0.00%	58.5 months	nm
Gosau et al.	2010	retrospective	34	36	Carnoy's	14	2	14.29%	45.6 months	0
					None	22	11	50.00%	67.40 months	nm
Chirapathomsakul et al.	2006	retrospective	31	63	Carnoy's	5	1	20.00%	41.9 months	nm
					None	17	4	23.53%		
Morgan et al.	2005	retrospective	40	40	Modified Carnoy's	15	1	6.67%	63.7 months	0
					None	22	8	36.36%		
Zhao et al.	2002	retrospective	255	255	Carnoy's	29	2	6.90%	93.6 months	0
					None	163	29	17.79%		
Schmidt et al.	2001	retrospective	26	26	Cryotherapy	26	3	11.54%	42 months	3
Stoelinga et al.	2001	prospective	80	82	Carnoy's	43	3	6.98%	141.6 months	0
					None	39	6	15.38%		
Chow	1998	retrospective	70	76	None	45	6	13.33%		1
					Cryotherapy	1	0	0.00%		
					Carnoy's	23	1	4.35%		
Pogrel	1993	retrospective	25	25	Cryotherapy	25	0	0.00%	75 months	1
Jensen et al.	1988	retrospective	22	25	Cryotherapy	13	5	38.46%	28.5 months	0
					None	12	4	33.33%	41.5 months	
Voorsmit et al.	1981	retrospective	103	92	Carnoy's	40	1	2.50%	12–120 months	0
					None	52	7	13.46%		

nm = not mentioned.

3.3. Risk of bias within studies included

The risk of bias within each study was assessed using the Newcastle – Ottawa Scale which is shown in Appendix 2. Each article was evaluated on a few key aspects in their selection of cases, comparability of the subjects as well as outcome reporting with a maximum score of 9 for each study. For the purpose of this review, a score of 7 or higher is identified as a high quality study. Of 28 articles, 6 received a score of less than 7 [23,31,36,39,43,45]. 4 articles scored 7 points [20,29,30,34], 13 articles scored 8 points [21,24,26,27,32,33,35,37,38,40–42,44], and 5 articles scored 9 points [22,25,28,46,47]. The full details of the critical appraisal according to the Newcastle – Ottawa scale are presented in Table 2.

3.4. Results of variable outcome

A regression analysis for recurrence percentage on mean follow up time for each chemical adjunct therapy, cryotherapy and no adjunct therapy was carried out in an attempt to identify any potential trends following the management of OKC with each treatment arm. The recurrence rate was measured against mean follow up time for each individual treatment arm and the sample size of each article is represented by the size of each data point. The regression graph is shown in Fig. 2.

From the regression analysis, it is observed that all treatment arms show a declining trend of recurrence as time passed with the majority of recurrences occurring before 60 months which is in accordance with previous observations that most recurrences occur within the first 5 years post-operatively.

It can also be observed from the regression plot, Carnoy's solution has the lowest recurrence rate at about 10% followed by a relative flat gradient with time. Cryotherapy has a slightly higher recurrence rate at about 30% but that is followed by a steep negative gradient over time. The same trend is observed with Modified Carnoy's solution with a recurrence rate of about 45% followed by a steep negative gradient. Regression analysis for the use of 5-FU was not possible as there was only 1 article who reported its use in 11 patients and reported no recurrences.

Care should be taken when interpreting trends from the far right side

Table 2
Critical appraisal of included studies.

Author	Year	Representation	selection for non exposed cohort	Ascertainment of exposure	demonstration that outcome of interest not present at start	control for NBCCS	Histological confirmation (parakeratinised)	assessment of outcome	follow up (1 year)	adequacy of follow up	score
Daroit et al.	2018	b	a	a	a	no	b	b	a	a	7/9
Alchalabi et al.	2017	b	no	a	a	a	b	b	a	a	8/9
de Souza Cruz	2017	b	no	a	a	no	b	b	a	a	6/9
Ledderhof et al.	2017	b	a	a	a	a	b	b	a	a	9/9
Ribeiro et al.	2017	b	a	a	a	a	b	b	a	a	8/9
Gupta et al.	2016	b	a	a	a	a	no	b	a	b	8/9
Leung et al.	2016	b	a	a	a	a	b	b	a	a	9/9
Dashow et al.	2015	b	a	a	a	a	b	b	a	a	9/9
Levorova et al.	2015	b	a	a	a	a	no	b	a	a	8/9
Carneiro et al.	2014	b	no	a	a	no	b	b	a	a	7/9
Rao et al.	2014	b	no	a	a	a	no	b	a	a	7/9
Rajeshkumar et al.	2013	b	no	a	a	no	no	b	a	b	6/9
Guler et al.	2012	b	a	a	a	a	no	b	a	a	8/9
Ribeiro et al.	2012	b	no	a	a	no	b	b	a	a	7/9
Titinchi et al.	2012	b	a	a	a	no	b	b	a	a	8/9
Zhao et al.	2012	b	a	a	a	a	no	b	a	a	8/9
Schussel et al.	2011	b	a	a	a	no	b	b	a	b	8/9
Tonietto et al.	2011	b	no	a	a	no	no	b	a	a	6/9
Gosau et al.	2010	b	a	a	a	a	no	b	a	a	8/9
Chirapathomsakul et al.	2006	b	a	a	a	no	no	b	a	c (no)	6/9
Morgan et al.	2005	b	a	a	a	a	b	b	a	c (no)	8/9
Zhao et al.	2002	b	a	a	a	a	no	b	a	a	8/9
Schmidt et al.	2001	b	no	a	a	no	no	b	a	a	6/9
Stoelinga et al.	2001	b	a	a	a	a	no	b	a	a	8/9
Chow	1998	b	a	a	a	no	b	b	a	a	8/9
Pogrel	1993	b	no	a	a	no	no	b	a	a	6/9
Jensen et al.	1988	b	a	a	a	a	b	b	a	a	9/9
Voorsmit et al.	1981	b	a	a	a	a	b	b	a	a	9/9

Please refer to Appendix 2 for full legend.

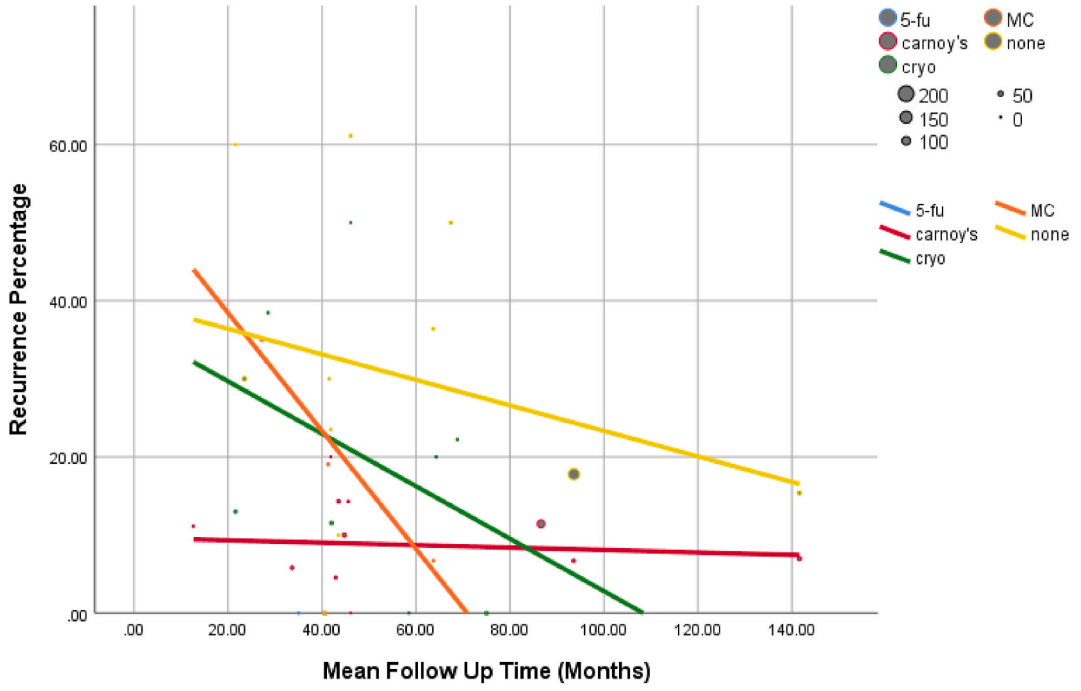

Fig. 2. Regression analysis of recurrence on mean follow up time for each adjunctive therapy.

of the graph due to the scarcity of data available resulting in a possible skewed observation. Furthermore, weighted regression analysis for each treatment arm as shown in appendix 3 all have the value of "0" included in the 95% confidence interval for B indicating that it is not statistically possible to distinguish the negative gradient of the fit lines from a "0" degree gradient line.

Of 28 articles, 8 articles contained detailed information on the management of a total of 159 OKCs and additional statistical analysis has been performed on these studies [21,22,26,31,34,36,37,46].

Of these 159 OKCs, 11 were managed with 5-FU with no recurrences with follow up period ranging from 8 to 63 months, 54 were managed with Carnoy's solution with 5 recurrences reported (9.26%) with follow

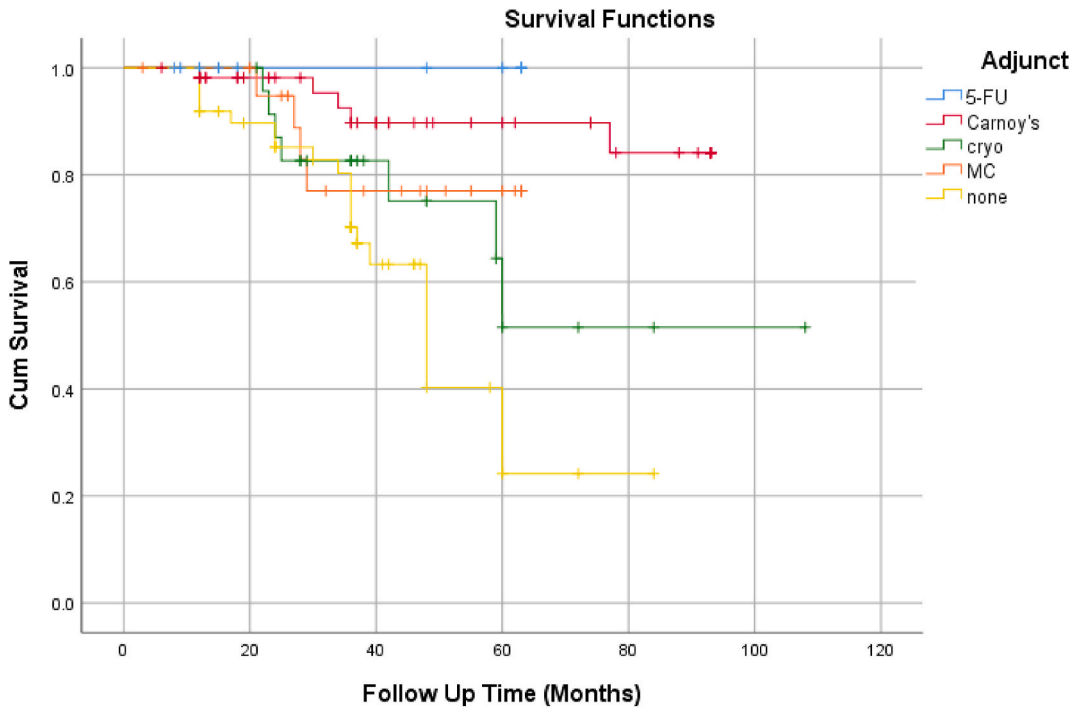

Fig. 3. Kaplan – Meier survival analysis.

up periods ranging from 12 to 93 months. 21 OKC were managed with Modified Carnoy's solution with a 4 reported recurrences (19.05%) over a follow up period ranging from 12 to 63 months. 24 OKC were managed with cryotherapy with 7 recurrences (29.17%) over follow up of 21–108 months. 49 OKCs were managed without any chemical adjunct or cryotherapy with 21 recurrences reported (42.86%) over a follow up period of 12–84 months.

Based on the data available from the cohort of 159 patients identified from these 8 studies, a Kaplan – Meier survival analysis was performed for each treatment arm and is shown in Fig. 3.

The Kaplan – Meier survival analysis shows the cumulative survival versus time for each treatment arm. Survival in this case is interpreted as no recurrence. Each "censured" point shows the point in time where the patient has been lost or excluded from follow-up. From the graph, we can observe that the omission of any chemical adjunct or cryotherapy is associated with lower survival rate. Although 5-FU is depicted as having a 100% cumulative survival rate it should be remembered that only 11 patients were managed with this treatment.

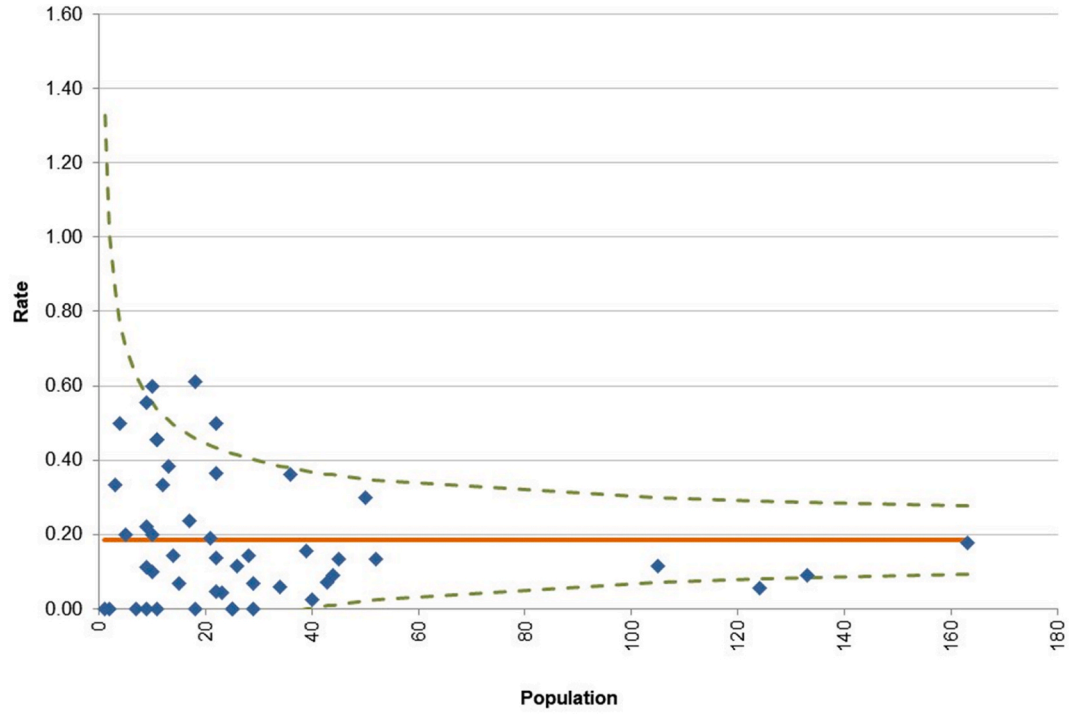

Fig. 4. Funnel plot, publication bias within included studies.

In addition to the Kaplan – Meier survival analysis, logistic regression analysis with generalised linear models of these 159 cases were performed and shown in appendix 4. The outcome shows that only Carnoy's solution produces a statistically significant result compared to no adjunct therapy. There is some evidence of the efficacy of Modified Carnoy's solution but no evidence to the efficacy of cryotherapy or 5-FU compared to no adjunct therapy. This result should be interpreted with caution however due to the small sample size for the treatment arms of 5-FU, Modified Carnoy's solution and cryotherapy.

Furthermore, it can also be observed the small p value of 0.034 for the follow up period indicates that the follow up period is an important covariate in regression analysis and variations in follow up periods will produce a statistically significant difference in regression analysis for recurrence.

3.5. Publication bias

A funnel plot was calculated in order to determine the presence for publication bias. The Cochrane handbook recommended that the standard error for the intervention effect should be plotted on the vertical axis instead of the sample size however this is not possible in this review based on the data available, hence the study population for each article was used. The funnel plot (Fig. 4) was markedly skewed. The lack of uniformity in the data could be due to the large variation in follow-up period or even skewed data sets as most articles publish relatively low recurrence rates resulting in a longer right arm and the skewed appearance. As this skewed population is difficult to distinguish between true publication bias, the presence of publication bias cannot be excluded.

4. Discussion

The treatment for OKC has been a subject of debate for many years with no clear resolution even today. The high recurrence rate reported after conservative surgery results in some surgeons advocating for radical treatment with resection, either marginal or segmental depending on the size of the pathology, as it demonstrates the lowest recurrence rates [11–15,48], while other surgeons argue that resection is too radical and an overtreatment especially since OKC is a benign pathology and should not be treated with oncology principles [16,17].

Management of OKC by enucleation although reported to have recurrences as high as 65% [49], is in the authors opinion, an outdated concept. The reports which describe these high recurrence rates are dated reports from the 1960s. As the field of surgery has advanced since then, the recurrence rates have also reduced significantly. Recent studies and systematic reviews have shown that the recurrence rates for OKC managed by enucleation alone is only about 25% and is reduced even further when adjunctive therapies are used [11,12,14,50,51].

4.1. Carnoy's solution

The main fixative agent in Carnoy's solution is absolute alcohol which dehydrates exposed cells by drawing out water. Chloroform acts as a lipid solvent allowing for enhanced and more rapid fixation action of alcohol. Glacial acetic acid, in addition to its fixative and haemostatic actions dissolve H-ions in tissues containing water allowing easier and more rapid penetration of alcohol creating a synergistic effect with chloroform to allow for the rapid fixative action of Carnoy's solution. Ferric chloride serves to enhance the haemostatic properties of the solution as well as to tan the tissues which serves as an optical aid to differentiate between treated and untreated tissues [52].

The use of Carnoy's solution in the management of odontogenic cysts was popularised by Voorsmit in 1984. He reported a recurrence rate of only 2.5% when Carnoy's solution was used to treat the bony cavity after enucleation [47]. Furthermore, he conducted in vitro studies that showed that application of Carnoy's solution for 5 min resulted in fixation of the entire keratocyst wall, penetration of 1.5 mm into cancellous bone but without penetration into the neurovascular bundle of the inferior alveolar nerve [52]. He theorised therefore that application of Carnoy's solution for 5 min to the bony cavity after enucleation of OKC is sufficient to fix any remnant epithelial lining or satellite cysts without nerve damage.

Despite his findings, sensory disturbance to the inferior alveolar nerve after exposure to Carnoy's solution was reported in the literature. In 1994, Frerich et al. reported a study using rabbits as a human analogue that showed significant sensory impairment at 3–5 min of exposure to Carnoy's solution and advocated for application of Carnoy's solution of no longer than 3 min [53]. Clinical studies appear to support this observation [34].

Management of OKC by enucleation and Carnoy's solution appears to result in the lowest recurrence rate when compared to other treatment modalities apart from resection [11–14,34,54,55]. This finding is also reflected in this study where regression analysis and Kaplan – Meier survival analysis for Carnoy's solution as an adjunct treatment shows the lowest recurrence and is the only treatment modality to show a statistically significant difference compared to no adjunct therapy.

The shortcoming for Carnoy's solution is the presence of chloroform which has been found by the Food and Drug Administration, USA to be a carcinogen in 1992 leading to a ban in certain countries of pharmaceuticals containing this compound.

4.2. Modified Carnoy's solution

Following the ban of Carnoy's solution, some surgeons have used a modified formulation of Carnoy's solution that does not contain chloroform. However, there is no standard formulation for Modified Carnoy's solution. Some practitioners purely subtract the chloroform compound from the solution (1g ferric chloride in 6 ml absolute alcohol and 1 ml glacial acetic acid) [24] while others use a formulation of 1g ferric chloride in 9 ml 95% alcohol and 3 ml glacial acetic acid [40,56]. Only the latter formulation has undergone any kind of laboratory testing on its potential clinical effect [56].

In his study, Hellstein demonstrated that modified Carnoy's solution demonstrated similar depth of penetration when compared with the original composition and concluded that the use of chloroform was not necessary. He also reported on the use of the non-chloroform containing compound in the management of 15 patients with similar recurrence rates to Carnoy's solution [56]. His findings have been called into question however with several laboratory studies showing superior penetration of Carnoy's solution containing chloroform versus without into both soft and hard tissue [57,58] as well as clinical studies reporting much higher recurrence rates in some cases up to 50% when using modified Carnoy's solution [22,28,40].

This finding is mirrored in this review where regression and Kaplan – Meier survival analysis showed markedly inferior performance when compared to conventional Carnoy's solution. Logistic regression analysis with generalised linear modelling showed that there was only weak evidence showing a statistical difference when comparing the outcome of modified Carnoy's solution to no adjunct therapy.

This outcome is somewhat to be expected as the protocol most practitioners adopt for the use of modified Carnoy's solution is the same as the application for conventional Carnoy's solution which is a 3-min application to the bony cavity after enucleation of the OKC. This approach is flawed in its logic as we cannot expect to remove a catalyst component of a formulation and expect it to work with the same efficacy as its conventional counterpart. Although Hellstein et al. showed a similar depth of penetration, it should be remembered that penetration of a compound does not correlate with degree of action of said compound. In addition, the safety profile for modified Carnoy's solution on other structures like vasculature and nerves has also not been tested in the same way Carnoy's solution has.

The authors therefore suggest developing in vitro and in vivo studies

to determine new protocols for the application of modified Carnoy's solution to allow for adequate exposure time for fixation of remnant epithelial lining or satellite cysts without compromising surrounding neurovascular structures.

4.3. Cryotherapy

Intense cold kills living tissue as is evident in frostbite. The principle of cryotherapy follows similar reasoning which is the induction of cell death by direct and indirect damage from ice crystal formation and, osmotic and electrolyte imbalance [59]. Temperatures below 20 °C have been observed to consistently cause cell death in mammalian cells [60]. Liquid nitrogen boils at a temperature of −196 °C which is more than sufficient to induce cell death.

Cryotherapy protocol calls for the rapid freezing of tissues by spraying liquid nitrogen for 1 min to cause maximum ice crystal formation followed by a slow thaw of 5 min resulting in maximum electrolyte imbalance which is then repeated 2 to 3 times to cause maximal cell lethal effects. The defect can then be grafted immediately and the overlying mucosa closed with watertight sutures [45,61,62]. The rationale for the use of cryotherapy to manage OKC is that liquid nitrogen is able to kill any epithelial remnants or satellite cysts while leaving the inorganic bone matrix intact as a scaffolding for osteogenesis [43].

The widespread use of cryotherapy in the field of surgery came about with the invention of Irving Cooper and Arnold Lee who created the prototype cryoprobe capable of delivering liquid nitrogen into deep surgical fields with minimal trauma to superficial structures [63]. In more recent times, the use of refrigerants such as a mixture of propane/butane/isobutene gas which results in temperatures of −50 °C has been described [23,30].

The advantage of cryotherapy is that the inorganic matrix of the bone is left untouched which allows it to act as a scaffold for osteogenesis after the pathology has been removed.

The use of cryotherapy as an adjunct therapy for OKC has been widely documented with relatively low recurrence rates of about 20% [11,14,43,45,46,62]. In this study, conflicting observations were made regarding the efficacy of cryotherapy as an adjunct therapy. Regression analysis and Kaplan – Meier analysis shows a higher recurrence rate when compared to Carnoy's and modified Carnoy's solution although lower compared to when no adjunct therapy was used. Logistic regression analysis also failed to show any statistically significant difference in outcome when comparing cryotherapy against no adjunct treatment. The data in this study must be interpreted with caution however as the small sample size could cause bias.

The limitation with cryotherapy is the difficulty in controlling the amount of liquid nitrogen that is introduced into the cavity. This makes the depth of penetration difficult to predict and also results in unpredictable post-operative swelling, pain and infection. Furthermore, inadequate protection of the surrounding soft tissues can lead to wound dehiscence and potentially post-operative infection of the surgical site. Cryotherapy can also weaken bone resulting in pathological fractures that can occur even 4 weeks postoperatively [43,45,46].

More recently, it is becoming increasingly difficult to obtain and store liquid nitrogen for regular use. In addition, cryotherapy is being used less and less in other fields of medicine so access to the cryoprobe is greatly limited. To get around the difficulties of access to liquid nitrogen and the cryoprobe, some surgeons have attempted to use various refrigerants such as a mixture of propane, butane and isobutene which is used to perform endodontic tests as a substitute. This mixture is reported to reach a temperature of −50 °C which is more than adequate to cause cell death and has the added advantage of being easily obtainable as well as being easy to apply and contain within the cavity as the product comes in a small container with a thin tube. The use of refrigerants as substitutes for liquid nitrogen is still new and untested but the results so far seem promising and should be investigated further.

4.4. 5-Fluorouracil

5-FU is an antimetabolite drug and was first formulated by Heidelberger, Pleven and Duschinsky in 1957 as a potential anti-tumour drug [64]. They based their formulation on an observation made by Rutman in 1954 that rat hepatomas utilised the nucleobase uracil more rapidly than normal tissues [65]. They then hypothesized that it was possible to formulate an anti-tumour drug that could inhibit the growth of tumours by interfering with the metabolism of uracil leading to the discovery of 5-FU.

5-FU which is a fluorinated analogue of uracil is suggested to exhibit anti-tumour effect by 2 mechanisms. Firstly, by a misincorporation of a fluorinated nucleoside into RNA and DNA instead of uracil. The second is by inhibiting the action of the nucleotide synthetic enzyme thymidylate synthase. It has since been used extensively as a chemotherapy agent for various cancers [66]. A recent study by Wang et al., in 2008 suggests that in addition to the mechanisms stated above, 5-FU also induces cell apoptosis by downregulating the hedgehog (HH) signalling pathway [67].

The role of the HH signalling pathway and the pathogenesis of OKC has been well established [68]. The HH signalling pathway is an essential process in normal tissue development. Loss-of-function mutations of the PTCH1 gene, which is the major receptor molecule for the HH signalling pathway and functions as a tumour suppressor gene results in dysregulation in the HH signalling pathway and overexpression of the SMO gene leading to tumorigenesis [68,69]. The HH signalling pathway dysregulation has been identified as the driving factor for the development of multiple basal cell carcinomas and OKC in patients with NBCCS [68].

The identification of the role the HH signalling pathway in the tumorigenesis of various cancers including basal cell carcinomas lead to the hypothesis that HH signalling pathway inhibitors could be a potential therapy for patients with NBCCS who frequently presented with multiple basal carcinomas and OKCs [70]. Furthermore, as new evidence emerged that the mutation in PTCH1 genes are significantly associated not just in NBCCS but also in sporadic OKCs, the concept of a targeted molecular therapy for OKC became more alluring [71,72].The use of HH signalling pathway inhibitors to treat OKC has been studied in both in vitro and in vivo settings with promising results. Cyclopamine and Vismodegib has been shown to significantly arrest the growth of OKC in a laboratory setting [73,74]. Vismodegib was also reported to cause a reduction in OKC size in patients in NBCCS in a recent clinical trial [75,76].

Following the observation that 5-FU results in a downregulation of the HH signalling pathway in hepatocellular carcinoma [67], it was theorised that the HH inhibiting action could also be used to treat syndromic and sporadic OKC [22]. In this review, 11 OKC were managed with the use of topical 5% 5-FU which was used to coat a ribbon gauze and placed in the cystic cavity after enucleation for 24 h. The authors reported no recurrences after a mean follow-up of 35 months. Furthermore, they reported that only 3 patients developed transient paraesthesia post-operatively which resolved after an average of 42 weeks [22].

Systemic use of 5-FU could result in serious adverse effects such as mucocitis, nausea, pancytopenia, cardiac toxicity, neuropathy and death [77]. Topical application of 5% 5-FU however appears to be safe as only approximately 2.5% of the applied dose is absorbed transcutaneously [78,79]. It should be noted that patients with dihydropyrimidine dehydrogenase (DPD) deficiency may present with severe systemic toxicity when treated with any form of 5-FU. This condition is reported to be present in approximately 3–5% of the general population and is most prevalent in African-American women with up to 12% of this demographic being deficient in DPD so care should be practised when treating this population with 5-FU [80].

Topical 5% 5-FU appears to have a wide margin of safety. A dose of 20 mg/kg is reported to be the threshold for developing toxicity [81].

Therefore, an average adult of 70 kg would have to be given approximately 1400 mg of 5-FU to develop toxicity. Topical 5% 5-FU preparations are sold in tubes of 20g or 40g. With the reported transcutaneous absorption of approximately 2.5%, a practitioner would theoretically have to use twenty-eight 40g tubes of topical 5% 5-FU in order to cause systemic toxicity in an average 70 kg adult. However, it should be noted that absorption rates differ between mucosa and skin so additional studies are needed to determine the safety of use of topical 5-FU.

The use of topical 5-FU to manage OKC appears so far to be an effective and safe method. However, more studies are needed before topical 5-FU can be accepted as a valid adjunct therapy for OKC. Lederhof et al. did not provide a rationale to their treatment protocol as to why they decided to leave the topical 5-FU in situ for 24 h, when topical application of 5-FU in the treatment of basal cell carcinoma requires several applications per day for up to two months. Furthermore, the degree of penetration of topical 5-FU into bone has not been established. Further studies are needed to determine the safety of topical 5-FU and the appropriate protocol to deliver it.

4.5. Limitation

The results of this review have to be interpreted with caution because of the study limitations.

Firstly, there is the absence of high quality prospective randomised controlled trials. However, the authors recognise it is not possible to conduct randomised controlled trials on this subject because blinding is not possible, nor is it ethical to subject patients to treatments that are less effective.

Secondly, confounding factors may have affected the results. The lack of uniformity in terms of treatment protocol, follow-up time and the way data is presented makes identifying the effects of these variables on the recurrence rates difficult to estimate. It was because of this that a meta-analysis was not performed. According to the Cochrane Handbook for Systematic Review of Interventions, the most appropriate way of summarizing time-to-event data is by using methods of survival analysis and to express the intervention effect as a hazard ratio [82]. The authors suggest that all future studies reporting on the treatment of OKC should attempt to perform survival analysis and publish the hazard ratios in their publications in order to allow meta-analyses to be performed in the future.

Thirdly, much of the series have small sample sizes with relatively short follow-up periods. This may have led to an underestimation of the recurrence rates as a longer follow-up period may result in higher recurrence rates. Understandably it is difficult to determine what length of a follow up is considered adequate as recurrences have been documented occurring as late as 41 years after surgery [83]. However, as most cases of recurrences have been reported to occur within 5 years after treatment, we suggest a follow-up period of at least 5 years.

Fourthly, we included studies that contained patients with NBCCS in this review while most others exclude these studies. The reason for exclusion is because patients with NBCCS have a predilection to develop new OKCs which may be mistaken as recurrences resulting in bias. We however have opted to include studies with patients with NBCCS as the exclusion of these studies would leave us with too little data to conduct any meaningful analysis. Furthermore, it could be argued that the population of people who benefits the most from this review are those with NBCCS as it is not practical to perform multiple radical treatments of OKC in this population. We suggest however that future studies publish a separate survival analysis and hazard ratio for this population to allow for meta-analysis to be conducted in the future.

Finally, there is a possibility that important studies were inadvertently excluded as the authors only included studies that were published in English. There was no attempt as well to access non-published data.

5. Conclusion

The results of this review appear to suggest that at present, Carnoy's solution shows the most evidence of efficacy in reducing the recurrence rates of OKC when used as an adjunct. However, it's availability is limited in some countries and the preparation and handling of its components such as glacial acetic acid requires a high volume fume cupboard which may not be available at every facility.

Further in vitro and in vivo studies on the fixative action and safety profile of modified Carnoy's solution could potentially result in better treatment outcomes which is beneficial in countries where Carnoy's solution is banned.

Cryotherapy with liquid nitrogen is falling out of favour due to the technical difficulties in obtaining the materials and equipment but the use of refrigerants as a substitute appears to be promising and should be investigated further.

The use of 5-FU or other HH signalling pathway inhibitors in the management of OKC is still new and untested although the logic behind its use, the wide availability of the drug and preliminary evidence of its efficacy is promising. Furthermore, with the lack of availability of Carnoy's solution, the untested efficacy of modified Carnoy's solution and the difficulty in performing cryotherapy, this avenue of treatment is potentially the best course of action in the future. Further studies on safety as well as appropriate protocols should to be conducted to further our understanding of this potential treatment.

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Declaration of competing interest

The authors confirm no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.adoms.2021.100116.

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