Efficacy and safety of beta carotones in treatment of oral leukoplakia: systematic review and meta-analysis.

Utilidad clínica de la Tomografía computarizada cone beam para definir conducta terapéutica en casos de mediana y alta complejidad endodóntica.

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Abstract: Objectives: A systematic review was conducted to evaluate effectiveness and safety of beta carotenes for the treatment of oral leukoplakia regarding clinical resolution and prevention of malignant transformation. Material and Methods: The systematic search was conducted in three electronic databases and the study's selection was performed according to pre-set eligibility criteria. Four studies evaluating the efficacy of beta carotenes in oral leukoplakia compared to placebo were included in the review; three of which were assigned for quantitative analysis. Data were extracted, tabulated, quality assessed and statistically analyzed. Results: The meta-analysis revealed that when comparing clinical resolution the beta carotene group favored was favored compared to placebo, with statistically significant difference. However, a meta-analysis comparing beta carotene and placebo groups regarding malignant transformation as a primary outcome failed to show any significant benefit. Furthermore, results showed evidence of beta carotene safety. Conclusion: the overall quality of evidence about efficacy of beta carotene in oral leukoplakia treatment was not high. However, given the obvious safety of this agent, data suggests it could have a promising effect in clinical improvement of oral leukoplakia lesions. However, no evidence supporting its benefits in reducing risk of malignant transformation in these lesions was found. Therefore, further long term, well designed randomized clinical trials are highly recommended.

Keywords: Beta carotene; leukoplakia; systematic review; precancerous conditions; cell transformation, neoplastic; administration, oral.

Resumen: Objetivos: Se realizó una revisión sistemática para evaluar la efectividad y la seguridad de los betacarotenos para el tratamiento de la leucoplasia oral en relación con la resolución clínica y la prevención de la transformación maligna. Material y Métodos: la búsqueda sistemática se realizó en tres bases de datos electrónicas y la selección del estudio se realizó de acuerdo con los criterios de elegibilidad preestablecidos. En la revisión se incluyeron cuatro estudios que evaluaban la eficacia de los betacarotenos en la leucoplasia oral en comparación con el placebo; tres de los cuales fueron asignados para el análisis cuantitativo. Los datos fueron extraídos, tabulados, su calidad evaluada y analizados estadísticamente. Resultados: El metanálisis reveló que al comparar la resolución clínica, el grupo de betacaroteno fue favorecido en comparación con el placebo, con una diferencia estadísticamente significativa. Sin embargo, un metaanálisis que comparó los grupos de betacaroteno y placebo con respecto a la transformación maligna como resultado primario no mostró ningún beneficio significativo. Además, los resultados mostraron evidencia de seguridad de betacaroteno. Conclusión: La calidad general de la evidencia sobre la eficacia del betacaroteno en el tratamiento de la leucoplasia oral no es alta. Sin embargo, dada la obvia seguridad de este agente, los datos sugieren que podría tener un efecto prometedor en la mejora clínica de las lesiones de leucoplasia oral. Sin embargo, no se encontraron pruebas que respalden sus beneficios en la reducción del riesgo de transformación maligna en estas lesiones. Por lo tanto, se recomiendan ensayos clínicos aleatorios bien diseñados a largo plazo.

Palabras Clave: Beta caroteno; leucoplasia; revisión sistemática; lesiones precancerosas; neoplasias; administración oral.

INTRODUCTION.

Oral leukoplakia is a common potentially malignant disorder with 1% prevalence and 2% to 3% annual malignant transformation rate.[1] The World Health Organization (WHO) in 2005 defined leukoplakia as "a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer".[2]

Oral leukoplakia is usually diagnosed in middle age, and its prevalence increases with age. Males are more often affected than females, probably owing to the greater prevalence of tobacco consumption by males.

However, the male-female ratio varies according to geographical area.[1] Overconsumption of tobacco and alcohol is considered the main risk factor for the development of oral leukoplakia. Elimination of such risk factors is essential in the treatment of oral leukoplakia but unfortunately, it is difficult to achieve.[1,3,4]

Generally, most oral leukoplakias are asymptomatic. Therefore, the main objective in their treatment is to prevent malignant transformation. Up to now there is lack of consensus on the most suitable management to prevent cancer development.[3]

The accumulation of oxidative damage has shown to underlie the mechanism of potentially malignant disorders. This explains the role of antioxidants in the prevention and management of these diseases.[5]

Beta carotene is a vitamin A precursor.[6,7] Many lines of evidence suggest a potential role for beta carotene in preventing oral cancer.[5] This role is possibly related to its anti-oxidizing properties.[8-10]

This function is accomplished through a ligation between beta carotene and oxygen, which is an unstable reactive molecule, thus diminishing the damaging effects of free radicals.[9-11] In addition, beta carotene seems to be safe as compared to other chemo-preventive agents such as retinoid, which demonstrates significant toxicity.[12-15]

Therefore, the objectives of this systematic review are to assess randomized controlled trials (RCTs) investigating the efficacy of beta carotene in the management of oral leukoplakia regarding prevention of malignant transformation, clinical resolution and to evaluate its safety.

MATERIALS AND METHODS.

Search strategy

Articles in this review were identified by searching the following electronic databases: *PubMed, Cochrane library* and *Scopus*. The following sources were hand searched: grey literature, the central library of Cairo University, library of faculty of dentistry and central library of national cancer institute.

The reference list of included studies and relevant reviews were manually checked.

The keywords used were: Leukoplakia, leukoplakias, leukokeratosis, leukoplakic, leukoplakic lesion, premalignant, potentially malignant, precancerous, premalignancy, betacarotene, beta carotene, ß-carotene, carotene, carotenoids, beta carotene, antioxidant, anticancer, anticancer agent.

Study eligibility criteria

We included randomized or quasi-randomized clinical trials (RCTs) discussing treatment with systemic beta carotenes compared to placebo in cases diagnosed with oral leukoplakia according to the definition of the WHO in 2005,[2] with no languages restrictions. We excluded studies with patients on concurrent supplements or taking any treatment for the lesion before the trial, cases with confirmed malignancy or if the diagnosis was suggestive of oral lichen planus or oral lichenoid reaction.

Primary outcome was malignant transformation demonstrated by histologic examination, while the secondary outcomes were clinical resolution in terms of thinning, reduction in size and decrease in number of lesions represented by scores according to proportion of lesion resolved. Also, the safety of the intervention was determined by the reporting of adverse effects.

Study selection and data extraction

The full search results from all databases were pooled after removal of duplicates. All results were screened independently by two of the authors (R.S and Y.F) to identify studies meeting the inclusion criteria. Any disagreement between the two review authors was resolved by discussion and consensus with the third author (B.A). Then, all eligible studies underwent data extraction using a data extraction form that was designed by the authors. Information extracted from

the included studies were:

1. Study design;

2. Characteristics of trial participants such as number, age, sex, ethnic group and history of smoking and drinking;

3. Characteristics of oral leukoplakia: clinical type, histologic type and site distribution;

4. Characteristics of intervention: drug dose, mode of administration, duration of treatment, overall study duration and participants compliance;

5. The outcomes measured in each study.

Quality assessment of included studies

Two review authors (R.A and B.A) independently assessed the risk of bias of each included study in duplicate using the Cochrane risk of bias assessment tool for RCT; any disagreement between the two review authors was resolved by discussion and consensus with the third author (Y.F). The Cochrane risk of bias assessment tool for RCT addresses the following domains: Random sequence generation (selection bias), allocation concealment (selection bias), blinding (performance and detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other sources of bias.

Each of these criteria was rated as "low risk of bias", *"high risk of bias"* or *"unclear risk of bias"*. The global validity of included trials was summarized as: low risk of bias, if low risk of bias in all criteria; unclear risk of bias, if unclear risk of bias in one or more criteria; and

high risk of bias, if high risk of bias in one or more criteria.

Data analysis

The primary outcome measured in this review was malignant transformation which was reported as dichotomous data; either the presence or absence of cancer development. However, clinical resolution is usually reported in an ordinal scale. Therefore, to be able to calculate treatment effect we dichotomized data about clinical resolution as: complete and partial response (overall response) *versus* no response or disease progression. Additionally, histologic changes were measured as: histo-logic improvement *versus* worsening or no change of histologic features.

Data were analyzed by calculating risk ratios. Forest plots were done using the Review Manager (RevMan) software (Version 5.1, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011).

Heterogeneity across studies was assessed represented by I2 statistic as a % and a *p*-value. When the *p*-value was *p*<0.05, then heterogeneity was considerable across the studies and the results should be taken cautiously. When heterogeneity was not significant meta-analysis was carried out using fixed effect model.

The quality of evidence

The overall quality of the body of evidence was assessed by the GRADE system (grade recommendation, assessment, development and evidence) using the GRADEpro software.

Figure 1. PRISMA 2009 Flow Diagram.

Identification

Records identified through database searching
(1st search+2nd search +3rd search)
n= (253(Pubmed) + 23 (Cochrane) + 95 (Scopus))=371

Additional records identified through other sources (n=1)

Screening

Records after duplicates removed (n = 338)

Records screened (n =338)

Records excluded (n =328)

Eligibility

Full-text articles excluded, Full-text articles assessed with reasons (n=7)

Studies included in qualitative synthesis (n=3)
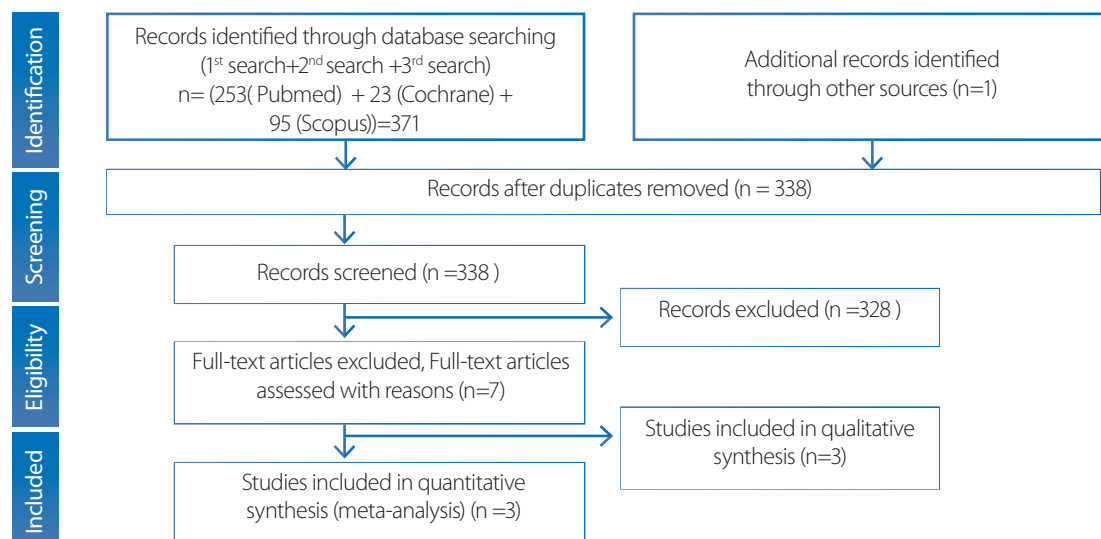
Included

Studies included in quantitative synthesis (meta-analysis) (n =3)

Figure 2. Forest plot of comparison: Comparison between beta carotene and placebo, outcome: 1.1 Malignant transformation.

Study or Subgroup	Beta Carotene		Placebo			Odds Ratio		
	Events	Total	Events	Total	Weight	M.H Fixed, 95% CI	Year	M.H, Fixed, 95%CI
Sankaranarayanan et al.[22]	2	46	4	43	60.3%	0.44 [0.08, 2.55]	1997	
Nagao et al.[23]	3	23	3	23	39.7%	1.00 [0.18, 5.56]	2015	
Total		**69**		**66**	**100.0%**	**0.66 [0.20, 2.22]**		
Total events	**5**		**7**					

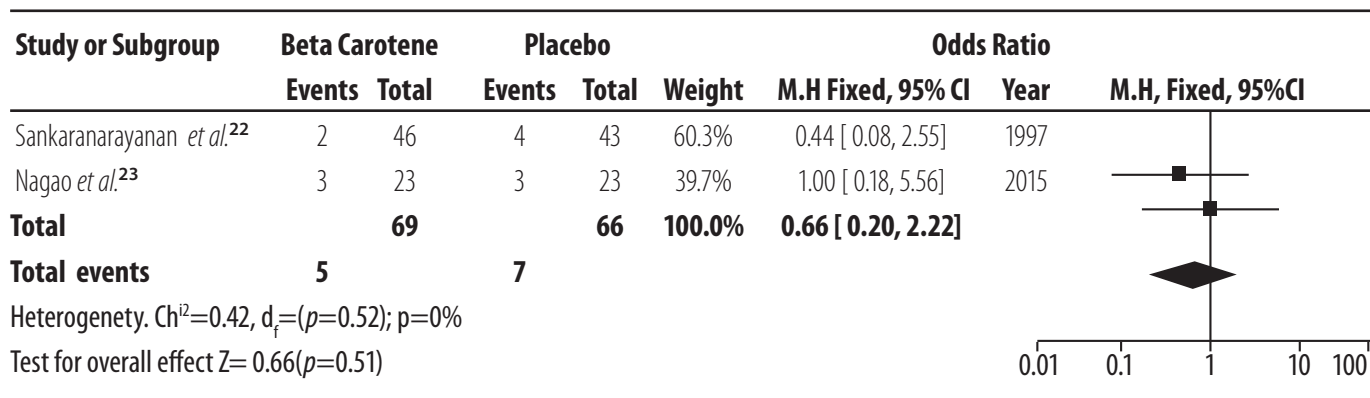Heterogenety. Chi^2=0.42, d_f=(p=0.52); p=0%

Test for overall effect Z= 0.66(p=0.51)

Figure 3. Forest plot of comparison: Comparison between beta carotene and placebo, outcome: 1.2 clinical improvement.

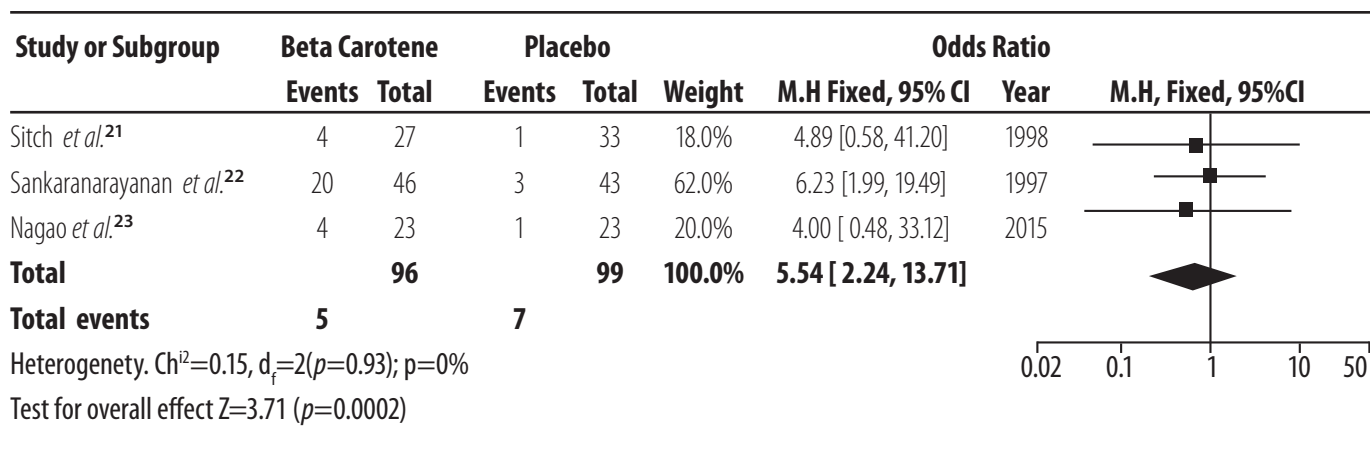| Study or Subgroup	Beta Carotene		Placebo			Odds Ratio		
	Events	Total	Events	Total	Weight	M.H Fixed, 95% CI	Year	M.H, Fixed, 95%CI
Sitch et al.[21]	4	27	1	33	18.0%	4.89 [0.58, 41.20]	1998	
Sankaranarayanan et al.[22]	20	46	3	43	62.0%	6.23 [1.99, 19.49]	1997	
Nagao et al.[23]	4	23	1	23	20.0%	4.00 [0.48, 33.12]	2015	
Total		**96**		**99**	**100.0%**	**5.54 [2.24, 13.71]**		
Total events	**5**		**7**					

Heterogenety. Chi^2=0.15, d_f=2(p=0.93); p=0%

Test for overall effect Z=3.71 (p=0.0002)

Table 1. Studies excluded from the review.

Reference	Year	Reason for exclusion
Malaker et al.[20]	1990	Intervention was compared to active drug, not placebo.
Lippman et al.[18]	1993	The patients randomized were a selected group of subjects who responded to one of the two drugs tested in the randomized phase (isotretinoin).
Liede et al.[10]	1998	Observational study.
Garewal et al.[15]	1990	Uncontrolled trials.
Toma et al.[19]	1992	Uncontrolled trials.
Lee et al.[17]	2000	Patients were given induction with high dose isotretinoin for 3 months followed by 9 months of maintenance treatment with either low dose isotretinoin or beta carotene.
Garewal et al.[16]	1999	Phase one of this study was the induction phase that was considered as a single arm study (uncontrolled). In the second phase, randomized patients were a selected group of participants (responders).

Table 2. Drinking, smoking and betel quid chewing habits of the participants.

Study	Year	Tobacco smoking			Betel quid chewing			Alcohol drinking		
		Smokers	Total number of participants	%	Chewers	Total number of participants	%	Drinkers	Total number of participants	%
Stitch et al.[21]	1988	62*	130	48*	110	130	85*	84*	130	65*
Sankaranarayanan et al.[22]	1997	41	160	9*	127	160	79*	72*	160	45*
Nagao et al.[23]	2015	15 former smokers	46	33*	N/A	46	N/A	18*	46	39*
Total		118*	336	35*	237*	336	70*	138*	336	41*

*: Calculated by authors of this review.   **N/A:** not available.

Table 3. Estimated potential risk of bias of the included studies. Risk of bias according to Cochrane Risk of Bias Tool for RCTs.[24]

Study	Year	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Other sources of bias
Stitch et al.[21]	1988	Not reported (unclear risk of bias)	Not reported (unclear risk of bias)	Double blind (participants and healthcare staff were blinded). However, author reported that yellow color of feces from patients receiving beta carotenes made it difficult to be truly blinded (low risk of bias)	18 out of 130 were lost due to death, illness or emigration; 111 (86%) continued to final analysis (low risk of bias)	Authors did not report tion which is an important outcome in the context of oral leukoplakia (unclear risk of bias)	No other sources of bias identified (low risk of bias)
Sankaranarayanan et al.[22]	1997	Method not reported (unclear risk of bias)	Not reported (unclear risk of bias)	Double blind: dentist and physician were blinded to treatment and to evaluate clinical response and side effects (low risk of bias)	131 out of 160 patients included in the study were included in the final analysis; others excluded due to lack of compliance (low risk of bias)	All outcomes were measured before and after (low risk of bias)	No other sources of bias identified (low risk of bias)
Nagao et al.[23]	2015	Allocation was carried out using computer-generated number sequencing with stratification, by blocking randomization according to presence or absence of dysplasia,(low risk of bias)	A trial coordinator not involved in the routine care of patients generated the allocation sequence and enrolled the participants. The central randomization by numbered containers was used for allocation concealment. (low risk of bias)	Double blind (low risk of bias)	High number of drop-outs 13/46 (28.2%), although not lost to follow-up and included in ITT analysis (high risk of bias)	All important outcomes were reported (low risk of bias)	No other sources of bias identified (low risk of bias)

Table 4. Outcome table (as reported by original papers)

Article	Year		Outcome 1 Clinical response		Outcome 2 Malignant transformation		Outcome 3 Intervention Safety
			Beta carotene	Placebo	Beta carotene	Placebo	Beta carotene
Stich, et al.[21]	1988	At 6 months	CR:4/27 PR: SR:19/27 P:4/27	CR:1/33 PR: SR:25/33 P:7/33	N/A	N/A	N/A
Sankaranarayanan et al.[22]	1997	At 1 year	CR:15/46 PR:5/46 SR:25/46 P:	CR:3/43 PR:0/43 SR:38/43 P:	2/46	4/43	3 headache 2 muscle pain Placebo: 1 dryness
Nagao et al.[23]	2015	At 1 year	CR:1/23 PR:3/23 SR:18/23 P:1/23	CR:0/23 PR:1/23 SR:20/23 P:0/23	At 1y 0/23 At86m 3/23	At 1y 1/23 At86m 3/23	No unwanted side effects were noted

Table 5. Outcome table, after data ware dichotomized.

Article	Year		Outcome 1 Clinical improvement		Outcome 2 Malignant transformation	
			Beta carotene	Placebo	Beta carotene	Placebo
Stich et al.[21]	1988	Overall response No response	4/27 23/27	1/33 32/33	N/A	N/A
Sankaranarayanan et al.[22]	1997	Overall response No response	20/46 25/46	3/43 38/43	2/46	4/43
Nagao et al.[23]	2015	Overall response No response	4/23 19/23	1/23 20/23	3/23	3/23

Table 5. Outcome table, after data ware dichotomized.

Outcomes	Anticipated absolute effects* (95% CI) Risk with placebo	Risk with beta carotene	Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)
Malignant transformation follow up: 2 years	106 per 1,000 (21 to 235)	70 per 1,000	RR 0.66 (0.20 to 2.22)	135 (2 RCTs)	⊕◯◯◯ VERY LOW1,2
Clinical resolution (overall response) follow up: range 6 months to 1 years	51 per 1,000	280 per 1,000 (113 to 692)	RR 5.54 (2.24 to 13.71)	195 (3 RCTs)	⊕◯◯◯ VERY LOW 1,2
Safety of intervention follow up: mean 1 years	In one study it caused side effects in about 11% of participants as; headache and muscle pain and no unwanted side effects were noted in a study supplementing 10mg/d for 1 year			–	(2 studies)

*: The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). **CI:** Confidence interval. **RR:** Risk ratio.
GRADE Working Group grades of evidence.
High quality: We are very confident that the true effect lies close to that of the estimate of the effect. **Moderate quality:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. **Low quality:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect. **Very low quality:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Supplementary table. Overview and characteristics of the included studies processed for data extraction.

| Study | Study design | Participants | | Drug dose and mode of administration | Control | Intervention Duration of treatment/ duration of study | Compliance control |
		Patient characteristics	Lesion characteristics				
Stich *et al.*[21] 1988 '	Quasi-randomized, parallel-group, double-blind, 3 arms clinical trial. Source of funding: unspecified	No: 130 (111 continued to final analysis) Ethnic group: Fishermen from Kerala (India) Habits : Betel quid chewing and alcohol drinking, 37% Chewing, drinking and smoking of bidis, 28% Chewing and smoking of bidis, 2% Betel quid chewing with no additional oral habit, 16%. Age :average age 48.8+_12.9	Clinical types: Homogenous 83% Speckled 2.9% Verrucous 5.7% Histologic types N/A Site distribution: Buccal mucosa 53.4% Commisures 23.3% Retromolar 9.3% Tongue 8.4% Lip 4.4% Other 1.4 %	1st arm: (35) betacarotene 30mg capsule (180 mg/ week) 2 capsules twice daily 2nd arm (60) betacarotene plus vitamin A (100.000 IU/week)	3rd arm :Placebo capsule containing dextrose & water dispersal beadlets were given twice daily	6 months	Yes capsules were taken under supervision of local nurse who confirmed that capsule was actually swallowed
Sankaran-arayanan *et al.*[22] 1997	Randomised, parallel-group, double-blind, 3 arms clinical trial. Source of funding unspecified	No: 160 (131 continued to final analysis) Ethnic group: Fishermen from Kerala (India) Habits : 127 (97 %) tobacco chewers; 41 (31%) smokers; 72 (55%) drinkers Age : average age 50.7 Sex : 47 females , 84 males	Clinical types: 69 homogenous 62 non homogenous; (erythroplakia, ulcerated, nodular, verrucous) Histologic types N/A Site distribution: N/A	1st arm : (n=55): Betacarotene (30 mg betacarotene/capsule) (360 mg/ week) 2nd arm: (n=50) Vitamin A (retinyl palmitate, 50,000 IU) (300,000 IU/week)	The placebo capsules were formulated to simulate the supplements as closely as possible	1 year 2 years (1 treatment, 1 follow up)	YES attendance of review and collection of refills
Nagao *et al.*23 2015	RCT, parallel-group, 2 arms. Source of funding: Supported by Butterfield Award of the Sasakawa Foundation GB and DSM Nutrition Japan	No: 46 Ethnic group: Japanese Habits : no current smokers; 31 never smoked, 15 former smokers; 18 regular drinkers; 28 non drinkers Age :median age 65 (range 38 to 80 years) Sex : 21 female; 25 male	Clinical types: 36 homogenous, 10 non-homogenous, Histologic types 33 no dysplasia 9 mild dysplasia 4 moderate dysplasia Site distribution: 24 gingiva/sulcus 3 palate 2 buccal mucosa	Experimental arm: 10 mg/d of beta carotene and 500 mg/d of vitamin C (23)	placebo arm: 0 mg/d of vitamin C (23)	1 year/ 1 year +open follow up	Not reported

RESULTS.

Search results

The search was carried out on August 1, 2018. A total of 371 articles were found from electronic databases and one from reference lists. After removal of duplicates, 338 articles were identified.

Primary exclusion was based on screening titles and abstracts for eligibility, which yielded 10 articles (Figure 1). Full texts were obtained for these articles. We excluded 7 studies 10,15-20 due to reasons presented in Table 1.

Study design and intervention

Two of the included studies had three-arm designs, which compared systemic beta carotene with placebo while the third arm was beta carotene plus vitamin A in one trial[21] and vitamin A alone in another.[22]

One RCT had a 2-arms design that compared beta carotene plus vitamin C with placebo that contained vitamin C hence, vitamin C was not considered a confounder.[23] (Overview of the included studies and characteristics processed for data extraction are presented as supplementary table)

Study population

Two out of three studies were conducted in India (fishermen from coastal areas),[21-23] and one is from Japan. The total number of participants was 336 patients. Their ages ranged from 17 to 84 with a male to female ratio of 1:1.5. The three studies reported histologic criteria of participants prior to the study.[21-23]

When we analyzed habits of tobacco smoking and alcohol drinking, which are important risk factors for oral leukoplakia and oral cancer, the total number of tobacco smokers of all studies was 118 (35%) participants. While, the total number of alcohol drinkers of all studies was 138 (41%) participants. (Table 2)

Quality assessment of included studies

We assessed risk of bias in the three RCTs included in our review according to criteria of Cochrane risk of bias tool for RCTs.[24] When we assessed selection bias; two studies[21,22] did not report method of random sequence generation or allocation concealment that was considered an "unclear risk of bias".

In Nagao *et al.,*[23] study allocation was carried out using computer generated number sequencing with stratification, by blocking randomization according to presence or absence of dysplasia. A trial coordinator not involved in the routine care of patients generated the allocation sequence and enrolled participants.

The central randomization by numbered containers was used for allocation concealment. This was judged as "low risk of bias". Regarding blinding, the three RCTs were double blinded[21-23] with "low risk of bias".

In the Stich *et al.,*[21] study, participants and staff were blinded; however, authors reported that yellow color of feces from patients receiving beta carotenes made it difficult to be truly blinded.

Two studies were free of selective reporting in which all outcomes were measured before and after treatment.[22,23]

Therefore, that was judged as "low risk of bias". However, the study by Stich *et al.,*[21] did not report data on histological outcome, which is an important outcome in context of management of oral leukoplakia with high risk of bias.

Assessing attrition bias in terms of incomplete outcome data, two studies were at "low risk".[21,22] In the study of Stich *et al.,*[21] 18 out of 130 participants were lost due to death, illness or emigration so 111 (86%) continued to final analysis, while in the study by Sankaranarayanan *et al.,*[22] 131 out of 160 patients (81.8%) were included in the final analysis, with patients excluded due to lack of compliance. Nagao *et al.,*[23] was evaluated as "high risk" due to the high number of dropouts, with only 71.8% of patients included in the final analysis.

Generally, no other sources of bias were identified and the overall assessment revealed that two RCTs had unclear risk of bias[21-23] and one was at high risk of bias. (Table 3)

Outcomes measured

All included studies evaluated clinical improvement in terms of size, number or thinning of lesion and photographically recorded.[21-23] All studies defined complete clinical response as complete regression or disappearance of the lesion.

Sankaranarayanan *et al.,*[22] added that this response was expected to last for at least one month. Disease progression was generally considered if there was an increase in size or if a new lesion appeared. Cancer development was only reported in two studies.

Effects of intervention and meta-analysis

1. Malignant transformation: When we performed a meta-analysis for the primary outcome (malignant transformation of oral leukoplakia lesions) in the two studies that reported it, there was no apparent heterogeneity between them (I_2= 0%, p=0.052).[22,23] Results failed to show any evidence of benefit for beta carotene as compared to placebo (RR 0.66, 95% CI 0.2 to 2.22, p=0.51). A forest plot is shown in Figure 2.

2. Clinical improvement: The three included RCTs reported the effect of beta carotene on clinical resolution.[21-23] As we dichotomized data about clinical outcome, heterogeneity between trials treatment out-come was tested which was found to be negligible (I_2=0%, p=0.93). Hence, findings were pooled into the meta-analysis and the fixed effect model was appropriate.

From the meta-analysis, there was an evidence of benefit of beta carotene when compared to placebo. The overall effect was statistically significant (RR 5.54, 95% CI 2.24-13.71, p= 0.000). (Figure 3)

3. Safety of intervention: Adverse effects of systemic beta carotene were reported by two studies. In both there was no significant toxicity observed. In the study by Sankaranarayanan *et al.,*[22] beta carotene caused side effects in about 11% of participants, including headache and muscle pain. Moreover, in Nagao *et al.,*[23] no unwanted side effects were demonstrated despite the supplementing dose of 10mg/d for 1 year. (Table 4 and Table 5) The quality of evidence: The results of the overall quality of evidence assessed by the GRADE system are summarized in Table 6.

DISCUSSION.

The reversal of potentially malignant disorders such as oral leukoplakia is an important step in cancer prevention. Currently, the standard treatment of oral leukoplakia is surgical removal. However, surgical management has not proved to eliminate or even reduce the risk of malignant transformation.[25]

Moreover, according to a Cochrane review of interventions for treating oral leukoplakia, there were no available RCTs on surgical management.[26] Furthermore, it is usually associated with recurrences.[27] Therefore, more conservative therapies have gained interest over surgical management and seem to be a more attractive alternative.[28] There is emerging evidence from epidemiologic

in vitro animal as well as clinical interventional studies about the potential benefit of beta carotene in oral leukoplakia management.[5]

Some phase II clinical trials without control groups have investigated the efficacy of beta carotene in oral leukoplakia treatment. Clinical resolution ranged from 44% to 71% with different doses and durations.[15,19] However, due to lack of control groups, small number of participants and short duration there are inherent weaknesses in these studies. Besides, histologic changes and malignant transformation were not the main outcomes considered.

In a multicenter trial in the United States of America,[16] subjects received beta carotene (60mg/day) for 6 months before they were randomized, and had a clinical response rate of 52% (95% CI 38% to 60%). Researchers performed biopsy on 46% of participants. There was no change in 61% of these and histologic improvement by at least 1 grade in 39%. Despite of the absence of blinding and adequate placebo control, the interesting finding was durability of response in a multi-institutional setting.

In the present review, the only outcome that was reported by all studies was clinical resolution. From the meta- analysis results, there was statistical evidence that systemic beta carotenes contributed to clinical resolution. However, the included RCTs ranged from high to unclear risk of bias. If we summed up results from previous uncontrolled trials, we can conclude that beta carotene has an obvious clinical benefit.

Nevertheless, the ultimate goal in the management of oral leukoplakia is cancer prevention. In our review, only two RCTs discussed reduction in cancer development risk,[22,23] which we set as a primary outcome. They ranged from high to unclear risk of bias that when pooled into meta- analysis, failed to show any evidence of benefit regarding this outcome.

Generally, when examining the efficacy of beta carotene, true blinding is difficult as patients taking the treatment are usually identifiable by yellow-orange color of skin or feces. Hence, results from true placebo controlled trials could be sometimes not possible.

The degree of epithelial dysplasia affects the decision about the most suitable treatment. Obviously, lesions

with severe epithelial dysplasia have a high malignant risk. Hence, surgical removal could be preferable.[29] This factor was not considered in the included studies.

None of the included studies demonstrated cessation of smoking or drinking habits, and such factors may affect the reliability of results. Additionally, variation of geographical area may affect implementation of results. Two of the included RCTs were betel quid chewers, which is common among Indians.[21-23] On the other hand, participants of the study from Japan did not significantly have this habit. Despite the fact that oral leukoplakia lesions have an increased risk of cancer, they are not yet associated with significant morbidity.[8,9,15,20,21,30]

This rationalizes the need for treatment with minimal adverse effects. From data in our review, beta carotene seems to be non-toxic and well accepted by patients. Especially, when we consider the significant toxicity demonstrated by other chemo preventive agents such as retinoids.[13,14,19] The reported side effects ranged from none to 11%. When we assessed the quality of evidence using GRADE, the overall quality of evidence was very low. However, the apparent safety and effectiveness in clinical improvement are encouraging for further evaluation of these chemo-preventive agents.

CONCLUSION.

Overall data suggests that beta carotenes are considered a promising candidate for reducing the risk of oral cancer as well as clinical resolution of lesions. However, a final conclusion about the efficacy of beta carotenes in oral leukoplakia treatment cannot be established. In addition, they are non-toxic agents demonstrating minimum adverse effects. These criteria indicate an interesting therapeutic alternative to surgical management.

However, further rigorously designed long-term, multi-centered placebo-controlled clinical trials with greater sample size are strongly recommended to reach a final conclusion about general validity of these agents.

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