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Mini-review

Betel quid–associated cancer: Prevention strategies and targeted treatment

Albert Min-Shan Ko[a], Chien-Hung Lee[b,c], Ying-Chin Ko[d,e,*]

[a] Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, China
[b] Department of Public Health and Research Center for Environment Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[c] Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[d] Environment-Omics-Disease Research Center, China Medical University Hospital, China Medical University, Taichung, Taiwan
[e] Graduate Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan

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ABSTRACT

Betel quid (BQ) and areca nut use are at risk of cancer. This review includes the latest evidence of carcinogenesis caused by BQ exposure, suggests possible prevention strategies. We conducted a systematic literature search in the PubMed and Web of Science databases to identify relevant articles published in the past 10 years according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria. Arecoline *N*-oxide, a metabolite of areca nut, is likely an initiator in carcinogenesis and is detoxified by *N*-acetylcysteine. Oral potentially malignant disorder and reactive oxygen species involved in carcinogenesis pathways may be treatable using antioxidants. Screening programs conducted by trained physicians are useful for identifying patients with early stages of oral cancer in high-risk groups. Anti-inflammatory medications may be used as chemopreventive agents in the disease-free stage after surgery. The association between survival and tumor somatic mutations in patients who chew BQ should be addressed in cancer studies. Current evidence on the natural course from BQ exposure to cancer occurrence and development provides information for developing primary, secondary, and tertiary prevention strategies against BQ-associated cancer at clinical or translational levels.

1. Introduction

Betel quid (BQ) is a mixture of substances, including dry or unripe areca nut (AN), slaked lime, catechu, betel leaf, tobacco, inflorescence of *Piper betel* L., and some unspecified spices, depending on regional and cultural backgrounds and the availability of specific ingredients [1]. AN is a common component of BQ, and arecoline, arecaidine, guvacoline, and guvacine are the major alkaloids in AN (approximately 2%) [2].

BQ and AN were considered safe for human consumption for thousands of years because they are natural products. In 1985, the first evaluation by the International Agency for Research on Cancer (IARC) recognized that there was inadequate evidence in humans for the carcinogenicity of BQ without tobacco [1]. However, in 2004, the IARC [2] reevaluated the subsequent data and concluded that both BQ and AN without tobacco were Group 1 carcinogens that cause oral cancer in humans. In 2012, the IARC reported that the main target organs of BQ were the oral cavity and esophagus [3]. The evidence of carcinogenicity of BQ and AN is based on consistent findings regarding the association of BQ exposure with cancer in three independent epidemiological studies conducted in Taiwan [4], Pakistan [5], and India [6], and this evidence provides stronger support for this association than an isolated observation from a single study. However, the findings in animal models have not been consistent with those of studies conducted on humans. In an earlier animal study, AN-induced oral cancer was demonstrated [7], but a subsequent study conducted using the same methods reported that no tumors were observed in the AN-treated group [8]. Animal studies have reported that only exposure to combinations of BQ or AN with known carcinogens, such as DMBA and 4-NQO, resulted in tumor initiation and development [8–11]. Therefore, AN may be considered a procarcinogen or tumor promoter; however, effect on tumor initiation remains unclear.

Alkaloids of AN are strongly suspected to be carcinogens; however, no evidence of arecoline-related cancer in humans has been provided; only one study reported the association between AN and cancer in an animal experiment [12]. Giri et al. identified 11 metabolites of arecoline and arecaidine in an animal model and recommended that subsequent studies investigate the toxicology of the newly discovered metabolites rather than that of arecoline itself [13]. In a subsequent study, the same authors demonstrated that the oxidation of arecoline produced 50% arecoline *N*-oxide and 10% arecoline *N*-oxide

* Corresponding author. Environment-Omics-Disease Research Center, China Medical University and Hospital, No. 2 Yude Road, Taichung, 40447, Taiwan.
E-mail addresses: ycko0406@gmail.com, d97913@mail.cmuh.org.tw (Y.-C. Ko).

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List of abbreviations

AN	areca nut
BQ	betel quid
IARC	International Agency for Research on Cancer
MNPN	3-methylnitrosaminopropionnitrile
OPMD	oral potential malignancy disorder
ROS	reactive oxygen species
SNPs	single-nucleotide polymorphisms
AUROC:	receiver operating characteristic curve
HNSCC	head and neck squamous cell carcinoma
COSMIC	Somatic Mutations in Cancer
OSCC	oral squamous cell carcinoma
OSF	oral submucous fibrosis
DMBA	7,12-dimethylbenz[a]anthracene
4NQO	4-nitroquinoline-1-oxide

mercapturic acid [14].

Stich et al. reported that 3-methylnitrosaminopropionnitrile (MNPN) was a nitrosamine that may be derived from AN. MNPN was not detected in the saliva of individuals who chewed BQ without tobacco in study populations sampled in Taiwan and India [15], while one study reported the presence of MNPN in saliva, as confirmed through mass spectroscopy [16]. On the basis of in vitro evidence, MNPN was classified as a possibly carcinogenic to humans (Group 2B), whereas *N*-nitrosoguvacoline, *N*-nitrosoguavacine, and 3-methylnitrosaminopropionaldehyde were classified as Group 3 (not carcinogenic to humans) by the IARC [2]. Although several nitrosamines related to AN have been suspected to be carcinogenic agents and have exhibited mutagenic activation in bacteria [17], Giri et al. reported that arecoline was metabolized to arecoline *N*-oxide by human flavin–containing monooxygenases 3 (not by cytochromes P450) and the AN-derived nitrosamines exhibited little or no carcinogenicity [14].

BQ use is associated with oral potential malignancy disorder (OPMD), oral submucous fibrosis (OSF), and leukoplakia [18–21] and is known to progress through malignant transformation into oral cancer [22,23]. Within a cohort of 1458 patients with OPMD, 44 patients developed oral cancer at the same site as the initial lesions with an overall malignant transformation rate of 3.0% and a mean follow-up time of 42.6 months [22]. A study on risk factors related to malignant transformation reported no effects of smoking cigarettes, drinking alcohol, or chewing BQ on malignant changes in OPMD [23].

Nair et al. demonstrated the formation of reactive oxygen species (ROS) in vitro with BQ ingredients and lime; the results suggested that ROS produced by BQ may be involved in the development of oral cancer [24]. The authors further suggested that the levels of meta-tyrosine and ortho-tyrosine should be measured in human saliva as markers of ROS [25]. Chewing two BQs (total of 60 mg/mL AN) with lime and betel leaf (or inflorescence) at a pH level of 7.5–8.5 for 15 min produced higher levels of ROS in human saliva than did chewing gum [26].

Arecoline *N*-oxide is suspected to be a potentially toxic substance, and MNPN, OPMD, and ROS production have been suggested to be underlying factors involved in the pathway of carcinogenesis. The primary prevention is the avoidance of BQ exposure and quits BQ use. No pharmacological evidence currently promotes the cessation of BQ use, and the effectiveness of counseling-based BQ-quitting programs is limited because of the addictive nature of BQ [27,28]. Therefore, the primary prevention strategy should identify the responsible carcinogens and methods of blocking the pathway of carcinogenesis. Treatment for OPMD and ROS production, even at the stage of tumor initiation, should be promoted.

In a cluster randomized controlled trial conducted in Kerala, India during 1996–2004, 96,517 and 95,356 participants were recruited as the intervention group and control group, respectively, to assess the effect of visual screening on oral cancer mortality. A significant reduction of 43% in oral cancer mortality was observed among the men with pan-tobacco and/or alcohol use in the intervention group compared with controls [29]. In a cohort study, 280 patients with oral cancer were followed up during an 8-year period, in that the overall 5-year survival rate was 58.5%, and multivariate analysis revealed that

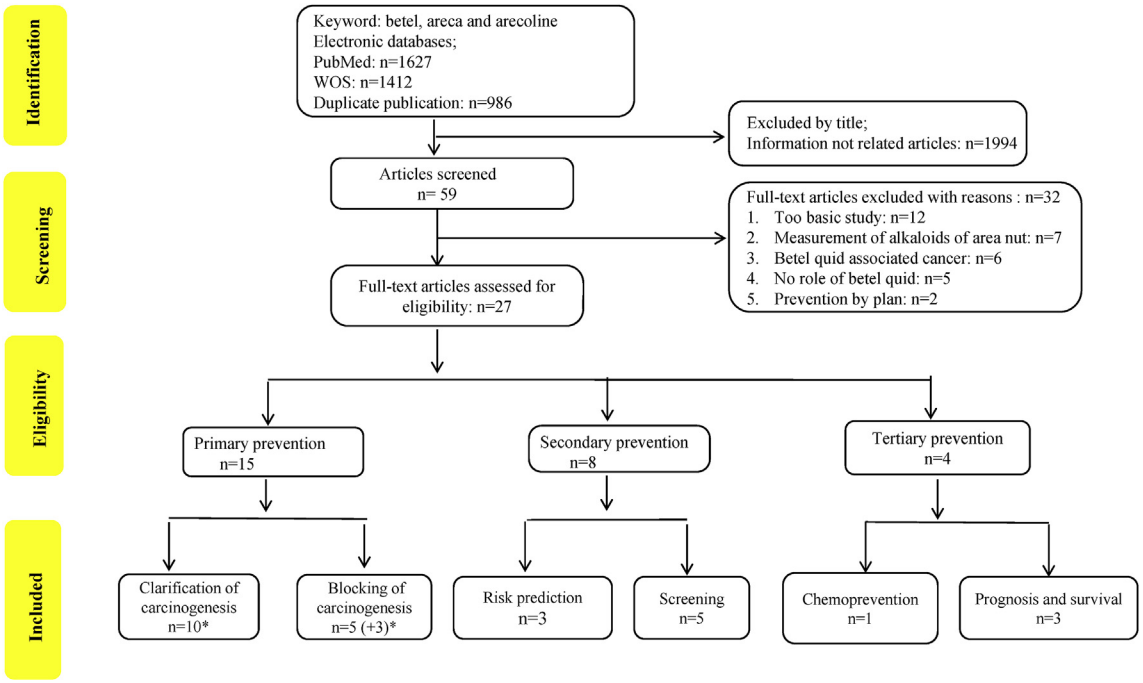

Fig. 1. Flow diagram on studies for review included for the prevention strategies and treatments targeting betel quid-associated cancer.
Note: * There are 3 articles repeatable.

age, clinical stage, and BQ-chewing status were independent prognostic factors for survival [30]. Thus, screening for early stages of cancer and prolonging survival were key secondary and tertiary prevention strategies for BQ-associated cancer.

2. Objectives

Almost 600 million people use BQ globally and are thus at risk of cancer. This study reviews the progression of BQ-induced cancer, the pathway of carcinogenesis, and the prediction, screening, chemoprevention, and survival of patients. We examined possible strategies to prevent BQ-associated cancer, including primary, secondary, and tertiary prevention strategies based on the natural progression from BQ exposure to cancer occurrence, development, and exacerbation at clinical or translational levels.

3. Methods

3.1. Search strategy

We performed a systematic literature search using the PubMed and Web of Science databases to identify relevant articles published between January 2010 and November 2019 according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria [31]. We searched for articles published in English using the keywords "betel," "areca," or "arecoline." Studies on cancer, prevention, chemoprevention, prediction, screening, and survival that met the eligibility criteria were assessed.

3.2. Study selection and data extraction

As illustrated in Fig. 1, the initial searches in both databases with the keywords yielded 3039 articles. Among the search results, 986 duplicate publications and 1994 unrelated articles were excluded on the basis of their titles and abstracts. Among the remaining 59 records that were screened using full-text retrieval, 27 records were eligible for inclusion. These included 10 articles that elucidated the carcinogen and pathway of carcinogenesis and five, three, five, one, and three articles respectively reporting the blocking pathway of carcinogenesis, risk prediction, screening, chemoprevention, and prognosis and survival.

4. Results

4.1. Primary prevention: clarification of carcinogens and carcinogenesis

Latest studies have not reported MNPN in saliva or urine after chewing of AN [32,33]; furthermore, no difference in the plasma levels of 8-hydroxy-2-deoxyguanosine, a marker of mutagenesis or carcinogenesis, was detected between BQ chewers and nonchewers [34]. Arecoline *N*-oxide is a suspected toxic substance. A toxicological study reported that an arecoline oxidation metabolite, arecoline *N*-oxide (1–1000 μg/plate), exhibited a visible dose-dependent mutagenic effect on *Salmonella* tester strains TA98 and TA100, whereas arecoline (dose up to 200 μg/plate) exhibited little or no mutagenicity only on TA100 after treatment with rat liver S9 [35]. Therefore, the mutagenic effects of arecoline may be due to one of the arecoline metabolites [35]. Kuo et al. observed higher collagen expression, more severe squamous hyperplasia, and a greater extent of leukoplakia in mouse tongue tissues treated with arecoline *N*-oxide than in those treated with arecoline [36]. In cultured oral fibroblasts, arecoline *N*-oxide exerted stronger OSF-related effects on the expression of inflammation and fibrosis-related genes [36,37]. In *vitro* and in *vivo* with arecoline and arecoline *N*-oxide studies have revealed that the mechanism of carcinogenesis involves the upregulation of P53, NOTCH1 and FAT1 protein expression by oral cancer cell and hyperplasia tissue of mice [38]. *CASP8* somatic mutations were identified from whole-exome sequencing of oral squamous cell carcinoma (OSCC) samples. Arecoline *N*-oxide induced oral squamous epithelial hyperplasia through the upregulation of caspase-8 in both immunodeficient and normal mice, which indicated the initial highly proliferative stage of oral carcinogenesis [37].

One study on arecoline-induced ROS in oral cancer cells used oral and esophageal cancer cells (OECM-1 and CE81T/VGH) obtained from patients in Taiwan as the experimental model to analyze the roles of ROS and AMP-activated protein kinase (AMPK) in arecoline-induced apoptosis [39]. Arecoline dose-dependently increased intracellular ROS levels and dose- and time-dependently inhibited AMPK phosphorylation [39]. Similarly, the exposure of normal human oral keratinocytes (HOKs) to arecoline (20 μg/mL) evoked concentration-dependent intracellular ROS generation; however, arecoline concentrations higher than 40 μg/mL significantly upregulated intracellular ROS generation in OECM-1 oral cancer cells [40].

A retrospective study reported transformation rates in OPMD malignancy development and associated factors [41]. Among 5071 patients with OPMD in the study, 219 developed oral cancer at the same site as the initial lesion at least 6 months after their initial biopsy, and these results are consistent with those of previous studies [22,23]. The overall transformation rate was 4.3% during 33.6 months, and findings confirmed that no significant effects of tobacco smoking, alcohol drinking, or BQ chewing were observed on the malignant transformation of OPMD [41].

4.2. Primary prevention: blocking pathway of carcinogenesis

Arecoline *N*-oxide is moderately mutagenic to *Salmonella* tester strains. This mutagenicity was potently and dose-dependently inhibited by the antioxidants glutathione, *N*-acetylcysteine, and cysteine but was not inhibited by methionine [35]. OECM-1 cells treated with DMSO alone or in combination with arecoline, *N*-acetylcysteine, or glutathione were analyzed through caspase-3 activity assay. Both *N*-acetylcysteine (10 mM) and glutathione (5 mM) effectively blocked the cytotoxicity of arecoline in OECM-1 and CE81T/VGH cells, and their dose-dependent inhibitory effects on the cytotoxicity of arecoline in OECM-1 were observed [39]. *N*-acetylcysteine and glutathione significantly inhibited arecoline-mediated caspase-3 activation in OECM-1 cells. OECM-1 cells were coincubated with pharmacological agents in the presence of 40 μg/mL arecoline [39]. The addition of *N*-acetylcysteine, curcumin, and epigallocatechin-3 gallate (doses not reported) significantly reduced arecoline-induced ROS generation in HOKs and OECM-1 cells [40].

Persistent fibroblast contraction may induce the fibrotic contracture of tissue and OSF. Arecoline (31 μg/mL) induced slight buccal mucosa fibroblast contraction [42]. AN extract (800 μg/mL) induced buccal mucosa fibroblast contraction and elevated ROS levels; however, catalase (500 U/mL), superoxide dismutase (1000 U/mL), and *N*-acetylcysteine (3 mM) exhibited no obvious effect on prompting AN extract to elicit buccal mucosa fibroblast contraction [42].

SIRT3 is a major mitochondrial deacetylase. One study indicated that AN (50 μg/mL) induced ROS generation and mediated ROS to stimulate SIRT3 expression and enzyme activity in HOKs, reporting that SIRT3 levels were elevated by AN in HOKs and reduced by *N*-acetylcysteine (10 mM) [43]. HOKs were exposed to AN component with or without inhibitors. AN-induced cytotoxicity was inhibited by catalase and enhanced by dicoumarol (an inhibitor of reductase), suggesting that AN component may contribute to the pathogenesis of OSF and oral cancer through the induction of aberrant differentiation, cytotoxicity, COX-2 expression, and PGE2 production [44]. Betel leaf and curcumin inhibited AN-induced inflammatory response; however, curcumin could not prevent AN-induced cytotoxicity [44].

In a randomized clinical trial conducted in India, Saran et al. reported the thrice-daily (TID) oral administration of lycopene (4 mg/TID) or curcumin (300 mg/TID) to 30 patients (in each group) with OSF for 3 months [45]. Their results revealed that the lycopene and

curcumin groups exhibited significant improvements in terms of ameliorating burning sensations and facilitating mouth opening [45]. In another randomized controlled clinical trial conducted in India, Piyush et al. reported the twice-daily (BID) oral administration of some substances, including lycopene (8 mg/BID), curcumin (300 mg/BID), and placebo (QD), in 30 patients (in each group) with OSF for 6 months and periodic follow-up of 9 months [46]. Their results indicated significant improvements in the lycopene and curcumin groups in terms of the amelioration of burning sensations, the facilitation of mouth opening and tongue protrusion, and increased cheek flexibility [46]. Table 1 presents the summary of update evidences and suggestions of clinical trials for prevention and treatment in betel quid-associated cancer.

4.3. Secondary prevention: risk prediction

One study of 128 patients with oral cancer in a cohort followed up for 13 years investigated somatic mutations and related genetic variants associated with the development and progression of oral cancer, in that patients who had *NOTCH1* somatic mutations had higher 5-year relapse-free recurrence and lower survival rates than other patients [47]. A *NOTCH1* genetic variant linked to somatic mutations was associated with higher OSCC incidence rates in conjunction with BQ chewing [47]. The findings indicated that BQ chewing was simultaneously associated with variants in *NOTCH1* and somatic mutations, suggesting that both mutants may potentially serve as early predictive and prognostic biomarkers for the occurrence and development of oral cancer.

Two studies have reported that genetic variants combined with BQ are highly predictive of oral cancer risk. One study recruited a total of 447 patients diagnosed with OSCC and 580 unrelated subjects, in that four-tag single-nucleotide polymorphisms (SNPs) significantly associated with OSCC occurrence were found in the genes *NOTCH1*, *BRCA1*, *COL9A1*, and *HSPA13* [48]. Genetic risk scores were calculated using the four tag SNP risk alleles, which showed an area under the receiver operating characteristic curve (AUROC) of 0.64, in that the AUROC was 0.91 for combined genetic risk scores and BQ chewing with a sensitivity of 88.6% and specificity of 86.7% [48]. A subsequent study reported that *FAT1* and *COL9A1* have high genetic scores for OSCC; the risk of OSCC was 0.10–0.43 without substance use and increased to 0.73–0.92 with substance use [49].

4.4. Secondary prevention: screening and early detection

Between 2000 and 2005, 7975 individuals using BQ and/or cigarettes were randomly assigned either to groups screened using toluidine blue (TBlue) or screened through visual examination [50]. After a 5-year follow-up, the incidence rates of oral cancer development were nonsignificantly different between the two groups [50]. From 2005 to 2010, 13,878 participants were enrolled in a visual screening study. Positive lesions were identified in 726 participants (5.2%), and 282 of those participants (2.1%) had confirmed oral cancer, in that the sensitivity and specificity of this study were 98.9% and 98.7%, respectively, and the study results confirmed the association between BQ use and oral cancer [51].

As mentioned, a cluster randomized controlled trial was conducted during 1996–2004 in Kerala, India [29]. After a 15-year follow-up, no significant reduction in oral incidence was noted, but a 24% reduction in oral cancer mortality was observed in users of pan-tobacco and/or alcohol in the intervention group after screening; a 38% reduction in oral cancer incidence and 81% reduction in oral cancer mortality in pan-tobacco and/or alcohol users were observed in the subgroup of patients who attended all four screening rounds [52]. The proportion of patients with confirmed stage I or II oral cancer increased from 24% after one screening round to 75% after four screening rounds [52].

Between 2004 and 2009, a total of 4,234,393 individuals older than 18 years in Taiwan (3,691,721 men and 542,672 women) with habitual cigarette smoking and/or BQ chewing were recruited as the eligible population for oral cancer screening, and 2,334,299 (55.1%) individuals participated in the first screening [53]. The participants were scheduled to receive visual inspection of the oral cavity by trained dentists or physicians. An average follow-up period of 4.5 years led to a 21% reduction in stage III or IV oral cancer diagnoses and a 26% reduction in oral cancer mortality, and the proportion of stage I or II cancer diagnoses increased from 40% to 47% [53].

In total, 27,717 patients with oral cancer enrolled in a population-based visual inspection program from 2004 to 2009 were followed up until the end of 2012 [54]. In the screening group, a greater number of cases of advanced-stage cancer were detected and diagnosed among patients with habitual BQ chewing; and among eligible high-risk screened patients, a significant mortality reduction of 15% was observed [54].

4.5. Tertiary prevention: chemoprevention

In a retrospective cohort study, 2334 patients with newly diagnosed oral cancer were followed up for over 5 years, in that the patients with different diseases taking selective COX-2 inhibitor (celecoxib)

Table 1
Summary of update evidences and suggestions of clinical trials for prevention and treatment in betel quid-associated cancer.

Study	Stage	Treatment	Effect
Saran et al., Indian [45]	**Primary prevention**	Thrice-daily oral administration of lycopene (4 mg/TID) or curcumin (300 mg/TID for OSF patients for 3 months.	Lycopene and curcumin groups exhibited significant improvements in terms of ameliorating burning sensations and facilitating mouth opening.
Piyush et al., India [46]	**Primary prevention**	Twice-daily oral administration of lycopene (8 mg/BID) or curcumin (300 mg/BID) for OSF patients for 6 months and periodic follow-up of 9 months.	Significant improvements were found in the lycopene and curcumin groups in terms of the amelioration of burning sensations, the facilitation of mouth opening and tongue protrusion, and increased cheek flexibility.
Nair et al. India [79]	**Tertiary prevention**	Erlotinib (150 mg/day), celecoxib (200 mg BID), the combination of erlotinib and celecoxib or observation alone for treatment-naïve operable oral cancer patients.	● Significantly higher 2-year survival was found in (erlotinib + combination) group (86%) than (celecoxib + control) group (60%). ● Preoperative targeted therapy with erlotinib and celecoxib combination can halt disease progression and downstage tumors, with a possible effect on survival.
Suggested double blind randomized controlled trial	**Primary prevention**	*N*-acetylcystein for chewers with betel use disorder (suggestion: 600 mg/day for at least 6 months).	**Expected**: decrease in the occurrence of oral cancer and OPMD in the early stage of carcinogenesis.
Suggested double blind randomized controlled trial	**Tertiary prevention**	Celecoxib for oral cancer patients with disease-free stage after surgery for oral cancer (suggestion: 200–400 mg/day for at least 6 months).	**Expected**: decrease in recurrence or mortality of oral cancer in the disease-free post-surgery stage for oral cancer.

Abbreviation: TID, thrice-daily; BID, twice-daily; OSF, oral squamous cells; OPMD, oral potentially malignant disorders.

medication for more than 5 years exhibited a significant reduction in oral cancer occurrence compared with those who did not receive celecoxib treatment [55]. A preclinical study suggested the mechanisms that celecoxib inhibited primary cultured BQ-related oral cancer cell growth, *EGFR* expression, and *NOTCH1* expression and inhibition of OSCC growth in murine xenograft model [55].

4.6. Tertiary prevention: prognosis and survival

A total of 162 patients with locally advanced head and neck squamous cell carcinoma (HNSCC) who received induction chemotherapy were analyzed for potential prognostic factors, in that the 2-year progression and overall survival rates were 37%/67% and 47%/71%, respectively, in patients with and without BQ-chewing history [56]. BQ-chewing history was independently associated with a poor response to induction chemotherapy, low progression-free survival rate, and low overall survival rate [56]. A total of 389 patients with oral cancer (78% with BQ use history) were analyzed, and the 5-year overall survival and disease-free survival rates in all patients were 61% and 49%, respectively [57]. Lymph node density, at a cutoff of 0.05, was an independent predictor of overall survival and disease-free survival, suggesting lymph node density is a reliable predictor of survival in BQ-chewing patients for postoperative adjuvant treatment, such as reoperation or adjuvant radiotherapy [57].

A study enrolled 25 patients from 2004 to 2008, among whom 60% were BQ users; 13 received first-line cetuximab plus chemotherapy, and 12 received second-line cetuximab with or without chemotherapy after the failure of cisplatin treatment [58]. No significant difference in median days with overall survival and time to progress to recurrent or metastatic HNSCC between the two groups was observed (the small sample size used in this study was noted as a limitation) [58].

4.7. Risk of bias

To prevent publication bias, we thoroughly screened the research articles collected from the PubMed and Web of Science databases at least twice. All articles were independently reviewed by two authors. The quality of the studies included in this review was evaluated in terms of study design, sample size, statistical method, and causality approach.

5. Discussion

5.1. BQ and other substance use and cancers

Studies have repeatedly confirmed the results reported by IARC, which demonstrated that chewing BQ increases the risk of oral, pharyngeal, and esophageal squamous cell carcinomas [2,59–63]. Among all types of BQ chewers, those using BQ with betel inflorescence and those who swallow areca fluid were at the greatest risk of the aforementioned cancers [59–62]. Although tobacco smoking and alcohol drinking multiplicatively modified the risk of oral and esophageal cancer [2,4,59,61], a large-scale study revealed that alcohol supra-additively modified the risk of BQ for oral, pharyngeal, and esophageal cancers [60]. Furthermore, studies have reported that the joint consumption of BQ, alcohol, and cigarette increased the risk of oral, pharyngeal, and esophageal cancers by at least 41-fold [4,59,62]. These findings revealed that the interplay of the use of BQ, alcohol, and tobacco, in conjunction with chewing habits may increase the cancer risk of anatomical sites of the upper aerodigestive tract.

5.2. BQ in affecting various hallmarks of cancer

Current evidences provide insight into primary, secondary, and tertiary preventive strategies based on the cancer hallmarks of natural course from BQ exposure to cancer progression. Arecoline was metabolized to arecoline *N*-oxide by human flavin–containing monooxygenases 3 [14], produced higher levels of ROS in human saliva [26]. In the proliferative and hyperplasia stage, arecoline *N*-oxide initiates a cascade of pathological changes after specific genetic interactions involving P53, NOTCH1, FAT1 and caspase-8 [37,38]. COX-2 levels were elevated in oral dysplastic lesions and in OSCC compared with oral

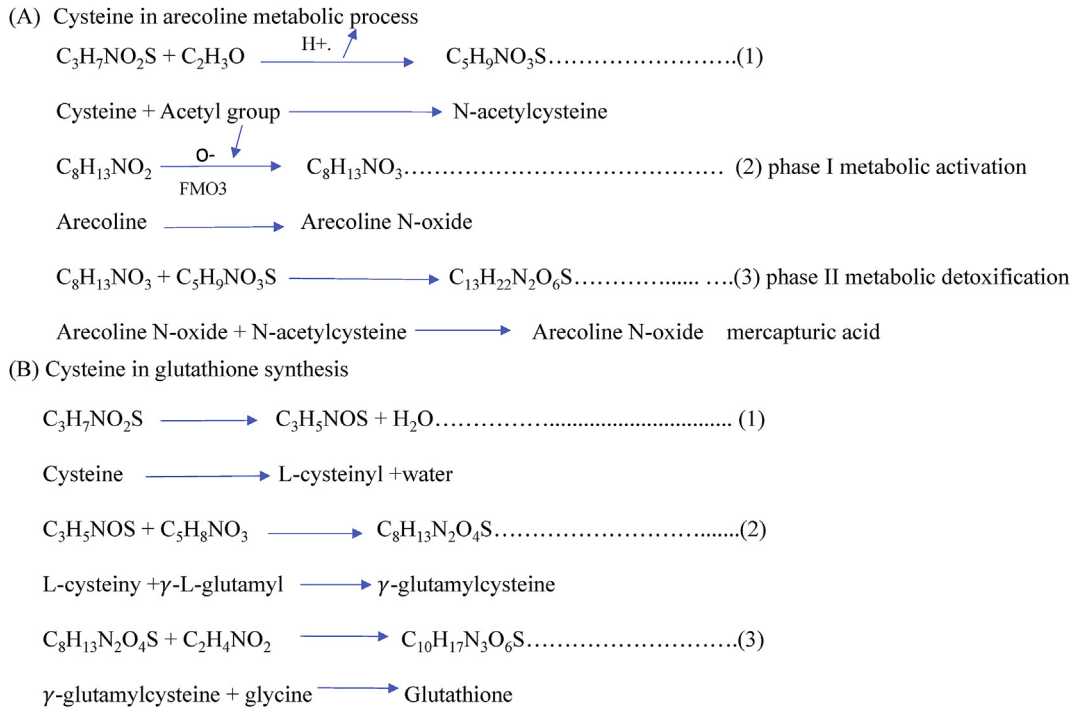

Fig. 2. Arecoline metabolic activation and detoxification on oral cells in the mouth via cysteine acetylation is hypothesized. Arecoline is oxidized into arecoline *N*-oxide in phase I metabolic activation and in phase II metabolic detoxification, arecoline N-oxide is conjugated and produces arecoline N-oxide mercapturic acid. Note: Cysteine involving in (A) arecoline metabolic process and (B) glutathione synthesis.

hyperplastic epithelium, suggesting that COX-2 is involved in the early stages of oral carcinogenesis [64]. The invasion and metastasis findings showed that COX-2 inhibitor is involved in the inhibition of EMT and cell mobility through blocking transcription factors (Slug, Snail and ZEB1), cytoplasmic mediators (focal adhesion kinase), vimentin and β-catenin), cell adhesion molecules (cadherins and integrins), and surface receptors (AMFR and EGFR) [55].

5.3. Primary prevention: clarification of carcinogen and carcinogenesis

AN metabolites were found in the urine (when provided by gavage and not chewing) in animal models, which indicated that AN was metabolized by the liver and kidneys. However, the IARC reported that sufficient evidence was not available to support the relationship of BQ use without tobacco with kidney, bladder, colorectal, and gastric cancers; furthermore, evidence of BQ use causing liver cancer was limited [3]. Therefore, research must focus on clarifying the AN metabolism and related carcinogenic processes in oral cells, which constitute the site of cancer initiation.

General chemical substances or xenobiotics in human cells are removed through a process of metabolic detoxification involving two distinct phases. Phase I is a metabolic phase involving enzymes that cause oxidation, reduction, and hydrolysis. Phase II involves conjugation of activated xenobiotic metabolites and their excretion. In phase I, arecoline is oxidized into arecoline *N*-oxide by FMO3, which controls the initiation of AN carcinogenesis [35–38]. In phase II, we hypothesize that arecoline *N*-oxide is conjugated with *N*-acetylcysteine through acetylation and produces arecoline *N*-oxide mercapturic acid, which is subsequently detoxified, indicating that it is likely noncarcinogenic.

5.4. Primary prevention: blocking pathway of carcinogenesis

N-acetylcysteine, glutathione, and cysteine effectively inhibit arecoline *N*-oxide induced-mutagenicity [35], and *N*-acetylcysteine inhibits arecoline-induced (40 μg/mL) cytotoxicity in normal oral keratinocytes and oral cancer cells [39,40]. One study reported that *N*-acetylcysteine demonstrated no obvious effect on AN-induced oral fibroblast contraction [42]. The disparity may be due to low-dose treatment with *N*-acetylcysteine. In fibroblast cell models, *N*-acetylcysteine was used at a dose of 3 mM [42], whereas the effect dose was 10 mM in other studies [39,40,43]. AN concentrations from 50 μg/mL to 800 g/mL were used for toxicological research. The dose range was wide, and the dose was small (800 μg/mL AN only contained 2–4 μg/mL arecoline) compared with the effective dose (40 μg/mL) in a study on arecoline. In fact, within several minutes of chewing BQ, AN was immediately metabolized into alkaloids and/or their metabolites, thus inducing toxicity in mouth.

Cysteine, found in human saliva, is also as a critical intermediate product during synthesis to glutathione [65]. Notably, it is also involved in phase II of the detoxification of arecoline *N*-oxide. We speculate that the underlying mechanism is that cysteine shifts to the synthesis of *N*-acetylcysteine and conjugates with arecoline *N*-oxide to form arecoline *N*-oxide mercapturic acid. Arecoline *N*-oxide in oral cells can be detoxified using high levels of *N*-acetylcysteine; this is a new strategy for chemoprevention of BQ-induced malignancy. If arecoline *N*-oxide is a carcinogen, then AN may be a promoter or procarcinogen, which would necessitate the reevaluation of its status as a carcinogen. The nontoxic mechanisms of arecoline *N*-oxide mercapturic acid must be determined. Fig. 2 illustrates our theory of arecoline metabolic processes. It outlines the acetylation that arecoline metabolizes into arecoline *N*-oxide, which is conjugated and produces arecoline *N*-oxide mercapturic acid through interaction with cysteine and *N*-acetylcysteine in the oral cavity.

BQ use is associated with OPMD, but not with OPMD malignant transformation. It is likely that BQ use disorder or dependent use play crucial roles in carcinogenesis [28,66,67]. Curcumin and lycopene appear to ameliorate symptoms of OSF but not cure the disease itself. A combination of surgery and therapy are probably required to block the malignant transformation and benefit patients. Curcumin is known to exhibit low bioavailability. Studies have reported the oral administration of a high dose of curcumin (900 mg/daily) over 3 months and of a lower dose (600 mg/daily) over 6 months for treating OSF [45,46].

5.5. Secondary prevention: risk prediction

Early prediction of BQ-associated cancer risk requires quantitative information regarding environmental and genetic factors to identify high-risk groups before oral cancer or OPMD occur. Epidemiological studies that involve comparisons as well as assessments of sensitivity, specificity, and AUROC have indicated that environmental factors have higher predictive ability than genetic susceptibility in cases of BQ-associated cancers [44]. A limited number of genetic variants are available for improving the predictability of OSCC occurrence risk [48,49]. One study indicated that somatic mutation-related variant in NOTCH1 is associated with oral cancer occurrence risk [47]. Highly predictive variants were found from referencing tumor somatic mutations, and AUROC analysis should be employed to produce more reliable results for estimating risk at the individual level [44].

5.6. Secondary prevention: screening and early detection

In a cluster randomized controlled screening study conducted in India [52], an 81% reduction in oral cancer mortality was observed in pan-tobacco and/or alcohol users who attended four screening rounds of visual inspection during a 15-year follow-up. In the short term, oral cancer incidence increased, and no significant changes were noted in the long term. In a screening study in Taiwan [53], a 26% reduction in oral cancer mortality in patients who chewed BQ and/or smoked cigarettes was observed during a 4.5-year follow-up through visual inspection. Findings regarding reductions in oral cancer incidence both in the short and long term were inconsistent with those of the study conducted in Indian, likely because participation was voluntarily in this large-scale practical program and self-awareness of risk was necessary. Nevertheless, both screening studies indicated that early detection of patients in stages I and II increased and led to a sustained reduction in oral cancer mortality; however, expecting a reduction in incidence was unreasonable unless the program increased the number of people who decided to quit chewing BQ.

Autofluorescence, chemiluminescence, and TBlue examination for screening of leukoplakia, erythroplakia, and dysplasia showed low sensitivity and specificity [68] and no effect on cancer incidence [50]. A study developed equipment specifically designed to detect OSCC in a mouse model [69]; however, doctors believed that they could detect the aforementioned conditions with very high sensitivity and specificity through visual inspection [51], laryngoscopy, or palpation, and that their experiences are more useful than an instrument [53]. Several studies have explored changes in protein levels among patients with OSCC [70], but cancer is already detectable by other means prior to any changes in protein levels. For early detection of OSCC, prediction and screening must focus on identifying high-risk groups before pathological changes occur or at the initial stages of pathological change. These general inspections take only a few minutes and do not require expensive equipment. Early detection of the primary tumor is a key factor for improving survival rates in patients with OSCC because most cases are currently detected during advanced cancer stages.

5.7. Tertiary prevention: prognosis and survival

Studies have shown that BQ use is an independent prognostic indicator and factor affecting recurrence and that quitting BQ use at any stage of OPMD and cancer development is necessary for prolonging survival [30,47,57,58]. MAO-A inhibitors have been suggested for

cessation therapy to quit BQ use [71,72]; however, additional clinical trial evidence is required.

A comparison of survival was reported between standard therapies (surgery, radiotherapy, and chemical drugs) and approved immunotherapy for late-stage patients with HNSCC. Cetuximab, an anti-EGFR monoclonal antibody, has been approved for use in combined treatment for patients with late-stage HNSCC, with the corresponding single-agent response rate being 10%–13% [73]. The survival period of patients treated with cetuximab is, on average, extended by 2–3 weeks (single therapy) to 10 months (combined therapy) [74].

Recently, HNSCC therapy has been conducted using immune checkpoint inhibitors. Nivolumab, an anti-programmed-death 1 (PD-1) monoclonal antibody, has been approved for treatment in patients with late-stage HNSCC on the basis of the results of the phase 3 CheckMate 141 study [75]. In this randomized, open-label, phase 3 trial, the median overall survival was 7.5 months in the nivolumab group and 5.1 months in the group that received standard therapy. Despite the modest therapeutic effect, overall survival was statistically significantly several months longer with nivolumab than with standard therapy.

The effect of nivolumab on human papillomavirus infection–associated oral cancer was assessed in subgroups [76]. Among patients with p16-positive tumors, the median overall survival was 9.1 months in the nivolumab group, which was significantly longer than the 4.4 months in the standard therapy group [76]. To date, no data are available regarding the effects of anti-EGFR and anti-PD-1 medications among subgroups of patients with BQ-associated cancers; anti-EGFR and anti-PD-1 medications could be considered treatment options for prolonging survival, particularly in patients with late-stage HNSCC.

5.8. Tertiary prevention: chemoprevention

Currently, the effects of several anti-inflammatory drugs, such as celecoxib, are being investigated and are undergoing clinical trials to determine their suitability for treating several types of human cancer. Celecoxib is used for anti-inflammation therapy in several diseases. Special application of celecoxib has been approved for use in patients with familial adenomatous polyposis [76]. Arecoline-induced COX-2 upregulation of human gingival fibroblasts was characterized [77], and an increase in COX-2 induction of oral keratinocytes following arecoline treatment was reported [78]. COX-2 levels were elevated in oral dysplastic lesions and in OSCC compared with oral hyperplastic epithelium, suggesting that COX-2 is involved in the early stages of oral carcinogenesis [64]. Sixty-four treatment-naïve operable patients were randomized into a four-arm window of opportunity study consisting of treatment with erlotinib (an anti-EGFR antibody), celecoxib, the combination of both, and observation alone, in that a significant difference was observed in the 2-year overall survival for celecoxib + control compared with erlotinib + combination groups (Table 1) [79]. Preoperative targeted therapy with erlotinib and celecoxib combination can halt disease progression and downstage tumors, with a possible effect on survival [79]. Therefore, combined treatment with celecoxib may be a potential chemopreventive option in the early stages to prevent the occurrence and progression of BQ-associated cancers.

Considering feasibility for long-term use, cost, and side effects, celecoxib may be suitable as a chemopreventive agent for patients in the disease-free stage after surgery or combined with anti-EGFR antibody drugs at the stage of exacerbation in BQ-associated cancer. A dose of 200–400 mg/daily for at least 6 months may be suitable guidelines for clinical trials (Table 1).

Curcumin dose-dependently inhibited arecoline-induced connective tissue growth factor protein expression in the buccal mucosal fibroblasts of OSF patients [80]. Curcumin and lycopene effectively ameliorated the symptoms of OSF [45,46]. Several clinical trials have been conducted to demonstrate the therapeutic effects of curcumin in the treatment of various diseases, including cancer; however, no randomized double-blind trial has been successful [81], possibly because of its low bioavailability and stability. If the bioavailability of curcumin is increased, it could nonetheless be considered an option for cotreatment as a potential anti-inflammatory supplement.

5.9. Clinical implications to be addressed for somatic mutations in BQ-associated cancer

In a study, which involved exome sequencing of tumor–normal pairs among 196 male patients with OSCC (95 BQ chewers and 101 non-BQ users), the authors explored the association between the sequences and BQ chewing [82]. Their results identified a BQ-associated mutational signature type and indicated mutagenic effects of BQ chewing in OSCC genomes [82]. The genetic landscape and mutational signatures of 15 cases of BQ-associated tongue cancer were compared with 82 cases of tongue cancer from the Cancer Genome Atlas, in that BQ chewers had a significantly higher frequency of mutation in *RASA1* than that of non-chewers [83].

The Catalogue of Somatic Mutations in Cancer (COSMIC) is an expert-curated database of somatic mutation mechanisms that cause human cancer that combines genome-wide sequencing results [84]. Data extracted from COSMIC regarding oral cancers revealed that the top five somatic tumor mutation rates were noted in *TERT* [85], *TP53* [38,86], *NOTCH1* [38,47,48,86], *FAT1* [38], and *CASP8* [37], which are reportedly related to BQ exposure. Ratios of oral cancer risk are 1.6- to 23-fold higher compared with all other cancers (Table 2). These findings provide insight regarding the use of personalized precise medicine to develop targeted drugs for treating patients with BQ-associated cancer.

6. Limitations

This review includes the effects of BQ-associated cancer. Fewer cases involving other known carcinogens related to oral cancer, such as tobacco, alcohol, and HIV infection, were included, although sometimes BQ, tobacco, and alcohol are all commonly used. Association studies have examined the relationships between genetic variants and oral cancer occurrence. Although numerous statistically significant findings in genetic susceptibility have been published, few studies have conducted analysis for sensitivity, specificity, and AUROC and were therefore omitted from this review because of the inability of their

Table 2
Top five tumor mutation rates in risk of betel quid associated-oral cancer compared with all other cancers.

Gene	Other cancer		Oral cancer		Oral vs. other cancer		Linking betel quid
	Mutation/Samples	Mutation rate (%)	Mutation/Samples	Mutation rate (%)	Rate ratios	(95% CI)	Reference
TP53	39541/146233	(27.04)	875/2058	(42.52)	1.57	(1.45–1.70)	[38,86]
FAT1	2017/48318	(4.17)	138/489	(28.22)	6.76	(5.57–8.21)	[38,48,49]
CASP8	491/50016	(0.98)	117/510	(22.94)	23.37	(18.76–29.11)	[37]
TERT	11564/88476	(13.07)	166/742	(22.37)	1.71	(1.45–2.03)	[85]
NOTCH1	4117/72955	(5.64)	162/812	(19.95)	3.54	(2.98–4.20)	[38,47,48,86]

Data extracts from catalogues of somatic mutations in cancer in 2018 was compared between oral cancer and other cancers.

Table 3
Summary of update evidence for prevention strategies in betel quid-associated cancer based on the natural course.

Stages	Questions raised	Findings	Key prevention strategies
Primary prevention			
1. Clarification of carcinogen and carcinogenesis	● Activation and detoxification of AN in the process of carcinogenesis? ● OPMD converted to malignant transformation?	● AN was a promoter; arecoline presented low mutagenicity ● Arecoline *N*-oxide controlled initiation of AN carcinogenesis ● OPMD, inflammation and ROS may involve in carcinogenesis	● Arecoline *N*-oxide was likely an initiator in carcinogenesis ● Arecoline *N*-oxide carcinogenesis evidences in mouth ● Arecoline *N*-oxide mercapturic acid nontoxic mechanism
2. Blocking pathway of carcinogenesis	● No pharmacology basis for BQ cessation therapy ● Limited effectiveness of counseling quitting programs	● ROS was found in oral cancer cells ● Antioxidant improved symptoms of OPMD ● Arecoline *N*-oxide was detoxified by *N*-acetylcysteine and glutathione	● *N*-acetylcysteine, glutathione, cysteine used for arecoline N-oxide toxicity to block carcinogenesis ● Antioxidants with surgery to be benefit for OPMD patients
Secondary prevention:			
1. Risk	● Can high risk group be predicted for betel quid-related cancers?	● Genetic factors have limited contribution	● Improvement of predictability by sensitivity, specificity and AUROC
2. Screening	● Can screening assist to find early stage of oral cancer and OPMD?	● Screening found oral cancer early stage in BQ and/or tobacco users	● Screening in high risk individuals for early detection
Tertiary prevention:			
1. Chemoprevention	● Have potential drugs that can be used for chemoprevention?	● Anti-inflammatory medications have been reported	● Anti-inflammatory agents used in early or disease-free stage
2. Prognosis and survival	● Has FDA approved new target drugs?	● Cetuximab and PD-1 used in later-stage prolonging short survival ● BQ was a prognostic factor in oral cancer mortality	● Stop BQ and/or tobacco use ● Combined use with anti-inflammatory agents ● Tumor somatic mutations in BQ-associated cancer to be addressed

Abbreviations: AN: areca nut; BQ: betel quid; OPMD: oral potential malignancy disorder; ROS: reactive oxygen species; AUROC: area under the receiver operating characteristic curve; FDA: food and drug administration.

findings to be applied in clinical practice.

7. Conclusion

The IARC determined that tobacco-free BQ/AN exposure is closely associated with OSF and is independently associated with cancers of the oral cavity, hypopharynx, oropharynx, and esophagus. Studies have provided evidence of BQ-associated cancers and strategies for their prevention through an understanding of the process from BQ exposure to the occurrence of oral premalignancy and cancer. A summary of questions, findings, and BQ-associated cancer prevention strategies is presented in Table 3. BQ/AN-derived arecoline N-oxide is probably an initiator of carcinogenesis that initiates a cascade of pathological changes after specific genetic interactions involving P53, NOTCH1, and FAT1. ROS and OPMD are also considered to play crucial roles in carcinogenesis. However, studies have found that BQ/AN-derived MNPN and nitrosamine exhibit negligible or no mutagenesis and carcinogenic activity [2,14]. As its primary prevention, *N*-acetylcysteine may be used to reduce the toxicity of arecoline *N*-oxide and prevent the occurrence of BQ-related cancers [39,40]. Antioxidant or anti-inflammatory dietary supplements, such as lycopene and curcumin, may be used for OPMD and ROS treatment. The secondary prevention strategy is early detection of oral premalignant and malignant cancers in high-risk groups. Screening activities by trained physicians or dentists and specific genetic testing may be conducted in tandem. However, the availability of genetic biomarkers that accurately predict the risk of BQ-associated cancers remains limited. The objective of tertiary prevention is to increase survival rates in patients with cancer. Anti-EGFR and anti-PD-1 are target drugs that have been approved by the Food and Drug Administration for use in the treatment of late-stage HNSCC, but they are prohibitively expensive. The use of combinations of anti-inflammatory medications in the early stages to prevent cancer development may be a cost-effective treatment option, but their clinical use for somatic mutations in BQ-associated cancer must be addressed. In preoperative targeted therapy, treatment with a combination of erlotinib and celecoxib was reported to significantly affect disease progression and downstage tumors. On the basis of our review, we suggest that future studies investigate the association between AN-derived metabolites in the oral cavity, tumor somatic mutations, and survival in patients with cancer who chew BQ and that the studies focus on comprehensively creating clinical practice guidelines for the prevention, early detection, and treatment of BQ-associated cancers.

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Author contributions

Study conception and design: AMS Ko, YC Ko. Acquisition, analysis or interpretation data: AMS Ko, CH Lee. Drafting the manuscript: AMS Ko, CH Lee, YC Ko. All authors approved the final manuscript for submission and final approval of the version to be published.

Declaration of competing interest

The authors declare no potential conflicts of interest.

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