A Comprehensive Analysis of the Role of Oxidative Stress in the Pathogenesis and Chemoprevention of Oral Submucous Fibrosis

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Abstract: Oral submucous fibrosis (OSMF) is a chronic oral potentially malignant disorder (OPMD). It is described as a scarring disease of the oral mucosa associated with excess oxidants and insufficient antioxidants. While it is becoming increasingly accepted that oxidative stress results in excessive accumulation of collagen and progressive fibrosis of the submucosal tissues, there is limited data regarding the moderation of oxidative stress to initiate or prevent OSMF. To assess the scope for mechanism-based approaches to prevent or reverse OSMF, we systematically evaluated the existing literature and investigated the role of oxidative stress in the pathogenesis and chemoprevention of OSMF. A search for relevant articles on PubMed and Scopus was undertaken using pre-defined inclusion and exclusion criteria. A total of 78 articles were selected in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines. The articles eligible for assessment investigated both OSMF and/or oxidative stress biomarkers or specific antioxidants. Both in vitro and human studies consistently demonstrated variations in oxidative stress biomarker levels in OSMF and revealed an increase in oxidative stress, paralleling the development of the disease. Furthermore, the use of antioxidant supplements was overall associated with an improvement in clinical outcomes. Having identified the significance of oxidative stress in OSMF and the therapeutic potential of antioxidant supplements, this scoping review highlights the need for further well-designed studies in the development of mechanism-based interventions for managing OSMF.

Keywords: oral submucous fibrosis; oxidative stress; reactive oxygen species; antioxidant supplements

1. Introduction

Oral submucous fibrosis (OSMF) is a chronic and subtle oral potentially malignant disorder (OPMD) affecting the oral cavity and oropharynx, in mainly the South-Asian populations [1]. Clinically, signs and symptoms of OSMF include but are not limited to, trismus, restricted tongue protrusion, burning mouth, marble-like appearance of the oral mucosa, xerostomia, recurrent ulceration, tongue papillae atrophy, and presence of
palpable fibrous bands [2]. These oral complications are fundamentally due to a juxtaepithelial inflammatory reactions leading to fibroelastic changes of the lamina propria, and subsequently to the stiffness of the oral mucosa, which significantly affects patients’ quality of life by reducing their ability to eat and speak.

Although OSMF is multifactorial in origin, there is overwhelming evidence that long-term areca-nut chewing (alone or in a mixed package known also as betel quid (BQ)), is considered the main etiological factor. Constituents of BQ have in fact been shown to generate substantial amounts of reactive oxygen species (ROS) [3], which may create a biological imbalance between oxidants and antioxidants [4], playing a significant role in OSMF pathogenesis through an excessive accumulation of free radicals and production of lipid peroxides (LPO) [5,6]. BQ chewing is a time-honoured tradition for 10–20% of the world’s population [7]; therefore, attempting to eradicate a habit that has been passed down through countless generations may not be realistic in the short term. Rather than imposing a change in culture and way of life, a novel approach may be to elucidate ways to transpose oxidative stress pathways in favour of antioxidants, to prevent or reverse OSMF. While oxidative stress in OSMF unequivocally results in excessive collagen accumulation and marked fibrosis, a comprehensive review of the role of oxidative stress and antioxidant pathways in initiating and preventing OSMF is yet to be considered.

The aim of this study was to evaluate the existing literature systematically to assess the role of oxidative stress in OSMF. It was concluded that excess oxidants may drive the pathogenesis, while an excess of antioxidants may play a fundamental role in the chemoprevention of OSMF and the potential reversal of its debilitating effects.

2. Materials and Methods
2.1. Protocol and Search Strategy

This scoping review was done according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines. A search for relevant articles on Pubmed and Scopus published up to June 2021 was completed using the search string: ‘(“oral submucous fibrosis” OR betel OR “piper betel” OR “areca nut” OR gutka OR paan OR “pan masala” OR “slaked lime”) AND (“Reactive Oxygen Species” OR “oxidat*” OR “free radicals” OR “ROS” OR “Superoxide Dismutase” OR “Catalase” OR “Glutathione peroxidase” OR “antiox*” OR “Lipid Peroxides”).

2.2. Inclusion and Exclusion Criteria

Inclusion criteria included studies with OSMF human subjects, in vivo or in vitro models of OSMF, as well as studies assessing oxidative stress biomarkers or molecules or using antioxidants in models of fibrosis. Exclusion criteria included non-English language and non-peer-reviewed studies, systematic reviews, meta-analyses, and books/book chapters.

2.3. Study Selection and Data Extraction

The selection process involved three stages (Figure 1); the search string was imputed into databases, inclusion and exclusion criteria were applied, and duplicates were manually removed; then, papers obtained from stage 1 were screened based on titles and abstracts according to the inclusion and exclusion criteria under the guidance of the senior author (NC); full texts were analysed according to the inclusion and exclusion criteria. During all the stages, manuscripts that held any uncertainty regarding their relevance to aims of this scoping review were brought to the senior author (NC) and discussed collegially to come to a final decision.

Data extracted from each article were the name of the first author, publication year, study type (in vitro, in vivo, human), PICO, oxidative stress biomarkers (if present), study design or model, assay methodology, details of treatment/intervention, if any.
3. Results

3.1. Overview of the Search Process

Details of the selection process are shown in Figure 1. A total of 549 and 489 (n = 1038) articles were retrieved from Scopus and PubMed, respectively. After removing 300 duplicates, the datasets were combined, giving a total of 738 manuscripts. A further 614 papers were excluded due to inconsistent titles and abstracts, leaving a total of 124 articles. After reading the full text, a further 45 manuscripts were excluded. The remaining 78 articles were deemed to be eligible for detailed assessment, of which 52 were laboratory-based studies (Table 1), 27 were clinical studies (Table 2), and 1 was a combination of the above-mentioned ones. There was often an overlap between clinical and laboratory-based studies, and these were included in the category that best represented the study. For example, the assessment of glutathione in a patient’s serum or saliva in the absence of clinical outcome measures was classified as a laboratory-based study.
Table 1. Overview of laboratory-based studies that were eligible for analysis.

| Author/s, Year | Relevant Biomarker(s) | OSMF Samples or Model | Intervention | Control/Comparison | Study Type |
|----------------|-----------------------|-----------------------|--------------|--------------------|------------|
| Aggarwal et al., 2011 | beta-carotene | blood samples from OSMF patients | — | age- and sex-matched controls | biochemical |
| Anuradha and Devi, 1995 | haemoglobin; ceruloplasmin; iron; copper; zinc | blood samples from OSMF patients; staged | — | age- and sex-matched healthy controls | biochemical |
| Avinash et al., 2014 | MDA; SOD | blood samples from OSMF patients | — | healthy subjects | biochemical |
| Bathi et al., 2009 | GSH; ceruloplasmin; MDA; GSTM1; GSTT1 | blood samples from OSMF patients | — | age-, sex-, and SES-matched controls | biochemical |
| Bale et al., 2017 | SOD2, Catalase, GLRX2, GSH, GPx, TXN2 | mitochondria purified samples taken from OSMF patients | — | mitochondrial antioxidants in healthy controls and OPMD | biochemical |
| Banerjee et al., 2020 | SOD2, Catalase, GLRX2, GSH, GPx, TXN2 | mitochondria purified samples taken from OSMF patients | — | mitochondrial antioxidants in healthy controls and OPMD | biochemical |
| Chang et al., 2001 | GSH; H$_2$O$_2$; mitochondrial membrane potential | oral OSF fibroblasts and oral KB epithelial cells treated with AN extracts and arecoline | — | baseline data (untreated control) | cell culture |
| Chang et al., 2002 | unscheduled DNA synthesis | gingival keratinocytes treated with AN extracts | Vit C, GSH, NAC, Deferoxamine | untreated control, other treatments | cell culture |
| Chang et al., 2013 | TGFβ1-induced CCN2 synthesis | buccal mucosal fibroblasts | EGCG; JNK, p38 MAPK, and ALK5 inhibitors | untreated control | cell culture |
| Chang et al., 2014 | PGE2, COX-2, CYP1A1, HO-1 | gingival keratinocytes exposed to AN extracts | piper betle leaf (PBL) extract, hydroxychavicol, dicoumarol, curcumin | untreated control, other treatments | cell culture |
| Chang et al., 2016 | 8-isoprostane, IL-1α; ADAM 17; PGE2; COX2 | primary human gingival keratinocytes treated with AN extracts and arecoline | α-naphthoflavone, aspirin, catalase; MEK, JAK, and Src inhibitors | untreated control, other treatments | cell culture |
| Chitra et al., 2012 | LPO; conjugated dienes; HO; SOD; H$_2$O$_2$; copper; calcium; magnesium; potassium; iron | saliva samples from OSMF patients | — | age- and sex-matched healthy controls | biochemical |
| Deng et al., 2009 | CCN2 | OSMF tissue samples; normal buccal mucosal fibroblasts treated with arecoline | NAC, curcumin | normal oral mucosa; untreated control | IHC, cell culture |
Table 1. Cont.

| Author/s, Year | Relevant Biomarker (s) | OSMF Samples or Model | Intervention | Control/Comparison | Study Type |
|----------------|------------------------|-----------------------|--------------|--------------------|------------|
| Divyambika et al., 2018 | LPO; GSH; SOD; GPx; Vit A, C and E | saliva samples from OSMF patients | — | age- and sex-matched healthy controls | biochemical |
| Francis et al., 2019 | — | OSMF cell lines | lycopen; quercetin | — | cell culture |
| Gupta et al., 2004 | MDA; ROS | blood samples from OSMF patients; graded | — | healthy controls | biochemical |
| Gurudath et al., 2012 | SOD, GPx | blood samples from OSMF patients | — | age- and sex-matched healthy subjects | biochemical |
| Guruprasad et al., 2014 | Vitamin C; iron | blood samples from OSMF patients | — | healthy patients | biochemical |
| Hou et al., 2017 | Cyclophilin A (CYP A) via 2D gel electrophoresis/mass spectrometry | tissue biopsy from OSMF patients | — | normal mucosal tissue | biochemical |
| Hsieh et al., 2015 | Egr1 | OSMF tissues; buccal mucosal fibroblasts treated with arecoline | NAC; EGCG; JNK, ERK inhibitors | untreated control | IHC, cell culture |
| Hsieh et al., 2017 | Egr1, COL1A1, COL1A2 | buccal mucosa fibroblast cultures stimulated with TGF-β | EGCG; ERK, JNK, p38 MAPK, ALK5, inhibitors | untreated control | cell culture |
| Hsieh et al., 2018 | TGFβ; ROS; CCN2, Egr-1 | human buccal mucosal fibroblasts treated with arecoline | EGCG; TGFβ inhibitor; antioxidant | untreated control | cell culture |
| Illeperuma et al., 2015 | ROS; GRO-α, IL-6, IL-8; DNA double strand breaks, 8-oxoG | OSMF tissues; immortalised human normal oral keratinocytes and AN-exposed fibroblasts | antioxidants; NOX1 and 4 silencing | normal oral mucosa; untreated controls | IHC, cell culture |
| Jeng et al., 2004 | mitochondrial membrane potential depolarization; GSH; ROS | oral KB epithelial cells treated with hydroxychavicol | NAC, SOD, catalase | untreated and DMSO-treated cells | cell culture |
| Jeng et al., 1994a | GSH, ATP, xanthine oxidase | normal oral mucosal fibroblasts treated with eugenol | — | untreated control | cell culture |
| Jeng et al., 1994b | DNA strand break | oral mucosal fibroblasts incubated with different BQ constituents | GSH, cysteine, mannitol, catalase, SOD | untreated controls | cell culture |
| Kapgate et al., 2020 | MDA | blood samples from OSMF patients | turmeric | healthy subjects | biochemical |
Table 1. Cont.

| Author/s, Year | Relevant Biomarker(s) | OSMF Samples or Model | Intervention | Control/Comparison | Study Type          |
|---------------|------------------------|-----------------------|-------------|--------------------|---------------------|
| Khan et al., 2015 | ROS, catalase activity | human keratinocytes and gingival fibroblasts treated with arecoline and AN extracts | Cu; GSH, SOD, NAC | untreated controls | cell culture        |
| Khanna et al., 2013 | copper; zinc; selenium and molybdenum | blood samples from OSMF patients | — | healthy subjects and OSCC patients | biochemical         |
| Kim et al., 2020 | Gro-α, IL-6, IL-8; EMT | HPV16 E6/E7-transfected immortalised human oral keratinocytes (IHOK) | EGCG; GSH; NAC | — | cell culture        |
| Kulasekaran et al., 2020 | 8-OHdG | OSMF tissues (very early, early, moderately advanced, and advanced) | — | normal buccal mucosa | IHC                |
| Lee et al., 2016 | ROS | OSMF buccal mucosa biopsy sample; normal oral fibroblasts | GSH; NAC; EGCG | normal buccal mucosa | IHC; cell culture   |
| Li et al., 2019 | ROS; PERK; collagen; glutathione | OSMF tissues; HHUVECs treated with arecoline; OSMF mouse model | verteporfin | normal buccal mucosa; untreated controls | IHC, cell culture, mouse model |
| Madhulatha et al., 2018 | glutathione GSTM1, GSTT1 | blood samples from OSMF patients | — | healthy subjects | biochemical         |
| Meera et al., 2020 | 8-isoprostane | blood and saliva samples from OSMF patients | — | OSCC and control patients | biochemical         |
| Nair et al., 1992 | ROS, DNA damage | Syrian golden hamsters exposed to various BQ components; OSMF patients | — | Untreated controls (no atropine); healthy subjects | Animal model, ICC    |
| Nandakumaar et al., 2020 | 8-OHdG | saliva samples from OSMF patients | — | age- and sex-matched healthy controls, OSCC patients | biochemical         |
| Pant et al., 2016 | TGF-β signaling (ATF2; pJNK); ROS | HaCaT and HPL1D epithelial cell lines exposed to AN extracts; OSMF tissues | — | normal buccal mucosa; untreated controls | cell culture, IHC    |
| Paulose et al., 2016 | MDA | blood samples from OSMF patients | — | age- and gender-matched healthy individuals | biochemical         |
| Pitiyage et al., 2012 | TIMP-1; TIMP-2 | early and advanced OSMF tissues; OSMF fibroblasts | — | age-matched healthy controls and paan users | cell culture, IHC    |
| Rai et al., 2010 | MDA, 8-OHdG | blood and saliva samples from OSMF patients | curcumin (1 g) | healthy patients | comparative, biochemical |
| Author/s, Year | Relevant Biomarker(s) | OSMF Samples or Model | Intervention | Control/Comparison | Study Type |
|---------------|----------------------|-----------------------|--------------|--------------------|------------|
| Rai et al., 2019 | 8-OHdG; 8-epi-PGF2α; Protein carbonyl | blood samples from OSMF patients | — | serum sample of healthy patients | biochemical |
| Rathod et al., 2018 | beta-carotene | blood samples from OSMF patients | — | age- and gender-matched control | biochemical |
| Sadaksharam, 2018 | NO; SOD | OSMF and OSCC patients | — | healthy controls | biochemical |
| Senghore et al., 2018 | 8-OHdG; 8-isoprostan | blood samples from OPMD male patients | — | plasma 8-OHdG and 8-isoprostan levels in OL | biochemical |
| Shah et al., 2017 | ceruloplasmin | blood samples from OSMF patients | — | blood samples from healthy controls | biochemical |
| Shakuntala et al., 2015 | MDA; antioxidant activity | OSMF patients | — | — | biochemical |
| Singh et al., 2015 | COX-2 | OSMF tissues, fibroblasts from OSMF, and normal oral fibroblasts treated with arecoline | — | healthy subjects; untreated controls | cell culture, IHC |
| Thangjam and Kondaiah, 2009 | heme oxygenase-1; ferritin light chain; G6PDH; GCLC; GSH; IL-1α; p38 MAPK | human keratinocyte cells (HaCaT cell line) treated with arecoline | — | untreated controls | cell culture |
| Tsai et al., 2009 | Heme Oxygenase-1 | OSMF tissues; fibroblasts from OSMF and normal oral fibroblasts treated with arecoline | — | normal oral tissues; untreated controls | cell culture |
| Yadav et al., 2020 | uric acid | blood samples from OSMF patients | — | healthy controls, leukoplakia, and OSCC | biochemical |
| You et al., 2019 | AT1R; Mas1; NOX4; IL-1β; α-SMA; collagen type 1; CCN2; NLRP3; ATIR; ACE; ACE2; H2O2 | OSMF tissues; animal model of OSMF in ALL Sprague-Dawley rats; normal oral fibroblasts treated with arecoline | VE0991 | normal oral tissues; positive and negative controls | cell culture |

—, not applicable; α-SMA, Smooth Muscle alpha-Actin; 8-epi-PGF2α, 8-epi-Prostaglandin F2alpha; 8-OHdG, 8-Hydroxydeoxyguanosine; ACE, Angiotensin-Converting Enzyme; ADAMs, A Disintegrin And Metalloproteinase; ALK, Activin Receptor-Like Kinase; AN, Area Nut; AOA, Aminooxyacetic Acid; AT1R, Angiotensin II Receptor Type 1; ATF2, Activating Transcription Factor 2; CCN2, Connective Tissue Growth Factor; COX-2, Cyclooxygenase-2; CYP1A1, cytochrome P450 1A1; EGCC, Epigallocatechin-3-Gallate; Eg1, Early Growth Response 1; ERK, Extracellular Signal-Regulated Kinase; G6PDH, Glucose 6 Phosphate Dehydrogenase; GCLC, Glutamate-Cysteine Ligase Catalytic subunit; GLR2X, Glutaredoxin 2; GSH, Glutathione; GSH Peroxidase; GPx; HO-1, hemeoxygenase-1; H2O2, Hydrogen Peroxide; HHUVECs, Human Umbilical Vein Endothelial Cells; IHOK, Immortalized Human Oral Keratinocytes; ICC, immunocitochemistry; IL, interleukin; JNK, c-Jun NH2-terminal Kinase; KGM-SFM, Keratinocyte Growth Medium; LPO, Lipid Peroxide; MAPK, Mitogen-Activated Protein Kinase; MDA, Malondialdehyde; NAC, N-Acetyl-L-Cysteine; NO, Nitric Oxide; NOX; NADPH Oxidase; OI, Oral Leukoplakia; OLP, Oral Lichen Planus; OPMD, Oral Potentially Malignant Disorders oculopharyngeal muscular dystrophy; OSCC, Oral Squamous Cell Carcinoma; OSMF, Oral Submucous Fibrosis; ROS-PERK, Reactive Oxygen Species—Protein Kinase RNA-like Endoplasmic Reticulum Kinase; SOD—Superoxide Dismutase; TGFβ, Transforming growth factor beta; TIMP, Tissue Inhibitor Matrix Metalloproteinases; TXN2, Thioredoxin 2. * Gupta et al. (2004) is a dual laboratory-based and clinical study.
| Author/s, Year           | Antioxidant(s)                                                                 | Clinical Parameters                                      | Control/Comparison                                                                 | Study Type |
|-------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------------------------------------|------------|
| Anuradha et al., 2017   | systemic (juice) and topical (gel) aloe vera                                  | BS; CF; MO; TP                                           | hydrocortisone; hyaluronidase; antioxidant supplements                        | RCT        |
| Arakeri et al., 2020    | lycopene (4 mg/day for 3 months)                                              | BS; MO                                                  | placebo capsule                                                              | RCT        |
| Baptist et al., 2016    | rebamipide (100 mg t.i.d. for 21 days)                                        | BS                                                       | betamethasone (4 mg/mL biweekly for 4 weeks)                                 | RCT        |
| Goel and Ahmed., 2015   | lycopene capsules (2 mg, b.i.d. for 6 months)                                 | MO                                                      | no treatment; betamethasone (4 mg/mL) diluted in 1 mL of 2% xylocaine (biweekly for 6 months) | RCT        |
| Gowda et al., 2011      | lycopene capsules with zinc, selenium, and phytonutrients (2000 µg, b.i.d. for 3–6 months) | BS; MO; healing of ulcers; mucosal color/texture       | baseline (before treatment); non placebo-controlled                         | RCT        |
| Gupta et al., 2004 *    | beta-carotene (50 mg); Vit A palmitate (2500 IU); Vit E acetate (10 IU); Vit C; zinc manganese; copper | MO; TP                                                  | age- and sex-matched healthy controls; baseline (before treatment)            | RCT        |
| Jiang et al., 2015      | allicin (1 mg TCM-046, 99% HPLC intralesional injection)                       | MO, BS                                                  | trimcinolone acetonide (intralesional injection)                              | RCT        |
| Johny et al., 2019      | lycopene (8 mg b.i.d for 3 months); lycopene (8 mg b.i.d for 3 months)/hyaluronidase (1500 IU twice/week for 3 months) | MO                                                      | placebo capsules                                                              | RCT        |
| Kalkur et al., 2014     | Pentoxifylline (400 mg/day)                                                   | BS, speech                                              | standard antioxidants                                                        | RCT        |
| Kapoor et al., 2019     | curcumin (400 mg/day for 3 months)                                            | pain, MO                                                | —                                                                            | RCT        |
| Karemore et al., 2012   | lycopene (4 mg b.i.d for 3 months)                                            | MO                                                      | placebo capsule                                                              | RCT        |
| Kholakiya et al., 2020  | pentoxifylline (400 mg t.i.d for 3 months)                                   | MO, BS, malignant transformation, relapse              | —                                                                            | retrospective |
| Kumar et al., 2007      | lycopene (8 mg b.i.d for 6 months); curcumin (300 mg b.i.d for 6 months)     | MO, BS                                                  | placebo capsule                                                              | RCT        |
| Patil et al., 2014      | oxitard (2 capsules/day); topical aloe vera (5 mg t.i.d for 3 months)        | MO, TP, swallowing, speech, pain                        | —                                                                            | RCT        |
| Patil et al., 2015a      | xitard (2 capsules b.i.d for 3 months)                                        | MO, TP, BS, pain, swallowing, speech                    | placebo capsules                                                              | RCT        |
Table 2. Cont.

| Author/s, Year   | Antioxidant(s)                                                                 | Clinical Parameters                  | Control/Comparison | Study Type |
|------------------|-------------------------------------------------------------------------------|--------------------------------------|--------------------|------------|
| Patil et al., 2015b | spirulina (500 mg/day for 3 months); topical aloe vera (5 mg t.i.d for 3 months) | MO, BS, pain, ulcers/erosions/vesicles | —                  | RCT        |
| Patil et al., 2018 | oxtard (2 capsules t.i.d for 3 months); lycopene (8 mg/day for 3 months)      | MO, TP, swallowing, speech, pain, BS  | —                  | RCT        |
| Pipalia et al., 2016 | turmeric (400 mg)/black pepper (100 mg) (2 capsules t.i.d for 3 months); nigella sativa (2 × 500 mg capsules t.i.d for 3 months) | MO, BS, CF, TP                       | —                  | RCT        |
| Piyush et al., 2019 | curcumin (300 mg b.i.d for 6 months); lycopene (8 mg b.i.d for 6 months)     | MO, BS, TP, CF                       | placebo capsules   | RCT        |
| Rajbhoj et al., 202 | aloe vera (~5 mg/day); curcumin gel (~5 mg/day)                              | MO, BS                               | —                  | RCT        |
| Rao PK, 2010       | alpha lipoic acid (once/day for 3 months) and betamethasone (1 mL) and hyaluronidase (1500 IU); once/week for 12 weeks | MO, BS                               | betamethasone (1 mL) and hyaluronidase (1500 IU); once/week for 12 weeks | RCT        |
| Saran et al., 2018 | lycopene (4 mg/day for 3 months); curcumin (300 mg t.i.d for 3 months)       | MO, BS                               | —                  | RCT        |
| Shetty et al., 2013 | Spirulina (500 mg b.i.d)                                                       | MO, BS                               | placebo capsules   | RCT        |
| Singh et al., 2016 | topical aloe vera (~5 mg t.i.d for 3 months)                                  | BS, MO, CF, TP                       | standard antioxidant capsule | RCT        |
| Sudarshan, 2012    | curcumin (2 × 300 mg/day for 3 months)                                        | BS, MO, TP                           | dexamethasone (4 mg) and hyaluronidase (1500 IU) | RCT        |

—, not applicable; 8-OHdG, 8-Hydroxydeoxyguanosine; µg, microgram; b.i.d., bis in die; BS, Burning Sensation; CF, Cheek Flexibility; g, grams; GPx, Glutathione Peroxidase; GSH, reduced Glutathione; GSTM1, Glutathione S-transferase Mu 1; GSTT1, Glutathione S-transferase Theta 1; H₂O₂, HPLC, High-Performance Liquid Chromatography; Hydrogen Peroxide; HO, Hydroxyl radicals; IU, international units; LPO, Lipid Peroxides; MDA, Malondialdehyde; mg, milligrams; ml, milliliter; MO, Mouth Opening; RCT, Randomised Controlled Trial; ROS, Reactive Oxygen Species; SES, Socioeconomic Status; SOD, Superoxide Dismutase; t.i.d., ter in die; TP, Tongue Protrusion; Vit, Vitamin. *Gupta et al. (2004) is a dual laboratory-based and clinical study.
3.2. Laboratory-Based Studies

Of the 51 laboratory-based studies and 1 dual study [8] included in this scoping review, 14 (26.9%) investigated the pathogenesis of OSMF, 13 (25%) demonstrated the significance of oxidative stress in the pathogenesis of OSMF, 7 (13.5%) investigated other biomarkers present in OSMF, 9 (17.3%) investigated antioxidant enzyme status in OSMF patients versus healthy controls, and ultimately 9 (17.3%) examined potential therapeutics for OSMF.

3.2.1. Pathogenesis of OSMF (Role of Betel Nut/Arecoline, Copper, and Eugenol)

Areca nut extracts (ANE) have been shown to have cytotoxic effects on hamster cheek pouch [3], normal mucosal cells [9,10], and gingival keratinocytes [11,12]. Consumption of BQ has been suggested to be associated with OSMF in oral epithelial cells [13,14], normal buccal mucosa fibroblasts (BMFs) [15], keratinocytes [16], gingival fibroblasts [17], HaCaT and HPL1D epithelial cell lines [18], and human umbilical vein endothelial cells [19]. Furthermore, copper has been shown to enhance the cytotoxicity of arecoline on epithelial cells [20], while eugenol is involved in the pathogenesis of OSMF in oral mucosal fibroblasts [21].

3.2.2. Oxidative Stress Biomarkers in the Pathogenesis of OSMF

Arecoline induces ROS generation and cell cycle arrest in human keratinocytes [22]. Compared to healthy subjects, OSMF patients had elevated levels of serum malondialdehyde (MDA) [8,23–26], copper [27,28], LPO, ceruloplasmin [28,29], nitric oxide [30], calcium, magnesium, potassium, iron, conjugated dienes, and hydroxyl radicals [28]; as well as elevated levels of salivary 8-Hydroxy-2′-deoxyguanosine (8-OHdG) [31], 8-isoprostane [32,33], lactoperoxidase, and total protein [34]. Conversely, OSMF patients had relatively decreased levels of hydrogen peroxide (H$_2$O$_2$) and sodium, compared to healthy controls [28].

On the other hand, there are conflicting results from the nine studies (17.3%) comparing antioxidant enzyme status in OSMF patients versus their healthy counterparts. Of these, three found decreased levels [28,35,36], while six found increased levels [8,23,34,37–39] of antioxidant enzymes. Banerjee and colleagues (2020) [40] evaluated mitochondrial antioxidant levels and found higher GSH and peroxiredoxins 3 levels, as well as lower glutaredoxin 2 and catalase levels. Five articles (55.5%) found more significant fluctuations with higher OSMF clinical grading and staging. Specifically, Sadaksharm (2018) [30] found lower levels of superoxide dismutase in OSMF samples, and Gupta and colleagues (2004) [8] found decreased serum β-carotene and vitamin E antioxidant levels. Lee and co-workers (2016) [41] found higher levels of transglutaminase-2 (TGM-2) expression.

3.2.3. Other Molecular Markers in OSMF

In OSMF serum samples, there were increased platelets, eosinophils, and erythrocyte sedimentation rates, and decreased haemoglobin, iron, ceruloplasmin, copper, and zinc levels, which were well correlated with disease progression [42]. There were also decreased levels of ascorbic acid and increased levels of fatty acids (FAA) [43], serum citrate, oxaloacetate, 8-OHdG [44], and 8-isoprostaglandin F2 alpha [43]. Other markers in OSMF include tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) [45], cyclophilin A [46], and serum uric acid level [47].

3.2.4. Potential Therapeutic Agents for OSMF

Chang et al. (2012) [48] found that N-Acetyl-L-Cysteine (NAC), apoptosis signal-regulating kinase 1 inhibitor thioredoxin, and c-Jun NH2-terminal kinase inhibitor SP600125 significantly reduced thrombin-induced connective tissue growth factor (CCN2) synthesis in human Bcl2-modified factors, while epigallocatechin-3-gallate (EGCG) completely inhibited thrombin-induced CCN2 synthesis. EGCG was later proposed as a potential therapeutic agent for OSMF [49–52]. In addition, EGCG, glutathione, and NAC were shown to be effective in inhibiting IL-6-induced epithelial-mesenchymal transition by ANE [53].
Other proposed antioxidants for managing OSMF include lycopene [54], curcumin [55], and angiotensin 1–7 [56].

3.3. Clinical Studies
Chemopreventive Effects of Nutrient Antioxidants as a Potential OSMF Treatment

A total of 26 clinical studies and one dual study [8] supported the chemoprotective role of antioxidant supplementation. Clinical improvements in at least one OSMF symptom (mouth opening, mucosal burning sensation, tongue protrusion, cheek flexibility, difficulty in swallowing and speech, pain associated with the lesion, oral health-related quality of life) were observed after administration of lycopene [57–65], alpha-lipoic acid [66], allicin [67], rebamipide [68], pentoxifylline [69,70], oxitard [59,71,72], aloe vera [71,73–77], curcumin [77–81], and spirulina [75,82] (Table 2).

4. Discussion
The aim of this review was to evaluate the existing literature and assess the role of oxidative stress in the pathogenesis and chemoprevention of OSMF. Overall, both laboratory-based and clinical studies consistently demonstrated variations in oxidative stress biomarker levels in OSMF, highlighting their important roles in OSMF pathogenesis and their potential as diagnostic, prognosis, or therapeutic biomarkers. Administration of nutrient antioxidants is a potentially efficacious treatment for OSMF by having chemopreventive effects with clinical improvement.

4.1. Oxidative Stress Biomarkers Is OSMF

Serum samples from OSMF patients revealed increased MDA [24], ceruloplasmin [29], LPOs [8], nitric oxide [30], and decreased levels of beta-carotene, vitamin E [8], and SOD levels [30]. Human studies largely demonstrated elevated levels of oxidative stress biomarkers serum MDA [8,23,25,26,83], salivary 8-hydroxy-2′-deoxyguanosine [31], salivary 8-isoprostane [33], salivary lactoperoxidase and total salivary protein [34], and serum copper [27,28], calcium, magnesium, potassium, iron, LPOs, conjugated dienes, and hydroxyl radicals [28]. MDA was found by two articles to be more significant with higher clinical OSMF staging and grading [8,83], while 8-OHdG (salivary oxidative stress biomarker) levels were almost double in OSMF patients [31]. Interestingly, studies investigating the level of antioxidant enzymes in OSMF have produced conflicting results. Lower antioxidant enzyme activity in OSMF patients found in some studies [28,35,36] may be due to depletion of antioxidant defence systems occurring as the consequence of overwhelming free radicals by the elevated levels of oxidative stress. Chitra et al. [28] also demonstrated decreased levels of H2O2 and sodium in OSMF.

Copper (in high levels in AN) initiates fibrinogenesis through the upregulation of lysyl oxidase, thereby inhibiting collagen degradation. High serum copper levels generate high levels of free radicals by metal-catalysed Haber–Weiss reaction, being one of the drivers of carcinogenesis in areca nut users. As a consequence, this compromises blood supply resulting in decreased flow of nutrients and ultimately will impact antioxidant levels. In line with this, Khan et al. (2015) [20] revealed that treating keratinocytes with arecoline and copper resulted in enhanced cytotoxicity, which becomes comparable to IC50 of ANE.

Glutathione S-transferase (GST), a family of Phase II detoxification enzymes that function to protect cellular macromolecules from attack by reactive electrophiles, are long-time known to protect cells from oxidative stress [84]. However, Bathi, Rao, and Mutalik (2009) [35] and Madhulatha et al. (2018) [36] did not observe strong associations between GST gene polymorphisms (GSTM1 and GSTT1) and OSMF. Compared to healthy controls, OSMF patients had considerably elevated levels of glutathione, ceruloplasmin, and malondialdehyde, but reduced levels of beta-carotene, vitamin E, and glutathione peroxidase (GPx). The three major families of SOD, with the availability of lowering the toxic effects of superoxide (O2⁻) and H2O2 by converting them into water, are copper/zinc, iron/manganese, and nickel type [85], and were found in decreased levels in
most studies [24,30,39], but increased in others [28]. Lower antioxidant enzyme activity in OSMF patients may be due to depletion of antioxidant defence systems occurring as a consequence of overwhelming free radicals by the elevated levels of oxidative stress. In particular, increased MDA levels in serum may serve as a valuable surrogate marker in the early diagnosis, treatment, and prognosis of OSMF. Indeed, Gupta et al. (2004) [8] demonstrated that beta-carotene and vitamin E levels in plasma, increased after 6 weeks of their oral administration to OSMF patients, along with decreased MDA levels associated with clinical improvement.

In summary, there is sound evidence that oxidative stress biomarkers are altered in OSMF tissues as well as in patients’ blood.

4.2. Chemopreventive Effects of Nutrient Antioxidants as an Efficacious Treatment for OSMF

Multiple compounds with antioxidant and anti-cariogenic properties were investigated to assess their effectiveness in managing or preventing OSMF. The mechanism of action of antioxidants may involve immune system stimulation and breakage of the free radical chain reactions [86].

Six articles investigated the efficacy of aloe vera in managing OSMF with substantial antioxidant vitamins and enzymes, topical aloe vera was effective in improving at least one of the following parameters: mouth burning and opening [71–74,76,77], tongue protrusion [71,73,76], cheek flexibility [73,76], and speech and swallowing [71].

Lycopene is a carotene, carotenoid pigment, and phytochemical with potent antioxidant and anti-carcinogenic properties. Documented as a non-invasive treatment option, it yields significant improvements in OSMF signs and symptoms [54]. Its administration improved mouth opening [57,58,60–65], tongue protrusion [58,61], cheek flexibility [61], and burning sensation [58–61,64,65]. When compared to betamethasone injections, Goel and Ahmed (2015) [57] found that lycopene was more effective in improving mouth opening in subjects with an initial mouth opening distance of less than 19 mm, but less effective with distances between 20–44 mm.

Curcumin, the main natural polyphenol found in Curcuma species [87], has been shown to target multiple signalling molecules. The current literature suggests that the use of curcumin (either as a topical gel or oral tablets) in OSMF patients improved burning sensation [77,79,80], mouth opening [77,79–81], and tongue protrusion [79]. Curcumin was found to be more effective than intralesional steroid injections in improving tongue protrusion and mouth burning [80]. Meanwhile, arecoline can stimulate CCN2, enhancing OSMF’s pro-fibrotic activity. Curcumin can block the arecoline-induced CCN2 expression, thus making it a potentially useful agent in controlling OSMF [16,55]. Furthermore, curcumin influences levels of oxidative stress markers and antioxidants: it decreased MDA and 8-oxo-2'-deoxyguanosine levels [78] while increasing salivary and serum vitamin C/E levels. Vitamin C plays a protective role in carcinogenesis as an antioxidant, reducing vitamin E degradation and enhancing detoxification via cytochrome P450 [88].

Epigallocatechin gallate (EGCG) is a plant-based potent antioxidant protecting against cellular damage caused by free radicals [53], with potential uses in OSMF. Hsieh and colleagues (2018) noted that EGCG dose-dependently inhibited arecoline-induced transforming growth factor 1 (TGFβ1) activation in BMFs. BMFs exposed to arecoline resulted in the generation of mitochondrial ROS, which activated latent TGFβ1, and in turn, stimulated CCN2 and early growth response-1 (Egr-1) synthesis. TGFβ1 may play a pivotal role in the pathogenesis of OSMF; thus, EGCG can be a useful agent in the chemoprevention and treatment of OSMF. EGCG blocks TGFβ1-induced CCN2 synthesis by suppressing c-JunNH2-terminal kinase (JNK), p38 mitogen-activated protein kinase, and activin receptor-like kinase 5 (ALK5) [49]. Hsieh and co-workers (2017) concluded that ALK5, Smad3, extracellular signal-regulated kinase, and JNK are involved in the TGFβ1-induced Egr-1 protein production in BMFs. Egr-1 mediates COL1A1 and COL1A2 mRNA expression and acid-soluble collagen production in BMFs. EGCG can block TGFβ1-induced collagen production by attenuating Egr-1 expression, which is a key mediator in the TGFβ1-induced
pathogenesis of OSMF. Hsieh et al., 2015, noted that arecoline induces an overexpression of Egr-1, which enhances the profibrotic activity seen in OSMF. EGCG was shown to completely block arecoline-induced Egr-1 expression in human buccal fibroblasts.

Lastly, other compounds such as alpha-lipoic acid (ALA) are worth noting due to their effects in alleviating burning sensation and improving mouth opening. Along with conventional intralesional steroid injections, ALA was indeed able to reverse higher clinical stages [66]. Jiang et al. [67] demonstrated that when compared to triamcinolone acetonide, allicin, a defence molecule derived from garlic exhibited greater and more stable augmentation in mouth opening, alleviation of mucosal burning sensation, and improvement in ‘Oral Health Related Quality of Life’ (OHRQoL) score. Rebamipide, an amino acid derivative of 2-quinolinone used for gastrointestinal mucosal protection was found effective in managing burning sensation [68]. Oral pentoxifylline, as an adjunct to surgical reconstruction, improves mouth opening, burning sensation, and relapse [70]. Oxitard capsules resulted in significant clinical improvements in mouth-opening, tongue protrusion, swallowing, speech pain associated with the lesion, and burning sensation when compared with placebo [72]. Turmeric/black pepper and Nigella sativa improved mouth opening, burning sensation, and SOD levels [89]. Lastly, spirulina, which has multiple antioxidant effects, was shown to improve mouth opening and burning sensation [82].

Eight papers compared the chemopreventive effects of nutrient antioxidants against each other. When compared to standard antioxidant capsules, aloe vera produced greater improvements in burning sensation and mouth opening [74], while pentoxifylline showed further reduction in dysphagia and burning sensation [69]. Aloe vera was less effective in improving mouth opening when compared to spirulina [75], and also less effective in improving swallowing, speech, and pain associated with the lesion when compared with oxitard capsules [71]. However, when compared to curcumin, aloe vera brought larger improvements in burning sensation, but the two were equally effective in increasing mouth opening [77]. Three studies compared lycopene to other antioxidants, yielding conflicting results. Saran et al. [58] found lycopene to be more effective than curcumin in improving mouth opening and burning sensation, while Piyush et al. [61] found no differences between the two. Patil et al. [59] found that lycopene was less effective than oxitard in improving mouth opening and tongue protrusion. Compared to Nigella sativa, turmeric/black pepper showed greater improvements in mouth opening, burning sensation, and SOD levels [89]).

In summary, results of clinical studies show that there is clear scope for investigating the preventive and therapeutic effects of antioxidants in betel nut chewers and patients with OSMF through well-designed, large, controlled studies.

4.3. Strengths and Limitations

By providing a broad overview of relevant studies, this scoping review has established the current understanding of the topic of interest while identifying gaps in the literature. However, we must acknowledge the possibility that we may have missed some relevant studies including non-English articles and articles with no full text. The balance of breadth and depth of the data reported in this article was also challenging due to the volume of articles identified and time constraints.

This present review was not aimed at providing a comprehensive pathogenic model of OSMF. While oxidative stress is indeed associated with OSMF, tissue inflammation is crucial for the induction of tissue fibrosis. The involvement of prostanoids and other inflammatory mediators [90,91] as well as immune cells (recently reviewed in [92]) is central to OSMF pathogenesis but was not in the scope of our review.

4.4. Future Directions

The current literature is in favour of antioxidants being used for OSMF prevention and management because it is relatively non-toxic and can be easily supplemented in the diet. Our findings have important public health implications for the management and reversal of OSMF’s debilitating effects. Additionally, our results can be used as a helpful precursor
for future systematic reviews to deepen knowledge of pathogenesis and chemoprevention of OSMF. This review may also serve as a framework for future studies to help inform the development of mechanism-based interventions and a clear guidelines for managing patients with OSMF using antioxidants.

5. Conclusions

Understanding the molecular profile of distinct BQ components and how these mediate the pathogenesis of OSMF is a key challenge if we are to develop mechanism-based preventive or therapeutic strategies for this potentially malignant disease [93,94]. This scoping review highlighted the role of oxidative stress in OSMF’s pathogenesis, as shown by altered levels of various oxidative stress biomarkers, including MDA and 8-OHdG. As such, there is the potential for these oxidative stress biomarkers to be used in OSMF diagnosis, prognosis, and potential therapeutic targets. Similarly, antioxidant enzymes, (e.g., serum SOD and ceruloplasmin) may also be used in OSMF diagnosis and prognosis as their levels were also changed in OSMF patients. Furthermore, this scoping review identified various nutrient antioxidants, (e.g., aloe vera, lycopene, curcumin, and EGCG) effective in improving the signs and symptoms of OSMF such as mouth opening, burning sensation, tongue protrusion, and cheek flexibility.

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