REVIEW

Editorial Process: Submission:10/10/2023 Acceptance:03/05/2024

Association of *COX-2, TNF-α, TLR4*, and *IKKα* With Survival of Patients With Oral Squamous Cell Carcinomas: A Systematic Review

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Abstract

Background: This review investigated the association of *COX-2, TNF-α, TLR4*, and *IKKα* with the survival of patients with oral squamous cell carcinoma (SCC). **Methods:** A systematic search was conducted in the databases PUBMED, Web of Science, LILACS, EMBASE, Scopus, and Cochrane Library. The studies should assess the expression of those proteins in the tumor and survival outcomes. **Results:** Twenty-one articles were included. The meta-analysis results leaned towards an association of *COX-2* overexpression with a lower overall survival. The estimated hazard ratio was 1.51 (95% CI 0.97, 2.33), but not statistically significant (p=0.07). A low heterogeneity was observed (I2=0%). Regarding *TNF-α, TLR4*, and *IKKα*, statistically significant results for the association with survival were presented, but there was not enough data to a meta-analysis. **Conclusion:** *COX-2* overexpression may be associated with a poorer prognosis in oral SCC. The insufficiency of studies about *TNF-α, TLR4*, and *IKKα* restrained their validation as predictors of prognosis.

Keywords: Oral cancer- Tumor necrosis factor α- Cyclooxygenase 2- Kinase IκB α- Toll-like receptor 4

Asian Pac J Cancer Prev, **25 (3)**, 757-766

Introduction

According to the latest GLOBOCAN, in 2020, 19.3 million new cases of cancer occurred worldwide and, around 10.0 million evolved to death [1]. Oral cancer holds the eighth position in the ranking of the most frequent tumors worldwide [2]. Squamous cell carcinoma (SCC) is the most prevalent malignant neoplasm in the mouth, with an incidence of up to three times higher in males [3, 4]. The association of extrinsic carcinogenic factors with genetic and epigenetic alterations produces the classical etiology of this tumor, however, the relationship with viral infections, such as human papillomavirus (HPV), and with inflammation has been extensively investigated [5, 6]..

Considered an aggressive tumor, oral SCC may cause important damage to anatomy, function, and esthetics. Many of these sequelae result from resective treatments, in addition to high doses of radiotherapy and chemotherapy [3, 5, 7]. The investigation of molecular markers of this neoplasm raises new perspectives for early diagnosis, and determination of prognosis, in addition to more predictable and less invasive treatments [8]. The relationship between inflammation and malignant tumors is increasingly researched. The hypothesis that a microenvironment, rich in inflammatory cells, growth factors, and agents that promote DNA damage may favor malignant transformation and tumor progression has been suggested [6, 9, 10]. This hypothesis is strengthened by the volume of research with positive results for the correlation between inflammatory cytokines and clinicopathological aspects in carcinomas in general [11-17].

The Nuclear Factor kappa B (NF-κB) family of transcription factors is highly researched, and involved in the regulation of inflammation, innate and adaptive immune responses, cell survival, and proliferation [18]. Such functions make this family the focus of research into the relationship between cancer and inflammation [19]. NF-κB signalers can be found in almost all multicellular organisms and are reactive to various stimuli. Most of these stimuli are originated by the Tumor Necrosis Factor receptor and Toll-like receptors [18]. Tumor Necrosis Factor-alpha (*TNF-α*) is a pro-inflammatory cytokine and mediates several aspects of inflammatory processes [10, 20]. Furthermore, it is related to cell differentiation, proliferation and apoptosis, and also to tumorigenesis [21]. It is suggested that the activation of NF-κB by *TNF-α* precedes the malignant transformation of oral dysplastic lesions .

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Among the Toll-like receptors, *TLR4* is investigated for its possible relationship with tumor progression and grading, as well as with the increase in levels of interleukin-6 (IL-6), interleukin-8 (IL-8), and vascular endothelial growth factor (VEGF) in head and neck tumors [13, 15]. *TLR4* is a transmembrane receptor, which is expressed on the surface of many cells of the immune system [22]. It can activate NF-κB through canonical or non-canonical pathways [23]..

After signaling on the cell surface, NF-κB primary regulation is associated with its inhibitors, IκB proteins [24]. The activation pathways of this family share the action of kinases IκB, including *IKKα* (kinase IκB α). This is the most important step in determining the NF-κB response to the initial stimulus [25]. *IKKα* is suggested as essential for the development of the epidermis and its derivatives, by controlling the production of the differentiation-inducing factor (kDIF) [26]. Its occurrence has an impact on the phenotypic differentiation of tumor cells with epithelial origin. Because of this, its use as a prognostic indicator for patients with oral SCC is suggested [12].

Another alternative to investigate the association between cancer and inflammation is the isoenzyme cyclooxygenase 2 (*COX-2*), possibly acting in carcinogenesis processes, such as angiogenesis, apoptosis, immunosuppression, and invasion [27]. *COX-2* is an isoform of cyclooxygenase, which is a bi-functional enzyme involved in the conversion of arachidonic acid into prostaglandins [28, 29].

COX-2 is undetectable in most of the normal tissues and it is induced by pro-inflammatory and mitogenic stimuli. When induced, there is an increase in prostaglandin synthesis in inflamed and neoplastic tissues [30, 31]. Non-selective *COX-2* inhibitors have been used for over a century to control symptoms of inflammation [28]. With the discovery of its isoforms and the development of drugs that act exclusively on one of the isoenzymes, the possibilities of action of these drugs seem to be infinite. Currently, the use of *COX-2* inhibitors in lesions at risk of malignant transformation or tumor recurrence is one of the great fields of pharmacological research [32-35].

Once oral SCC is a malignancy with expressive rates of morbidity and mortality and has a possible association with inflammation, the identification of molecular markers in this lesion is of great interest. These markers can help determine the prognosis and to establish more effective treatments. Given the large number of studies investigating the relationship between inflammatory markers and oral cancer and the heterogeneity of results, this study carried out a systematic review of the literature and meta-analysis to investigate the evidence regarding the association of *TNF-α*, *TLR4*, *IKKα*, and *COX-2* with survival of patients with oral SCC.

Materials and Methods

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) platform under the number CRD42021203672 and followed the recommendations of the Cochrane Collaboration Handbook and the parameters of the PRISMA-P [36].

A systematic review of observational studies that evaluated the association of the expression of *COX-2*, *TNF-α*, *TLR4*, and *IKKα* with the survival of patients with oral SCC was conducted to elucidate the following question:

"Does the overexpression of Tumor Necrosis Factor α, Cyclooxygenase 2, Kinase IκB α, or Toll-like receptor 4 influence the survival of patients with oral squamous cell carcinoma?"

The following information configures the "PECO" question:

Population

Patients with oral squamous cell carcinoma

Exposition

Overexpression of *IKKα, COX-2, TLR4 e TNF-α*

Control

Low expression of *IKKα, COX-2, TLR4*, and *TNF-α*

Outcome

Survival

Search Strategy

Searches were carried out in the databases PubMed, Web of Science, LILACS, EMBASE, Scopus, and Cochrane Library. The search strategy containing the terms (Squamous Cell Carcinoma of the Head and Neck) AND (Tumor Necrosis Factor-alpha) OR (Cyclo-Oxygenase II) OR (B Kinase, I-kappa) OR (Toll-Like Receptor 4) AND (Prognoses) and their Mesh Terms was developed on the PubMed platform and adapted to the other databases. Manual searches were also performed among the references of the most recent review articles on the subject and in the gray literature. The last search was executed on November 11th, 2022.

Selection of the Articles

The Rayyan software (http://rayyan.qcri.org) was used to select the articles [37] independently by two different evaluators (FFB, AMF). After a detailed reading of titles and abstracts of retrieved articles, publications unrelated to the topic were excluded. Studies considered potentially eligible were read in full and evaluated by both reviewers concerning inclusion and exclusion criteria. In cases of disagreement between researchers, a third researcher (FGS) was requested.

Eligibility Criteria

Prospective and/or retrospective cohort studies were selected. The studies included in this review matched the following criteria: a) use of oral SCC samples; b) evaluation of the expression of one or more of the inflammatory markers *IKKα*, *COX-2*, *TLR4*, and/or *TNF-α* in primary tumor; c) description of the evaluation technique used; d) analysis of survival outcomes.

Outcome Measures

The primary outcomes of this review were: overall survival, disease-free survival, disease-specific survival, probability of survival, and five-year survival. Overall survival, measured in months of life, is counted from the time of diagnosis of the disease until the patient's death, regardless of the cause. In disease-specific survival, survival time is assessed from the moment of diagnosis to disease-related death. Disease-free survival is counted from the time the disease is eliminated until recurrence. Probability of survival uses sets of prognostic predictors to determine the percentage of patients alive within a given time frame after diagnosis. Finally, five-year survival calculates the percentage of alive patients after five years of diagnosis of the disease.

Data Extraction

The following information was extracted from the selected articles by two independent researchers: name of the first author, country and year of publication, number of patients, anatomical site of the tumor, method of marker detection, cut-off value on marker overexpression (cut-off level), tumor staging and survival information. Reported statistical results (estimated hazard ratio [HR], 95% confidence interval [CI], and p-value) for the assessed survival outcomes were extracted from selected studies.

Risk of Bias

To assess the methodological quality of the studies included in the systematic review, an adapted model from the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK), developed by Almangush et al. [38] was used. The criteria are described in Table 1.

Meta-Analysis

To estimate the impact of *COX-2* expression on overall survival (OS), hazard ratio (HR) values were used, with a 95% confidence interval. I^2 statistic was used to investigate the heterogeneity among the studies. When the I^2 test showed a result <50%, the presence of low heterogeneity was considered. Then, the individual estimated HR values were combined and calculated using the fixed effect model. The results were presented in the form of a forest plot, developed in the Review Manager software (5.4 version, Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration).

Results

A total of 3223 articles emerged from our primary search. After duplicate removal, 2716 articles remained. Subsequently the screening process, 81 titles were submitted for the eligibility procedure. Afterward, 21 articles fulfilled the eligibility criteria for the review (Figure 1). Among the selected titles, 15 investigated the inflammatory marker *COX-2*, two approached the *IKKα*, three researched the TLR-4, and one, the *TNF-α*. Only three articles were included in the meta-analysis due to presenting the HR data related to the overall survival outcome.

General Characteristics of the Studies

The total number of patients with oral SCC included in the review was 1608. In 1130 cases the *COX-2* marker was analyzed, the *TLR-4* was studied in 244 patients, and *IKKα* and *TNF-α* were investigated in 158 and 76 cases, respectively. The sample varied considerably among the

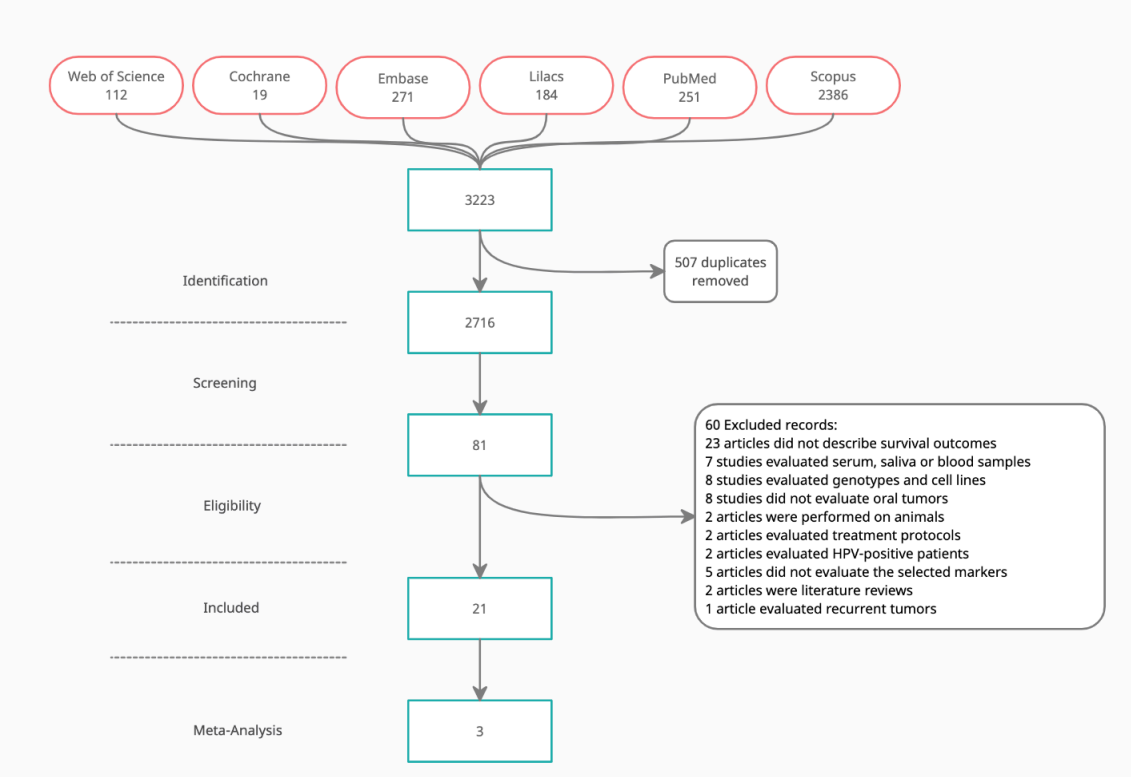

Figure 1. Flowchart of the Search Strategy and Studies Selection

Table 1. Criteria for Assessing the Quality of the Included Studies (REMARK Adaptation)

Checklist	Criteria
1 - Samples	Retrospective or prospective cohort study with a well-defined sample Medical treatments applied to patients explained. Authors detailed whether all patients received the same treatment
2 - Clinical Information	Basic clinical information such as age, sex, clinical stage and histopathological grade were provided
3 - Immunohistochemistry	Detailed immunohistochemical protocol, or referred to the article used as a model
4 - Prognostication	The survival outcomes analyzed were defined (e.g., overall survival, disease-free survival) Cut-off points used to define risk groups were specified
5 - Statistics	Estimated effect (CI, HR) describing the relationship between the assessed biomarker and the outcome. Appropriate statistical analyzes (e.g. Cox regression model) were performed to adjust the estimate effect of the biomarker on known prognostic factors
6 - Classic Prognostic Factors	Prognostic value of classic prognostic factors reported The association between the evaluated biomarker and classic prognostic factors reported

studies, the largest being 160 [39] and the smallest, 22 participants [40]. Nineteen articles had samples of less than 100 patients (Table 2).

Regarding the anatomical regions of the tumors, six articles did not specify the location of the samples [12, 41-43, 16, 44], three evaluated only SCC of the tongue [45-47] and twelve studies used samples from different sites (Table 3). Most studies did not report the staging of the tumors individually, describing them only in the form of groups.

Regarding the analysis of the markers, the method most frequently used was immunohistochemistry (IHC) [48, 30, 12, 39, 31, 41, 42, 45, 49, 50, 43, 16, 47, 51, 52, 44, 53] Ali Yang et al., 2018;. The technique of reverse transcription followed by polymerase chain reaction (RT-PCR) was used in three studies [40, 12, 44]. In two studies, both analysis methods, IHC and RT-PCR [12, 44], were used.

To quantify the expression of the markers, different rating scales were used. The cut-off points for determining overexpression of the markers ranged from 5% to 50%. However, ten articles used qualitative scales and did not disclose quantitative information to determine the scores.

Regarding the methodological quality of the articles, items 1, 5, and 6 were omitted by six different studies. Item 3, on the details of IHC and RT-PCR techniques, was described by all the articles included in the review. The results are summarized in Figure 2.

Association of COX-2 with Survival Outcomes

Fifteen articles included in this review analyzed the association between *COX-2* expression and survival in patients with oral SCC. In the studies by Morita et al. [46] and Ryott et al. [45], only tongue carcinoma specimens were analyzed. Another 10 articles included specimens from different locations, with the tongue being the most prevalent site, followed by the gingiva [30, 40, 39, 31, 49-51, 53, 48] (Ali et al., 2018). Three articles did not detail the anatomical sites of the neoplasms [41, 42, 16].

The main findings of the *COX-2* studies are described in Table 4.

Association of TLR4 with Survival Outcomes

Three articles analyzing this marker met the criteria of the systematic review. The immunohistochemistry technique for the detection of *TLR4* in tumor samples was

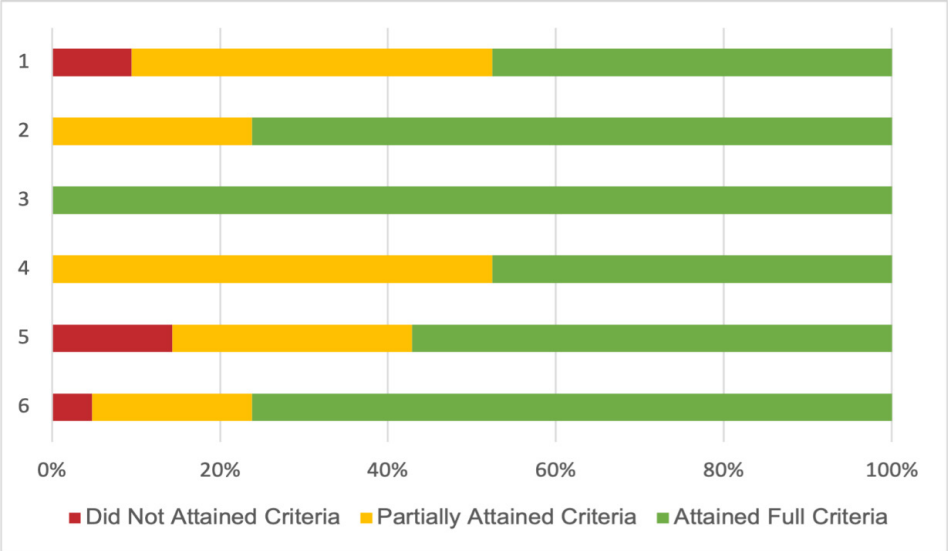

Figure 2. Evaluation of the Quality of the Studies

Table 2. Characteristics of the Studies Included in the Systematic Review

First Author	Country	Year of Publication	Patients (n)	Marker	Percentage of Positive Cases	Method of Evaluation	Tumor Staging	Survival Outcome
Itoh et al.	Japan	2003	72	*COX-2*	65.2%	IHC	I-IV	DFS / OS
Kyzas et al.	Greece	2004	68	*COX-2*	44.1%	IHC	I-IV	OS
Søland et al.	Norway	2007	53	*COX-2*	87%	IHC	I-II	DFS
Pannone et al.	Italy	2007	22	*COX-2*	68.1%	RT-PCR	I-IV	OS / DSS
Sakurai et al.	Japan	2007	160	*COX-2*	100%	IHC	I-IV	OS
Saba et al	USA	2009	54	*COX-2*	62.9%	IHC	III-IV	OS
Cha et al.	South Korea	2010	103	*COX-2*	41.7%	IHC	I-IV	OS
Ryott et al.	Sweden	2010	76	*COX-2*	100%	IHC	I-IV	DFS
Kim et al.	South Korea	2011	96	*COX-2*	40%	IHC	I-IV	Probability of Survival
Kono et al.	Japan	2013	60	*COX-2*	66%	IHC	I-IV	DSS
Morita et al.	Japan	2013	40	*COX-2*	100%	IHC	I-IV	OS
Byatnal et al.	India	2015	75	*COX-2*	67.3%	IHC	-	DSS
Baghban et al.	Iran	2016	57	*COX-2*	100%	IHC	-	OS
Sano et al.	Japan	2018	94	*COX-2*	37.2%	IHC	III-IV (a, b, c)	OS / DSS
Ali et al.	Pakistan	2018	100	*COX-2*	55%	IHC	I-IV	OS / DFS
Maeda et al.	Japan	2007	64	*IKKα*	79.6%	IHC / RT-PCR	I-IV	DSS
Lv et al	China	2019	94	*IKKα*	63.8%	IHC / RT-PCR	I-IV	OS / DDFS
Mäkinen et al.	Finland	2014	73	*TLR4*	97%	IHC	I-IV	OS / DFS / DSS
Ren et al.	China	2014	61	*TLR4*	100%	IHC	I-IV	DSS
Yang et al.	China	2016	110	*TLR4*	100%	IHC	I-IV	OS / DFS / DSS
Dantas et al.	Brazil	2019	76	*TNF-α*	-	IHC	I-IV	5 year survival

Note: "-", data not provided; DFS, disease-free survival; OS, overall survival; DSS, disease-specific survival; DDFS, distant disease-free survival; IHC, immunohistochemistry; RT-PCR, reverse transcription followed by polymerase chain reaction.

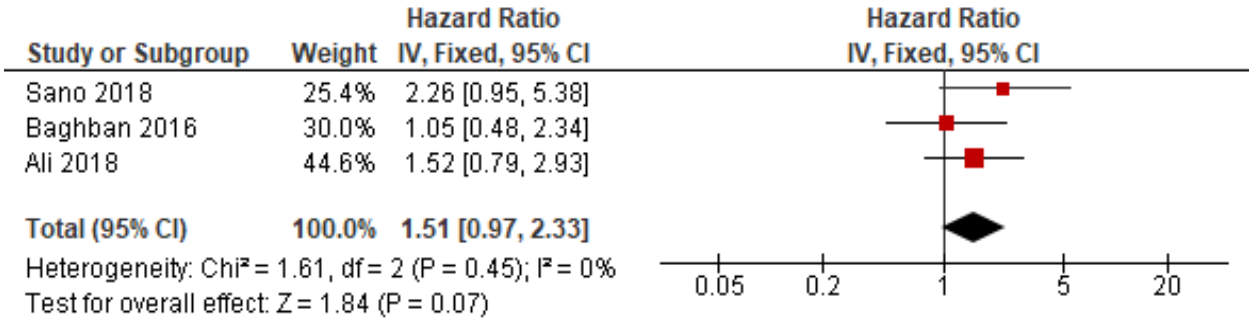

Figure 3. Forest Plot of *COX-2* association with Overall Survival

Table 3. Anatomical Sites of Tumors Included in the Systematic Review

Location of Tumors	
Not provided	509
Tongue	545
Gingiva	227
Buccal Mucosa	115
Floor of Mouth	112
Lower Lip	38
Retromolar Region	21
Alveolar Mucosa	17
Hard Palate	15
Larynx	9
Total	1608

used in all studies. Mäkinen et al. [47] used SCC samples from the tongue, while Yang et al. [54] evaluated tumors from different sites in the oral cavity. Ren et al. [43] did not specify the tumor locations.

Mäkinen et al. [47] showed the *TLR4* expression in normal tissues and malignant tumors, with the difference that the expression was nuclear in normal tissues and cytoplasmic in malignant neoplasms. This finding is in line with those of Ren et al. [43], who found cytoplasmic immunodetection of the marker, demonstrating that the worse the degree of differentiation, the higher the level of expression. Yang et al. [55] reported that the cytoplasmic expression of the marker was higher in oral SCC samples when compared to normal mucosal epithelial tissue.

Despite similar results regarding overexpression in malignant tumors, Mäkinen et al. [47] reported that there was no significant association with survival outcomes,

Table 4. Association of *COX-2* Expression with Survival Outcomes in Patients with Oral Squamous Cell Carcinoma (SCC) in the 15 Studies Included in the Revision.

Authors	n	Outcomes
Ali et al.	100	Percentage of *COX-2* overexpression (55%) in the samples was higher compared to the literature. There was a significant association between *COX-2* immunodetection and OS and DFS outcomes (p=0.013 and 0.001). Patients positive for *COX-2* expression had lower indices of OS. The marker could only be considered an independent prognostic factor when associated with DFS.
Baghban et al.	57	*COX-2* expression was cytoplasmic and there was no significant association between the marker and OS (p=0.896). 36 samples showed diffuse expression, 16 had moderate expression and 5 had low expression. *COX-2* was not considered a prognostic indicator for oral SCC.
Byatnal et al.	75	*COX-2* overexpression was observed in 58 samples. The marker was detected in stromal cells, including macrophages, lymphocytes, neutrophils, fibroblasts and endothelial cells. Kaplan Meyer analysis was used to assess DSS, but HR or p-value data are not described. There was no significant relationship between *COX-2* expression and OS outcome.
Cha et al.	103	In most of the samples (58.3%), *COX-2* was not immunodetected. 62.1% from positive samples had low expression and 37.9% had high immunodetection. Through univariate analysis, significant results were obtained between the overexpression of *COX-2* and OS (p=0.053). In the multivariate analysis, it was not possible to obtain the same results (p=0.201).
Itoh et al.	72	*COX-2* immunoreactivity was cytoplasmic in tumor cells, and was also observed in stromal cells, including macrophages, neutrophils, fibroblasts, and endothelial cells. In cases with overexpression of *COX-2*, OS and DFS were significantly lower (p=0.039 and p=0.043). Univariate analyzes showed that *COX-2* overexpression predicts a worse DFS (p=0.002) and OS (p=0.047) index. Multivariate analyzes confirmed the effect of a prognostic factor independent of *COX-2* overexpression on DFS (p=0.015), but did not obtain the same result for OS (p=0.109).
Kim et al.	90	Survival rates were lower in *COX-2* positive samples, but the results were not significant in univariate and multivariate analyzes (p=0.392 and p=0.66).
Kono et al.	60	*COX-2* diffuse cytoplasmic expression was observed in 40 specimens (66%). Patients with overexpression of this marker had shorter DSS rates (p=0.001). Multivariate analyzes showed that DSS was influenced by *COX-2* overexpression (p=0.032) in patients with lymph node metastases.
Kyzas et al.	68	30 samples analyzed showed high levels of *COX-2*. Immunodetection was cytoplasmic and diffuse, with a heterogeneous pattern. Overexpression of *COX-2* was rare in tumors located in the lower lip, contrary to what was observed in tumors of the mouth and larynx. Univariate analysis between survival outcomes and *COX-2* overexpression was described as non-significant, but the p-value was not presented. On the other hand, the multivariate analysis involving the association between *COX-2* overexpression, VEGF-C overexpression and the presence of positive lymph nodes showed statistically significant results (p=0.006).
Morita et al.	40	In 22 samples the immunodetection of *COX-2* was weak and in 18, intense. There was no significant difference between the groups with and without *COX-2* overexpression, in relation to OS (p=0.09412), in the univariate analysis, as well as in the cox regression model (p=0.0962), in which other clinicopathological variables were inserted.
Pannone et al.	22	Analyzes demonstrated an overexpression of *COX-2* mRNA in 15 samples. There was a statistically significant association between *COX-2* and DFS (p value <0.05), but not with OS (p value>0.05). It was also possible to verify that the overexpression occurred more in gingival and tongue carcinomas than in the other sites.
Ryott et al.	76	All samples were positive for *COX-2* expression. The intensity of immunohistochemical staining increased according to the staging of the tumors. No statistically significant association was observed with the DFS outcome, however the authors did not describe data related to HR and p-value.
Saba et al.	54	A positive association was observed between *COX-2* overexpression and OS outcome (p=0.026). Through multivariate analysis, it was demonstrated that the expression of *COX-2* can be a predictor of survival, with significant results (p=0.033).
Sakurai et al.	160	80 patients with oral SCC with lymph node metastasis and 80 patients without metastasis were compared. In cases of larger lesions and with involvement of lymph nodes, a significant increase in *COX-2* expression was detected. OS rates were significantly lower in patients with *COX-2* overexpression. However, this association was found when overexpression was detected in metastatic lesions (p<0.005) (both tissues were analyzed, metastasis and primary tumor).
Sano et al.	94	35 samples showed overexpression of *COX-2*. Univariate and multivariate analyzes were performed, and the expression of *COX-2* showed no statistically significant association with OS (p=0.86 and p=0.63) and DSS (p=0.65 and p=0.49).
Søland et al.	53	*COX-2* immunodetection was observed in most tumors. The authors divided the analyzes by area: tumor invasion front and central/superficial areas. Most of the tumors showed no immunodetection on the invasion front. No significant results were obtained between the overexpression of *COX-2* and DFS neither for the invasion front (p=0.94), nor for the central/superficial areas (p=0.22).

OS, overall survival; DFS, disease-free survival; DSS, disease specific survival; SCC, squamous cell carcinoma; HR, hazard ratio

but they did not specify the data regarding p-value and HR. On the other hand, Yang et al. [54] observed that in carcinoma specimens with overexpression of *TLR4*, patients succumbed to a more aggressive disease. A significant association between overexpression of that marker and OS, DFS, and, DSS (p<0,001) was observed through univariate analyses. In multivariate analyses, Yang et al. [55] obtained similar results, concluding that *TLR4* expression is an independent prognostic predictor associated with the outcomes of DFS, DSS, and OS (p=0.001, p=0.005 and p=0.006, respectively).

Ren et al. (2014) found, through univariate analyses, a significant association between *TLR4* overexpression and a shorter DSS in patients with oral SCC of the mouth (p=0.004). The authors point out that *TLR4* expression may play a key role in the long-term prognosis of those patients.

Association of IKKα with Survival Outcomes

Two articles investigating *IKKα* expression in the survival of patients with oral SCC were included and both used immunohistochemistry and RT-PCR techniques. Maeda et al. [12] reported that *IKKα* was expressed in the nucleus of basal cells in normal oral epithelium. *IKKα* expression was weak in eight samples of carcinomas and absent in 13 specimens. Its detection was significantly lower in less differentiated carcinomas. Through analysis of the Kaplan-Meyer survival curve, a statistically significant association between *IKKα* and DSS time was observed (p=0.001). Through multivariate analysis, it was found that *IKKα* acted as an independent predictor of survival (p=0.0293).

Lv et al. [44] observed that patients with oral SCC positive for *IKKα* had an unfavorable prognosis for DDFS and OS. In both, univariate and multivariate analyses, the association between *IKKα* expression and DDFS was significant (p=0.043 and p=0.041), as well as the association with OS (p=0.049 and p=0.048).

Association of TNF-α with Survival Outcomes

Only one article analyzing the *TNF-α* expression met the inclusion criteria for this systematic review. Using the multinomial logistic regression model, Dantas et al. [52] found that patients with *TNF-α* overexpression had lower 5-year survival rates (p=0.021). Another interesting data from the study was the association between low educational level and high expression of the *TNF-α*. When grouped, patients with both factors had lower 5-year survival rates when compared to the other groups (p=0.003).

Meta-Analysis

A meta-analysis was performed with three articles that investigated the association between *COX-2* and overall survival. An estimated hazard ratio (HR) of 1.51 (95% CI 0.97, 2.33) was found, however not significant (p = 0.07). Low heterogeneity was observed between studies (I^2 = 0%, Figure 3).

Data related to other markers and outcomes did not present enough studies to carry out a meta-analysis.

Discussion

The factors that contribute to the development and progression of oral SCC are complex. Inflammation is one of the first reactions to cancer, however, the role of inflammation in tumor initiation, growth, and progression [51], as well as in the survival of patients with oral cancer, is not yet fully understood. In the present study, the markers *TNF-α* and *TLR4* were investigated for participating in the activation process of the canonical or non-canonical NF-κB pathways from specific receptors on the cell's surface [14, 23]. The *IKKα* kinase has been studied because it is responsible for the phosphorylation of inhibitor proteins of the NF-κB [26] complex. After all, *COX-2* is related to the induction of pro-inflammatory and mitogenic stimuli [27]. Therefore, in this review, evidence was collected on markers related to the activation and functioning of the NF-κB complex and the *COX-2* iso-enzyme, present in inflammatory processes. Their association with the survival of patients with oral SCC was studied.

Most of the studies included in this review investigated the *COX-2* marker. The results of these studies are still conflicting, even though the role and influence of *COX-2* in the inflammatory process are widely researched. Some of the studies support the possibility that *COX-2* is a predictor of prognosis [48, 40-42, 50], while others present opposite results [30, 45, 49, 46, 16, 53, 51]. These disagreements may be explained by different analyses in the detection of *COX-2*, different types of survival outcomes, small sample sizes, bias in patient selection, use of different scoring systems for the marker, and different antibodies, as cited by Sakurai et al. [39]. Another factor that may explain those differences is the use of tumor samples from different locations of the mouth, which have different mechanisms of carcinogenesis. The way of synthesizing and evaluating the data can also contribute to conflicting results. When univariate and multivariate analyses are applied in the same study, the results can be different, as in the study by Cha et al. [42]. The analysis of multiple variables can resignify the value of the marker expression.

Although the results were not statistically significant, our meta-analysis showed a tendency for *COX-2* overexpression to be related to a lower overall survival rate. These results are in agreement with the meta-analysis performed by Yang et al. [54] However, their study included head and neck tumors from different origins, without differentiating the sites during the analyses. It should also be considered that the present study used only HR data to analyze the associations with the evaluated outcomes and that there was low heterogeneity, suggesting that the included studies had similar methodological approaches. Our findings can be considered promising regarding the use of *COX-2* as a prognostic predictor in cases of oral cancer.

COX-2 immunoreactivity can be observed not only in neoplastic cells but also in stromal cells such as macrophages, lymphocytes, neutrophils, fibroblasts, and vascular endothelial cells [48, 16]. In a retrospective study, Segawa et al. [56] demonstrated that *COX-2* expression increased according to the progression of precursor lesions of oral SCC, suggesting its influence on the process of malignancy and tumor growth. The immunoreactivity of this marker can be modulated by the interaction between stromal cells and cancer cells during tumor invasion.

Sano et al. [51] emphasize that the occurrence of *COX-2* in the tumor can be influenced by systemic inflammation and that the prognostic impact of this marker may depend on host factors and tumor characteristics. The authors credit the high percentage of *COX-2* immunodetection to risky habits of the researched population, which include chewing tobacco, areca nut, gutka, and naswar, agents that can cause inflammation in the oral mucosa.

Regarding *IKKα*, both studies included in the review showed significant results for the association between the marker and survival outcomes. However, some particularities need to be highlighted. Maeda et al. [12]

suggest that the higher the expression of the marker, the more differentiated and less aggressive the tumor. Contrary to this statement, Lv et al. [44] demonstrated that *IKKα* detection was negatively associated with prognosis meaning that, overexpression of the marker was associated with worse rates of OS and DDFS. Its role in the formation of the epidermis may justify the results found in the first study, taking into account that the higher the occurrence of this marker, the more differentiated the tissue involved can be. Nevertheless, its role in activating the NF-κB complex in inflammatory responses may support the results of the second author. The authors also describe the *IKKα* as a possible independent prognostic indicator.

The studies that investigated *TLR4* immunodetection [47, 43, 54] used different cut-off points to determine the expression of the marker. This fact made it difficult to compile data to assess its influence on the survival of patients with oral SCC. According to Yang et al. [55] and Ren et al. [43], who had significant results for the association between overexpression and worse survival outcomes, *TLR4* can be considered as a prognostic indicator in patients with oral SCC. However, more evidence is needed to confirm this claim.

In the only study investigating the *TNF-α* marker, [52] evaluated the sociodemographic aspects of patients with oral SCC associated with the *TNF-α* immunoreactivity, to assess the 5-year survival rate. Participants with high *TNF-α* immunoreactivity and low levels of education had a lower survival rate. This fact may be due to the probable relationship of the marker with cell differentiation, proliferation, and apoptosis [21], and the fact that people with low education have less access to health care. The direct association between the marker and the survival outcome showed statistically significant results, but these data are insufficient to determine the prognostic value of *TNF-α*.

The articles included in this revision showed distinct and, sometimes, conflicting results. As mentioned earlier, such variations can be attributed to the different analysis methods applied, protocols of the immunohistochemistry technique and PCR processing, and the different survival measures used. The samples of most of the included studies were small, except for the studies by Cha et al. [42], Sakurai et al. [39], and Yang J. et al. [54] which exceeded the number of 100 participants. To validate evidence related to the prognostic value of the markers surveyed, studies with a greater number of participants may provide statistically significant results more frequently. On the other hand, it is relevant to use a homogeneous and well-defined population, which can be made difficult by very large samples. Smaller groups can collaborate to prevent information bias and ensure more substantial results on the evaluated outcome.

Another limitation of this study was that the specific location of the tumors was not described in several included studies. This information is imperative, since the carcinogenesis process may occur in distinct ways in different oral sites [39]. The lack of standardization regarding the techniques for quantification of the evaluated markers, and the different scales applied in the studies, hampered the synthesis of the data. Likewise, the absence of a detailed description of the statistical analyses, performed to estimate the effect of the marker on the evaluated prognostic factor (i.e. HR and 95% CI), frustrated the development of a more extensive meta-analysis. None of the studies published after 2012 used the REMARK [57] recommendations tool, available since this year. In addition, part of the articles included in this study, and published before the existence of that tool, omitted information for the evaluation of markers. This fact makes the evidence presented weaker and exposes risks of bias in the studies evaluated.

In summary, there is an interest in the use of molecular markers for oral SCC prognostication and the results of this systematic review should act as an incentive. The scarcity of studies relating the potential markers *TNF-α*, *TLR4*, and *IKKα* with survival outcomes in patients with oral SCC constrained their validation as predictors of prognosis. Regarding *COX-2*, both the results of this systematic review and those obtained through the meta-analysis lean towards a positive association between overexpression and lower survival rates in patients with oral SCC. It is noteworthy that the use of guidelines such as REMARK to develop future research on prognostic markers in malignant tumors would reduce the omission of fundamental information. Larger multicenter cohort studies are also indicated to help validate the prognostic use of the markers evaluated in this review.

Author Contribution Statement

FFB and FGS: study conception and design; FFB, AMF, and GCL: acquisition, analysis, and interpretation of data for the work; FFB, KC, and FGS: drafting the work and reviewing it critically. All authors have approved the final of the manuscript.

Acknowledgements

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - – financial code 001.

Conflict of Interest

The authors declare no conflict of interest.

Study Registration

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) platform under the number CRD42021203672.

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