REVIEW

Prognostic Ability of Expression of Myofibroblasts in Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

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Abstract

   Aim: To systematically review the existing scientific literature in providing a comprehensive, quantitative analysis on the prognostic ability of Cancer Associated Fibroblasts (CAFs) in Oral Squamous Cell Carcinoma (OSCC) a novel meta-analysis. **Methods:** Review was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and registered in PROSPERO – CRD CRD42023467899. Electronic databases were searched for studies having data on effect of CAFs on overall survival rate and disease prognosis in patients with OSCC, oral epithelial dysplasia (OED) compared to normal healthy controls. Quality assessment of included was evaluated through Newcastle Ottawa scale (NOS) for included studies through its domains. The hazard ratio (HR) and risk ratio (RR) was used as summary statistic measure with random effect model and p value <0.05 as statistically significant. **Results:** Twenty studies fulfilled the eligibility criteria and were included in qualitative synthesis and eighteen studies for meta -analysis. Included studies had moderate to low risk of bias. It was observed through the pooled estimate that overall survival rate - (HR) =2.30 (1.71 – 3.10) was lesser in group with high CAFs compared to low CAFs while pooled estimate through RR =1.53 (0.73 – 3.19) and RR = 5.72 (2.40 – 13.59) signified that overall survival rate was lower n OSCC patients with high CAF compared to patients with OED and healthy controls. Publication bias through the funnel plot showed asymmetric distribution with presence of systematic heterogeneity indicating presence of publication bias. **Conclusion:** Abundance of CAFs in tumor stroma of OSCC patients is associated with overall poor survival rate and poor disease prognosis. CAFs acts as a good prognostic and therapeutic marker in disease progression and advancements and should be assessed early to reduce patient's mortality and morbidity.

   Keywords: Cancer- immunohistochemistry- myofibroblast- systematic review- survival rate

Introduction

   Oral squamous cell carcinoma (OSCC), one of the 10 most prevalent cancers worldwide, has a global annual incidence of approximately 300,000 new cases and 145,000 deaths, with considerable geographical and environmental risk factor difference [1]. Overall, the 5-year survival rate is approximately 50%, which has remained unchanged over recent decades. Markers for early detection, differentiating low and high-risk groups, personalizing treatment plans and post-therapeutic monitoring are urgently required [2].

   During OSCC invasion, tumour cells induce a series of modifications in the adjacent stroma, composed of mast cells and fibroblasts especially myofibroblast. The growth of OSCC cells is influenced by its stroma. Stromal cells such as mast cells (MCs), cancer associated fibroblasts (CAFs) and tumour associated macrophages (TAMs) [3].

   Myofibroblasts or reactive fibroblasts play a key role in inflammatory, growth, and wound repair processes, which are responsible for further progression of tumorigenesis [4]. Myofibroblasts are classified into two types; first type proto myofibroblasts, a partially differentiated fibroblast that contains actin stress fibers but no immunohistochemically detectable alpha smooth muscle actin (α SMA). The second type expresses α SMA and is considered a mature myofibroblast [5]. In the cancer stroma, CAFs undergo changes in protein expression that

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represents an "activated" myo-fibroblastic phenotype, which typically involves the upregulation of markers such as α SMA [6]. There is no specific marker for CAF, but alpha smooth muscle actin (α-SMA) is the most used and reliable marker for detecting CAF through histochemical analysis [7]. Studies of different cancer types have shown that CAFs are located in the vicinity of tumour cells and are able to enhance tumour growth through the secretion of growth factors and angiogenic factors but the one that is most consistently shown to have an adverse effect on prognosis is myofibroblasts [8].

Previous studies have demonstrated that increased density of CAF in the stroma of OSCC correlated with higher mortality [9, 10]. Further analyses revealed that CAF promote tumorigenesis of OSCC cell lines via an enriched and specific secretome, [10] provided evidence that the presence of CAF in the stroma of OSCC is a stronger predictor of mortality than the classical TNM staging. However, other studies [11] did not find a significant association between CAF and survival of OSCC patients. There is no clear consensus regarding the relative importance of various prognostic histopathological parameters, hence it is also importance to recognize the role of myofibroblasts in prognosis of patients with OSCC with respect to survival outcomes.

Going through evidences, till date no study has provided a comprehensive, quantitative analysis on the prognostic ability of CAFs in OSCC. Therefore, we updated our research for related articles and conducted a systematic review with the aim to provide the evidence on the high quantity of CAFs and its relation with overall survival rate through a meta-analysis.

Materials and Methods

Protocol development

This review was conducted and performed in according to the preferred reporting items for systematic review and meta-analysis (PRISMA) statement [12] and registered in Prospective Registration of Systematic Review (PROSPERO)- CRD42023467899.

Study design

The following focused research question in the Participants (P), Exposure (E), Comparison (C) and Outcome (O) format was proposed "What is the effect of presence of high quantity of CAFs in patients with OSCC on overall survival rate (OSR) or disease prognosis?"

The PICO criteria for this review were as follows:

P (Participants) – patients with OSCC
E (exposure) – patients with high quantity of CAFs
C (Comparison) – patients with less quantity of CAFs
O (Outcome) – effect of CAFs on overall survival rate and disease prognosis

Eligibility Criteria: studies were selected based on the following eligibility criteria's

a) Inclusion Criteria: following were the inclusion criteria

1) Articles published in English language
2) Articles published between January 2000 – April 2023 and having relevant sufficient data on the effect of CAFs on overall survival rate and disease prognosis in patients with OSCC, oral epithelial dysplasia (OED) compared to normal healthy controls.

3) Comparative and cross-sectional studies were selected

4) Articles reporting the study outcomes in terms of risk ratio (RR) and hazard ratio (HR).

b) Exclusion Criteria: following were the exclusion criteria

1) Any studies conducted before 2000
2) Articles in other than English language
3) Case control study, cohort study, cross-sectional study, reviews, abstracts, letter to the editor, editorials, animal studies and in vitro studies were excluded

Search Strategy

A comprehensive electronic search was performed till April 2023 for the studies published within the last 23 years (from 2000 to 2023) using the following databases: PubMed, google scholar and EBSCOhost to retrieve articles in the English language. Grey literature was explored using Google Scholar, Greylist, and OpenGrey.

Appropriate key words and Medical Subject Heading (MeSH) terms were selected and combined with Boolean operators like AND. The relevant data was searched using the following keywords and their combinations: "Cancer" (MeSH term) AND "malignancy" (MeSH term); "myofibroblast" (MeSH term) AND "cancer-associated fibroblasts" (MeSH term); "immunohistochemistry" (MeSH term) AND "alpha smooth muscle actin" (MeSH term) AND tumour stroma (MeSH term); "survival rate" (MeSH term) AND "oral squamous cell carcinoma" (MeSH term) AND "prognosis" AND "association and risk" AND "cross-sectional study" (MeSH term); "comparative study" (MeSH term).

In addition to the electronic search, a hand search was also made, and reference lists of the selected articles were screened. The reference lists of identified studies and relevant reviews on the subject were also scanned for possible additional studies.

Screening Process

The search and screening, according to previously established protocol were conducted by two authors. A two-phase selection of articles was conducted. In phase one, two reviewers reviewed titles and abstracts of all articles. Articles that did meet inclusion criteria were excluded. In phase-two, selected full articles were independently reviewed and screened by same reviewers. Any disagreement was resolved by discussion. When mutual agreement between two reviewers was not reached, a third reviewer was involved to make final decision. The final selection was based on consensus among all three authors. The corresponding authors of study were contacted via email where further information was required.

Data extraction

For all included studies, following descriptive study details were extracted by two independent reviewing

authors and using pilot-tested customized data extraction forms in Microsoft excel sheet with the following headings included in the final analysis: author(s), country of study, year of study, sample size, study design, outcome assessed and conclusion.

Quality assessment of included studies

The quality of included studies for observational studies was evaluated based on Newcastle Ottawa Scale and accordingly a numeric score (NOS Score) was assigned [13]. It was designed to evaluate bias based on participant selection, study group comparability in cross-sectional study, attainment of exposure in case-control studies and outcome of interest in cohort study. It is a valid and reliable tool for assessing the quality of non-randomized studies, supported by the Cochrane Collaboration for the quality appraisal of non-randomized trials. The NOS uses a nine-star rating system with a maximum of four points available for selection, two for comparability and three for the assessment of the outcome or exposure. The tool was deemed acceptable for the appraisal of cross-sectional studies as the effectiveness of an intervention was not being measured. Quality appraisal of the included studies was undertaken by the two authors and a third author was consulted in the event of any discrepancy. A study with a score from 7 to 9 will be considered as high quality, 4 to 6 will be considered as moderate quality and 0 to 3 will be considered as low quality or very high risk of bias.

Statistical analysis

The hazard ratio (HR) and risk ratio (RR) with 95% CI was calculated for dichotomous outcomes [14] through random effects model using the RevMan 5.3 and keeping the significance level at p<0.05.

Assessment of heterogeneity

The heterogeneity was assessed by means of Cochran's test and the I2 statistics [15]. A rough guide to the interpretation of I2 is as follows: (1) from 0 to 40%, the heterogeneity might not be important; (2) from 30% to 60%, it may represent moderate heterogeneity; (3) from 50% to 90%, it may represent substantial heterogeneity; (4) from 75% to 100%, there is considerable heterogeneity.

Investigation of publication bias

Publication bias was investigated using Begg's funnel plot [16]. Asymmetry of funnel plot indicate publication bias and other biases related to sample size, although asymmetry may also represent a true relationship between trial size and effect size.

Results

Study Selection

After duplicates removal, reference list of included studies was screened. Of which 121 studies were excluded. After this full text articles were assessed for eligibility and articles that did not meet inclusion criteria were excluded. Nineteen studies fulfilled eligibility criteria and were included in qualitative synthesis and seventeen studies were included in meta – analysis. A flowchart of identification, inclusion and exclusion of studies is shown in Figure 1.

Study Characteristics

A summary of descriptive characteristics of all included studies is shown in Table 1. Data was evaluated from twenty studies [17, 18, 9, 10, 4, 11, 5, 19, 3, 20, 6-8, 21, 1, 2, 22-25] from an aggregate of 1803 samples for the evaluation of high content of CAFs being subjected to immunohistochemistry using α SMA antibody in tumour stroma as a prognostic marker in OSCC in comparison to OED and normal healthy controls. All included studies had comparative and cross-sectional study design. Among studies, six studies [20-25] were conducted in India, three studies [17, 11, 3], three studies [9, 4, 2] were conducted in Finland, two studies [5, 6] and Kelner et al. [19]; Bagordakis et al. [8]were conducted in China and Brazil respectively. One study [18] was conducted in Iran; Marsh et al. [10] in UK; Luksic et al. [7] in Croatia and Parajuli et al. [1] in Norway respectively. It was concluded that high quantity of CAFs in stroma is an excellent prognostic indicator of disease progression and for assessing overall survival rate. All of the studies found a strong association between increased CAF density and higher mortality in OSCC and analysis of α-SMA expression for MF proliferation can be used as a stromal marker for predicting behaviour in oral precancer and cancer.

Assessment of methodological Quality of included studies

Among the included studies, only four studies [10, 19, 21, 2] reached the maximum score of the Newcastle Ottawa scale. Four studies [10, 19, 21, 2] gained the maximum score in the selection criteria and was considered to have the highest level of quality with an estimated low risk of bias; seven studies [18, 4, 6, 7, 1, 25], had the lowest score in the comparability outcome and was considered to have the lowest level of quality with an estimated high risk of bias; and all the studies had a partial score in the exposure outcome while only two studies [3, 22] had the lowest score for exposure outcome having the lowest level of quality with an estimated high risk of bias. Risk of bias of included studies through Newcastle Ottawa scale is depicted in Figure 2

Synthesis of results

The meta-analysis was performed for assessing the effect of presence of high quantity of CAFs in patients with OSCC on overall survival rate or disease prognosis as shown in Figures 3-8.

A) Overall survival rate compared between patients with high and low CAFs

Twelve studies [17, 9, 10, 4, 11, 5, 19, 3, 6-8, 2] were included in analysis. As shown in Figure 3. the Hazard Ratio (HR) is 2.30 (1.71 – 3.10) and the pooled estimates signifies that overall survival rate or disease prognosis on an average was 2.30 times lesser in group with high CAFs compared to low CAFs (p<0.05).

At the pooled estimate, the highest and lowest weightage was seen for Li et al., 2015 and Ding et al.,

Table 1. Showing Descriptive Characteristics of Included Studies

Author, years of study	Country	Study design	Sample size	Outcome assessed	Conclusion
Kawashiri et al. [17]	Japan	Comparative study	84	extent of connective tissue in the tumour stroma and whether myofibroblasts play a role in assisting cancer invasion and metastasis.	There were high levels of stromal collagen fibers in invasive tumours. Myofibroblast appearance increased with increasing tumour invasiveness
Seifi et al. [18]	Iran	Comparative study	oral epithelial dysplasia (N=18) and oral squamous cell carcinoma (N=18)	to review the frequency and the distribution pattern of myofibroblasts(αSMA-positive) in the stroma of OSCC and to compare with with OED and hyperkeratosis	increase in number of myofibroblasts and change in their distribution pattern occurs during carcinogenesis
Bello et al. [9]	Finland	Cross-sectional study	128	CAFs, a parameter of the tumour microenvironment in the prognosis of patients with OSCC	strong association between increased CAF density and higher mortality in OSCC
Marsh et al. [10]	UK	Cross-sectional study	282	To assess whether stromal features are predictive of disease mortality in oral cancer patients	myofibroblastic stroma is the strongest predictor of OSCC mortality
Dayan et al. [4]	Finland	Cross-sectional study	64 patients with OSCC	to investigate the possible existence of a molecular crosstalk between cancer cells and CAFs.	CAFs is a good prognostic and disease indicator of disease progression
Fujii et al. [11]	Japan	Comparative study	108	to evaluate the distribution of CAFs in OSCC, focusing on clinicopathological factors and patient prognosis, as well as cancer invasion	CAFs may be potential prognos tic predictors of OSCC.
Ding et al.[5]	China	Cross-sectional study	50	CAF (α-SMA positive fibroblasts) as a prognostic marker of oral squamous cell carcinomas.	CAF is associated with tumour stage, lymph node metastasis and OS at multivariate level
Kelner et al. [19]	Brazil	Cross-sectional study	110	whether CAFs could be predictive markers for occult lymph node metastasis in OSCC	immunodetection of CAFs can be useful for prognostication of OSCC, revealing lower overall survival.
Matsuoka et al. [3]	Japan	Cross-sectional study	60	to determine the clinical significance of CAFs and TAMs in OSCC	TAMs are a potential biomarker for the expression status of CAFs and TAMs may be useful for making treatment decisions to improve survival of OSCC patients
Gupta et al. [20]	India	Comparative study	normal mucosa (10), OED (14), and OSCC (25) were subjected to immunohistochemistry using α-SMA antibody for detection of MFs	to evaluate and compare the distribution of MFs using alpha smooth muscle actin (α-SMA) in OL, OSMF, and various histopathological grades OSCC	MFs with the creation of a permissive environment for tumour invasion in OSCC. Hence the presence of MF is a prognostic marker
Li et al. [6]	China	Comparative study	178	to determine the role of CAFs in the development and progression of OSCC.	CAFs have the ability to promote the growth, proliferation, metastasis of OSCC cells.
Luksic et al. [7]	Croatia	RCT	152	To assess significance of MF appearance in OSCC on the occurrence of occult regional metastases, distant metastases, and survival	myofibroblast proliferation facilitates tumour invasion, the occurrence of occult neck disease, and distant metastasis. The survival rate was poorer in patients with abundant myofibroblasts.
Bagordakis et al. [8]	Brazil	Comparative study	14	Prognostic ability of CAFs in OSCC	CAFs is an indicator of outcome of OSCCs
Joshi et al. [21]	India	Comparative study	60	to analyze the distribution and possible association of MF in OED and OSCC by immunohistochemistry	Analysis of α-SMA expression for MF proliferation can be used as a stromal marker for predicting behaviour in oral precancer and cancer
Parajuli et al. [1]	Norway	Comparative study	healthy volunteers (24), and HNSCC (106)	To investigate integrin a11 expression and its correlation with the expression CAF, a-SMA, in HNSCC	Integrin a11 was overexpressed in HNSCC stroma and colocalized with a-SMA.
Sundquist et al. [2]	Finland	Comparative study	128	CAFs as a prognostic indicator in differentiating well and moderately differentiated OSCC	Stromal TNC and, especially, FN expressions differentiate patients into low- and high-risk groups
Bhattacharjee et al. [22]	India	Cross-sectional study	20 histologically confirmed cases of OSCC, 20 cases of oral dysplastic epithelium, and 10 cases normal oral mucosa	to compare the frequency and distribution of αSMA immune-expression in OED and OSCC	The expression of MFs increases as the disease progresses from high-grade epithelial dysplasia to invasive OSCC

a- SMA, alpha smooth muscle actin; CAF, cancer associated fibroblast; MDOSCC, moderately differentiated oral squamous cell carcinoma; MF, myofibroblasts; OED, oral epithelial dysplasia; OSCC, oral squamous cell carcinoma; OSMF, oral submucous fibrosis; PDOSCC, poorly differentiated oral squamous cell carcinoma; TAM, Tissue associated macrophages; WDOSCC, well differentiated oral squamous cell carcinoma

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Table 1. Continued

Author, years of study	Country	Study design	Sample size	Outcome assessed	Conclusion
Annamalai et al. [23]	India	Cross-sectional study	Group I- OSMF(20), Group II-OSCC (20) and Group III – normal buccal mucosa (10) taken as control group	To evaluate the expression of myofibroblasts in oral submucous fibrosis (I), oral squamous cell carcinoma (II) and normal mucosa (III) by Immunohistochemical analysis.	Staining Intensity was seen more in group I (30%) than in group II (20%) and higher staining intensity was observed in well differentiated oral squamous cell carcinoma.
Pinishetti et al.	India	Comparative study	OED (n = 15), OSCC (n = 15) and 5 cases as the control.	to evaluate the presence of myofibroblasts quantitatively in oral epithelial dysplasia, oral squamous cell carcinoma	presence and absence of MFs in the stroma reveals that these cells play role in cancer cell invasion and progression
Gandhi et al. [25]	India	Comparative study	40 cases each of WDOSCC, MDOSCC, PDOSCC and controls	To assess the involvement of myofibroblasts in the invasive process of OSCC using α-SMA antibody	higher expression of myofibroblast was observed in OSCC group in comparison with the control group

a- SMA, alpha smooth muscle actin; CAF, cancer associated fibroblast; MDOSCC, moderately differentiated oral squamous cell carcinoma; MF, myofibroblasts; OED, oral epithelial dysplasia; OSCC, oral squamous cell carcinoma; OSMF, oral submucous fibrosis; PDOSCC, poorly differentiated oral squamous cell carcinoma; TAM, Tissue associated macrophages; WDOSCC, well differentiated oral squamous cell carcinoma

2013 respectively.

The funnel plot did show significant asymmetry, indicating presence of publication bias as shown in Figure 4.

B) Overall survival rate in OSCC and OED with high CAFs
Six studies [18, 20-24] were included in analysis

As shown in Figure 5. the RR is 1.53 (0.73 – 3.19) and the pooled estimates signifies that overall survival rate or the prognosis of disease on an average is 1.53 times is lesser in OSCC patients with high CAF compared to patients with OED (p>0.05). At the pooled estimate, the highest and lowest weightage was seen for Bhattacharjee et al. [22] and Gupta et al. [20] respectively, The funnel plot did show significant asymmetry, indicating presence
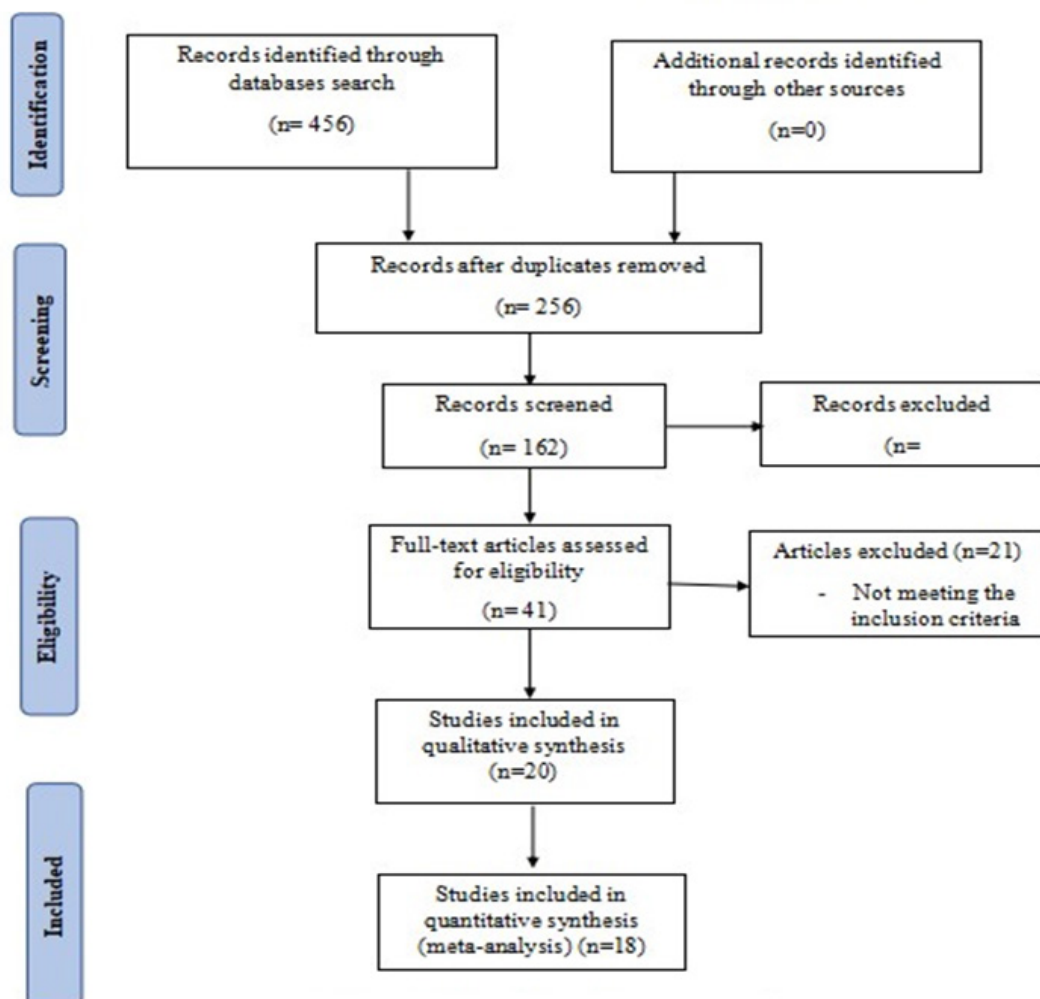

Figure 1. PRISMA Flow Diagram

Figure 2. Risk of Bias of Included Studies through Newcastle Ottawa Scale

Author, Year	Selection(Max = 4)	Comparability(Max = 2)	Exposure(Max = 3)	Overall quality score(Max = 9)
Kawashiri et al., 2009	**	**	**	6
Sufi et al., 2010	***	*	**	6
Bello et al., 2011	**	**	***	7
Marsh et al., 2011	****	**	***	9
Dayan et al., 2012	**	*	**	5
Fujii et al., 2012	**	**	**	6
Ding et al., 2013	***	**	**	7
Kelner et al., 2014	****	**	***	9
Matsuoka et al., 2014	**	**	*	5
Gupta et al., 2015	**	**	**	6
Li et al., 2015	**	*	**	5
Luksic et al., 2015	***	*	**	6
Bagordakis et al., 2016	**	**	***	7
Joshi et al., 2016	****	**	***	9
Parajuli et al., 2017	**	*	**	5
Sundquist et al., 2017	****	**	***	9
Bhattachatjee et al., 2018	**	**	*	5
Annamalai et al., 2019	**	**	**	6
Pinishetti et al., 2022	**	*	**	5
Gandhi et al., 2023	***	*	**	6
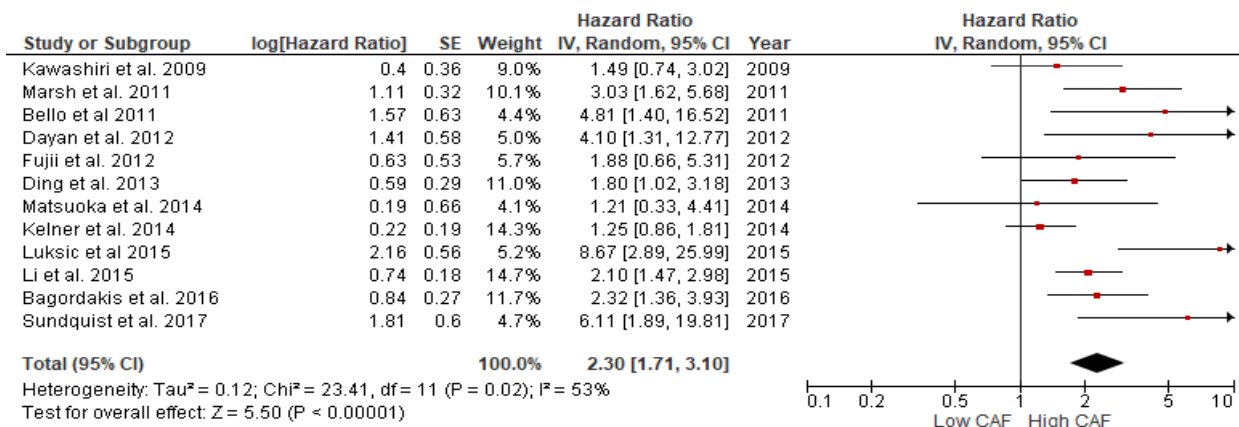
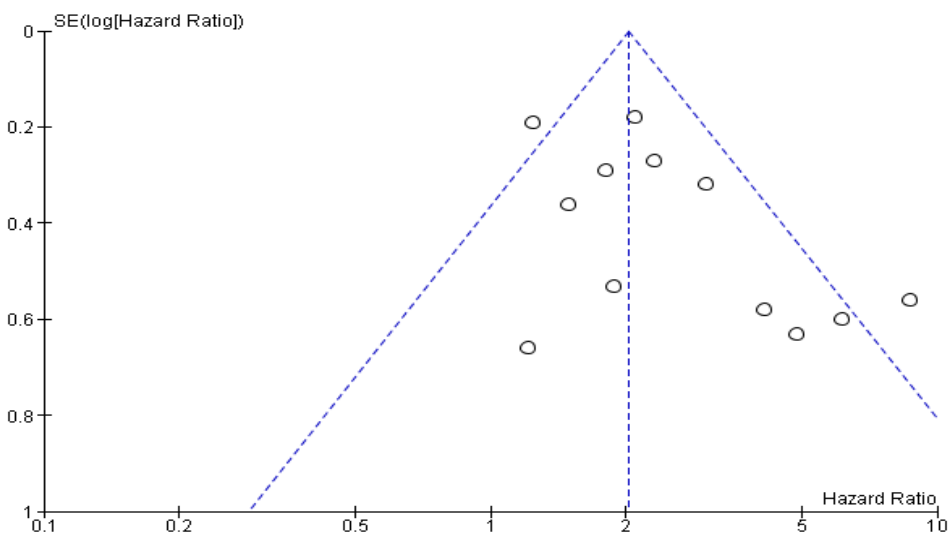Figure 3. Overall Survival Rate Compared between Patients with High and Low CAFs

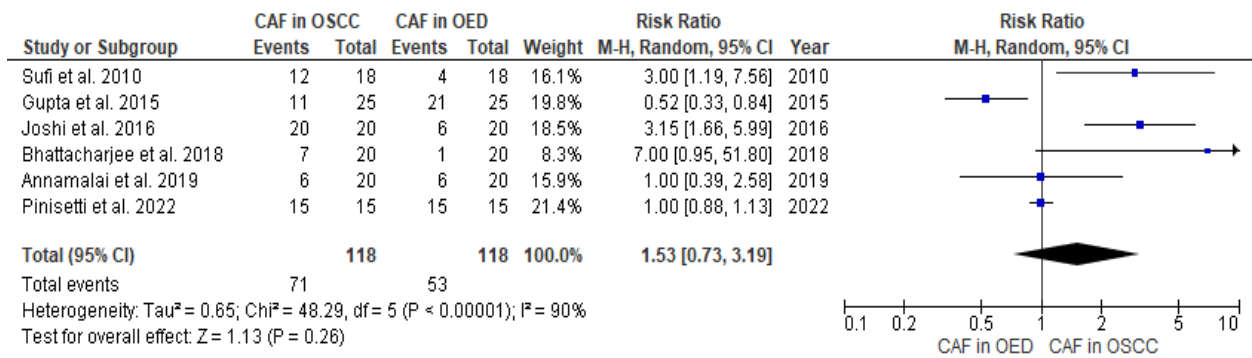Figure 4. Showing Begg's Funnel Plot Demonstrating a Presence of Publication Bias

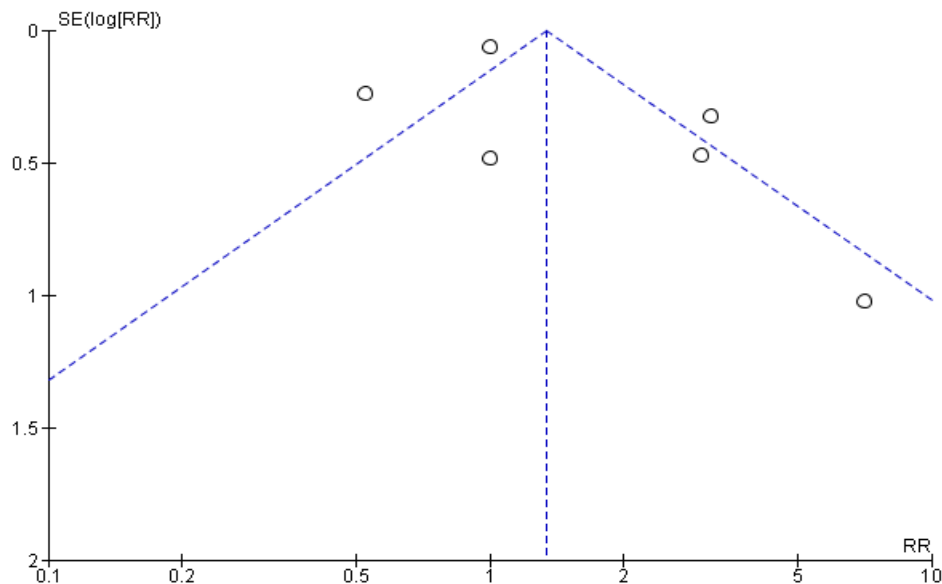Figure 5. Overall Survival Rate in OSCC and OED with High CAFs

Figure 6. Showing Begg's Funnel Plot Demonstrating a Presence of Publication Bias

of publication bias as shown in Figure 6.

C) Overall survival rate in OSCC with control

Four studies [20-23] were included in analysis. As shown in Figure 7. the RR is 5.72 (2.40 – 13.59) and the pooled estimates signifies that overall survival rate or the prognosis of disease on an average is 5.72 times is lesser in OSCC patients with high CAF compared to normal healthy controls (p<0.05). The funnel plot did show significant asymmetry, indicating presence of publication bias as shown in Figure 8.

Discussion

The biological properties and functions of myofibroblasts in tumour progression and metastasis have been extensively reported in several studies. Owing to the substantial weight of evidence indicating a pro-tumorigenic role, myofibroblasts have been suggested as a promising therapeutic target in various cancers. However, data on the prognostic value of myofibroblasts particularly in OSCC are limited.

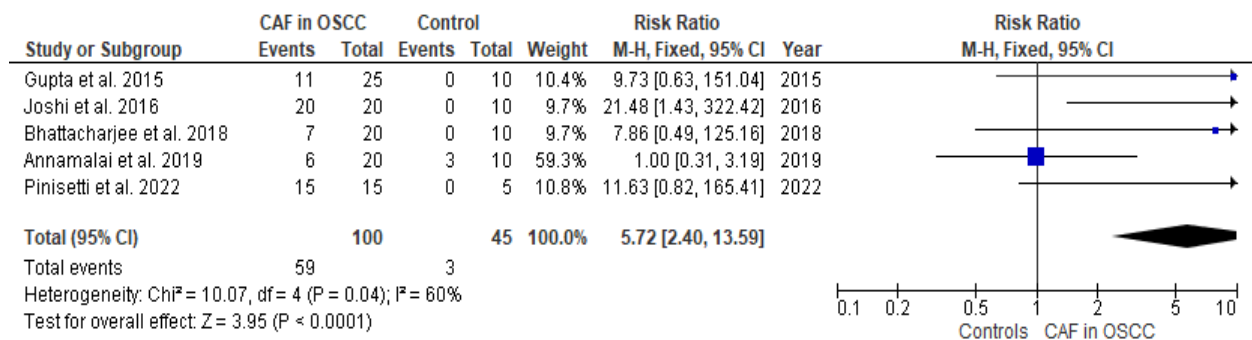In the current systematic review, we included articles

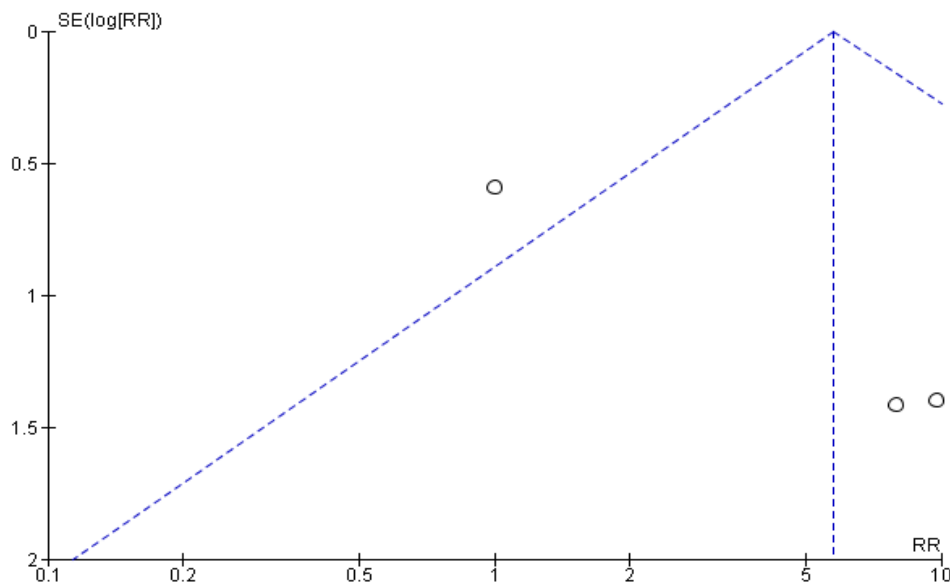Figure 7. Overall Survival Rate in OSCC and OED with High CAFs

Figure 8. Showing Begg's Funnel Plot Demonstrating a Presence of Publication Bias.

evaluating the role of myofibroblasts in prognosis (survival outcome) of OSCC patients. An electronic search was done comprehensively involving studies of last 23 years. Twenty studies fulfilled the eligibility criteria's and were included in qualitative analysis and eighteen studies for meta-analysis. From the result of our review, it was concluded that high quantity of CAFs in stroma is an excellent prognostic indicator of disease progression and for assessing overall survival rate. All of the studies found a strong association between increased CAF density and higher mortality in OSCC and analysis of α-SMA expression for MF proliferation can be used as a stromal marker for predicting behaviour in oral precancer and cancer.

However, our meta – analysis was done in three phases, at first we analysed an OSR in patients with high and low CAFs. Pooled estimate through HR showed that that overall survival rate or disease prognosis on an average was 2.30 times lesser in group with high CAFs compared to low CAFs. This was in accordance with Seifi et al. [18] who found that increase in number of myofibroblasts and change in their distribution pattern occurs during carcinogenesis.

After that we analysed the OSR in OSSC patients with OED and healthy controls. The RR revealed that OSR or the prognosis of disease on an average is 1.53 times is lesser in OSCC patients with high CAF compared to patients with OED and overall survival rate or the prognosis of disease on an average is 5.72 times is lesser in OSCC patients with high CAF compared to normal healthy controls. Funnel plot revealed significant heterogeneity with presence of publication bias.

This study is limited by overall quality of included studies. Further standardised comparative analytical studies that minimises potential sources of bias through rigorous design, conduct and reporting are needed. Future research must focus on the prognostic ability of myofibroblasts by employing larger sample size with clear

and robust methodology. Dourado et al. [26] performed a meta-analysis to assess whether CAF is a prognostic marker in OSCC. Immunohistochemical studies assessing the prognostic relevance of CAF (alpha smooth muscle actin (α-SMA)-positive fibroblasts) in patients with OSCC were reviewed systematically. The outcomes assessed were in terms of overall survival (OS) and disease-free survival (DFS) with HR as summary statistic measure. It was found that presence of high levels of CAF in the stroma of OSCC predicted shortened time to DFS (HR= 3.32) and an overall decrease in survival (HR: 2.16). Moreover, it was concluded that high presence of CAF was frequently reported in association with parameters that worsen the prognosis in OSCC.

Sekhon et al., [27] conducted a systematic review to evaluate the role of myofibroblasts in progression of oral cancer. It was found that immunohistochemistry tests have correlated the presence of high myofibroblast count in oral cancer stromal cells and concluding that myofibroblasts playing a significant role in oral cancer invasion and progression. Joshi et al., [28] carried out systematic review with an objective to recognize the role of myofibroblasts in prognosis or survival of patients with OSCC. Most of the studies assessed myofibroblasts using immunohistochemistry and was found that the presence of high levels of myofibroblasts in the stroma of OSCC patients predicted shortened time to progression of the disease and an overall decrease in survival. Also, high presence of myofibroblasts in association with various histopathological prognostic parameters including advanced disease stage (TNM staging), recurrence, tumour grade, depth of invasion, vascular, lymphatic and neural invasion, and extra-nodal metastatic spread was noted.

The adherence to the PRISMA guidelines, the thorough unrestricted literature search, utilization of reliable methodology with regard to the qualitative synthesis of data, the quality assessment of evidence with the Cochrane risk of bias tool for randomized

controlled trials strengthens this systematic review. The quality assessment of all the included studies showed low-moderate risk of bias whereas overall quality was high, specifying lack of potential and inevitable sources of bias with limited variability and reporting deficiencies.

A systematic review is a transparent and repeatable procedure for identifying, selecting and critically assessing published or unpublished data to address a well-defined research question. Meta-analyses, a statistical analysis that incorporates numerical data from related studies, are frequently paired with systematic reviews. The best evidence is generally regarded as systematic reviews and meta-analyses. However, the calibre of the included studies has an impact on how strong the evidence is from a systematic review and meta-analysis. In the current systematic review, sufficient studies with a brief observation period and a known risk of bias were included. As a result, the presently available evidence is sufficient to make therapeutic recommendations in response to the current systematic review's focus question.

In conclusion, it was found that high quantity of CAFs in tumor stroma of OSCC patients is associated with overall poor survival rate and poor disease prognosis. Therefore, CAFs acts as a good prognostic and therapeutic marker in disease progression and advancements and should be assessed early to reduce patient's mortality and morbidity. Further studies should be carried out with larger sample size to obtain an overall good quality evidence.

Author Contribution Statement

Concepts, manuscript preparation, manuscript review, manuscript editing.. Anagha Shete, Kirti Buva, Pallavi Channe and Mrinal Shete manuscript preparation, manuscript editing, manuscript review. Rashmi Sapkal and Ashwini Nerkar Rajbhoj analysed the data and reviewed the manuscript. Mrinal Shete wrote the first draft of the manuscript. All authors contributed to the critical revision.

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