RESEARCH ARTICLE

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Meta-Analysis of Prognostic Significance of Cancer Stem Cell Markers in Oral Squamous Cell Carcinoma

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Abstract

Objective: Researchers have shown significant interest in cancer stem cells in recent years. *CD44, CD24, CD133*, and *ALDH* serve as indicators of cancer stem cell-like cells in Oral Squamous Cell Carcinoma. However, the prognostic significance of these Cancer Stem Cell markers in Squamous Cell Carcinoma is still debated. This study employed meta-analysis to evaluate the prognostic significance of cancer stem cells about Oral Squamous Cell Carcinoma. **Methods:** *CD44, CD133, CD24*, and *ALDH* markers were analyzed in 19 retrospective studies to determine their relationship with prognosis and clinicopathological parameters. Risk ratios (RRs) and odds ratios (ORs) were calculated for 3-years survival rates and clinicopathological parameters, respectively, using a fixed-effects model. **Result:** The finding of our study based on extracted survival rates showed that cancer stem cell markers, *CD133* expression was related with the poor prognosis (RR= 1.62 ,95% CI = 1.08-2.44, P= 0.02). *ALDH* expression significantly correlated with lymph node metastasis (OR= 4.13, 95% Cl= 1.88-9.10, P<0.001) and clinical staging (OR= 2.26, 95% CI= 1.05-4.88, P= 0.04). **Conclusion:** The findings indicate that CSC markers could be used to predict oral cancer prognosis. Our study contributes to the literature on survival outcomes of Oral Squamous Cell Carcinoma. These findings offer a structure for the advancement of cancer treatments that specifically target cancer stem cells. Conducting additional studies with a broader group of patients will help confirm the role of cancer stem cells as dependable predictors of prognosis.

Keywords: oral squamous cell carcinoma- cancer stem cell marker- prognosis- clinicopathological parameters

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Introduction

Oral squamous cell carcinoma (OSCC) ranks as the sixth most prevalent malignancy globally [1]. The global incidence of oral cancer in 2020 was expected to be 377,713 cases, resulting in 177,757 deaths [2]. There has been a continuous increase in new cases of OSCC worldwide every year. Oral cavity cancer is quite prevalent in India, representing approximately 30% of the total global cases [3].

Smoking and alcohol consumption are the primary risk factors for oral cancer in developed countries, while chewing tobacco is the predominant cause of oral cancer in South Asia, particularly India [4]. Conventionally, lymph node metastasis (number and size of lymph nodes and extra nodal extension) is the furthermost significant prognostic factor, resulting in a 50% decrease in survival [5]. Additional characteristics that influence the prognosis include the size of the tumor, the degree of differentiation, the presence of perineural invasion (PNI), and lymphovascular invasion (LVI) in the initial lesion [6, 7]. Over the years, improvements in surgery, radiotherapy techniques, new chemotherapeutic agents, and monoclonal antibodies have improved the prognosis of this cancer.

Despite current treatment modalities, the prognosis for OSCC remains poor, as more than half of the patients die within five years [8]. A subset of patients with early-stage disease has poor outcomes, and some advanced stages of cancer have relatively better outcomes in terms of recurrence and survival. A small minority of patients do not exhibit a response to chemotherapy and radiotherapy, or they acquire resistance to these treatments. CSC exhibits strong tumorigenicity, self-renewal, and differentiation capabilities which maintain cancer. Additionally, these cells are capable of drug resistance and immune evasion. Owing to their rapid immunophenotypic changes, CSCs are difficult to identify using specific markers in tissue and blood [9]. These cells may serve as important targets for the oral cancer therapy, considering their critical roles in tumor induction, progression & metastasis [10].

Although the treatment of oral cancer has been extensively investigated, some questions remain

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unanswered. It is challenging to predict OSCC behavior using standard clinical and histological indicators. CSC is one such area that has recently received attention from researchers and is believed to be an important driver of tumor biology, which can predict metastasis, recurrence, and survival. There has been growing interest in these CSC markers for oral cancer in recent years, such as *ALDH*, *CD133*, *CD44*, and *CD24* [11].

Therefore, the present study targeted to identify the association amongst CSC marker expression (*CD133*, *CD24*, *CD44*, and *ALDH*), survival and tumor characteristics. This meta-analysis is expected to provide insights into cancer stem cells as promising prognosticators and quantitatively explore which of these CSC markers are more clinically relevant.

Materials and Methods

Data sources and search strategy

A literature search of studies on samples of human subjects was conducted through online databases, including PubMed, ClinicalTrials.gov, Science Direct, Embase and Scopus, using a web-based search in Google Scholar until the 1st of August 2021. Two independent authors (AT and MS) separately searched titles and abstracts based on predefined inclusion criteria to identify eligible studies. The search was run using both MeSH thesaurus and the combination of the following main keywords: "Oral Squamous Cell Carcinoma," "Cancer Stem Cell Markers," "*ALDH*," "*CD133*", "*CD44*", "*CD24*", "Prognosis," and "Survival." The search strategy is summarized in the figure (Figure S1). Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) were followed to select studies for the proposed meta-analysis.

Criteria for inclusion and exclusion of articles

The studies incorporated in this meta-analysis fulfilled the subsequent inclusion criteria : [i] original articles published in English with full texts available;[ii] patients should be histologically confirmed cases of OSCC;[iii] the expression of oral cancer CSC markers should be detected in a primary cell or surgically dissected primary tumour using immunohistochemistry (IHC) or fluorescence-activated single-cell sorting (FACS);[iv] studies demonstrating the relationship of expression of *ALDH*, *CD133*, *CD24*, and *CD44* with survival, i.e., overall survival (OS), disease-free survival (DFS), and clinical and pathological parameter of OSCC;[v] Studies should provide sufficient data for the calculation of risk ratio (RR), odds ratio (OR), disease-free survival (DFS), hazard ratio, or overall survival at 95% confidence interval (CI). The exclusion criteria were as follows: [i] studies involving animal specimens or other cell lines; [ii] reviews, meta-analyses, systematic reviews, case reports, letters, editorials, book chapters, conference abstracts, or expert opinions; [iii] studies not focusing on the risk of OSCC; [iv] duplicate publications; [v] studies not related to the topic of interest; and [vi] studies that did not provide enough information about prognosis.

Data extraction

Literature review table for the initial screening of articles was prepared and discrepancies were resolved through mutual discussion. Two authors (AT and PM) isolated the prognostic and tumour characteristic data from relevant literature and compiled them in a table. For each of the included studies, the following descriptive data were collected: author name, year of publication, period of sample collection, sample size, disease specified, cancer stem cell markers included, the technique used for isolation of markers, clinicopathological features measured, survival data (Kaplan-Meier curves), and the total number of cancer stem cell marker-positive and negative populations. PRISMA was used for the study selection process (Figure 1). Using the criteria for inclusion and exclusion, a comprehensive evaluation was conducted on a total of 88 papers that were deemed relevant. Ultimately, a total of 19 papers were incorporated into the meta-analysis. All included studies had an observational study design. Web Plot Digitizer software v.4.4 was used to extract data from KM curves. The 3-year survival rate was calculated by digitizing the Kaplan-Meier (KM) curves reported in each study. The extracted data were statistically analyzed. Events were calculated based on the survival rates obtained. PM, MS, and AT cross-checked all extracted data.

Reporting quality assessment

The assessment of the reporting quality of all eligible studies was conducted using the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria (Table S1). Articles with a reporting quality score of six or higher were considered high-quality studies and included in a meta-analysis. Disagreements were resolved by discussion.

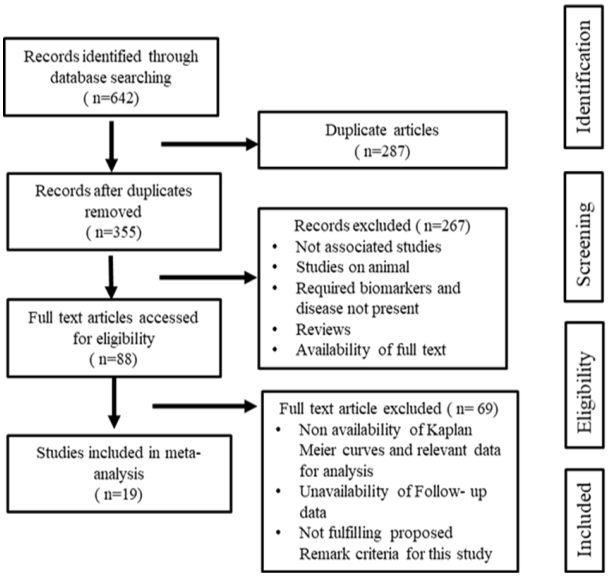


Figure 1. 642 Records Identified through Database Searching. After removing duplicates, 355 records were screened, and 267 were excluded. 88 full-text articles were assessed, with 69 excluded. 19 studies were included in the meta-analysis.

Statistical analysis

The primary endpoint of this study was the relationship with CSC marker expression and Survival (OS and DFS) in subjects with OSCC. Secondary endpoints included the association between tumour characteristics (tumour differentiation, lymph node metastases, clinical TNM staging, and tumour size) and CSC marker expression. Review Manager software v.5.4.1 was used to calculate the forest plots. The heterogeneity of the odds ratios (ORs) for tumor characteristics and relative risks (RRs) for survival rates was determined using a fixed-effects model at a 95% confidence interval, and a random-effects model was processed when the heterogeneity was significant. The threshold for statistical significance was established at a p-value of less than 0.05. The Egger test with a funnel plot was conducted to evaluate publication bias using JASP software v.0.14.1. Furthermore, a p-value of less than 0.10 indicated publication bias and asymmetry.

Results

Study characteristics

Among the 19 research that were part of the meta-analysis, 9 studies were on *CD44* [12–20], 6 studies were on *ALDH* [20–25], 5 studies were on *CD24* [19,23,26–28], and 3 studies were on *CD133* [16, 29, 30]. All studies were published between 2000 and 2020. The total sample size of the studies was 1410, with a range of 20-150, and the mean was 74.21. Out of the 19 studies, 17 expressed overall survival, one expressed disease-free survival, and one expressed disease-specific survival. Geographically, most studies were conducted in China and India, including four (21.05%) from each country, three from Japan (15.78%), three from Brazil (15.78%), one from the United States of America (5.26%), Germany (5.26%), Iran (5.26%), Sri Lanka (5.26 %), and South Korea (5.26%). Ten studies expressed tumor differentiation (52.6%) and lymph node metastasis (52.6%), five studies expressed clinical TNM staging (26.3%), and four studies expressed tumor size (21.05%). Flow cytometry and immunohistochemistry (IHC) were used to characterize cancer stem cell markers. There were 2 studies on tongue squamous cell carcinoma (TSCC) and 17 studies on OSCC. The characteristics of the studies are displayed in Table 1.

Correlation of cancer stem cell marker expression and tumour characteristics

Lymph node Metastasis

The expression of *CD24* (OR= 1.32, 95% CI= 0.55-3.15, P= 0.53) and *CD44* (OR= 1.19, 95% CI= 0.31-4.60, P= 0.80) was not associated with Lymph node metastasis. Moreover, expression of *ALDH* (OR= 4.13, 95% CI= 1.88-9.10, P< 0.001) was related with the presence of lymph node metastasis (N+) with no heterogeneity. The combined pooled OR of all the studies expressing cancer stem cell markers was not associated (OR=2.02, 95% CI= 0.98-4.15, P= 0.06) with the presence of lymph node metastasis (N+). However, considerable heterogeneity (I^2= 74 %, P_h < 0.001) was observed for lymph node metastases (Figure 2a).

Clinical TNM staging

Clinical staging (Stage III+IV) was not associated with CD 44 expression (OR= 1.70, 95% CI= 0.54-5.38, P= 0.37). However, considerable heterogeneity (I^2= 76%, P_h= 0.005) was observed. In contrast, *ALDH* expression (OR= 2.26, 95% CI= 1.05-4.88, P= 0.04) was considerably related with higher clinical TNM staging (stage III + IV), with non-significant heterogeneity (I^2= 0%, P_h= 0.93). Statistically, no association was found among the expression of CSC markers and clinical staging in the combined pooled OR of all the studies expressing CSC markers (OR= 1.81, 95% CI=0.84-3.91, P= 0.13) with a significant heterogeneity (I^2= 64%, P_h = 0.02) (Figure 2b).

Tumor Differentiation

The ORs for tumor differentiation for individual cancer stem cell biomarkers are depicted in Figure 3a. The pooled OR for *CD24* (OR= 0.65, 95% CI= 0.11-3.65, P= 0.62), *CD44* (OR= 0.94, 95% CI= 0.40-2.22, P= 0.89), *CD133* (OR= 0.81, 95% CI= 0.17-3.91, P= 0.79) and *ALDH* (OR= 1.93, 95% CI= 0.86-4.34, P= 0.11) showed that their expression was not connected with tumor differentiation. Considerable heterogeneity was observed in the studies for the markers, *CD24* (I^2= 76%, P_h= 0.02) and *CD44* (I^2= 72%, P_h=0.001) respectively. In the combined pooled OR of the studies, CSC marker expression and tumor differentiation (OR=0.99, 95% CI=0.57-1.69, P= 0.96) were not statistically related, despite of considerable heterogeneity (I^2= 63%, P_h= 0.0005).

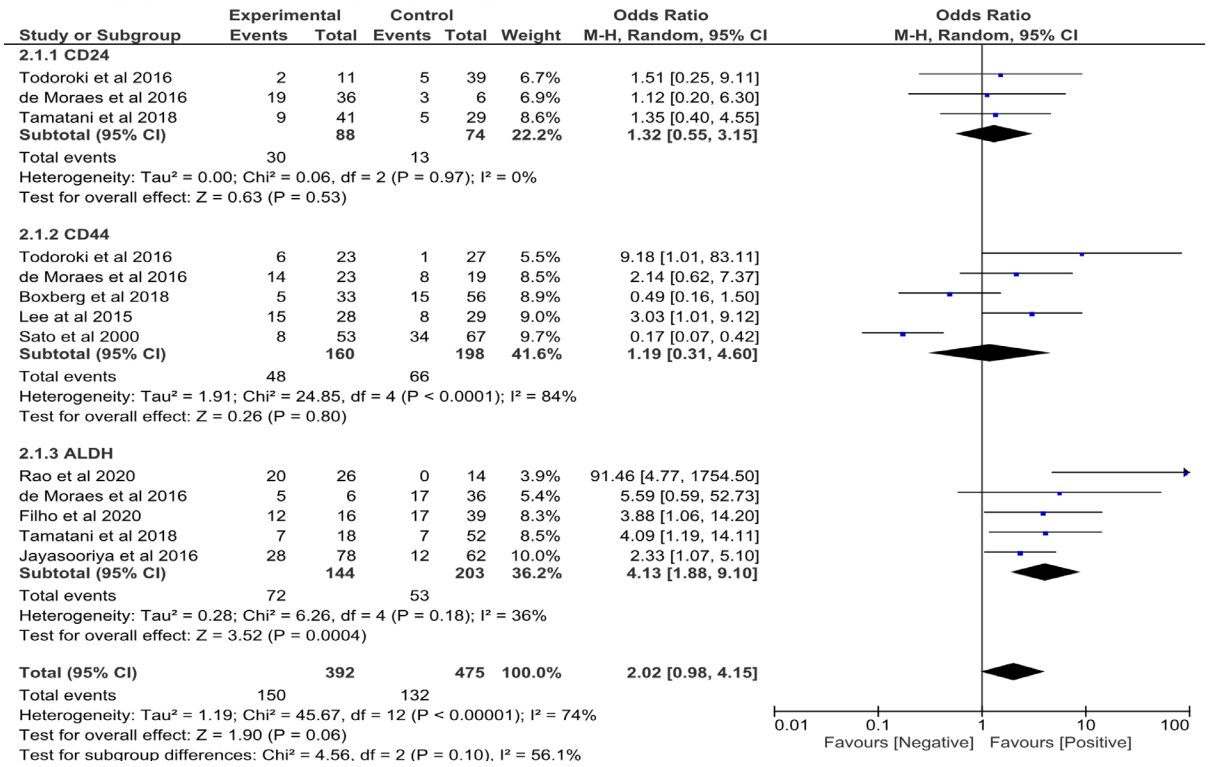
Tumor size

No significant association was found between *CD24* (OR= 0.73, 95% CI= 0.29-1.84, P= 0.50) and *ALDH* (OR= 1.45, 95% CI= 0.58-3.60, P= 0.43) expression and tumour size. No heterogeneity was observed between these studies expressing *CD24* and *ALDH* (I^2= 13 %, P_h= 0.28 and I^2= 0%, P_h=0.64), respectively. The combined pooled analysis of all studies showed that CSC marker expression was not related with tumor size (OR= 1.04, 95% CI= 0.55-1.95, P= 0.91). No significant heterogeneity (I^2= 0%, P_h= 0.50) was observed between the studies related to tumour size (Figure 3b).

Association between the expression of cancer stem cell markers and the overall survival rate after 3 years

The pooled risk ratio (RR) of the studies expressing *CD44* was 1.32 (95% CI= 0.81-2.13, P= 0.26), *CD24* was 0.83 (95% CI= 0.53-1.28, P= 0.39), *CD133* was 1.62 (95% CI= 1.08-2.44, P= 0.02), and *ALDH* was 1.20 (95% CI= 0.95-1.52, P= 0.12). Accordingly, our observation displayed the over expression of *CD44*, *CD24*, and *ALDH* was not significantly connected with overall survival. Though, a statistically significant association was only observed for *CD133* expression. Heterogeneity was significant between studies expressing *CD44* (I^2= 60%, P_h = 0.01) but not between those expressing *CD24* (I^2= 52%, P_h= 0.10), *CD133* (I^2= 56%, P_h=0.10), and *ALDH* (I^2= 48%, P_h= 0.10). Although, as per the forest plot illustration, three out of the four markers supported a poor prognosis, *CD24* expression was associated with a good prognosis. *ALDH*, *CD44*, and *CD133* expressions were

(a)



(b)

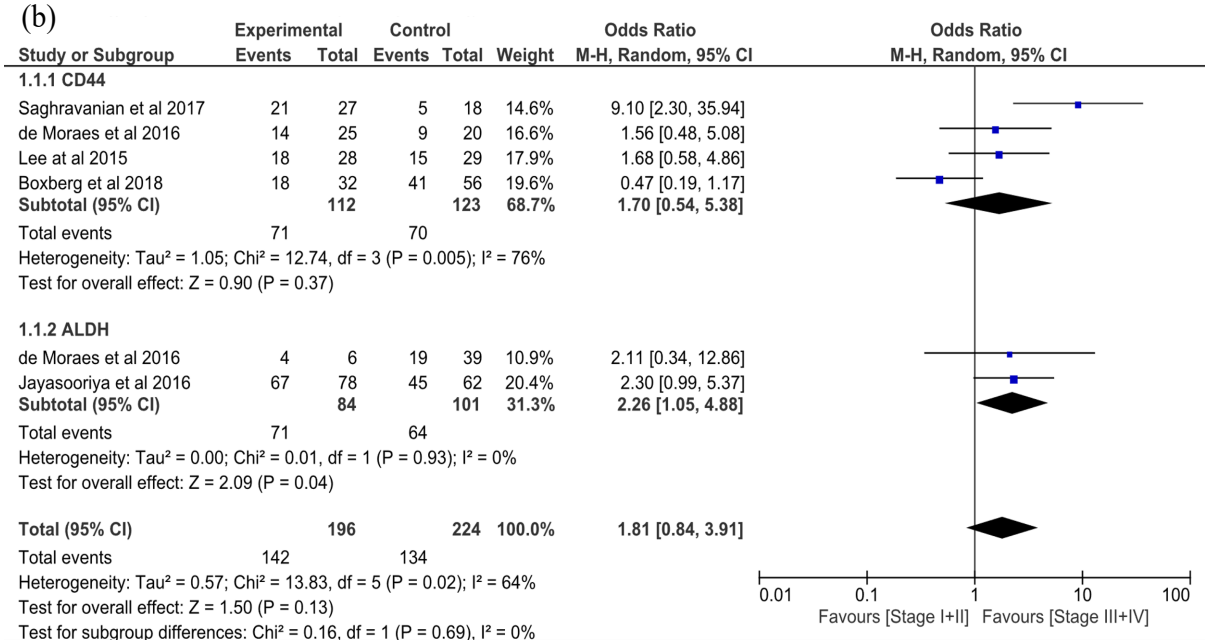


Figure 2. The Forest Plot of OR was assessed for the association between Cancer Stem Cell Marker and Tumour Characteristics, Analysis of *CD 24, CD44*, and *ALDH* expression and Lymphnode metastasis in OSCC patients (a); Analysis of *CD44*, and *ALDH* expression and clinical staging in OSCC patients (b).

associated with lower overall survival rates (Figure 4).

Assessment of Publication Bias

Egger's regression tests revealed non-significant publication bias for each CSC marker in terms of tumour size; however, in terms of clinical staging and tumour differentiation, *CD44* expression showed a significant publication bias. *CD44* and *ALDH*, in contrast to *CD24*, revealed considerable publication bias in the case of lymph node metastasis (Table S2). Graphically, the funnel plots in the meta-analysis results of 3-year overall survival rates of the studies expressing *CD24*, *CD44*, and *CD133* did not exhibit any asymmetry (Figure S2), and Egger's regression test didn't reveal any significant

Table 1. Overview of Studies on Oral Squamous Cell Carcinoma, Including Cancer Stem Cell Markers, Methods Used, Sample Sizes, Survival Metrics, Risk Ratios, p-values, Clinicopathological Features, and Quality Assessments.

Reported studies	Year of data collection	Disease	Cancer Stem cell marker	Technique	Total sample	Survival measure	RR/OR (95%CI)	P-Value	Clinicopathological features included	Quality Score
Rao et al [22]	ND	OSCC	ALDH	IHC	40	OS	6.00[0.89,40.63]	0.0664	Lymphnode Metastasis	8
Chiou et al. [30]	ND	OSCC	CD133	FACS, IHC	52	OS	1.98[0.96,4.10]	0.0662	Tumour size, Tumour Differentiation, Lymphnode Metastasis	6
Fugle et al. [26]	ND	OSCC	CD24	ND	52	OS	0.52[0.28,0.96]	0.0364	ND	6
Mishra et al.[28]	ND	OSCC	CD24	IHC	50	OS	2.17[0.81,5.80]	0.1217	ND	6
Tamtani et al. [23]	ND	OSCC	ALDH, CD24	IHC	70	DFS	NA	NA	Tumour size, Tumour Differentiation, Lymphnode Metastasis	6
Boxberg et al. [12]	2007-2012	OSCC	CD44	IHC	108	OS	1.56[1.06,2.29]	0.0252	Tumour Differentiation, Lymphnode Metastasis, Clinical TNM staging	6
Saghravanian et al. [13]	ND	OSCC	CD44	IHC	45	OS	2.67[1.06,6.69]	0.0365	Tumour Differentiation, Clinical TNM staging	6
Sato et al. [14]	ND	TSCC	CD44	IHC	120	OS	0.25[0.09,0.07]	0.0077	Tumour Differentiation, Lymphnode Metastasis	6
deMoraes et al. [15]	2000-2005	OSCC	CD44	IHC	52	DSS	NA	NA	Tumour Differentiation, Lymphnode Metastasis, Clinical TNM staging	7
Jayasooriya et al. [21]	2009-2012	OSCC	ALDH	IHC	140	OS	1.26[0.79,2.00]	0.3414	Clinical TNM staging, Lymphnode Metastasis	7
Filho et al. [25]	1970-2013	OSCC	ALDH	IHC	63	OS	1.29[0.65,2.55]	0.4718	Tumour size, Tumour Differentiation, Lymphnode Metastasis	6
Wu et al. [24]	2008-2010	OSCC	ALDH	IHC	78	OS	0.90[0.70,1.16]	0.4308	ND	6
Oliveria et al. [16]	1990-2009	OSCC	CD44, CD133	IHC	150	OS	1.75[0.93,3.31]	0.0852	ND	8
							1.87[1.10,3.17]	0.0214		
Lee et al. [18]	2004-2014	OSCC	CD44	IHC	57	OS	2.29[0.66,7.92]	0.1922	Tumour Differentiation, Lymphnode Metastasis, Clinical TNM staging	7
Wang et al. [29]	2000-2010	OSCC	CD133	IHC	144	OS	0.08[0.00,1.54]	0.0929	ND	7
Mohanta et al. [17]	2010-2013	OSCC	CD44	FACS	53	OS	0.19[0.01,3.11]	0.2452	Tumour Differentiation	7
Ghuwalewala et al. [27]	ND	OSCC	CD24	FACS, IHC	20	OS	0.67[0.23,1.92]	0.4528	ND	6
Todoroki et al. [19]	2007-2008	OSCC	CD24, CD44	FACS, IHC	50	OS	0.64[0.17,2.49]	0.523	Tumour size, Tumour Differentiation, Lymphnode Metastasis	6
							1.64[0.60,4.48]	0.3321		
Huang et al. [20]	2003-2006	TSCC	ALDH, CD44	IHC	66	OS	1.33[0.57,3.12]	0.5076	ND	7
							1.26[0.58,2.77]	0.5595		

Abbreviations: OSCC, Oral squamous cell carcinoma; TSCC, Tongue squamous cell carcinoma; IHC, Immunohistochemistry; FACS, Fluorescence activated cell sorting; OS, Overall Survival; DSS, Disease-specific survival; DFS, Disease free survival; NA, applicable; ND, Not defined.

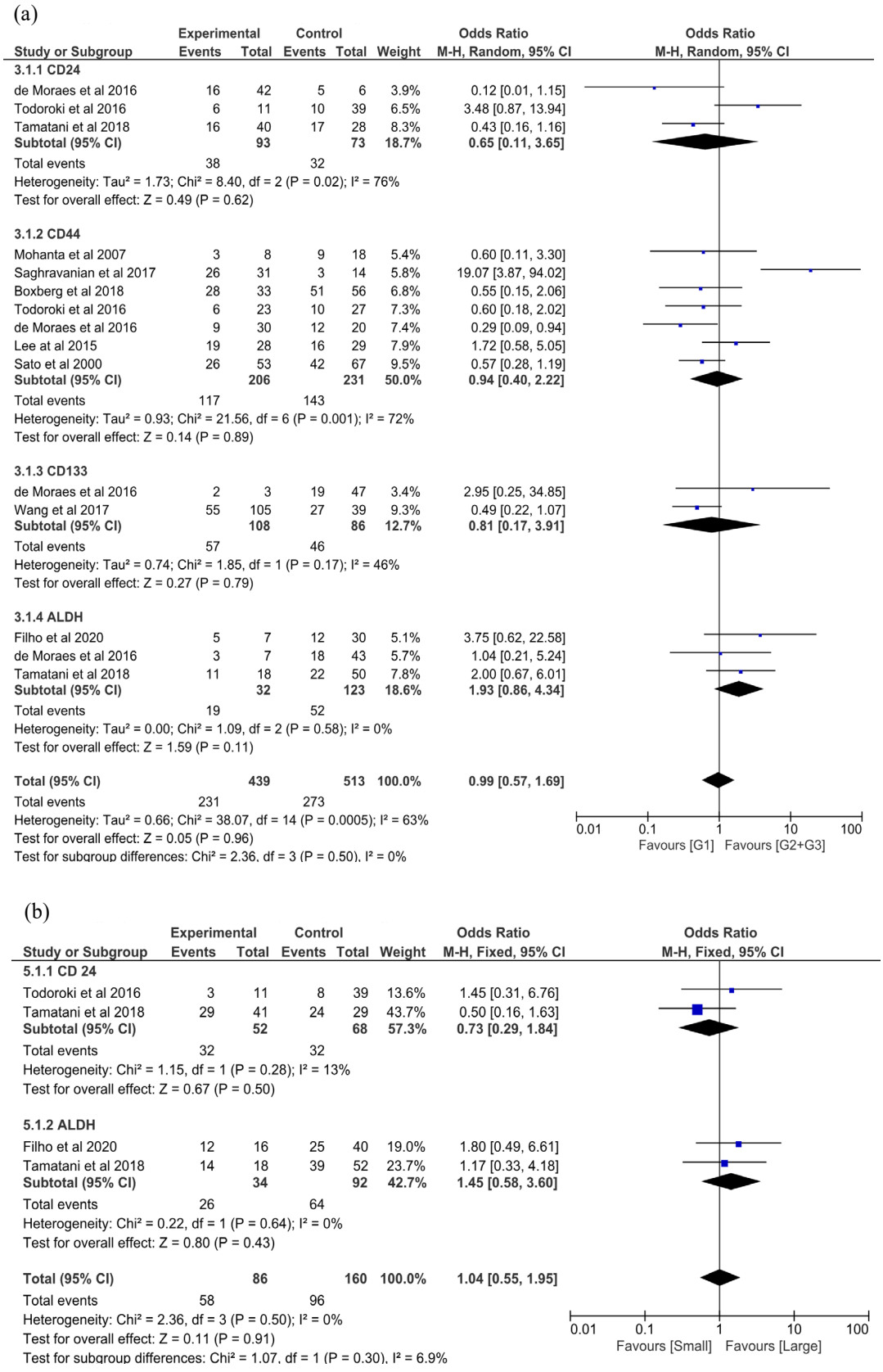


Figure 3. The Forest Plot of OR was Assessed for the Association between Cancer Stem Cell Marker and Tumour Characteristics, Analysis of *CD 24, CD44, CD 133* and *ALDH* Expression and Tumor Differentiation in OSCC Patients (a); Analysis of CD 24, and ALDH expression and tumor size in OSCC patients (b).

publication bias. In contrast, *ALDH* showed funnel plots with asymmetry and significant publication bias in Egger's regression test (Table S3).

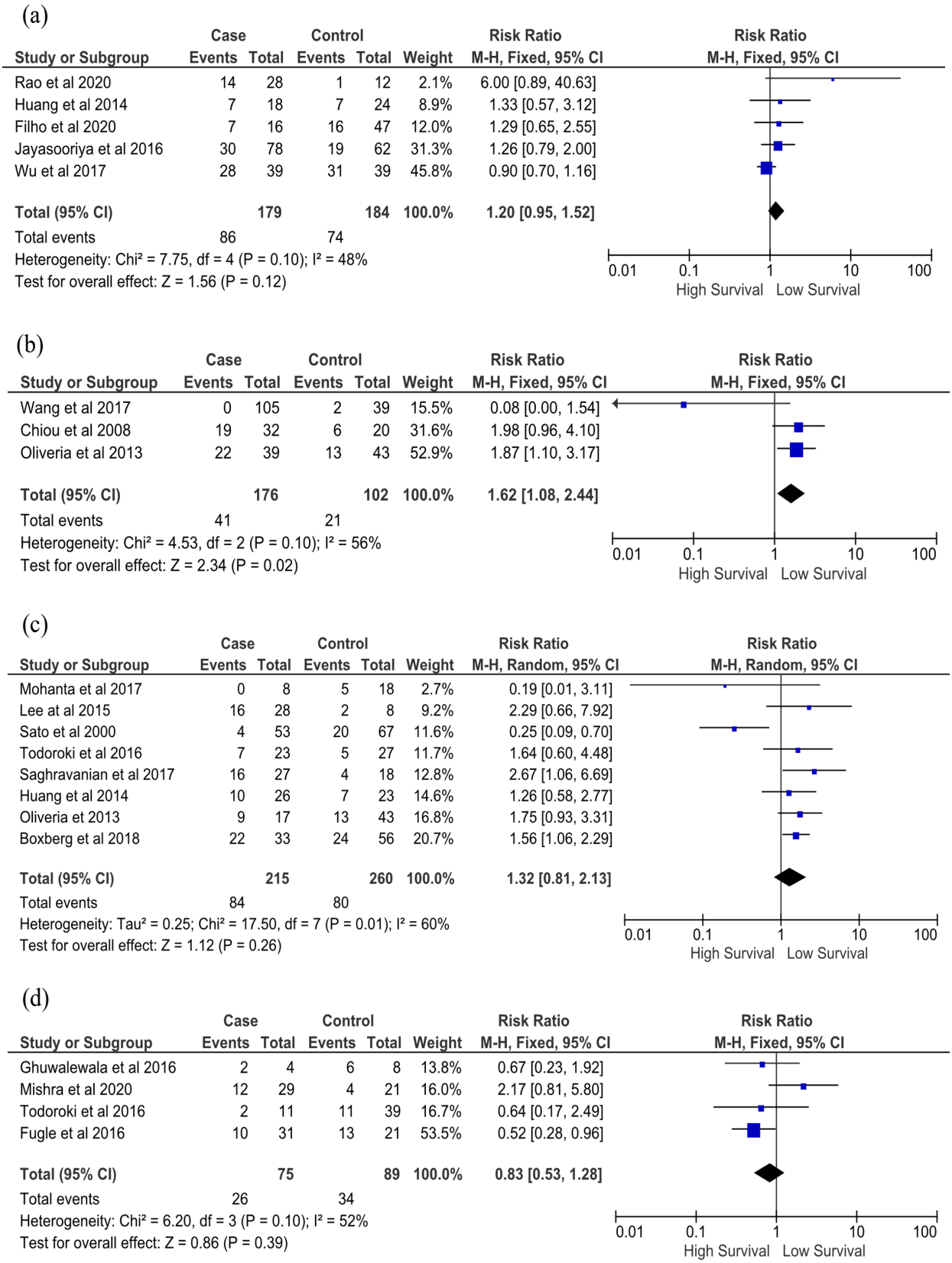


Figure 4. Analysis of *ALDH* (a), *CD 133* (b), *CD 44* (c), and *CD24*(d) Expression and Survival of OSCC Patients, Forest Plot of RR was assessed for the association between Cancer Stem Cell Markers and Survival.

Discussion

Recently, several treatment options have been developed for oral cancer, but still, it has poor outcomes. One of the reasons for metastasis and recurrence of cancer is believed to be CSCs within tumors. Cancer stem cells can be identified using CSC markers to study their influence on tumor formation and growth [9, 31] .

This meta-analysis investigated the correlation among CSC marker expression and tumour characteristics and their function in predicting the survival of subjects with oral carcinoma. Our study provides the first comprehensive assessment by comparing prevalent CSC markers (*ALDH*, *CD24*, *CD133* and CD 44) in oral cancer. We found an association between these markers and tumor characteristics, suggesting that they have an adverse prognostic impact.

The presence of *CD133* is highly correlated with a worse outcome in individuals with oral cancer, consistent with prior well-documented studies [32]. Research indicates that cancer stem cells possess the ability to undergo self-renewal, which is believed to be a contributing element to their ability to form tumors [33]. Some other studies done in colon and lung cancer also showed similar result [34, 35]. Due to their chemotherapeutic resistance, *CD133*-positive cancer stem-like cells have been hypothesized to be accountable for tumor development and reappearance [36], leading to a poor prognosis. Conversely, one of the included studies, Wang et al. established the *CD133* expression was connected with a higher survival rate [29]. These results could be attributed to selection bias because they only included patient population with early-stage oral cancer.

Our investigation found no significant correlation between the expression of *CD44*, *ALDH*, and *CD24* with survival. *CD44* expression has been associated with an unfavorable outcome in many types of cancer [37, 38]. The impact of *CD44* expression on the prognosis of oral squamous cell carcinoma (OSCC) is still uncertain, as conflicting findings have been reported [39]. *ALDH* expression has been linked to worse outcomes in different types of cancers, such as breast [40] and colorectal cancer [41]. However, one study showed similar results to our study [42]. Among the included studies, only one study by Rao et al. showed low survival with the expression of *ALDH* [22]. However, our findings on *CD24* expression are consistent with those of another study on OSCC [16,43]. *CD24* expression has been linked to survival in breast cancer [44] and hepatocellular carcinoma [45], whereas our findings contradict these studies. Previous studies have indicated that *CD24* has a detrimental impact on cell migration and epithelial-mesenchymal transition. This implies that cells with a *CD44* low and *CD24* high phenotype exhibit comparable behavior to non-cancer stem cell features [27, 46]. Several laboratory investigations conducted in test tubes and living organisms have demonstrated that low levels of *CD24* expression in oral cancer are linked to a negative prognosis [47].

According to previously reported findings, we have confirmed that the expression of *ALDH* is associated with the dissemination of cancer cells to the lymph nodes and a more advanced stage of the disease. Several studies have established a correlation between *ALDH* expression and aggressive tumors, lymph node metastases, and advanced tumor stage [42, 48, 49]. Nevertheless, there was no discernible association observed between the expression of *ALDH* and the size or differentiation of the tumor. Furthermore, Szafarowski et al. found that *CD24* was linked to the spread of cancer to the lymph nodes in head and neck cancer, but it did not have an impact on survival [50]. Owing to inadequate information, *CD24* has not been evaluated for clinical tumor staging. Even though CD 24 has a distinct function in the preservation of cancer stem cells, not many studies evaluate this marker, and publication bias still exists; we believe that *CD24* is not a specific prognostic marker for Oral Cancer.

In our study, *CD133* was not connected with tumour differentiation; however, *CD133* expression was correlated with poorly differentiated tumors in some studies [51]. Insufficient data was available to study *CD133* expression for all tumour characteristics, except for tumour differentiation. Our findings revealed that *CD44* expression was not to be related with lymph node metastasis, TNM staging, and tumour differentiation; although some studies were incongruent, most supported these findings. *CD44* is associated with tumors differentiation. This discrepancy could be explained as *CD44* has two isoforms, *CD44v* and *CD44s*, which are involved in cancer's cellular function and pathogenesis [52–54].In head and neck cancers, *CD44v3* levels are elevated, leading to increased cell migration. Low expression of the *CD44v9* variant is related to higher survival, according to Sato et al. [14]. In our study, we did not characterize *CD44* based on its isoforms. Various *CD44* isoforms exhibit different cellular characteristics when co-expressed with other CSC markers [55]. The findings of our investigation indicate that *CD44* expression in oral tumour should be studied based on its isoforms and its co-expression with other CSC markers. We were unable to analyze *CD44* expression in relation to tumor size because of the lack of relevant studies.

Our results are in line with previous studies with some discrepancies. It is possible that these differences are due to the fact that previous studies included early-stage oral cancer, different subtypes of oral cancer, different antibodies for analysis, and had different patient demographics. According to Forest Plot, CD 24 is the only marker that supports a good prognosis, while CD 133 showed a statistically significant poor prognosis out of the four markers. Expression of all four cancer stem cell markers supports lymph node metastases (N+), higher clinical TNM stages (stage III + IV), large tumor size, and tumor differentiation grade (G2+G3); however, only *ALDH* showed statistical significance with lymph node metastasis and clinical stage. The sample size was comparatively small, perhaps diminishing the data's ability to identify any pattern. Due to the scarcity of studies that fit our specific criteria, only a small number of papers were included in the current investigation. However, we believe that strict inclusion and exclusion criteria led to high-quality and more relevant results. Finally, the events were calculated using data digitalization of Kaplan-Meier curves, which may have resulted in minor differences with the actual data due to manual errors. Despite this limitation, one of the key strengths of this meta-analysis is that we only included studies which examined cancer stem cell markers in patient derived primary tumour, which made it more clinically relevant. Patient-derived samples can retain heterogeneity and tumour microenvironment, so they have more translational value than the secondary

cell line.

In conclusion, overall, increased research and understanding of CSC markers may allow clinicians to better prognosticate and choose more personalized treatment options for patients with oral cancer. Co-expression of these markers should also be considered so we can get a specific phenotype or panel of markers to understand the prognostic role of CSC markers in oral cancer. Our study contributes to the literatures on survival outcomes and tumor characteristics of patients with OSCC. Further extensive, well-designed cohort studies using primary human tumor cells must be required to explore their role in clinical factors & treatment-related outcomes in oral cancer.

Author Contribution Statement

Abhilasha Tripathi (M. Pharm.) and Dr. Vijay Kumar (MCh)conceptualization; Dr. Prabhaker Mishra (PhD) methodology, software analysis, data interpretation, and statistical analysis; Abhilasha Tripathi, Mohit Singh (M.Sc.) and Dr. Prabhaker Mishra data curation, writing-original draft preparation, prepared the figure, charts, and tables; Abhilasha Tripathi and Dr. Naseem Fatima (PhD) writing, reviewing, and editing. Each author contributed to the manuscript and reviewed the final draft before submission.

Acknowledgements

Ethical Consideration/Approval

This meta-analysis, approved by the Institutional Ethics Committee, KGMU, Lucknow (Ref no 1460/ Ethics/R.Cell-16) as part of an approved student thesis, was conducted in accordance with institutional ethical standards and the Declaration of Helsinki. The study involved no patients, public, or animals, and was designed independently by the authors, waiving the need for informed consent.

Data Availability

All data generated or analyzed during this study are included in this article.

Conflict of Interest

The authors declare no potential conflicts of interest.

Abbreviations

OSCC: Oral Squamous Cell Carcinoma
CSC: Cancer Stem Cell
OS: Overall Survival
DFS: Disease Free Survival
DSS: Disease-Specific Survival
ORs: Odds Ratios
RRs: Risk Ratios
IHC: Immunohistochemistry
MeSH: The Medical Subject Headings (MeSH)
FACS: Fluorescence-activated single-cell sorting
REMARK: Reporting Recommendations for Tumour Marker Prognostic Studies
KM: Kaplan-Meier

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