SUBMITTED 7 AUG 23
REVISION REQ. 10 SEP 23; REVISION RECD. 28 SEP 23
ACCEPTED 12 OCT 23
ONLINE-FIRST: OCTOBER 2023
DOI: https://doi.org/10.18295/squmj.10.2023.062

Correlation between Vascularity and Advancing Histological Grades of Oral Submucous Fibrosis with a Plausible Role in Malignisation

Systematic review of a persisting matter of conflict

***Deepak Pandiar,[1] Suvarna K. Nair,[1] Ronell Bologna-Molina,[2] Reshma P. Krishnan,[1] Naina Sivakumar,[3] Rahul Anand,[4] Sahil Choudhari,[5] Pooja Sharma[6]**

[1]Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India; [2]Department in Diagnostics in Oral Pathology and Oral Medicine, University of the Republic, Montevideo, Uruguay; [3]Division of Oral Pathology & Microbiology and Forensic Odontology, CDER, All India Institute Of Medical Sciences, New Delhi, India; [4]Department of Oral Pathology and Microbiology Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, Sant-Tukaram Nagar, Pimpri, Pune, Maharashtra, India; [5]Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India; [6]Department of Oral and Maxillofacial Pathology, King George's Medical University, Lucknow, India.

**Corresponding Author's e-mail: deepakpandiar1923@yahoo.com*

Abstract

Objectives: Recent studies showed that as the stage advances there is no significant change in the vascularity as opposed to the conventional concept, thus, the present was designed to quantify the vascularity in histological grades of OSMF and to assess if there is any connection between vasculogenesis and malignisation. ***Methods:*** A comprehensive database search was done for published articles on vascularity in oral submucous fibrosis following PRISMA guidelines

without date constrains; the search was done till December 2022. The review was registered in Prospero. After screening 607 articles, a total of 13 studies were finally included for systematic evaluation. ***Results:*** A total of 607 cases were included, with a definite predilection for the male gender. 11/13 studies evaluated mean vascular density; in more than half, the vascularity decreased as the stage advanced. Similar results were obtained for endothelial cells /square μm, mean vascular area percentage & mean vascular area. ***Conclusion:*** The present review supports the prevailing concept that vascularity decreases with advancement of the stage of OSMF, denying systemic absorption of carcinogens into the circulation with resultant longer exposure of compromised epithelium and malignisation.

Keywords: Malignisation; Mean Vascular Density; Oral Submucous Fibrosis; OSMF; Vascularity.

Introduction

The earliest mention of oral submucous fibrosis (OSMF) probably dates back to ancient Indian medical literature by 'Sushruta' as Vidari showing features such as reduced mouth opening, pain on eating food and depigmentation of the oral mucosa.[1] OSMF is usually a habit-related enigmatic, insidious, chronic yet potentially malignant oral, oropharyngeal and esophageal condition seen mainly in natives of Southeast Asian countries particularly the Indian subcontinent, which is always associated with juxta-epithelial inflammatory reaction followed by progressive stromal fibro-elastic changes such as hyalinization and homogenization of collagen bundles, altered vascularity and epithelial atrophy resulting in varied degrees of mucosal stiffness and compromised functional activities.[1-3] It has been estimated that OSMF affects around 0.5 million people in the Indian subcontinent and the highest prevalence is noted in the Kerala state of South India. It has also been reported among people of Indian origin across the world.[2,4,5]

Vasculature in OSMF has always been a debatable territory with highly variable results yielded from case-control studies.[3,6,7] The prevailing concept being that there is hyperplasia of blood vessels in the very early/early histological grades of OSMF and blood vessels and luminal diameter reduce as the disease progresses.[2] But few recent studies have challenged this concept and have shown that there is either vascularity remains unaltered as the stage advances or there is

a significant increase in the number of blood vessels.[6-8] In a morphometric analysis Rajendran et al were the pioneers to demonstrate that mean vascular density does not alter as the stage advances; also the luminal diameter and area percentage showed an increasing trend.[6] these finding were confirmed individually by Desai et al, immunohistochemically[7] and Fang et al, morphometrically.[8]The varied results are further complicated by variegated methods of assessing vascularity or angiogenesis. While morphometry is used in some studies on H&E-stained sections, vascularity was else-wise assessed by various immunohistochemical markers in the other studies. Further, studies have demonstrated that as OSMF turns malignant through dysplastic changes in epithelium, the vascular density increases, depicting a temporal shift in the microenviroment.[3]

Irrespective of all, angiogenesis and vascularity are indeed the key factors in the malignant transformation and progression of the disease. As there is conflict of information in the existing literature regarding vascularity with advancement of stage in OSMF and if there is any connection between vasculogenesis and malignisation, the present systematic review was planned to systematically gather and abridge the available data on vascularity and angiogenesis in oral submucous fibrosis to update the current cognizance of the disease progression and malignant transformation in a nutshell.

Material and Methods

Protocol and registration

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were used to design the present systematic review. It was registered at the International Prospective Register of Systematic Reviews (PROSPERO) database CRD42021226351. The research question was 'Does vascularity changes with increasing histological grades of oral submucous fibrosis and if it has any correlation with malignant transformation?' The PICO for the present review are as follows: Population: Oral submucous fibrosis; Comparison: Assessment of vascularity in OSMF with normal healthy controls; Outcome: Evaluation of vascularity in histological grades of OSMF and its correlation in malignant transformation.

Eligibility Criteria

All the papers were included in the review if they met the following criteria: (a) Full-length original articles published in the English language only, (b) Studies that included a quantitative assessment of vascularity and/or angiogenesis in oral submucous fibrosis irrespective of the method employed for quantification.

Information sources and search strategy

Two authors independently searched the electronic databases namely, MEDLINE by PubMed, SCOPUS, Web of Science, EMBASE and Google Scholar for the following keywords singly or in combination: (ALL ("oral submucous fibrosis"/"OSMF") AND ALL ("vascularity/angiogenesis", "morphometric", "CD31", "CD34", "bFGF", "mast cells", "CD105", "VEGF", "von Willebrand factor", "angiogenic markers")). Articles that ascertained the aforementioned eligibility criteria were included and appraised further to obtain the data.

Selection and data collection process

DP and SKN individually screened the titles and abstracts of all the articles. The papers which did not meet the eligibility criteria were excluded followed by eligibility evaluation by reading the complete articles and the reasons for exclusions were recorded. Any disagreements were resolved by discussion in a consensus meeting with other authors. The following information was extracted from the included articles: country of origin, author(s), year of publication, number of cases and controls, histological classification followed and the method used to assess vascularity/angiogenesis. The parameters were mean vascular density (MVD), mean vessel luminal diameter (MVLD), mean vessel area percentage (MVAP), mean vascular perimeter (MVP) and total vascular area (TVA). Briefly, MVD is defined as the mean of the vessel count in the most vascularized areas from three to five high power fields. MVLD and MVP are estimated in a similar way utilizing an image software, where cursor is used to draw the outline of blood vessels at high magnification and mean is estimated. MVAP signifies evaluation of the area occupied by blood vessels in the entire field and finally TVA is the total of areas of all traced vessels at 400X magnification. Additionally, studies were recorded where oral squamous cell carcinoma arising from OSMF were included for comparative evaluation.

Summary Measures

The main outcome was the quantification of vascularity/angiogenesis in histological grades of oral submucous fibrosis

Data synthesis and statistical analysis

The quantitative data were tabulated and processed in Microsoft Excel (Microsoft Corporation. 2013). IBM SPSS statistics software version 25 (IBM Analytics, Armonk, New York, U.S.) was used to analyze the data.

Risk of bias analysis

The Joanna Briggs Institute (JBI) critical appraisal checklist for analytical cross-sectional studies was used to assess the quality of the included studies where eight questions were evaluated and answered for various points with "Yes", "Not clear," and "No".[9] Finally the studies are categorized into three groups: a) low risk of bias (at least 70% of the quality criteria are fulfilled) b) moderate risk of bias (between 50% and 70% of the quality criteria are fulfilled), and c) high risk of bias (< 50% of the quality criteria are fulfilled). Two authors judged the risk of bias on each domain of the tool independently. Any discordance was resolved by a consensus meeting.

Results

The search strategy identified 98 articles published until 2022 from various electronic databases as aforementioned. After the removal of 21 duplicate articles, the remaining 77 articles were reviewed through the titles and abstract. Forty-three articles were excluded with appropriate reasoning, resulting in 34 articles. These 34 articles were selected for the eligibility evaluation, which was carried out by reading the full text by the authors (DP&SKN). At this stage, 21 articles were further excluded due to the lack of quantification of vascularization in different grades of OSMF. Finally, thirteen articles were selected for the present review.[3,6,7,10-19] The PRISMA flowchart is given in Figure 1.

Characteristics of the selected studies

Data extracted from all 13 studies including the details of the country of origin, authors, number of cases and controls incorporated, classification system followed, methodology used, parameters assessed, and results are provided in Table 1.[3, 6, 7,10-19]

The included studies were conducted in India between 2005 and 2022. A total of 607 OSMF cases and 110 controls were included, along with 5 cases of OSMF with dysplasia, 2 OSMF turning to oral squamous cell carcinoma (OSCC) and 30 OSCC (well differentiated, WDSCC) were included as comparison groups. 53.8% of the selected studies used immunohistochemical markers such as CD34, factor VIII, VEGF for quantitative assessment of the vascularity at varied stages of OSMF. 46.2% of the studies used hematoxylin and eosin-stained slides for the same. Among the included studies, 3 (23.1%) did not use any control groups [10,12,18] and only 2 (15.4%) studies added comparison groups other than control groups.[3,13]

Demographic data

The demographic details of cases and controls were retrieved from 8 studies;[3,11,12,14,15,17-19] while five studies did not provide any such details.[6,7,10,13,16] Thirteen selected studies included a total sample size of 607 OSMF cases and 110 controls. However, the demographic details were specified only for 368 cases, out of which 285 (77.4%) were males and 83 (22.6%) were females (M:F::3.44:1). Of the included 13 studies, only 4 mentioned the habit history and duration of the habits.[3,11,14,15]

Mean Vascular Density of different grades of OSMF

Eleven of thirteen studies (84.6%) evaluated the Mean Vascular Density (MVD) in different grades of OSMF.[3,6,7,11-17,19] Six of the 11 included studies (54.5%) reported a decrease in MVD as the grades of oral submucous fibrosis (OSMF) advanced.[3,12-14,17,19] Pandiar *et al* [3] proposed that MVD reduced from normal mucosa to advanced OSMF and further increased to OSMF with dysplasia and OSMF with OSCC (N (Normal-40.08)>E (Early OSMF-20.48)>MA (Moderately advanced OSMF-17.40)>A (Advanced OSMF-14.85)<OSMF-D (OSMF with dysplasia-22.04)<OSMF-OSCC (OSMF turning malignant-42.30), however, the other 4 studies showed an increase of MVD from normal mucosa to early OSMF and then decreased to Advanced OSMF (N<E>MA>A).[13,14,17,19] Four studies failed to establish a statistically significant variation in MVD between different grades of OSMF and the control group.[6,7,11,16] One out of eleven included studies showed a discordant data set, hence categorized separately in this review. [15]

Endothelial cells /square μm

Two studies specifically computed the number of endothelial cells /square μm and thus were categorized separately.[10,18] Irrespective of the parameter used both articles reported that the number of endothelial cells decreased from very early to advanced OSMF similar to MVD reported in other studies.

Mean vascular area percentage (MVAP) & mean vascular area (MVA)

In total, 7 studies evaluated MVA/MVAP in different grades of OSMF.[6,7,10,11,13,18,19] Four studies showed a decrease in MVA/MVAP from early to advanced OSMF.[10,13,18,19] Murgod *et al* [13] included WDSCC as a comparison group, and demonstrated that MVA/MVAP gradually declined from early to advanced OSMF and further increased to WDSCC. On the contrary, increased MVAP in advanced OSMF cases when compared to early OSMF was reported by Rajendran R et al (Control-0.16; Early OSMF-0.32 and advanced OSMF-1.02).[6] Two studies did not find any significant difference in MVAP between different grades of OSMF.[7,11]

Mean Vascular Luminal Diameter (MVLD)

Seven of 13 studies evaluated MVLD.[6,7,10,11,13,14,18] Four studies concluded that as the grades of OSMF advanced, the MVLD also reduced.[10,13,14,18] Further, among these four studies, Nitheash *et al* [14] reported maximum MVLD in moderately advanced OSMF (2.38 ± 1.10) but rest 3 studies reported maximum MVLD in early OSMF. Conversely, 1 study group showed an increase in MVLD along with the advancing grades of OSMF,[6] and 2 studies could not put forth any statistically significant difference in MVLD as the advancing grades of OSMF.[7,11]

Mean Vascular Perimeter (MVP)

Two studies (2 of 13) evaluated the MVP and its variability among different grades of OSMF and normal tissue.[11,14] One of these studies proposed a significant reduction of MVP in advanced OSMF when compared to early OSMF (maximum in Moderately advanced OSMF)[14] while the other research failed to establish any statistically significant variation in different grades of OSMF.[11]

Total Vascular Area

Only one study assessed this parameter and showed that more total vascular area is found in early OSMF when compared to advanced OSMF.[19] The studies which have included normal tissue samples as comparison groups, all of them showed an increase in MVD in Early OSMF when compared to normal mucosa, except one study which showed higher MVD in normal tissue than Early OSMF.[3]

Risk of bias within the studies

The results of the quality assessment of all the included studies are displayed in Figure 2. Except three studies, all the included studies showed high quality of estimation and a low risk of bias in which unclear risk was estimated in two domains.[12,17,18]

Discussion

Oral submucous fibrosis is one of the most common oral potentially malignant diseases in Southeast Asia, especially in the Indian subcontinent. The vascularity of OSMF has always been a conjecture. The vascularity of OSMF varies according to the advancement of grades. According to the conventional concepts, the increased and altered fibroblast proliferation in oral submucous fibrosis results in extensive fibrosis in the connective tissue stroma causing the blood vessels to obliterate, resulting in claudication of the vascularity and tissue hypoxia.[20] However, recent studies challenge the prevailing concept and suggest there is no significant decrease in vascularity with the advancement of OSMF. The present review was orchestrated to shed light on equivocality of vascularity with the advancement of stages.

The present study confirmed the fact that OSMF is a habit related progressive disease. Wherever available the most common habits included areca nut chewing, betel quid with tobacco, *paan*, or commercially available products. It has been previously found that the severity and duration of the habits correlated with increased histopathological grades of oral submucous fibrosis.[21] In line with the literature, the present review reiterates a preponderance in male gender. Interestingly, all the studies were from India.

In the present review 54.5% of the included studies supported that the mean vascular density decreases as the advancement of oral submucous fibrosis.[3,12-14,17,19] This reinforces the conventional theory that the increase in fibrosis is the result of increased TGF-β mediated fibroblastic proliferation.[22,23] One research group confirmed that arecoline promotes CD147 expression in oral keratinocytes via the TGF-β1 signaling pathway[22], who also opined that CD147 overexpression in OSMF is responsible for the progression of disease. TGF-β1 appears to play the major role in the fibrotic pathway while cytokine TGF-β2 acts as the contributor.[23] Areca nut chewing with or without slaked lime through various pathways activates tissue inhibitors of matrix metalloproteinases and induces copper-mediated activation of lysyl oxidases altogether contributing to the increased cross linking of collagen and further proliferation of fibroblasts. This further increases the fibrosis and results in hyalinization leading to obliteration of the blood vessels, thus reducing vascularity as the grade advances.[3] Four studies included in the present review did not find any statistically significant variation of MVD between the groups of OSMF.[6,7,11,16] This lack of significant variation could be attributed to hypoxia induced neovascularization in advanced OSMF cases. Hypoxia activates HIF-1 which further leads to VEGF mRNA, resulting in angiogenesis.[6] Another reason for such equivocal results could be number of samples included in the study, type of method used for quantification and variation in classification for grading of OSMF. It must be noted that two of these studies used clinical staging.[6,7] It must be mentioned here that previous studies have found no significant correlation between clinical and histopathological grading explaining the discordance regarding vascularity. [21,24,25]

The present systematic review of existing data depicts that the sequence of vascularity with advancing stages of OSMF is mostly consistent with increased angiogenesis in very early and early stages and reduction as the stage advances with a temporal shift in the nature of the inflammatory reaction. The view put forwarded by Tilakratne *et al* holds true here that desmoplasia and reduced vascularity of the corium, in the presence of altered cytokine activity, generates a microenvironment for carcinogens of areca nut such as arecoline and arsenic and/or tobacco.[26] The role of cytokines in fibrosis is well established in other body parts. It has been previously reported that mRNA expression of collagen (I&III) and fibronectin is upregulated in cultured lung fibroblasts through IL-1β and TNF-α.[27] Few studies have shown contrasting results

however, later research demonstrated that TNF-α inhibits adherence and phagocytosis of collagen.[28-30] Role of these cytokines is also demonstrated in OSMF.[31-33] As the fibrosis increases with concomitant spatial shift in nature of the inflammatory reaction and reduced vascularity, an important query arises regarding increased vascularity in OSMF with dysplasia and in malignant transformation which is discussed in subsequent section.

In the most recent systematic review and meta-analysis, malignant transformation rate (MTR) in OSMF has been reported to be 6% with wide heterogeneity among the different nations and ethnic groups.[34] Indian and Pakistani cohort showed the highest MTR as compared to Chinese and Taiwanese population.[34] As OSMF is a progressive condition, all the cases should be speculated as a potential candidate for malignisation. Further, most if not all cases undergoing transformation have been reported as well differentiated with low incidence of nodal dissemination.[35-36] In a recent paper we reported 21 cases of OSCC arising in a background of OSMF and hypothesized a putative role of copper in fibroplasia and vasculogenesis, a phenomenon reported as 'cuproplasia'.[1] As the disease advances the fibroblastic activity is stabilized resulting in fibrosis along with collapsed blood vessels explaining the reduced vascularity and decreased systemic absorption of known carcinogens compromising the atrophied epithelium. Few studies have however, shown no significant change in mean vascular density in the advanced stages with extreme contrasting results from other studies.[6,7] As aforementioned, this may be attributed to the methodology, type of assessment tool employed to quantify vasculature and sample size. However, when there is malignant transformation, the role of copper gets reversed, and has been hypothesized to be more protective through copper mediated autophagy, cuproptosis. This opens possibilities of application of copper in therapeutics in the early stages of OSMF where it bears a role in fibroplasia and vasculogenesis.

Conclusion

In conclusion, the present review of existing data supports the prevailing concept regarding vasculature of OSMF that with advancement of stage of OSMF the vascularity decreases, denying systemic absorption of carcinogens into the circulation with resultant longer exposure of compromised epithelium and malignisation.

Conflict of Interest

No conflict to disclose

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contribution

DP: Acquisition of data, Conception and design, analysis and interpretation of data, and drafting of the manuscript; SKN: Acquisition of data, literature review, interpretation of data; RBM & RPK: Article screening, interpretation of data, final revision of the article; NS & RA: assessment of risk bias and preparation of images, review of manuscript and language editing; SC & PS: preparation of PRISMA flow chart and final revision. All the authors approved the final version.

References

1. Pandiar D, Krishnan RP, Ramani P, Anand R, Sarode S. Oral submucous fibrosis and the malignancy arising from it, could best exemplify the concepts of cuproplasia and cuproptosis. J Stomatol Oral Maxillofac Surg. 2023 Feb;124(1S):101368. https://doi.org/10.1016/j.jormas.2022.101368
2. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol. 1966 Dec;22(6):764-79. https://doi.org/10.1016/0030-4220(66)90367-7
3. Pandiar D, Shameena P. Immunohistochemical expression of CD34 and basic fibroblast growth factor (bFGF) in oral submucous fibrosis. J Oral Maxillofac Pathol. 2014 May;18(2):155-61. doi: 10.4103/0973-029X.140718
4. Chiu CJ, Chiang CP, Chang ML, Chen HM, Hahn LJ, Hsieh LL, et al. Association between genetic polymorphism of tumor necrosis factor-alpha and risk of oral submucous fibrosis, a pre-cancerous condition of oral cancer. J Dent Res. 2001 Dec;80(12):2055-9. https://doi.org/10.1177/00220345010800120601
5. Misra SP, Misra V, Dwivedi M, Gupta SC. Oesophageal subepithelial fibrosis: an extension of oral submucosal fibrosis. Postgrad Med J. 1998 Dec;74(878):733-6. https://doi.org/10.1136/pgmj.74.878.733

6. Rajendran R, Paul S, Mathews PP, Raghul J, Mohanty M. Characterisation and quantification of mucosal vasculature in oral submucous fibrosis. Indian J Dent Res. 2005 Jul-Sep;16(3):83-91.
7. Desai RS, Mamatha GS, Khatri MJ, Shetty SJ. Immunohistochemical expression of CD34 for characterization and quantification of mucosal vasculature and its probable role in malignant transformation of atrophic epithelium in oral submucous fibrosis. Oral Oncol. 2010 Jul;46(7):553-8. https://doi.org/10.1016/j.oraloncology.2010.04.004
8. Fang CY, Han WN, Fong DY. A morphometric study on the microvessel in oral submucous fibrosis. Hunan Yi Ke Da Xue Xue Bao. 2000 Feb 28;25(1):55-7.
9. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Chapter 7: Systematic reviews of etiology and risk. JBI Manual for Evidence Synthesis for analytical cross-sectional studies. JBI, 2020. https://doi.org/10.46658/JBIMES-20-08
10. Debnath S, Mitra B, Paul B, Saha TN, Maity A. Morphometric analysis of oral submucous fibrosis and its correlation with histological staging and clinical severity of trismus. Egypt J Ear Nose Throat Allied Sci 2013;14:85-90. https://doi.org/10.1016/j.ejenta.2013.04.005
11. Garg N, Mehrotra R R. Morphometric analysis of epithelial thickness and blood vessels in different grades of oral submucous fibrosis. Malays J Pathol. 2014 Dec;36(3):189-93.
12. Thakkannavar SS, Naik VV. Histochemical and Immunohistochemical Analysis of Collagen Fibers and Microvascular Density in Various Grades of Oral Submucous Fibrosis. Iran J Pathol. 2019 Spring;14(2):127-134. https://doi.org/10.30699/ijp.14.2.127
13. Murgod VV, Kale AD, Angadi PV, Hallikerimath S. Morphometric analysis of the mucosal vasculature in oral submucous fibrosis and its comparison with oral squamous cell carcinoma. J Oral Sci. 2014 Jun;56(2):173-8. https://doi.org/10.2334/josnusd.56.173
14. Nitheash P, Bastian TS, Cyriac MB, Selvamani M, Malini P. Epithelial and Connective Tissue Changes in Oral Submucous Fibrosis – A Morphometric Analysis. Ann Clin Lab Res. 2021; Vol.9 No.9:370.
15. Pammar C, Nayak RS, Kotrashetti VS, Hosmani J. Comparison of microvessel density using CD34 and CD105 in oral submucous fibrosis and its correlation with clinicopathological features: An immunohistochemical study. J Cancer Res Ther. 2018 Jul-Sep;14(5):983-988. doi: 10.4103/0973-1482.181186

16. Sabarinath B, Sriram G, Saraswathi TR, Sivapathasundharam B. Immunohistochemical evaluation of mast cells and vascular endothelial proliferation in oral submucous fibrosis. Indian J Dent Res. 2011 Jan-Feb;22(1):116-21. doi: 10.4103/0970-9290.80009.
17. Sharma E, Tyagi N, Gupta V, Narwal A, Vij H, Lakhnotra D. Role of angiogenesis in oral submucous fibrosis using vascular endothelial growth factor and CD34: An immunohistochemical study. Indian J Dent Res. 2019 Sep-Oct;30(5):755-762. doi: 10.4103/ijdr.IJDR_186_17
18. Singh M, Chaudhary AK, Pandya S, Debnath S, Singh M, Singh PA, Mehrotra R. Morphometric analysis in potentially malignant head and neck lesions: oral submucous fibrosis. Asian Pac J Cancer Prev. 2010;11(1):257-60.
19. Tekade SA, Chaudhary MS, Tekade SS, Sarode SC, Wanjari SP, Gadbail AR, et al. Early Stage Oral Submucous Fibrosis is Characterized by Increased Vascularity as Opposed to Advanced Stages. J Clin Diagn Res. 2017 May;11(5):ZC92-ZC96. doi: 10.7860/JCDR/2017/25800.9948
20. Sirsat SM, Pindborg JJ. The vascular response in early and advanced oral submucous fibrosis. Acta Pathol Microbiol Scand. 1967;70(2):179-84. doi: 10.1111/j.1699-0463.1967.tb01280.x
21. Pandya S, Chaudhary AK, Singh M, Singh M, Mehrotra R. Correlation of histopathological diagnosis with habits and clinical findings in oral submucous fibrosis. Head Neck Oncol. 2009 May 2;1:10. https://doi.org/10.1186/1758-3284-1-10
22. Wang W, Xiong H, Hu Z, Zhao R, Hu Y, Chen W, Han Y, Yang L, Hu X, Wang C, Mao T, Xia K, Su T. Experimental study on TGF-β1-mediated CD147 expression in oral submucous fibrosis. Oral Dis. 2018 Sep;24(6):993-1000. https://doi.org/10.1111/odi.12845
23. Kamath VV, Krishnamurthy S, Satelur KP, Rajkumar K. Transforming growth factor-β1 and TGF-β2 act synergistically in the fibrotic pathway in oral submucous fibrosis: An immunohistochemical observation. Indian J Med Paediatr Oncol. 2015 Apr-Jun;36(2):111-6. doi: 10.4103/0971-5851.158842
24. Motgi AA, Shete MV, Chavan MS, Diwaan NN, Sapkal R, Channe P. Assessment of correlation between clinical staging, functional staging, and histopathological grading of oral submucous fibrosis. J Carcinog. 2021 Oct 7;20:16. doi: 10.4103/jcar.jcar_8_21

25. Kanneganti S, Kattappagari KK, Tanuja K, Chandra K L, Poosarla C, Baddam VR. Oral submucous fibrosis: Clinical and histopathological correlation of collagen fibers using Masson's trichrome and Van Gieson stains. J NTR Univ Health Sci 2018;7:181-4. doi: 10.4103/JDRNTRUHS.JDRNTRUHS_78_17
26. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8. https://doi.org/10.1016/j.oraloncology.2005.08.005
27. Zhang Y, Lee TC, Guillemin B, Yu MC, Rom WN. Enhanced IL-1-beta and tumour-necrosis-factor-alpha release and messenger-RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos exposure. J Immunol 1993; 150: 4188–96.
28. Dayer JM, de Rochemonteix B, Burrus B, Demczuk S, Dinarello CA. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. J Clin Invest. 1986 Feb;77(2):645-8. doi: 10.1172/JCI112350
29. Mauviel A, Heino J, Kähäri VM, Hartmann DJ, Loyau G, Pujol JP, et al. Comparative effects of interleukin-1 and tumor necrosis factor-alpha on collagen production and corresponding procollagen mRNA levels in human dermal fibroblasts. J Invest Dermatol. 1991 Feb;96(2):243-9. https://doi.org/10.1111/1523-1747.ep12462185
30. Chou DH, Lee W, McCulloch CA. TNF-alpha inactivation of collagen receptors: implications for fibroblast function and fibrosis. J Immunol. 1996 Jun 1;156(11):4354-62. https://doi.org/10.4049/jimmunol.156.11.4354
31. Haque MF, Meghji S, Khitab U, Harris M. Oral submucous fibrosis patients have altered levels of cytokine production. J Oral Pathol Med. 2000 Mar;29(3):123-8. https://doi.org/10.1034/j.1600-0714.2000.290304.x
32. Haque MF, Harris M, Meghji S, Barrett AW. Immunolocalization of cytokines and growth factors in oral submucous fibrosis. Cytokine. 1998 Sep;10(9):713-9. https://doi.org/10.1006/cyto.1997.0342
33. Kaur J, Jacobs R. Proinflammatory cytokine levels in oral lichen planus, oral leukoplakia, and oral submucous fibrosis. J Korean Assoc Oral Maxillofac Surg. 2015 Aug;41(4):171-5. doi: 10.5125/jkaoms.2015.41.4.171.

34. Murthy V, Mylonas P, Carey B, Yogarajah S, Farnell D, Addison O, et al. Malignant Transformation Rate of Oral Submucous Fibrosis: A Systematic Review and Meta-Analysis. J Clin Med. 2022 Mar 24;11(7):1793. doi: 10.3390/jcm11071793
35. Sarode SC, Sarode GS. Better grade of tumor differentiation of oral squamous cell carcinoma arising in background of oral submucous fibrosis. Med Hypotheses. 2013 Oct;81(4):540-3. doi: 10.1016/j.mehy.2013.07.001
36. Chaturvedi P, Vaishampayan SS, Nair S, Nair D, Agarwal JP, Kane SV, et al. Oral squamous cell carcinoma arising in background of oral submucous fibrosis: a clinicopathologically distinct disease. Head Neck. 2013 Oct;35(10):1404-9. https://doi.org/10.1002/hed.23143

Table 1: Clinicopathological details and data pertaining to quantitative assessment of vascularity in OSMF cases retrieved from 13 included studies; NOM- normal oral mucosa, IHC-immunohistochemistry, H&E- haematoxylin and eosin, MVAP- Mean vascular area percentage, MVA -mean vascular area, MVLD- Mean Vascular Luminal Diameter, MVP- Mean Vascular Perimeter

Sl No	Author & year	Country	Classification	Cases	Control	Comparison	Method	Parameter	Statistical test used	Results
1	Rajendran et al 2005 [6]	India	Haider Et Al (2000) Clinical	20 Early-8 Advanced-12	10 NOM	Nil	H&E	MVD, MVAP, MVLD	ANOVA	MVD: No significant difference between groups (P>0.05) MVAP:Normal < Early < Advanced (P<0.001) MVLD-Normal < Early < Advanced (P<0.01)
2	Desai et al 2010 [7]	India	Lai Dr (1995) Clinical	30 Stage 2- 4 Stage	10 NOM	Nil	IHC (CD34)	MVD, MVAP, MVLD	ANOVA	MVD- No significant difference between

				3- 17 Stage 4- 9						groups(P>0.05) MVAP-No Significant difference between groups(P>0.05) MVLD- No Significant difference between groups(P>0.05)
3	Singh et al 2010 [18]	India	Sirsat And Pindborg (1967)	83 Very Early-9, Early-32, Moderately Advance-39 Advanced -3	None	Nil	H&E Van Gieson's picric acid, acid fuchsin stain, Masson's Trichrome	No of endothelial cells/LPF, MVAP, MVLD	CHI-SQUARE	1) No of endothelial cells/LPF: Very Early> Early> Moderately Advanced> advanced (VE &E P=0.051) (MA & A P=0.000) 2) MVA: Very Early< Early> Moderately Advanced> advanced (VE &E P=0.051) (MA & A P=0.000) 3) MVLD:Very Early< Early> Moderately Advanced> advanced (VE &E P=0.051) (MA & A P=0.000)
4	Sabrinath et al 2011[16]	India	Sirsat And Pindborg (1967)	30 Very Early-9, Early-14, Moder	10 NOM		IHC (Factor VIII)	MVD	ANOVA, T test	1. MVD:Normal < Very Early< Early< Moderately Advanced (P>0.05 between the groups)

				ately Advanced-7						2. MVD: Normal <OSMF (P<0.05)
5	Debnath et al 2013 [10]	India	Sirsat And Pindborg (1967)	100 Very Early-36, Early-29, Moderately Advanced-28, Advanced-7	None	Nil	H&E	No Of Endo Cells/Squm, MVAP, MVLD	ANOVA	1. No of endothelial cells/sq um: Very Early> Early> Moderately Advanced> Advanced (P<0.001) 2. MVAP: Very Early< Early> Moderately Advanced> Advanced (P<0.001) 3. MVLD: Very Early< Early> Moderately Advanced> Advanced (P<0.001)
6	Garg et al 2014 [11]	India	Sirsat And Pindborg (1967)	35 Very Early-7, Early-14, Moderately Advanced-9, Advanced-5	10 NOM		H&E	MVAP, MVLD MVP	ANOVA	1. MVAP: No significant difference between groups (P=0.55) 2. MVD: No significant difference between groups(P=0.83) 3. MVP: No significant difference between groups (P=0.90)

7	Pandiar et al 2014 [3]	India	Sirsat And Pindborg (1967)	30 Early-11, Moderately Advanced-17, Advanced-2	10 NOM	OSMF-dysplasia- 5, OSMF-OSCC-2	IHC (CD34)	MVD	ANOVA	1. MVD: Normal > OSMF (P=0.000 2.Normal> Early> Moderately Advanced> Advanced (P=0.000) 3. Normal> Early> Moderately Advanced> Advanced <OSMF-D<OSMF-M (P=0.000)
8	Murgod et al 2014 [13]	India	Sirsat And Pindborg (1967)	60 30 Early, 30 Advanced	10 NOM	30 WDSCC	H&E	MVD, MVA, MVAP, MVLD		1. MVD: Normal< Early>Advanced < WDSCC (P<0.001) 2. MVA: Normal< Early>Advanced < WDSCC (P<0.001) 3. MVAP: Normal< Early>Advanced < WDSCC (P<0.001) 4. MVLD: Normal< Early>Advanced < WDSCC (P<0.001)
9	Tekade et al 2017 [19]	India	Lai Dr (1995) Clinical	45 15-Stage 1, 15-Stage	15 NOM	Nil	IHC (CD34)	MVD, MVA, TVA	Kruskal wallis	1. MVD: Normal< Stage 1> Stage 2> Stage 3 (P<0.00) 2. MVA: Normal>Stage

				2, 15- Stage 3						1> Stage 2> Stage 3 (P<0.00) 3.TVA: Normal<Stage 1> Stage 2> Stage 3 (P<0.00)
10	Pammar et al 2018 [15]	India	Lai Dr (1995) Clinical Sirsat And Pindborg (1967)	30 Stage 2- 23, Stage 3- 6, Stage 4-1 (CLINICAL) Early- 3, Moderately advanced- 18, Advanced-3	15 NOM	Nil	IHC (CD34 CD 105)	MVD	Chi-Square	1. MVD: Early > Moderately Advanced > Advanced (P value - not mentioned) 2. MVD: Normal> OSMF (P value not mentioned)
11	Sharma et al 2019 [17]	India	Sirsat And Pindborg (1967)	30 Very Early- 0, Early- 10, Moderately Advanced- 10, Advanced- 10	10 NOM	Nil	IHC (VEGF , CD34)	MVD	ANOVA, Independent t test	1. MVD: Very Early < Early > moderately Advanced >Advanced (P<0.001) 2. MVD: Normal < OSMF (P<0.001)
12	Thakkannavar et al 2019 [12]	India	Sirsat And Pindborg	40 Early- 20, Advan	None	Nil	IHC (Factor VIII)	MVD	Fischers exact test	1. MVD- Early > Advanced (p= 0.00)

			(1967)	ced-20						
13	Nitheash et al 2021[14]	India	Sirsat And Pindborg (1967)	75 Very Early-0, Early-25, Moderately Advanced-25, Advanced-25	10 NOM	Nil	H/E	MVD, MVLD, MVP	ANOVA	1. MVD: Normal< Early > Moderately advanced > Advanced ($p<0.05$) 2. MVLD: Normal > Early < Moderately advanced> Advanced ($p<0.05$) 3. MVP: Normal < Very early, < Moderately advanced> Advanced ($p<0.05$)

446

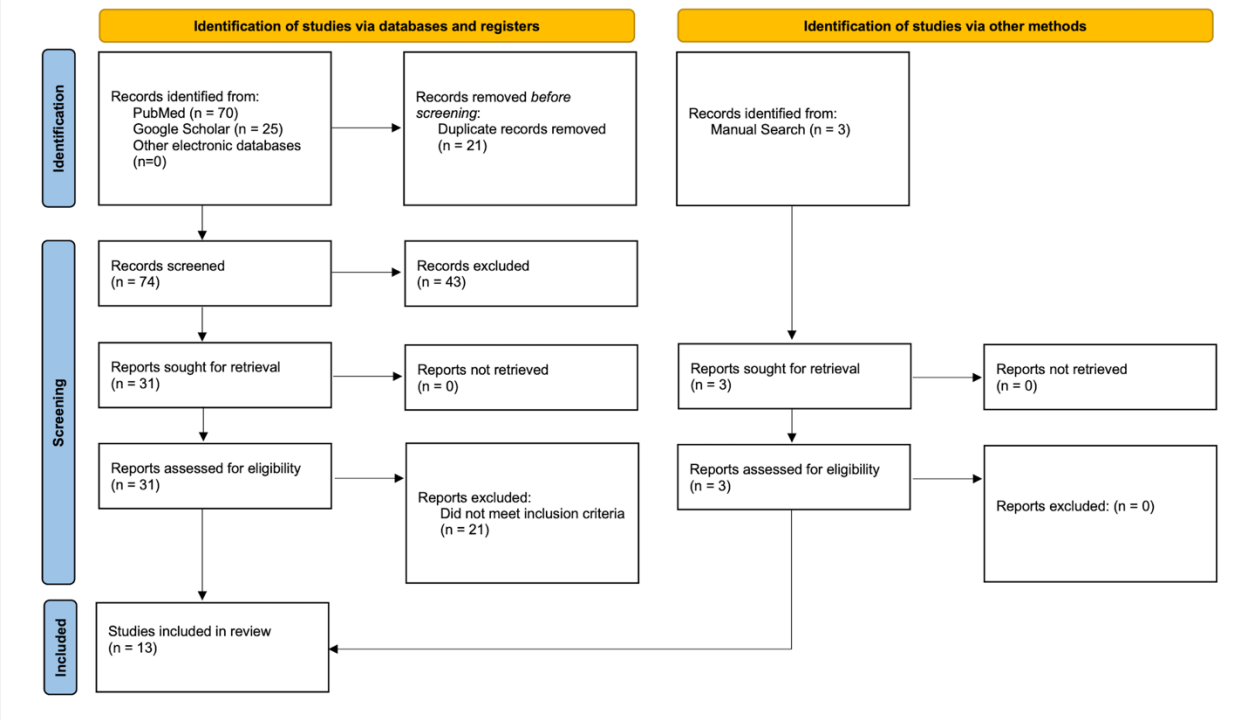

Figure 1: Flow chart of study selection adapted from PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and meta-Analysis)

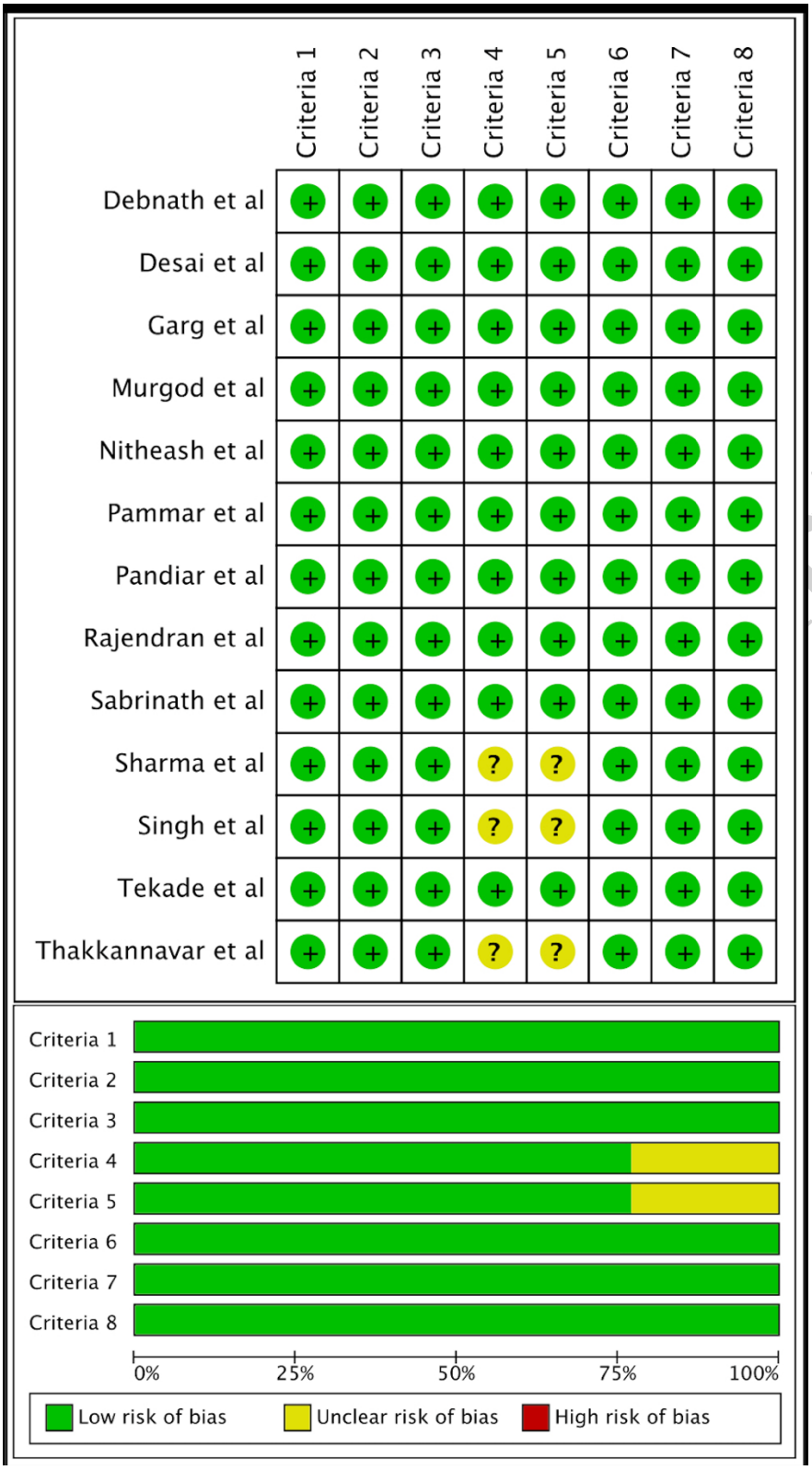

Figure 2: Risk of bias summary and graph (assessed by JBI critical appraisal checklist for analytical cross-sectional studies)