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 Chaudhari,⁵ Pooja Sharma⁶

¹Department of Oral Pathology and Microbiology, Saveetha University, Chennai, India; ²Department in Diagnostics in Oral Pathology and Oral Medicine, University of the Republic, Montevideo, Uruguay; ³Division of Oral Pathology & Microbiology and Forensic Odontolog, All India Institute Of Medical Sciences, New Delhi, India; ⁴Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Pune, India; ⁵Department of Conservative Dentistry and Endodontics, Saveetha University, Chennai, India; ⁶Department 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. 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. 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.

Vascularity in OSMF has always been a debatable territory with highly variable results yielded from case-control studies. 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. 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. 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. These
finding were confirmed individually by Desai et al, immunohistochemically\textsuperscript{7} and Fang et al, morphometrically.\textsuperscript{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 microenvironment.\textsuperscript{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
assessments 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”. 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. 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. 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 and only 2 (15.4%) studies added comparison groups other than control groups.  

**Demographic data**

The demographic details of cases and controls were retrieved from 8 studies, while five studies did not provide any such details. 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.

**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. Six of the 11 included studies (54.5%) reported a decrease in MVD as the grades of oral submucous fibrosis (OSMF) advanced. Pandiar et al 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). Four studies failed to establish a statistically significant variation in MVD between different grades of OSMF and the control group. One out of eleven included studies showed a discordant data set, hence categorized separately in this review.

**Endothelial cells /square µm**

Two studies specifically computed the number of endothelial cells /square µm and thus were categorized separately. 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. Four studies showed a decrease in MVA/MVAP from early to advanced OSMF. Murgod *et al* 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). Two studies did not find any significant difference in MVAP between different grades of OSMF.

**Mean Vascular Luminal Diameter (MVLD)**

Seven of 13 studies evaluated MVLD. Four studies concluded that as the grades of OSMF advanced, the MVLD also reduced. Further, among these four studies, Nitheash *et al* 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, and 2 studies could not put forth any statistically significant difference in MVLD as the advancing grades of OSMF.

**Mean Vascular Perimeter (MVP)**

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

**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. 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 pathway22, 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. 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. Four studies included in the present review did not find any statistically significant variation of MVD between the groups of OSMF. 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. 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. It must be mentioned here that previous studies have found no significant correlation between clinical and histopathological grading explaining the discordance regarding vascularity.

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 Tilakaratne 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. 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. Few studies have shown contrasting results however, later research demonstrated that TNF-α inhibits adherence and phagocytosis of collagen. Role of these cytokines is also demonstrated in OSMF. 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. Indian and Pakistani cohort showed the highest MTR as compared to Chinese and Taiwanese population. 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. 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’. 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. 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

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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**


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

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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 | India   | Sirsat and Pindborg (1967) | 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 | India   | Sirsat and Pindborg (1967) | 10 NOM OSM F-dysplasia-5, OSM F-OSCC-2 IHC (CD34) | MVD | 1. MVD: Normal > OSMF (P=0.000)  
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1. MVD: Normal< Early> Advanced < WSCC (P<0.001) 2. MVA: Normal< Early> Advanced < WSCC (P<0.001) 3. MVAP: Normal< Early> Advanced < WSCC (P<0.001) 4. MVLD: Normal< Early> Advanced < WSCC (P<0.001)
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<th>MVD Details</th>
<th>Statistical Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>et al 2018</td>
<td>India</td>
<td>Stage 2-23, Stage 3-6, Stage 4-1 (CLINICAL) Early-3, Moderately advanced-18, Advanced-3</td>
<td>NOM (CD34 CD105)</td>
<td>Square</td>
<td>&gt; Moderately Advanced &gt; Advanced (P value - not mentioned) 2. MVD: Normal &gt; OSMF (P value not mentioned)</td>
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<tr>
<td>Sharma et al 2019</td>
<td>India</td>
<td>Very Early-0, Early-10, Moderately Advanced-10, Advanced-10</td>
<td>NOM</td>
<td>IHC (VEGF, CD34)</td>
<td>ANOVA, Independent t test</td>
<td>1. MVD: Very Early &lt; Early &gt; moderately Advanced &gt; Advanced (P&lt;0.001) 2. MVD: Normal &lt; OSMF (P&lt;0.001)</td>
</tr>
<tr>
<td>Thakkannavar et al 2019</td>
<td>India</td>
<td>Early-20, Advanced-20</td>
<td>Nil</td>
<td>IHC (Factor VIII)</td>
<td>Fischers exact test</td>
<td>1. MVD: Early &gt; Advanced (p=0.00)</td>
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<tr>
<td></td>
<td>Nitheash et al 2021(^{14})</td>
<td>India</td>
<td>Sirsat And Pindborg (1967)</td>
<td>75 Very Early-0, Early-25, Moderately Advanced-25, Advanced-25</td>
<td>10 NOM</td>
<td>Nil</td>
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<tr>
<td>1. MVD:</td>
<td>Normal</td>
<td>Early</td>
<td>Moderately advanced</td>
<td>Advanced (p&lt;0.05)</td>
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<tr>
<td>2. MVLD:</td>
<td>Normal</td>
<td>Early</td>
<td>Moderately advanced</td>
<td>Advanced (p&lt;0.05)</td>
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<tr>
<td>3. MVP:</td>
<td>Normal</td>
<td>Very early</td>
<td>Moderately advanced</td>
<td>Advanced (p&lt;0.05)</td>
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</table>
**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)