Leaving no stone unturned: Role of profibrotic genes in oral submucous fibrosis – A systematic review

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Abstract

Introduction: Understanding the molecular pathogenesis of an entity helps in devising the mode of progression as well as mode of therapy. Even with years of research to claim the understanding of the molecular pathogenesis of oral submucous fibrosis (OSMF) is limited. More deeper knowledge of the genes responsible for this will help in understanding and managing this disease better.

Materials and Methods: The articles published during a time period of 1990–2020 were chosen in accordance with the inclusion and exclusion criteria according to the PRISMA guidelines.

Results: From a total of 80 articles obtained from both electronic search of PUBMED, EMBASE, MEDLINE and Cochrane registry as well as the manual search only 21 articles were selected and analyzed.

Conclusion: Careful analysis of the samples revealed that transforming growth factor-beta may be a potential biomarker or a candidate for targeted therapy in OSMF.

Keywords: Oral cancer, oral submucous fibrosis, profibrotic genes, SMAD, transforming growth factor-beta

INTRODUCTION

Genes play a vital role in determining the course of fibrosis. Numerous genes are either upregulated or downregulated during the fibrotic disease process. Pro-fibrotic genes propel the disease toward fibrosis and include growth factors such as transforming growth factor-beta (TGFβ) and fibroblast growth factor (FGF2), collagen genes like collagen type I alpha 1 (COL1A1), COL1A2, tumor necrosis factor (TNF), connective tissue growth factor (CTGF), lysyl oxidase, tissue inhibitors of matrix metalloproteinases (TIMP) and matrix metalloproteinases (MMP11, MMP12, MMP19 and MMP23).

Oral submucous fibrosis (OSMF) is a potentially malignant disorder with an increased prevalence in Asian countries, especially India. OSMF is a chronic, insidious disease that affects the lamina propria of the oral mucosa, and as the disease advances, it involves tissues deeper in the submucosa of the oral cavity with resulting loss of fibroelasticity. This disease is attributed to the use of areca nut and slaked lime. Earlier many factors such as chili, infections, autoimmunity were all considered but at present, arecoline is identified as the sole causative trigger in the development of OSMF. With decades of studies, still the pathogenesis of this condition is like a deep pit which refuses to be filled. Recently, many researchers have given

Access this article online

Quick Response Code: Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_102_21

How to cite this article: James A, Jayan L, Ramadoss R, Arunachalam P. Leaving no stone unturned: Role of profibrotic genes in oral submucous fibrosis – A systematic review. J Oral Maxillofac Pathol 2022;26:228-35.
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their valuable time to try and fill this void. In this review, our aim is to analyze the literature to assess the role of profibrotic antifibrotic genes in the pathogenesis of OSMF.

MATERIALS AND METHODS

Criteria used for selection of studies

Types of studies
Original researches (randomized control trials, case control, cohort, etc.) evaluating the role of profibrotic genes in the pathogenesis of OSMF were included, whereas overviews, narrative reviews, letter to editors, short communications, case reports and case series were excluded from the study.

Types of participants
Both in vitro and in vivo studies were analyzed in the existing literature for inclusion in this systematic review.

Outcomes of the study

Primary outcome
Effect of profibrotic genes in the pathogenesis of OSMF.

Secondary outcome
1. Evaluation of the role of these genes in malignant transformation of OSMF
2. Evaluation of the ability of these genes to act as potential targets in the management of OSMF.

Search strategy
Systematic review is the gold standard for answering any medicine-related question, for assessing association between a disease and cause or intervention or outcome. Detailed search strategies were developed for inclusion of studies for this review in accordance with the PRISMA checklist for systematic review as well as the Cochrane Highly Sensitive Search Strategy. The databases PUBMED, MEDLINE, Embase, LILAC and Cochrane Library were searched from 1990 to September 2020. The computer search strategy included two components where the first component focused on identifying the disease (OSMF) and the second component for the pathogenic factor (profibrotic genes). The search strategy was modified in accordance with the database that is searched to account for the difference in the vocabulary as well as the syntax rules. The first strategy utilizes the keywords fibrosis, OSMF, oral fibrosis or combination of the above for identifying the studies done on these conditions. The second strategy identified studies utilizing the profibrotic genes using the keywords profibrotic genes, CTGF, TGF-beta, SMAD, hypoxia-inducible factor 1 (HIF)-alpha, MMP, TIMP, TNF, etc., either alone or in various combinations. Broad search pathway was ensured by not including any keywords related to outcomes as well as study design. Manual search for researches was also performed for identifying relevant researches.

Databases searched for the samples
The electronic databases reviewed for the selection of the studies were the Cochrane Oral Health Group Trials Register, the Cochrane Central Register of Controlled Trials, MEDLINE through OVID, EMBASE through OVID, PUBMED CENTRAL, LILAC and Cochrane Library. In addition, manual search of articles was also conducted. Only articles published in English languages were considered. All articles published from 1990 to September 2020 were included in this review.

Exclusion criteria
Articles published in languages other than English were excluded. Articles that evaluated other pathogenic factors of fibrosis were excluded. Articles that included studies on other pathological conditions using these gene molecules were excluded.

Collection and analysis of data from the study samples
Two individual reviewers evaluated the title and abstract of each of the articles obtained from the electronic search engines to assess the eligibility. A third reviewer was included in case of disagreement in the eligibility of a study by the two reviewers. Full-text copies of all the eligible as well as potentially eligible studies were further evaluated next by all the reviewers. From this, the studies which did not meet the inclusion criteria were excluded. Any disagreement was resolved by discussion among all the authors.

Data extraction and management
The data were independently extracted by the two review authors. The reviewers were not blinded to the details of the study and its authors. All the data were extracted in accordance with the guidelines provided by the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011). For clarification or requirement of additional details, the authors of the studies were contacted through E-mail.

Assessment of risk of bias in included studies
Four main categories of bias are commonly encountered in a systematic review
1. Selection bias
2. Performance bias
3. Attrition bias
4. Reporting bias.

Risk was then categorized as high, low and unclear.
RESULTS

The search strategy using the combination of keywords yielded a total of 80 articles, from this, 59 articles were excluded in accordance with the PRISMA guidelines which included a series of systematic screening of the articles [identification, screening, eligibility and inclusion] giving a final sample size of 21 articles which satisfied all the inclusion and exclusion criterion. The search strategy of inclusion and exclusion of articles is illustrated in Figure 1. The specific study characteristics are recorded in Table 1 which depicts the evidence level of the studies included in the review. The profibrotic genes included in the current study are illustrated in the form of a pie chart [Figure 2].

DISCUSSION

OSMF is one of the most researched potentially malignant disorders as it has high malignant potential and even with years of research, there are still many stones left unturned which has impeded the understanding of this entity. Many esteemed researchers have made many enlightening breakthroughs in the molecular pathogenesis of OSMF. At present, profibrotic and antifibrotic genes have gained a lot of attention in the pathogenesis of OSMF. In our study, we have cumulated the literature for researches that have analyzed the role of profibrotic genes in OSMF. Out of the 21 studies included in our systematic review, 10 studies were conducted to evaluate either the role of TGF-beta in the pathogenesis or as a potential biomarker in the detection of the condition.

Sukumaran et al. from their study to analyze the TGF-beta polymorphisms in OSMF inferred that polymorphism of 5’UTR C-T in TGF-beta has a significant association in OSMF. They also suggested that although the exact role of this region in the progression or presentation of the disease is uncertain, at the same time is an area with potential for further research. Khan et al. used tissue microarray to identify and validate the genes expressed in OSMF. They also attempted to show the regulation of some of these genes by TGF-b and arecoline in keratinocytes and fibroblast cells. They showed that there is upregulation of TGF-b1, TGFBIp, THBS1, SPP1 and TIG1 and downregulation of BMP7. According to them, upregulation of profibrotic genes or cytokines and downregulation of antifibrotic molecules by TGF-beta may be the possible mechanism by which OSMF develops. The same authors published another paper in 2012, studying the gene expression profile in epithelial cells and fibroblasts following treatment with areca nut extract. They concluded that the expression of the profibrotic genes, especially TGF-beta induced by the polyphenols and alkaloids in areca nut, had little influence on the profibrotic molecule expression from fibroblast but induced increase expression in the epithelium. They proposed that the areca nut has a causative role in triggering profibrotic genes in the epithelial cells which further influence the underlying stroma to elicit the fibrotic response. Kale et al. evaluated the expression of TGF-beta in OSMF and its role in

Figure 1: Diagrammatic representation of selection of articles for the systematic review using PRISMA checklist

Figure 2: Profibrotic genes included in this systematic review
Early stages showed more intense evidence of PCNA, c-myc, EGFR, TGF-alpha in OSMF. Aim was to clarify whether the functional nucleotide polymorphism with different promoter activity of MMP3 related to the susceptibility and the Disease progression of OSCC and OSF. Identification of the expression profile in both fibroblasts and endothelial cells in OSMF and comparison of the level of the expression in various grades of OSMF. Evaluation of expression of FGF in both fibroblasts and endothelial cells in OSMF and comparison of the level of the expression in various grades of OSMF.

| Author and year of study | Profibrotic gene studied | Research objective | Evidence level of study | Sample size | Analytic method followed | Inference |
|-------------------------|-------------------------|--------------------|------------------------|-------------|-------------------------|-----------|
| Srinivasan et al. (2001) | TGF-alpha | Evaluate the expression of PCNA, c-myc, EGFR, TGF-alpha in OSMF | Level IV (Cohort) | OSMF: 15 OSCC: 10 | IHC evaluation | Increased expression of TGF-alpha is evident in the basal and parabasal or the proliferative layers of epithelium |
| Tu et al. (2006) | MMP-3 | Evaluation of expression profile in epithelial cells (HaCat) following treatment with areca nut extracts and the TGF-beta-induced gene expression profile | Level IV | Control-98 Case-OSMF: 71 OSCC: 150 | Polymerase chain reaction based genotyping | The 5A promoter group was more frequent in OSMF, but no association was found between 5A genotype in MMP3 promoter and site or lymph node metastasis as well as stage of OSCC |
| Bishen et al. (2008) | FGF | Evaluation of the expression of FGF in both fibroblasts and endothelial cells in OSMF and comparison of the level of the expression in various grades of OSMF | Level IV | Control-5 Case-30 | IHC evaluation | Increased expression of FGF in fibroblasts and endothelial cells with strong positivity in the early stages of the disease and reduced in advanced stages may be an immunomodulator in the initial stages of the disease. At the same time, it also contributes to the reduced vasculature and hypoxia with the progression of the disease |
| Tilakaratne et al. (2008) | HIF-1alpha | Evaluation of role of hypoxia in progression and malignant transformation of OSCM | Level IV | Control-10 Case-48 | Real-time polymerase chain reaction | The levels of HIF-1 alpha were elevated at both protein and mRNA levels, and the resulting hypoxia contributes to the fibrosis and progression of OSMF and eventually lead to malignant transformation |
| Mishra and Ranganathan (2010) | MMP-1 | To evaluate the role of MMP-1 in OSMF | Level IV | Control-10 Case-40 | IHC evaluation | The level of MMP-1 is elevated in OSMF, but there was no correlation between the levels of MMP-1 and the grade of the disease |
| Rajendran et al. (2010) | TGF-beta | Evaluation of polymorphisms in TGF-beta associated with OSMF | Level IV | Control-50 Case-50 | Quantification of DNA was performed with spectrophotometer and the analysis of polymorphism by Genotyping Microarray and real-time polymerase chain reaction to identify the genes in the sample | The polymorphism in 5’UTR C-T in TGF beta 1 gene has a significant association With OSMF, being a prime determinant in the pro-angiogenic pathway which has got direct bearing with the pathophysiology of the disease |
| Khan et al. (2011) | TGF-beta | Identification of differentially regulated genes in tissue microarray and analyze the role of TGF-beta and arecoline in regulating other genes present in keratinocytes and fibroblasts | Level IV | Control-11 (microarray) 10 (IHC) Case-16 (microarray) 11 (IHC) | Quantification of DNA was performed with spectrophotometer and the analysis of polymorphism by Genotyping Microarray and real-time polymerase chain reaction to identify the genes in the sample | The SMAD2 positivity has shown that TGF-beta activation is an important mediator in OSMF. On treating keratinocytes and fibroblasts with TGF-beta, there was upregulation of CTGF, TGM2, THBS1 at the same time downregulation of BMP7. This indicates that in OSMF, there is activation of TGF-beta and at the same time, there is downregulation of BMP-7. Polyphenol and alkaloid fractions in areca nut induces TGF-beta signaling and its downstream targets in epithelial cells but not in fibroblasts. This indicates that the arecoline-induced activation of SMAD2 upregulates and activates TGF-beta in OSMF and contributes to its progression. Early stages showed more intense TGF-beta staining than in advanced stages with loss of adipose tissue increasing as the grade progresses. The findings suggest that TGF-beta plays a key role in causing lipodystrophy in OSMF |
| Khan et al. (2012) | TGF-beta | Comparison of the gene expression profile in epithelial cells (HaCat) following treatment with areca nut extracts and the TGF-beta-induced gene expression profile | Level IV | 16 gingival tissues 17 human keratinocytes | Real-time polymerase chain reaction, microarray | |
| Kale et al. (2013) | TGF-beta | Correlation of the role of TGF-beta in the loss of adipose tissue in OSMF | Level IV | 84 OSMF (24-early stages, 60-advanced stages) | IHC evaluation | |
| Shrestha and Carmelo (2013) | MMP-2 TIMP-2 | Evaluation of expression and distribution of MMP-2 and TIMP-2 in various grades of OSMF | Level IV | Control-10 Case-30 | IHC evaluation | |

Contd...
### Table 1: Contd...

| Author and year of study | Profibrotic gene studied | Research objective | Evidence level of study | Sample size (each group) | Analytic method followed | Inference |
|--------------------------|--------------------------|--------------------|------------------------|-------------------------|--------------------------|-----------|
| Sodhi et al. (2014)      | TNF-alpha                | Evaluation of the levels of TNF-alpha in OSF and to correlate the levels of TNF-alpha with disease severity | Level IV                | Control-25 Case-25      | Sandwich enzyme immunosorbent assay | The levels of TNF-alpha were increased in OSF, and there was positive correlation with the levels of TNF alpha and the severity of OSF |
| Kamath et al. (2014)     | TGF-beta                 | Assessment of correlation between levels of TGF-beta with stages and grades of OSF | Level IV                | Control-10 Case-OMSF: 58 Scar tissue: 5 | IHC analysis | The expression of TGF-beta in moderate grade of OSFM was comparable to that of scar or keloid tissue. Thus, fibrosis in both these conditions may be linked through the TGF-beta pathway |
| Patil et al. (2015)      | CTGF                     | Estimate the levels of CTGF in OSFM and to correlate the value with progression of the disease (stage and grade) | Level IV                | Control-40 Case-40      | Enzyme-linked immunosorbent assay | CTGF is elevated in OSFM group in comparison to the control group and at the same time should statistically significant elevation with the progression of stage or grade of the disease |
| Kamath et al. (2015)     | TGF-beta 1,2             | Correlate the levels of TGF-beta 1,2 with various stages of OSF | Level IV                | Control-10 Case-OMSF: 128 (58 for TGF-beta 1; 70 for TGF-beta 2) Scar tissue: 4 | IHC evaluation | The level of TGF-beta 1 was higher than TGF-beta 2 in OSFM. TGF-beta 1 is the promoter of the fibrosis in OSFM whereas TGF-beta 2 plays a contributory role |
| Maria et al. (2016)      | TGF-beta 1               | Areca nut and pan masala extracts where used to induce OSFM and then the expression of TGF-beta 1 is assessed | Level IV                | Control-10 Case-20 (10 in each group) | Real-time polymerase chain reaction | Significant upregulation of TGF-beta 1 was reported, and a statistically significant elevation was noticed with the progression of the disease |
| Pant et al. (2016)       | TGF-beta                 | Assessment of regulation of TGF-beta signaling by areca nut in epithelial cells and the evaluation of the mechanism by which it is induced in the epithelial cells in OSFM | Level VI                | HeLa cell lines are used | Immunoblotting technique | TGF-beta signaling can be induced by areca nut extract which leads to fibrosis in OSFM |
| Iyengar et al. (2017)    | TGF-beta                 | Evaluation of the presence of COX-2 and TGF-beta in different stages and grades of OSFM | Level IV                | 35 subjects with OSFM | IHC evaluation | Progressive increase in the levels of TGF-beta and COX-2 with advancement of the disease both stage and grade. Both the molecules play an important role in the progression of the disease |
| Katarkar et al. (2018)   | MMP-9                    | To investigate whether SNPs in promoter and coding region of MMP-9 gene may constitute the risk for OSFM and to elucidate the mechanism by which MMP-9 and its genetic variants may influence the pathogenesis of OSFM | Level IV                | Control-196 Case-189    | RT-PCR, Western blotting technique | Genotypic and functional study revealed definitive role of MMP-9 Coding SNPs R279Q, P574R and R668Q in the pathogenesis of OSFM with strong predictive and prognostic value to determine OSFM at early stages in the areca chewers. Pathologically, overexpression of MMP-9 leads to decrease in collagen type-IV and epithelial thinning which contribute to basement membrane degradation along With continuous accumulation of collagen type-I enhanced by MMP-9; 1562C>T, R279Q, P574R and R668Q SNPs, resulting into early onset of OSFM The mRNA expression of all isotypes of TGF-beta and receptors were increased in OSFM. This indicates that the molecule plays a very important role in the molecular pathogenesis of OSFM |
| Rai et al. (2020)        | TGF-beta                 | Evaluation of role of TGF-beta in pathogenesis of OSF | Level IV                | Control-10 Case-33      | Real-time polymerase chain reaction | SMAD-7 expression is higher in OSCC and OSFM. It is a promotor for the progression of OSFM and development of OSFM |
| Hu et al. (2020)         | SMAD-7                   | Evaluation of the role of SMAD-7 in the progression of OSMF and OSCC | Level IV                | Control-12 Case-OMSF: 69 OSCC: 28 | IHC evaluation and cancer genome atlas analysis | |
reduction of adipose tissue seen in this condition. They showed that the TGF-beta promotes lipodystrophy and inhibits angiogenesis. It is suggested that the reduction of adipose tissue may also contribute to the stiffness of mucosa and sunken appearance of the cheeks.\[11\] Kamath et al. on correlating the levels of TGF-beta with different stages and grades of OSMF using IHC found a progressive increase TGF-beta with advancing grades of OSMF and also noted a substantial increase in scar tissue. This suggested that the fibrotic change in OSMF may be a reparative process in response to the injury by the noxious agents in areca nut, and thus, targeting this molecule may help in controlling the progression of the disease.\[13\] Pant et al. showed in their study that Maria et al. injected Sprague-Dawley mice with extracts of pan masala and areca nut, producing OSMF like lesions with similar clinical and histopathological features. The levels of TGF-beta were analyzed using quantitative real-time PCR which showed a significant increase in the levels, confirming that areca nut and pan masala induce the production of TGF-beta which induces the fibrotic change in OSMF.\[18,24\] Iyengar et al. aimed at evaluating the role of TGF-beta and COX-2 in the pathogenesis of OSMF to analyze potential targeted therapeutic applications. The levels of COX-2 were found to be higher in the early and moderate stages and grades of the disease progression whereas the levels of TGF-beta showed that progressive increase is noted with the progression of the disease.\[19\]

Rai et al. conducted a case–control study evaluating the expression of TGF-beta in OSMF to understand its role in the molecular pathogenesis of OSMF by RT-PCR which was confirmed with IHC evaluation. They inferred that TGF-beta 1 was most expressed isoform of the molecule and that the receptors 1, 2 were also elevated. Increase in the mRNA for all three isoforms was also noticed, confirming that it plays a major role in the molecular pathogenesis of OSMF.\[21\]

Connective tissue growth factor

CTGF or CCN2 is a matricellular associated heparin-binding protein. It plays important role in cell adhesion, migration, proliferation, angiogenesis, wound healing and skeletal development. Studies conducted on various fibrotic lesions revealed active participation of this gene, but this gene is not expressed in oral cavity under normal conditions. OSMF is the only condition where this gene is expressed and this may be a potential target for therapy. In this current review, only one study was included. Patil et al. estimated the serum levels of CTGF in OSMF, correlating the level of this gene with the different grades of the condition. It was inferred that the levels of CTGF levels were increased progressively from Grade 1 to Grade 3.\[13\] Blocking the activation of this profibrotic gene may pave a way in managing this condition better.

SMAD

It comprises a family of structurally similar proteins which act as signal transducers for receptors of the TGF-beta, regulating cell development and growth. Two researches were included in our current review. Hu et al. investigate the expression and function of SMAD7 in the progression of OSMF and OSCC. The SMAD7 levels were consistently upregulated in OSMF and OSCC, and the subsequent bioinformatics evaluation revealed that there was no mutation in the SMAD7 protein encountered in HNSCC and was elevated in OSCC.\[22\] Another study conduction in our institution by Zagabathina et al. in 2020 revealed that the levels of SMAD2 were more in OSMF than in reactive lesions or normal tissue. As mentioned before, SMAD2 being the initiator of transcription of TGF-beta further emphasizes the role of this cytokine if fibrogenesis in OSMF.\[23\]

Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases

Matrix metalloproteinase or collagenase is a group of enzymes produced by fibroblasts for degrading

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### Table 1: Contd...

| Author and year of study | Profibrotic gene studied | Research objective | Evidence level of study | Sample size | Analytic method followed | Inference |
|--------------------------|--------------------------|--------------------|-------------------------|-------------|--------------------------|-----------|
| Zagabathina et al. (2020) | SMAD-2 | Compare the expression of SMAD-2 in OSMF and reactive lesions | Level IV | Control-20 Case-OSMF: 40 Reactive lesions: 40 | Quantitative Sandwich enzyme-linked immunosorbent assay | There was a statistically significant elevation in the levels of SMAD2 in OSMF in comparison with control group. However, the levels of this molecule were not statistically significant. This indicates that SMAD plays a role in accentuating fibrosis in OSMF. |

TGF: Transforming growth factor, CTGF: Connective tissue growth factor, EGFR: Estimated glomerular filtration rate, OSMF: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma, MMP: Matrix metalloproteinases, OSF: Oral submucous fibrosis, FGF: Fibroblast growth factor, HIF: Hypoxia inducible factor, mRNA: Messenger RNA, IHC: Immunohistochemical, TIMP: Tissue inhibitors of matrix metalloproteinases, TNF: Tumor necrosis factor, SNP: single nucleotide polymorphism, PCNA: proliferating cell nuclear antigen
the extracellular matrix, and TIMPs are the enzymes responsible for inhibiting the action of MMPs. Chaudhary et al. analyzed the role of functional polymorphism of MMP-2 and 9 promoters in OSMF and HNSCC. The results concluded that SNPs in MMP-2 (-1306 C/T) and MMP-9 (-1562 C/T) promoter region may be associated with susceptibility to HNSCC, and addiction habits such as areca nut chewing and tobacco smoking may enhance the polymorphic association of C/T allele of the MMP-2 and MMP-9 gene polymorphisms in an Indian population. This polymorphism could be a prognostic maker in head-and-neck cancer.[28] Katarkar et al. concluded that a definitive role of MMP-9 coding SNPs has predictive and prognostic value in determining OSMF and can promote to the basement membrane degradation and epithelial atrophy.[29] Mishra et al. evaluated the role of collagenase 1 in OSMF. The intensity of MMP1 expression was decreased as the grade of the disease progressed.[7]

Fibroblast growth factor
FGF belongs to a family of cell signaling proteins necessary for normal development. One study conducted by Bishen et al. in 2008 was included in this review. The study evaluated the role of bFGF in the progression of OSMF and to study the changes in the stroma with increase in the severity of OSMF. The increase in bFGF expression in early stages of OSMF is parallel to the stage of injury caused by areca nut consumption. [20] This might be a contributory event or element in the molecular alteration at a cellular event in OSMF.

Tumor necrosis factor-alpha
TNF alpha is a cytokine which plays an important role in mediating inflammatory reactions in the body. Sodhi et al. evaluated the levels of TNF alpha with increase in disease severity. They found that the levels of TNF alpha increased with the severity of the disease.[13]

Hypoxia inducible factor 1 alpha
This gene is located on chromosome 14 and codes for a transcription factor that controls cellular responses to reduced oxygen concentrations within tissues. HIF-1α regulates numerous profibrotic mediators and contributes to fibrosis. TGF-β1 induces HIF1A stabilization in fibroblasts even without prominent hypoxic conditions. Tilkaratne et al. tested the role of hypoxia in the progression as well as malignant transformation of OSMF. They found that as the grade of dysplasia increased, the mRNA as well as the protein levels of HIF-1 alpha also increased. It may act as a potential marker for malignant transformation in OSMF[6].

In our study, we focused on the literature which has been done to evaluate the role of profibrotic genes in OSMF. We had collected 21 articles including one conducted in our institution and reviewed all of the samples. Out of these 21 articles, 10 were conducted using TGF-beta, all of the studies evaluated showed an increase in the molecule as the disease progresses. All the studies conducted had shown a positive response with each of these molecules showing elevation with progression of the disease. Since the research obtained for the other genes was scanty, the available literature supports the statement that TGF-beta plays an important role in the promotion of the disease. The role of these profibrotic genes has potential as both a promoter and a potential biomarker in OSMF. The expression of the genes when closely monitored may also be useful in analyzing the progression to a malignancy. The exact inference was difficult to obtain as there is no standardized procedure used, and many studies used IHC to study the molecules whereas others utilized PCR for the quantification of these genes. In addition to all this, if the exact function of these genes can be blocked, it may help in stopping the progression of the disease. Thus, profibrotic genes may be the future of research in OSMF as it may provide answer to the dilemma of the management of OSMF.

CONCLUSION
TGF-beta is a key mediator of the fibrotic cascade in OSMF. Careful analysis of the samples revealed that TGF-beta may be used as a potential biomarker or a candidate for targeted therapy and could aid in early diagnosis as well as predict the malignant transformation in OSMF.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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