Review Article

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

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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).[1,2]

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

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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 using TGF-beta, 4 on MMP and TIMPs, 2 on SMAD including one study conducted in our institution and one each on TGF-alpha, TNF-alpha, HIF-1 alpha, FGF.

Transforming growth factor-beta

TGF-beta is a multifunctional cytokine of the TGF superfamily which includes three isoforms 1, 2, 3. It is produced by all white blood cells. It is the most important cytokine implicated in the pathogenesis of OSMF. Research has shown that it is produced by the epithelium in the early stages of the disease and later, the stroma begins to produce with disease progression, possibly by the epithelial-mesenchymal interactions mediated by the signaling molecules seen in normal as well as the lesional mucosa. In our 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.[8] 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.[9] 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.[10] Kale *et al.* evaluated the expression of TGF-beta in OSMF and its role in

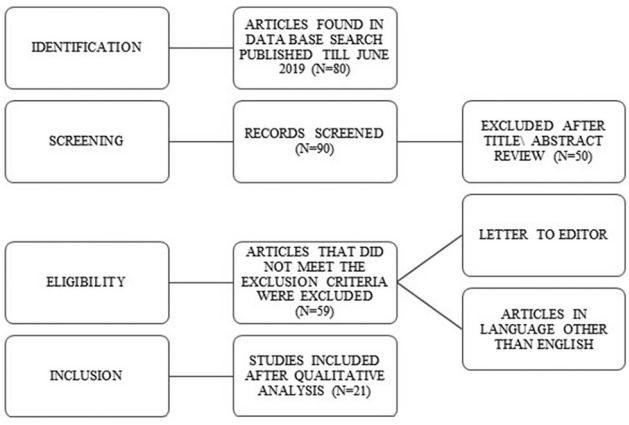

Figure 1: Diagrammatic representation of selection of articles for the systematic review using PRISMA checklist

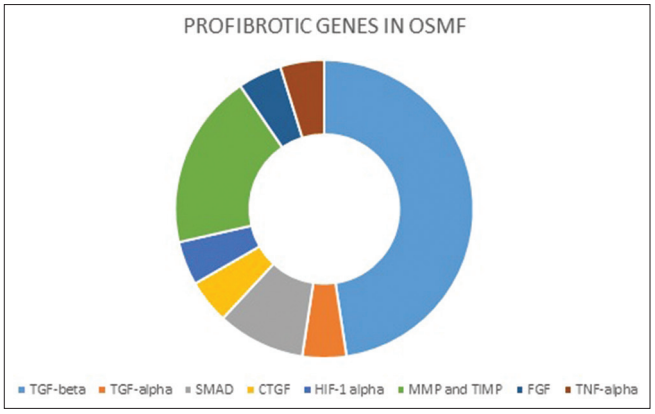

Figure 2: Profibrotic genes included in this systematic review

Table 1: Overview of the studies included in the review

Author and year of study	Profibrotic gene studied	Research objective	Evidence level of study	Sample size	Analytic method followed	Inference
Srinivasan *et al*. (2001)[3]	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)[4]	MMP-3	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	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)[5]	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)[6]	HIF-1alpha	Evaluation of role of hypoxia in progression and malignant transformation of OSMF	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)[7]	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)[8]	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	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)[9]	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)	Microarray and real-time polymerase chain reaction to identify the genes in the specimen IHC evaluation for the identification of the genes.	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
Khan *et al*. (2012)[10]	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	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
Kale *et al*. (2013)[11]	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	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
Shrestha and Carnelio (2013)[14]	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	Both MMP-2 and TIMP-2 were elevated with progression of the disease and may indicate their role as important mediators in the development and progression of the disease

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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
Sodhi *et al.* (2014)[12]	TNF-alpha	Evaluation of the levels of TNF-alpha in OSMF 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 OSMF, and there was positive correlation with the levels of TNF alpha and the severity of OSMF
Kamath *et al.* (2014)[13]	TGF-beta	Assessment of correlation between levels of TGF-beta with stages and grades of OSMF	Level IV	Control- 10 Case- OSMF: 58 Scar tissue: 5	IHC analysis	The expression of TGF-beta in moderate grade of OSMF 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)[15]	CTGF	Estimate the levels of CTGF in OSMF 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 OSMF 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)[16]	TGF-beta 1,2	Correlate the levels of TGF-beta 1,2 with various stages of OSMF	Level IV	Control- 10 Case- OSMF: 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 OSMF. TGF-beta 1 is the promoter of the fibrosis in OSMF whereas TGF-beta 2 plays a contributory role
Maria *et al.* (2016)[17]	TGF-beta 1	Areca nut and pan masala extracts where used to induce OSMF 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)[18]	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 OSMF	Level VI	HeLa cell lines are used	Immunoblotting technique	TGF-beta signaling can be induced by areca nut extract which leads to fibrosis in OSMF
Iyengar *et al.* (2017)[19]	TGF-beta	Evaluation of the presence of COX-2 and TGF-beta in different stages and grades of OSMF	Level IV	35 subjects with OSMF	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)[20]	MMP-9	To investigate whether SNPs in promoter and coding region of MMP-9 gene may constitute the risk for OSMF and to elucidate the mechanism by which MMP-9 and its genetic variants may influence the pathogenesis of OSMF	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 OSMF with strong predictive and prognostic value to determine OSMF 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 R688Q SNPs, resulting into early onset of OSMF
Rai *et al.* (2020)[21]	TGF-beta	Evaluation of role of TGF-beta in pathogenesis of OSMF	Level IV	Control-10 Case-33	Real-time polymerase chain reaction	The mRNA expression of all isotypes of TGF-beta and receptors were increased in OSMF. This indicates that the molecule plays a very important role in the molecular pathogenesis of OSMF
Hu *et al.* (2020)[22]	SMAD-7	Evaluation of the role of SMAD-7 in the progression of OSMF and OSCC	Level IV	Control-12 Case- OSMF: 69 OSCC: 28	IHC evaluation and cancer genome atlas analysis	SMAD-7 expression is higher in OSCC and OSMF. It is a promoter for the progression of OSMF and development of OSMF

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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)[23]	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

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.[15] 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 instituition by Zagabathina *et al.* in 2020 revealed that the levels of SMAD2 were more in OSMF than in reactive lesions or normal tissues. 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

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.[25] 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.[20] 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.[5] 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.[12]

Hypoxia inducible factor 1 alpha

This gene is located on chromosome14 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 as 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.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Fagone P, Mangano K, Mammana S, Pesce A, Pesce A, Caltabiano R, *et al.* Identification of novel targets for the diagnosis and treatment of liver fibrosis. Int J Mol Med 2015;36:747-52.
2. Karsdal MA, Nielsen SH, Leeming DJ, Langholm LL, Nielsen MJ, Manon-Jensen T, *et al.* The good and the bad collagens of fibrosis – Their role in signaling and organ function. Adv Drug Deliv Rev 2017;121:43-56.
3. Srinivasan M, Jewell SD. Quantitative estimation of PCNA, c-myc, EGFR and TGF-alpha in oral submucous fibrosis--an immunohistochemical study. Oral Oncol 2001;37:461-7.
4. Tu HF, Liu CJ, Chang CS, Lui MT, Kao SY, Chang CP, *et al.* The functional (-1171 5A-->6A) polymorphisms of matrix metalloproteinase 3 gene as a risk factor for oral submucous fibrosis among male areca users. J Oral Pathol Med 2006;35:99-103.
5. Bishen KA, Radhakrishnan R, Satyamoorthy K. The role of basic fibroblast growth factor in oral submucous fibrosis pathogenesis. J Oral Pathol Med 2008;37:402-11.

6. Tilakaratne WM, Iqbal Z, Teh MT, Ariyawardana A, Pitiyage G, Cruchley A, *et al*. Upregulation of HIF-1alpha in malignant transformation of oral submucous fibrosis. J Oral Pathol Med 2008;37:372-7.
7. Mishra G, Ranganathan K. Matrix metalloproteinase-1 expression in oral submucous fibrosis: An immunohistochemical study. Indian J Dent Res 2010;21:320-5.
8. Sukumaran A, Vidyadharan R, Banerjee M, Rajendran R, Harish R. Transforming growth factor-β-1 polymorphisms are infrequent but exist at selected loci in oral submucous fibrosis. Indian J Dent Res 2010;21:413.
9. Khan I, Agarwal P, Thangjam GS, Radhesh R, Rao SG, Kondaiah P. Role of TGF-β and BMP7 in the pathogenesis of oral submucous fibrosis. Growth Factors 2011;29:119-27.
10. Khan I, Kumar N, Pant I, Narra S, Kondaiah P. Activation of TGF-β pathway by areca nut constituents: A possible cause of oral submucous fibrosis. PLoS One 2012;7:e51806.
11. Kale AD, Mane DR, Shukla D. Expression of transforming growth factor β and its correlation with lipodystrophy in oral submucous fibrosis: An immunohistochemical study. Med Oral Patol Oral Cir Bucal 2013;18:e12-8.
12. Sodhi S, Sodhi JS, Khambete N, Kumar R, Marthala M, Sodhi NK. Expression of tumor necrosis factor α and its correlation with severity of oral submucous fibrosis: A case-control study. Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:704-8.
13. Kamath VV, Satelur KP, Rajkumar K, Krishnamurthy S. Transforming growth factor beta 1 in oral submucous fibrosis: An immunohistochemical study – Understanding the pathogenesis. J Dent Res Rev 2014;1:75.
14. Shrestha A, Carnelio S. Evaluation of matrix metalloproteinases-2 (MMP-2) and tissue inhibitors of metalloproteinases-2 (TIMP-2) in oral submucous fibrosis and their correlation with disease severity. Kathmandu Univ Med J (KUMJ) 2013;11:274-81.
15. Patil AA, Bhavthankar JD, Barpande SR, Mandale MS. Estimation of serum connective tissue growth factor in oral submucous fibrosis patients and its clinico-pathologic correlation. Journal of International Oral Health 2015;7:84-90.
16. Kamath V, Krishnamurthy S, Satelur K, 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;36:111.
17. Maria S, Kamath VV, Satelur K, Rajkumar K. Evaluation of transforming growth factor beta1 gene in oral submucous fibrosis induced in Sprague-Dawley rats by injections of areca nut and pan masala (commercial areca nut product) extracts. J Cancer Res Ther 2016;12:379-85.
18. Pant I, Rao SG, Kondaiah P. Role of areca nut induced JNK/ATF2/Jun axis in the activation of TGF-β pathway in precancerous Oral Submucous Fibrosis. Sci Rep 2016;6:34314.
19. Iyengar AR, Kumar V, Karnam S, Patil S, Simon SD, Zainab S, *et al*. The expression of cox-2 and TGF β in oral submucous fibrosis. International Journal of Development Research 2017;7.
20. Katarkar A, Prodhan C, Mukherjee S, Ray JG, Chaudhuri K. Role of matrix metalloproteinase-9 polymorphisms in basement membrane degradation and pathogenesis of oral submucous fibrosis. Meta Gene 2018;16:255-63.
21. Rai A, Ahmad T, Parveen S, Parveen S, Faizan MI, Ali S. Expression of transforming growth factor beta in oral submucous fibrosis. J Oral Biol Craniofac Res 2020;10:166-70.
22. Hu X, Xiong H, Wang W, Huang L, Mao T, Yang L, *et al*. Study on the expression and function of smad family member 7 in oral submucous fibrosis and oral squamous cell carcinoma. Arch Oral Biol 2020;112:104687.
23. Zagabathina S, Ramadoss R, Ah HP, Krishnan R. Comparative evaluation of SMAD-2 expression in oral submucous fibrosis and reactive oral lesions. Asian Pac J Cancer Prev 2020;21:399-403.
24. Brembilla NC, Dufour AM, Alvarez M, Hugues S, Montanari E, Truchetet ME, *et al*. IL-22 capacitates dermal fibroblast responses to TNF in scleroderma. Ann Rheum Dis 2016;75:1697-705.
25. Chaudhary AK, Pandya S, Mehrotra R, Singh M, Singh M. Role of functional polymorphism of matrix metalloproteinase-2 (-1306 C/T and -168 G/T) and MMP-9 (-1562 C/T) promoter in oral submucous fibrosis and head and neck squamous cell carcinoma in an Indian population. Biomarkers 2011;16:577-86.