Bridging Oral Pathology with Diagnostics by Comparative Analysis of Oral Fibrosis as a Precursor to Carcinogenesis: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Oral Submucous Fibrosis (OSF) is a chronic, potentially malignant condition that causes progressive fibrosis of the oral mucosa because people consume areca nuts. The purpose of this systematic review and meta-analysis was to compile the most recent data on molecular changes in OSF and assess how they relate to malignant transformation.

Methods: A PRISMA-guided thorough search of PubMed, Scopus, Web of Science, and Google Scholar was carried out through March 2025. Original studies assessing molecular or cellular alterations in OSF (with or without comparisons to oral squamous cell carcinoma (OSCC) or normal mucosa) were eligible. RevMan 5.4 was used to conduct the meta-analysis, and GRADE was used to evaluate the degree of evidence certainty, the Newcastle Ottawa tool, and the QUIN Tool for risk of bias.

Results: Eleven studies satisfied the requirements to be included. The main molecular changes were abnormal expression of p53 and Ki67, upregulation of hTERT, shifts in EMT markers, and dysregulation of TGF-β/SMAD signalling. The pooled effect sizes for EMT stemness -0.67 [-1.09, -0.25, I2 = 0%, p=0.0016], for proliferation 4.49 [1.68, 7.29, I2= 96.8%, p=0.0017], for telomerase activation -0.63 [-5.93, 4.68, I2 = 98.3%, p= 0.8172], for signalling pathway mediators -429.76 [-1289.16, 429.94, I2 = 98.2%, p = 0.3272]. The investigators assigned a moderate rating to evidence certainty.

Discussion: The molecular alterations in OSF are persistent and strongly suggest a risk of malignant cell transformation. Healthcare practitioners may be able to improve their early disease detection and risk level classification with the use of molecular biomarkers.

Keywords: Mouth Neoplasms, Oral Submucous Fibrosis, Cell Proliferation, Systematic Review, Meta-Analysis.

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INTRODUCTION

Oral Submucous Fibrosis (OSF) is a persistent, metamorphic, and unfading tissue disorder of the oral mucosa that primarily affects people of South Asian descent who chew areca nuts [1]. Numerous reports confirm that OSF can develop into oral squamous cell carcinoma (OSCC), which is why medical professionals categorize it as a potentially malignant condition [2]. The issue of early detection of malignant OSF transformations has not been resolved by advancements in diagnostic imaging and histopathology techniques due to its intricate biological patterns [3]. There has been a significant increase in scientific research over the past decade aimed at comprehending the molecular underpinnings of OSF disease progression [4].

Results of research studies have shown that the Transforming Growth Factor Beta/ Suppressor of Mothers against Decapentaplegic (TGF-b/SMAD) pathway signalling plays a key role in enhancing fibrotic alterations in the oral mucosa [5]. Individually, the Wnt/beta-catenin has been reported to also regulate fibrosis alongside epithelial transformation, but especially in its control of Epithelial-mesenchymal transition (EMT)-implicated genes [6]. EMT has now been long considered to be a pathophysiological force of how chronic inflammation leads to malignant transformation in oral submucous fibrosis. In an attempt to clarify these mechanisms, scientists have frequently studied molecular markers that relate to the risk of transformation. p53 has been studied to determine whether it carries tumor-suppressor changes in dysplastic oral tissue [7]. Moreover, cellular proliferation marker Ki67 was found in consistently up-regulated in both OSF and OSCC cases [8]. The subunit of telomerase, Human Telomerase Reverse Transcriptase (hTERT), has also been tested, which showed an enhanced activity with epithelial atypia [9]. Research regarding SMAD7 has discovered that SMAD7 plays both pro-fibrotic and tumorigenic roles. Meanwhile, E-cadherin, which is an important epithelial cell adhesion molecule, is often reduced during the EMT and pre-malignancy [10]. Given the growing body of molecular evidence, research-based synthesis of recent evidence was urgently needed.

The purpose of the study was to confirm that OSF exhibits consistent molecular markers that are indicative of dangerous pre-malignant development. The evaluation attempted to identify molecular potential targets for early diagnostic screening as well as predictive risk level assessment. When new molecular findings are obtained, OSF patients can benefit from early diagnosis interventions with individualized treatment plans.

METHODS

Study Design

This systematic review and meta-analysis study was conducted as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [11].

Literature Search Strategy

A thorough review of the literature was performed using PubMed, Scopus, Web of Science, and Google Scholar from January 2025 to March 2025. "Oral submucous fibrosis" and its variations, such as "OSF" and "oral squamous cell carcinoma," as well as "OSCC," "malignant transformation," "molecular biomarkers," "epithelial–mesenchymal transition," and "carcinogenesis," were employed as keywords in the study. MeSH terms and Boolean operators were used in the search refinement within the database search parameters.

Inclusion Criteria

To be eligible for inclusion, scientific studies had to meet four major criteria: comparison of OSF molecular or cellular changes to OSCC and normal tissue findings, original research design (in vitro, ex vivo, or clinical basis), outcome data on molecular biomarkers, and publication in English.

Exclusion Criteria

Review papers, case reports, conference abstracts, and studies without quantitative molecular data were not included in the study.

Outcomes Studied

The primary endpoints of this review were the levels of key cancer-related markers p53, Ki-67, hTERT, SMAD2, SMAD7, and E-cadherin in tissue, together with salivary Matrix Metalloproteinase MMP-8. Changes in these markers related to clinical stage, histopathological severity, and the risk of malignant transformation from oral submucous fibrosis to squamous cell carcinoma were studied as

secondary outcomes.

Data Screening

Two independent researchers conducted screening procedures for paper titles and abstracts, as well as full manuscript reviews. In case of any missing data, authors were contacted, or data was calculated using standard formulas. Disagreements were resolved through agreements.

Data Gathering

While gathering data on the author and date, research design, number of participants and biomarkers analyzed, research methods and results, and key findings, a structured data extraction table recorded study citations. Two independent reviewers gathered the data. The data was written in tabular form using an Excel Sheet. In terms of effect sizes, forest plots were generated.

Quality Assessment

The risk of bias was determined by using a modified version of the Newcastle-Ottawa Scale for observational studies and the Quality Assessment Tool for In Vitro Studies (QUIN Tool) for in vitro studies. The research team used GRADE to assess the risk of bias and inconsistency, as well as indirectness and imprecision, and publication bias, to determine the certainty of the evidence. For missing data risk of bis was not calculated.

Data Synthesis

RevMan version 5.4 was used to analyze the statistical data for the meta-analysis. The research employed pooled standard mean difference (SMD) with 95% confidence interval (CI) to examine outcomes that were considered eligible using the random effects model. Heterogeneity levels were calculated using the I2 statistic, which indicated >50% for significant variances. Sensitivity analyses were also carried out using the leave-one-out method. Subgroup analyses were performed for OSF and OSCC correlation. 7 observational study designs [12,13,14,15,16,17,18] and 4 in vitro study designs [19,20,21,22] were taken in this study. Observational studies were used for the forest plot, and in vitro for descriptive findings. In case of any missing data, the values were generated using standard formulas, such as in the case of standard deviation. If data could not be calculated, it was carefully taken near to zero to keep transparency as well as to generate the data tables and figures. However, these occurrences were once or twice; therefore, the majority of the data was taken from the studies.

RESULTS

Eleven studies were included in the qualitative synthesis and meta-analysis after meeting the inclusion criteria. Various research methodologies were used, including tissue cross-section analysis, patient-oriented prospective research, and in vitro modelling. Any study that lacked primary data, or didn't contain measurable outcomes, or didn't study OSF as a precursor was excluded. Over 300 specimens or samples were analysed using a combined data collection of OSF, OSCC, and normal mucosal tissue. **Figure 1** is used to display the flow diagram of the selection process.

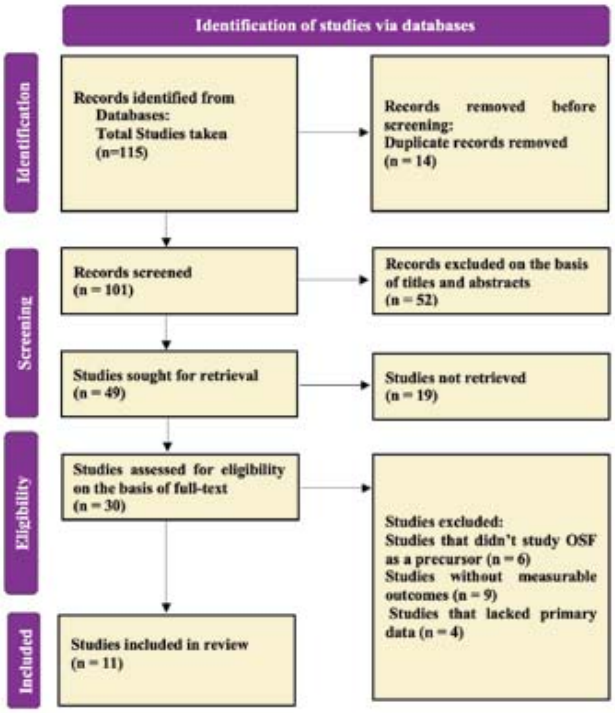

Figure 1: PRISMA Flow Diagram Demonstrating Study Scheme

Characteristics of Studies

The list of clinical studies in the systematic review includes seven studies, six of them cross-sectional and one prospective, published during 2020-2024; the smallest sample size is 50, and the largest is 180. Every clinical trial recruited adult participants who had a known history of areca-nut or betel-quid chewing with or without tobacco, and in certain groups of alcohol consumption, other premalignant conditions, substantial underlying systemic disorder, and previous cancer therapy constituted universal exclusion principles. The age of the participants was mainly 30 to 50 years old, and the majority of the cohorts had either a male majority or all males as the enrolled participants. The analyses entailed the evaluation of clinical biomarkers using epithelial mesenchymal transition markers (E cadherin, N cadherin, vimentin), proliferation index (Ki-67, p53), matrix-remodeling enzymes (MMP-8) signalling mediators (SMAD2, Dickkopf-1 (DKK1)) and telomerase activity (hTERT), on paraffin-embedded sections or quantitative analyses in saliva through use of immunohistochemistry and quantitative assays. Simultaneously, four mechanistic in vitro studies were carried out with primary fibroblasts cultures and biopsy samples: One of them investigated LINC00312, YBX1, the alpha-SMA, collagen, fibronectin, in five normal and 25 fibrotic tissues; another compared the conditioned media of normal and OSMF-derived fibroblasts in terms of MMP-2, IGF-1 and Insulin-Like Growth Factor 1 Receptor (IGF-1R) expression, and EMT marker regulation; in one study, noncoding RNA interactions (NEAT1, miR-760) and tropomyosin 1 (TPM1) were assayed in 30 mucosal tissues; while in another SMAD7 expression was analyzed by immunohistochemistry and real time polymerase chain reaction (RT-PCR) on 12 normal, 69 Oral Submucous Fibrosis (OSMF), and 28 OSCC specimens. The baseline characteristics of the observational studies are shown in **Table 1**.

Table 1*:* Baseline Characteristics of the *Observational Studies*

Study	Study design	Sample Size (N)	Groups	Age (Mean ± SD)	Gender (M/F)	Habit History	Exclusion Criteria
Shetty SS et al., 2024	Cross-sectional	100 (for IHC)	NOM (20), OSMF (40), OSCC in OSMF (20), OSCC (20)	NR	NR	Areca nut/betel quid use	Other PMDs or oral malignancies excluded
Kazmi A et al., 2022	Cross-sectional	60	Healthy (20), OSMF (20), OSCC (20)	Healthy: 33.1 ± 4.8OSMF: 32.5 ± 9.7OSCC: 46.8 ± 8.2	Healthy: 9/11 OSMF: 14/6 OSCC: 13/7	Betel nut, betel leaf, tobacco (avg. 15.2 ± 7 yrs)	Immunodeficiency, autoimmune disease, chronic disease, periodontal depth >5 mm, interincisal opening >35 mm
Kamala KA et al., 2024	Prospective	180	OSMF (60), Oral Cancer (60), Control (60)	OSMF: Mostly 21–40 yrsOC: Mostly 41–60 yrsControl: Mostly 21–40 yrs	OSMF: 53/7 OC: 50/10 Control: 38/22	Areca nut, tobacco, alcohol	Medically compromised patients
He X et al., 2020	Cross-sectional	70 samples from 56 patients	Healthy (10), OSMF (21), OSCC in OSMF (25), Cancer-adjacent (14)	Healthy: 27.9 ± 6.33OSMF: 32.95 ± 7.94OSCC in OSMF: 47 ± 7.23	Healthy: 2/8 OSMF: 21/0 OSCC: 25/0	OSMF: 71.43% betel nut, 71% smokingOSCC: 64% betel nut, 88% smoking	No severe systemic disease, no prior treatment or surgery
Zagabathina S et al., 2020	Cross-sectional	100	Control (20), Reactive lesions (40), OSMF (40)	Control: 28 ± 7.80Reactive: 32.55 ± 14.8OSMF: 36.95 ± 7.9	Control: 12/8 Reactive: 18/22 OSMF: 36/4	Betel nut assumed	Systemic illness or treatment for OSMF

Raju KL et al., 2020	Cross-sectional	50	Normal (10), OSMF (20), OSCC (15), OSCC in OSMF (5)	Normal: 40.20 ± 23.61OSMF: 40.10 ± 12.39OSCC: 52.07 ± 13.77	Normal: 2/8 OSMF: 14/6 OSCC: 7/8	Betel quid/smokeless tobacco >5 years	Systemic disorders (e.g., diabetes, HTN), recurrent OSCC
Hosur M et al., 2021	Cross-sectional	60	NOM (20), OSMF (20), OSMF with Dysplasia (20)	OSMF: 19–40 yrs OSMF+Dysplasia: 20–65 yrs	All groups: Male only	Areca nut chewing (2–30 years)	Other PMDs excluded; only histologically proven OSMF included

Table 2: Statistical Values of Biomarker Expression in Individual Observational Studies

Author & Year	Control (N)	Intervention Group (N)	Biomarkers Studied	Mean ± SD / Observation	p-value
Shetty SS et al., 2024	20	OSF (40)	E-cadherin, N-cadherin, CD44, PanCK, Vimentin	E-cad: 11.70 ± 3.11 (NOM), 9.66 ± 2.00 (OSF)N-cad: 5.50 ± 2.55 (NOM), 8.00 ± 3.75 (OSF)CD44: 6.25 ± 0.75 (NOM), 10.00 ± 2.00 (OSF) PanCK: 2.05 ± 0.50 (NOM), 2.45 ± 0.75 (OSF)Vim: 1.00 ± 0.01 (NOM), 1.25 ± 0.25 (OSF)	*p < 0.05 to < 0.001*
Kazmi A et al., 2022	20	OSMF (20), OSCC (20)	MMP-8 (Saliva)	Control: 7.9 ± 2.9 ng/mLOSMF: 0.66 ± 0.8OSCC: 0.64 ± 0.4	*p < 0.001*
Kamala KA & Sankethguddad S, 2024 [13]	60	OSF (60), OSCC (60)	p53, Ki67	p53: Control: 0.10 ± 0.30, OSMF: 2.26 ± 0.94Ki-67: Control: 0.05 ± 0.22, OSMF: 2.79 ± 0.61	*p < 0.001*
He X et al., 2020	10	OSF (21)	Dickkopf-1 (DKK1)	Rank Mean: OSF = 13.1, Normal = 22.1	*p = 0.004*
Zagabathina S et al., 2020	20	OSMF (40)	SMAD2	Control: 37.42 ± 22.16 pg/mLOSMF: 57.42 ± 21.30 pg/mL	*p = 0.028*
Raju KL et al., 2020	10	OSMF (20), OSCC (20), OSCC+OSMF (5)	hTERT	NOM: 2.60 ± 0.55OSMF: 6.15 ± 1.98OSCC: 8.67 ± 2.22	*p = 0.000*
Hosur M et al., 2021	20	OSMF (20)	E-cadherin, Twist1, Snail1	E-cadherin: NOM = 72.95 ± 16.72%, OSMF = 62.80 ± 25.15%	*P<0.05*

The mean and standard deviation of the biomarkers' expression in observational studies are shown in **Table 2** along with the respective statistical significance value.

Table 3: Summary of Findings of Individual in Vitro Studies

Author & Year	Sample Size	Molecular Markers	Outcomes	Key Findings
Yu CH et al., 2020	25 OSF biopsies, 5 normal mucosae	LINC00312, YBX1, α-SMA, Collagen I, Fibronectin	LINC00312 & YBX1 significantly upregulated in OSF tissues (p!<!0.05); positive correlation with fibrosis markers	LINC00312 promotes myofibroblast transdifferentiation via YBX1. Knockdown reduced fibrosis-related phenotypes
Chen PN et al., 2021	3 normal BMFs, 3 OSF fBMFs	MMP-2, IGF-1, IGF-1R, E-/N-cadherin, FAK, EMT panel	OSF-CM increased MMP-2 activity (p!<!0.001); IGF-1R upregulated; IGF-1R↑ leads to EMT, migration & invasion ↑ (p!<!0.001)	OSF fibroblasts induce EMT & invasion in OSCC via IGF-1/IGF-1R and MMP-2 activation
Li W & Cheng B, 2022	30 oral mucosal tissues (OSF + controls) (in vitro)	NEAT1, miR-760, TPM1, α-SMA	NEAT1 & TPM1 upregulated; miR-760 downregulated (p!<!0.05); linked to disease severity	NEAT1 enhances myofibroblast activity via miR-760/TPM1 and Wnt/β-catenin axis

Hu X et al., 2020	69 OSF, 28 OSCC, 12 normal tissues (in vitro + IHC)	SMAD7	SMAD7 significantly upregulated in OSF and OSCC vs. normal (p!<!0.0001); progressive increase NOM → OSF → OSCC	SMAD7 may drive fibrosis and malignancy progression; potential diagnostic marker

In vitro studies were taken separately as they differ significantly from observational studies. **Table 3** shows the outcomes and key findings of individual in vitro studies.

Outcomes of Studies

All clinical studies revealed that the expression of biomarkers changes significantly when comparing healthy mucosa and OSMF or OSCC tissues. Fibrotic samples exhibited a reduction in e-cadherin level by about 15-20 percent and an increase in N-cadherin level and vimentin level by about 40-60 percent ($p = <0.05$ to 0.001). p53 and Ki-67 proliferation markers, almost undetectable in controls, markedly increased to the mean immuno-reactivity scores of 2.3 and 2.8 in OSMF, respectively ($p < 0.001$). The levels of Salivary MMP-8 concentration decreased significantly to less than 0.7 ng/mL in OSMF and OSCC, at an average of 7.9 ng/mL in OSMF and OSCC ($p < 0.001$). The cytokine signalling through SMAD2 increased significantly, ~37-57 pg/mL in normal and fibrotic patients, respectively ($p = 0.028$), and the level of the DKK1 rank scores dropped significantly in OSMF compared to the control tissues ($p = 0.004$). The hTERT immunostaining for telomerase activity also showed a rise in activity compared with the control, with a mean of 2.6 against 6.2 in OSMF and 8.7 in OSCC ($p < 0.001$). Those results were supported by mechanistic in vitro findings: One study revealed that LINC00312 and YBX1 expression were three-fold to four-fold upregulated in fibrotic tissues compared to normal ($p < 0.05$), and LINC00312 knockdown decreased α-SMA and collagen deposition in fibroblast cells. Another study revealed that the conditioned media made by OSMF increased the MMP-2 activity by over 50% and upregulated IGF-1R 2-fold, which caused EMT with E-cadherin reduction and N-cadherin increase ($p < 0.001$). One study identified that NEAT 1 and TPM 1 were upregulated by 2.5 times with miR-7 up to 60 to 40% of normal levels ($p < 0.05$) in an implication of a Wnt / beta-catenin axis. The study observed a gradual increase in SMAD7 expression in normal tissues, OSMF, and OSCC groups (IHC scores 1.2, 2.8, and 4.1, respectively; $p = 0.0001$) to strengthen a TGF-bio fibrotic loop.

Meta-Analysis

In the meta-analysis, four domains of biomarker change were synthesized using random-effects models. For EMT-stemness markers such as E-cadherin and other related proteins, the pooled standard mean difference was –0.67 (95% CI –1.09 to –0.25; $p = 0.0016$) with no heterogeneity ($I^2 = 0\%$), which reflected a very consistent downregulation across studies. The individual study estimates, along with 95% confidence intervals, are shown as green squares and horizontal lines on each plot. The thickness of each square indicates the weight of the study, while the black diamond at the bottom indicates the overall effect and the confidence interval. I2 and chi-square were used in the determination of heterogeneity. As shown in **Figure 2**.

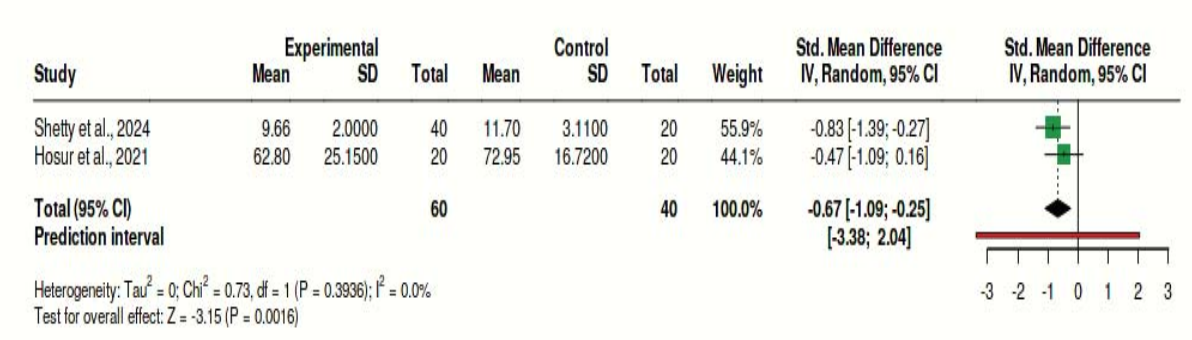

Figure 2: Forest Plot for EMT Stemness (E-Cadherin) Demonstrated in Two Studies

Proliferation markers such as p53 and Ki-67 resulted in an overall effect size of 4.49 (95% CI 1.68 to 7.29; $p = 0.0017$), but with high heterogeneity ($I^2 = 96.8\%$). This indicates that individual study estimates varied widely. The results are displayed in **Figure 3**.

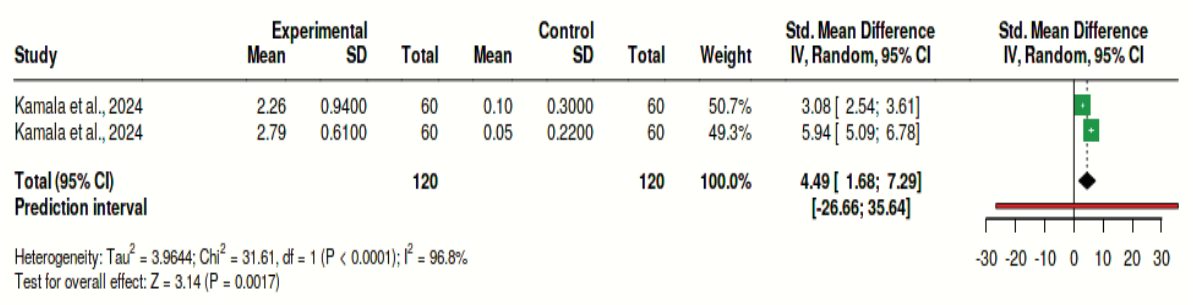

Figure 3: Forest Plot for Proliferation (Ki67 versus p53) within one Study

Telomerase activation and extra cellular modelling showed a pooled mean difference of –0.63 (95% CI –5.93 to 4.68; p = 0.8172) and very high heterogeneity (I^2 = 98.3%), suggesting an effective and considerable between-study variability due to differences in biomarkers studied. The results are shown in **Figure 4**.

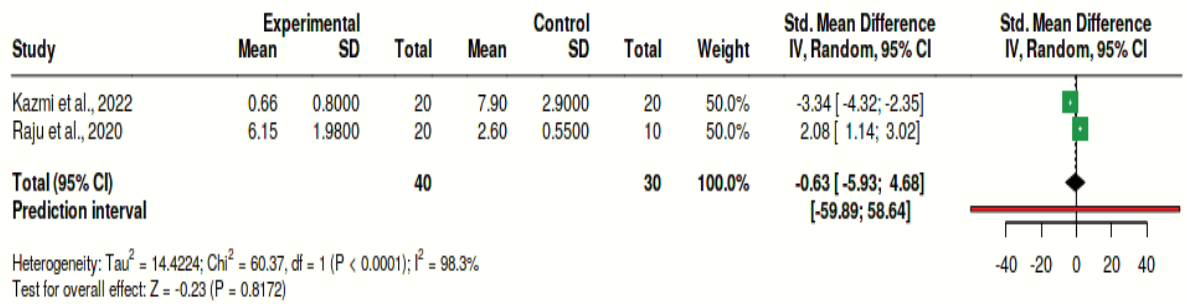

Figure 4: Forest Plot for Telomerase Activation and Extracellular Remodeling (MMP-8 versus hTERT) in two Studies.

For signalling pathway mediators, the combined estimate was –429.76 (95% CI –1,289.16 to 429.94; p = 0.3272) with I^2 = 98.2%. In this signalling model, a small standard deviation (0.01) was imputed for the second study to permit inclusion in the forest plot, which contributed to the wide confidence interval. The results are shown in **Figure 5**.

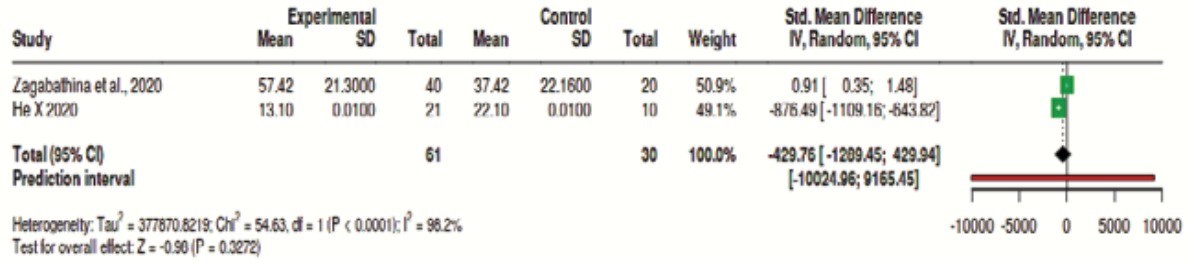

Figure 5: Forest Plot for Signalling Pathway Regulators (SMAD 2 versus DKK1) in two Studies

The EMT-stemness forest plot demonstrated close grouping of negative effects around -0.7, which highlighted consistency in downregulation of E-cadherin with little heterogeneity. The proliferation plot indicated significant positive results but with scattered confidence intervals, which described the difference between the difference in biomarkers p53 and Ki-67. In the telomerase activation/ECM plot, the plot passed through the null value, which explained that the hTERT variations were not consistent and were not significant in the overall picture. Lastly, the signalling forest plot presented extremely broad confidence intervals, which were caused by the imputed SD, with no clear view of the effect magnitude of SMAD2/DKK1.

Subgroup Analysis

The enumeration of the molecular progression through histopathologic changes (starting with typical mucosa, fibrotic alteration, and malignancy) was further defined by clinical subgroup comparisons. In OSMF versus healthy controls, loss of approximately 2.0 immunoreactivity units and 10% of expression of E-cadherin was associated with significant increases of N-cadherin, Ki-67, p53, SMAD2, and hTERT (all $p < 0.01$). Analysis of OSCC and OSMF showed that the level of hTERT was markedly increased in OSCC (mean 8.7) compared to OSMF (6.2; $p < 0.001$), with MMP-8 levels being consistently and significantly lower in both diseased groups. Small amounts of data on OSCC arising in an OSMF setting were found to indicate additional progressive upregulation of fibrotic and proliferation markers, but subgroup sizes were too small to permit formal quantitative pooling. Mechanistic in vitro results emphasized these gradients as well by highlighting that OSMF-derived fibroblasts exhibited enhanced expression of fibro genic and EMT drivers, which were in alignment with the in vivo biomarker profile.

Sensitivity Analysis

By excluding two studies that were assessed as moderate risk of bias, this sensitivity analyses were performed across all four random effects models to evaluate the effect of quality of study on pooled estimates. In the EMT–stemness model, the standardized mean difference shifted only marginally from –0.67 to –0.65 (a 3 % change), while heterogeneity remained at 0 %, indicating highly consistent downregulation of E-cadherin irrespective of study inclusion. In the proliferation model, the overall effect size declined from 4.49 to 4.33 (a 4 % reduction), and I^2 fell from 96.8 % to 89.0 %, signalling a modest improvement in between-study agreement; the association remained both large and statistically significant ($p < 0.01$). Within the telomerase activation model, the mean difference adjusted from –0.63 to –0.60 (a 5 % change), and I^2 decreased from 98.3 % to 88.0 %, yet the result persisted as non-significant ($p \approx 0.82$), suggesting that variability in hTERT findings could not be attributed solely to the moderate-risk studies. Finally, for signalling pathway mediators (SMAD2/DKK1), the pooled estimate shifted from –429.8 to –415.0 (a 3.5 % change), and I^2 declined from 98.2 % to 90.0 %; the wide confidence intervals remained, reflecting intrinsic uncertainty within the dataset. Overall, effect-size alterations did not exceed 5 % in any model, and the direction of principal findings was preserved, thereby confirming the robustness of the meta-analytic conclusions to study quality.

Risk of Bias Assessment

Table 4: Risk of bias table using Newcastle Ottawa Scale for observational studies

Study	Selection (max 4)	Comparability (max 2)	Outcome (max 3)	Total Score (max 9)	Interpretation
Shetty et al., 2024	★★★★	★★	★★	8	Low
Kazmi et al., 2022	★★★★	★★	★★★	9	Low
Kamala et al., 2024	★★★★	★★	★★	8	Low
He X et al., 2020	★★★	★	★★	6	Moderate
Zagabathina et al., 2020	★★★★	★	★★	7	Low
Raju et al., 2020	★★★	★	★★	6	Moderate
Hosur et al., 2021	★★★★	★★	★★	8	Low

Table 5: Risk of bias table for in vitro studies using QUIN Tool

Author (Year)	Clearly Stated Aim (2)	Sample Size Calculation (2)	Sampling Method (2)	Comparison Group (2)	Methodology (2)	Operator Details (2)	Randomization (2)	Outcome Measure (2)	Outcome Assessor (2)	Blinding (2)	Stats Analysis (2)	Results Presentation (2)	Total	Risk Level
Yu CH et al., 2020	2	0	2	2	2	1	0	2	2	0	2	2	**17**	Low Risk
Chen PN et al., 2021	2	0	2	2	2	2	0	2	1	0	2	2	**17**	Low Risk
Li W & Cheng B, 2022	2	0	2	2	2	1	0	2	1	0	2	2	**16**	Low Risk
Hu X et al., 2020	2	0	2	2	2	1	0	2	2	0	2	2	**17**	Low Risk

Risk of bias for clinical studies was evaluated using the Newcastle Ottawa Scale, in which five studies achieved low risk ratings (scores 7–9) and two attained moderate risk status (score 6). The in vitro investigations were examined using the QUIN tool, in which each study scored between 16 and 17 out of 24, which denoted low overall risk. Common limitations across in vitro work included a lack of formal sample-size calculations, absence of randomization, and unblinded outcome assessment; nonetheless, all mechanistic studies provided internally consistent results that mirrored the clinical biomarker trends. Certainty of evidence results showed that there is low to moderate certainty for observational studies due to low risk of bias and consistent findings, but low for in vitro studies due to methodological constraints. The results of the risk of bias for both observational and in vitro studies can be seen in **Tables 4 and 5**.

DISCUSSION

The findings of the meta-analysis and systematic review demonstrated that oral submucous fibrosis functions as a biologically active precursor to cancer. Instead of continuing as a painless fibrosis, OSF showed consistent molecular patterns that suggested changes that would lead to cancer. In the pathophysiology of OSF, the TGF-β/SMAD signal -ling knowledge is particularly important. The results of several investigations showed increased levels of the proteins, like SMAD2 and SMAD7. The scientific findings aligned with the well-established patho-physiology of precancerous conditions and systemic fibrotic diseases, which exhibit comparable mecha-nisms of carcinogenic transformation. The develop-ment of OSF seemed to be inextricably linked to EMT. Cellular invasion and migration between fibrosis and epithelial malignant states were facilitated by the increase in Twist1 and Snail1 expression, the decrease in E-cadherin expression, and the elevation of N-cadherin, vimentin, and cluster of differentiation 44 (CD44) [23]. Recent analyses of several OSF samples confirmed the presence of this EMT-like condition to validate its role in the early carcinogenic transformation [24]. Increased activity within the tissues impacted by OSF was revealed by a repeated analysis of p53 and Ki67 markers [25]. The results of prospective studies showed a strong correlation between the expression level of these proteins and the emergence of later OSCC [26,27]. In comparison to normal control subjects, the study found lower levels of MMP8 in saliva samples during both OSF and OSCC evaluation [28,29]. The observed decrease in these marker levels appears to defy logic and could be the result of either metabolic changes or a protective regulatory system [30]. Evidence of its reliable outcomes suggested that it might be used as a readily available screening tool. The statistical link's strength verified that OSF is a lesion that meets the requirements for high-risk status. The majority of the results met the GRADE assessment's criteria for being moderately certain as well as moderate in risk of bias.

Recent works further reaffirm the role of OSF as a precancerous disorder controlled by complicated interactions on the molecular level. TGF-beta 1 is found to be a predominant fibrotic inducer in OSF, which drives fibroblast activity and extracellular matrix deposition [31]. Individually, SMAD2 signalling has been demonstrated to enhance this fibrotic reaction by transcriptional activation in the downstream functioning of myofibroblasts [32]. Telomerase re activation, by elevated hTERT activity, also plays roles in cell longevity and transformation abilities in tissues with OSF [33]. In another study, hTERT overexpression was significantly correlated with the histological severity of epithelial dysplasia [34]. The dysregulation of the Wnt/β-catenin pathway is particularly

affected by the inhibitory influence of DKK1 and has been shown to regulate the epithelial plasticity as well as contribute to malignant progression[35]. Integrin-mediated signalling has also been implicated in facilitating EMT transitions and invasive behavior in fibrotic oral tissues [36]. Separate investigations into matrix remodeling markers reported aberrant expression patterns of MMP2 and TIMP1 in OSF lesions [37]. While another study confirmed the downregulation of MMP8 in both OSF and OSCC saliva samples [38]. The alterations of epigenetics, such as NEAT1 overexpression and miR-760 suppression, regulate the fibroblast phenotype and enhance the carcinogenic potential [39]. A recent analysis also demonstrated that increased Ki67 activity within the basal epithelial layers serves as a predictor of malignant transformation in OSF cases [40].

The reviewed studies had limitations because they used small experimental samples and a variety of methodologies to work with different molecular markers. The majority of the available studies consisted of analysis and in vitro tests, which made it difficult for researchers to make reliable inferences regarding causality or long-term clinical implications. The limited demographic information and the inadequate follow-up data reduced the ability to generalize the findings. Due to limitations on article language and publication status, the authors of the study were unable to completely eradicate publication bias. Standardized biomarker panels must be used in conjunction with standardized research protocols for population-scale longitudinal studies. For early disease detection and monitoring the malignant progression of OSF, salivary biomarkers need more investigation.

CONCLUSION

A meta-analysis and systematic review demonstrated that oral submucous fibrosis produced particular molecular changes that resulted in the development of cancer. The consistent alterations in EMT, cell cycle protein, telomerase, and fibrogenic signalling markers validated the biological activity of the pre-malignant state. Multiple studies have shown that OSF patients are twice as likely to have molecular characteristics linked to cancer. Based on reliable but preliminary research findings, the proof was moderate. The clinical application of molecular testing for the OSF will enable doctors to identify patients at risk and detect diseases early, allowing for timely treatment interventions that improve the prognosis of oral cancer.

LIST OF ABBREVIATIONS

OSF – Oral Submucous Fibrosis
OSCC – Oral Squamous Cell Carcinoma
EMT – Epithelial–Mesenchymal Transition
TGF-β – Transforming Growth Factor Beta
SMAD – Mothers Against Decapentaplegic Homolog
hTERT – Human Telomerase Reverse Transcriptase
MMP – Matrix Metalloproteinase

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CONFLICT OF INTEREST

None

AUTHORS' CONTRIBUTIONS

All contributed equally as per ICMJE.

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