RESEARCH ARTICLE

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Salivary Expression of Cyclophilin A and FK506-Binding Protein 51 in Oral Submucous Fibrosis Versus Healthy Controls: A Cross-Sectional Analysis

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Abstract

Background: Immunophilins, a group of proteins comprising FK506-binding proteins (FKBPs) and cyclophilins (Cyps), have been increasingly recognized as key contributors to fibrosis development in multiple organs, including the lungs, liver, heart, and bone marrow. The identification of immunophilins as potential therapeutic targets opens avenues for the development of inhibitory agents designed to impede both the initiation and progression of fibrosis. **Aim and Objective:** This research aims to assess the levels of two vital salivary immunophilins- Cyclophilin A (CyP A) and FK506-binding protein 51 (FKBP 51), in OSMF patients. The central goal is to quantify and then juxtapose the salivary concentration of CyP A and FKBP 51 in this specific patient group and healthy controls. **Material and Methodology:**Saliva was collected from 32 subjects in the study group and 32 subjects in the healthy control group using an unstimulated spitting method. Salivary CyP A and FKBP51 levels were measured using sensitive and precise enzyme-linked immunosorbent assay (ELISA) kits. Statistical analysis was performed using the independent samples t-test to determine statistical significance. **Results:** The study demonstrated a statistically significant elevation in CyP A levels among subjects with OSMF (mean ± SD: 45.414 ± 9.69 ng/mL) compared to healthy controls (mean ± SD: 8.243 ± 4.32 ng/mL; $p < 0.001$). It also showed a statistically significant increase in FKBP 51 levels in patients with oral submucous fibrosis (mean ± SD: 23.857 ± 5.45 ng/mL) compared to healthy individuals (mean ± SD: 5.829 ± 2.82 ng/mL; $p < 0.001$). **Conclusion:** This study substantiates that the elevated salivary concentrations of CyP A and FKBP 51 in OSMF patients suggest their potential utility as a markers for diagnosis and prognosis of the same, offering the potential for timely intervention, more effective treatment planning, and improved patient outcomes.

Keywords: Oral Submucous Fibrosis- Immunophilins- Cyclophilin A- FKBP-51

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Introduction

Oral submucous fibrosis (OSMF) is a chronic, debilitating, and potentially malignant disorder of the oral cavity, characterized by progressive fibrosis of the submucosal tissues. It carries a malignant transformation risk ranging from 7.6% to 13%, underscoring its significance as a high-risk oral potentially malignant disorder (OPMD) [1, 2]. Despite decades of research, OSMF remains one of the most poorly understood and therapeutically challenging diseases in oral pathology. The reported prevalence in India ranges between 0.2% and 0.5%, largely attributed to widespread use of areca nut and its derivatives [2, 3].

Several molecular pathways are believed to govern the progression of OSMF, with dysregulation of normal cellular signaling mechanisms occurring at various stages of the disease [4, 5]. It is postulated that distinct molecular alterations differentiate normal oral mucosa from OPMDs, contributing to malignant transformation. [1, 4] In this context, biomarkers defined as objectively measurable indicators of normal biological processes, pathogenic mechanisms, or therapeutic responses have gained prominence in the diagnosis and monitoring of OSMF [6, 7].

Among emerging molecular biomarkers, immunophilins have attracted considerable interest. These include FK506-binding proteins (FKBPs) and cyclophilins (Cyps), both of which exhibit peptidyl-prolyl cis-trans isomerase (PPIase) activity.[8] This enzymatic function facilitates protein folding and modulates the assembly or disassembly of protein complexes, essential for maintaining cellular homeostasis [5, 8, 9]. Notably, FKBP51 and Cyclophilin A (CypA), primarily mitochondrial in localization, undergo nuclear translocation under oxidative stress, acting as protective agents in cellular stress responses and playing roles in cell differentiation and survival [1, 8].

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The role of immunophilins in the pathogenesis of fibrosis in various organs such as the liver, lungs, and kidneys is well established and extensively studied [8, 9]. However, their involvement in the fibrotic mechanisms of OSMF remains largely unexplored. Given their role in fibrosis and cellular stress regulation, immunophilins represent potential therapeutic targets. FKBP51 is known to bind immunosuppressive agents such as tacrolimus and rapamycin (sirolimus), while cyclophilins bind to cyclosporin [1, 8, 9] These interactions highlight the therapeutic potential of immunophilin inhibitors, which could modulate fibrotic pathways and offer novel treatment strategies for OSMF [7, 8].

Progressive oral diseases with cancer potential necessitate immediate, widespread minimally invasive diagnosis. Salivary biomarkers offer a fast, non-invasive, accurate, and well-accepted diagnostic approach [3, 6, 10]. This study is the first to investigate the potential role of immunophilins, specifically Cyclophilin A and FKBP51, in the salivary profiles of OSMF patients.

Materials and Methods

The study received approval from Krishna Vishwa Vidyapeeth Ethics Committee of Karad, India (Protocol no.- 396/2023-2024) . In accordance with ethical guidelines, written informed consent was obtained from all participants prior to saliva collection.

A. Recruitment of Study Subjects

The study included 32 cases of OSMF and 32 healthy controls, all recruited from the Outpatient Department of School of Dental sciences, Karad. Demographic details and medical history were collected. Subjects were excluded if they had any infectious diseases within a month prior to saliva sampling, active dental abscesses, systemic illness, collagen vascular diseases, or were undergoing any form of medical or dental treatment. Individuals who have taken or are currently receiving treatment for oral potentially malignant disorders were also excluded. None of the control participants presented with oral lesions.

B. Sample collection

Saliva samples were obtained in the morning between 9:00 and 12:00, under non-stimulatory conditions. Participants were instructed to abstain from eating, chewing, or drinking for at least one hour prior to collection. A total of 4 to 5 milliliters of saliva was collected from OSMF patients before any therapeutic interventions. The samples were then centrifuged immediately to remove cellular debris, and the supernatant was stored at −80°C for subsequent analysis.

C. Estimation of CypA & FKBP-51

Cyp A & FKBP-51 concentrations in saliva were quantified using a commercial ELISA Kit from Bioassay Technology, following the manufacturer's instructions. The assay was performed according to the provided protocol, with absorbance measured at 450 nm using a Robonik ELISA plate reader. The results were expressed in units of nanograms per milliliter (ng/ml) for saliva samples.

Statistical Analysis

All data were analysed using IBM SPSS Statistics (version 25; IBM Corp., Armonk, NY, USA). For continuous variables, means and standard deviations were calculated. Group comparisons between OSMF patients and healthy controls for salivary Cyclophilin A and FKBP51 levels were conducted using the independent samples t-test. Normality and homogeneity of variance assumptions were verified prior to analysis. Pearson's correlation coefficient was used to assess the relationship between biomarker levels and clinical severity grades. A p-value of <0.05 was considered statistically significant.

Results

Key Insight 1(As shown in Table 1 & Figure 1A&1B)
A) Cyclophilin A

The study revealed a statistically significant increase

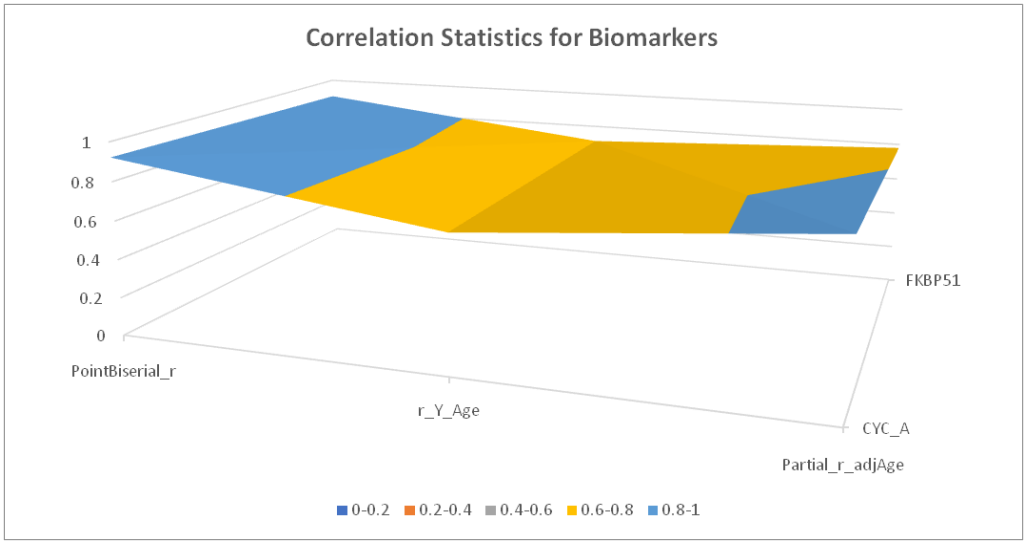

Figure 1. Correlation Statistics for Salivary Biomarkers in OSMF and Healthy Controls

Table 1. Descriptive Statistics for Healthy and OSMF Groups

Biomarker	Mean in Healthy	SD in Healthy	Mean in OSMF	SD in OSMF	Estimated Age- adjusted Mean Diff
CYC_A	8.243	4.322	45.414	9.692	15.816
FKBP51	5.829	2.820	23.857	5.456	7.161

Table 2. Effect Size Metrics for Biomarkers

Biomarker	Unadjusted Hedges' g	95% CI (Lower)	95% CI(Upper)	Estimated Age-adjusted Mean Diff
CYC_A	4.893	3.903	5.884	15.816
FKBP51	4.101	3.228	4.973	7.161

in Cyclophilin A (CYC A) levels in subjects affected by OSMF (mean ± SD: 45.414 ± 9.69 ng/mL) compared to healthy individuals (mean ± SD: 8.243 ± 4.32 ng/mL; $p < 0.001$).

B) FKBP51 (FK506 Binding Protein 51)

The study demonstrated a statistically significant augmentation in FK506-binding protein 51 (FKBP51) concentrations within the cohort afflicted by oral submucous fibrosis (mean ± SD: 23.857 ± 5.45 ng/mL) when juxtaposed with the control group of healthy individuals (mean ± SD: 5.829 ± 2.82 ng/mL; $p < 0.001$).

Key Insight 2 (As shown in Table 2-4 & Figures 2A & 2B, 3) A) Age positively correlated with

The statistical inquiry unveiled a notable direct association between chronological age and the concentrations of both Cyclophilin A (CYC A) and FK506-binding protein 51 (FKBP51), with Pearson correlation coefficients (r) of 0.695 ($p < 0.001$) and 0.709 ($p < 0.001$),

Table 3. Multivariate Analysis of Variance (MANOVA) Results for Salivary CYC_A and FKBP51 in OSMF and Healthy Groups

Statistic	Value	F Value	Num DF	Den DF	Pr> F
Wilks' Lambda	0.0634	459.49	2.0	62.0	<0.0001
Pillai's Trace	0.9366	459.49	2.0	62.0	<0.0001
Hotelling's Trace	14.765	459.49	2.0	62.0	<0.0001
Roy's Largest Root	14.765	459.49	2.0	62.0	<0.0001

Table 4. Clinical Interpretation Table Combining All Key Diagnostic Metrics

Biomarker	Optimal Cutoff	Sensitivity	Specificity	PPV	NPV	AUC
CYC_A	22.79	1.00	1.00	1.00	1.00	1.000
FKBP51	13.78	1.00	1.00	1.00	1.00	0.998

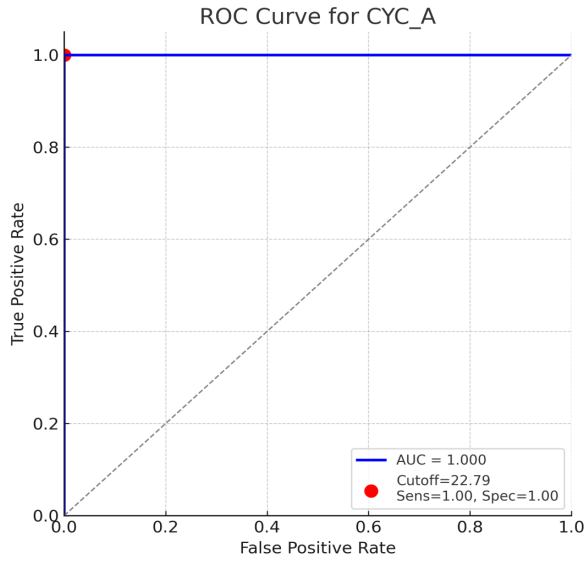

Figure 2. ROC Curve for CYC A

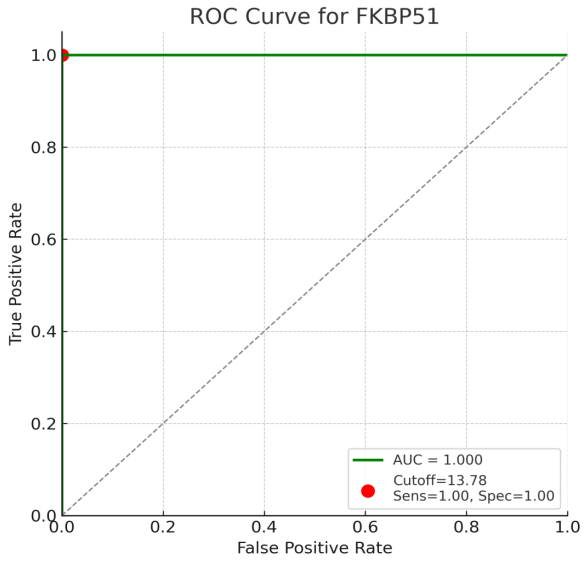

Figure 3. ROC Curve for FKBP51

respectively. This strong correlation indicates that as age increases, the levels of these two immunomodulatory proteins within the study population also rise.

B) CYC A and FKBP51

These biomarkers are closely linked, possibly reflecting a common pathway or shared role in OSMF pathology.

Discussion

Immunophilins are recognized modulators of fibrosis in organs such as the liver, lungs, and kidneys [8, 9] Their influence on collagen synthesis, protein folding, and cellular stress responses positions them as potential contributors to the fibrotic changes seen in OSMF [5, 9] However, their role in OSMF has not been explored until now.

The present study demonstrated significantly elevated salivary levels of Cyclophilin A (CypA) and FKBP51 in individuals with Oral Submucous Fibrosis (OSMF) compared to healthy controls, aligning with and extending the limited existing literature on the subject. Yuan et al. [11] and Hou et al. [12] identified CypA overexpression in fibrotic oral tissues using proteomic approaches, suggesting its potential as a biomarker and therapeutic target. Our findings corroborate these observations and further demonstrate that CypA can be detected non-invasively in saliva, highlighting its diagnostic potential [10, 11] Wang and Tang [1] emphasized the immunopathogenic mechanisms of areca nut–induced fibrosis, including upregulation of proteins like CypA, thus reinforcing the biological plausibility of our results. However, most previous studies have focused on tissue samples, while our study is one of the very few to investigate salivary immunophilin expression in OSMF. Furthermore, the involvement of FKBP51 in OSMF has been unexplored. The recent review by Alqudah et al. [8] discussed the role of FKBP family proteins in fibrosis broadly, but without specific reference to OSMF.

Romano et al. [13] did an extensive review on FKBP51 and concluded that it plays a critical role in promoting cancer cell survival, malignancy, and therapy resistance through NF-κB activation and other signaling pathways. They highlighted FKBP51 as a potential oncogene and an important molecular target for anticancer therapies, particularly those involving rapamycin and its analogs [1, 13, 14] Our study fills this gap by providing the first evidence of elevated salivary FKBP51 levels in OSMF patients. Given the scarcity of published data on salivary immunophilins in OSMF, this study offers novel insights and supports their utility as promising, non-invasive biomarkers for disease detection and monitoring [15, 16, 17].

Interestingly, the study found a strong positive correlation between age and salivary levels of both CypA ($r = 0.695$) and FKBP51 ($r = 0.709$). This association may reflect cumulative exposure to etiological agents like areca nut, or age-related decline in oxidative stress defense, resulting in enhanced immunophilin expression. These findings align with the general understanding that fibrosis and impaired wound healing become more pronounced with advancing age, further reinforcing the potential diagnostic value of these proteins in age-related fibrotic risk assessment [18, 19].

Moreover, the close association between CypA and FKBP51 levels indicates that these proteins may be co-regulated or part of a shared molecular pathway in OSMF. This raises the possibility of a synergistic effect, where both proteins contribute to the initiation and perpetuation of fibrosis via complementary mechanisms, including collagen stabilization, TGF-β signaling, and stress-mediated fibroblast activation.

Clinical Implications

The findings from this study open new avenues for non-invasive early diagnosis and monitoring of OSMF using salivary biomarkers [15, 16]. Given the simplicity, safety, and patient compliance associated with saliva-based sampling, these biomarkers could potentially be integrated into mass screening protocols, especially in high-risk populations with prevalent areca nut habits.

Furthermore, the implication of immunophilins in OSMF pathogenesis raises the prospect of targeted therapy using immunophilin inhibitors. Drugs such as cyclosporin analogues and rapamycin derivatives, already in clinical use for other fibrotic disorders, might be repurposed or studied for their efficacy in halting or reversing OSMF progression.

Limitations and Future Directions

This study, while novel and insightful, is not without limitations. Grade-wise correlation with Cyclophilin A (Cyp A) and FKBP51 levels was not assessed in this study. The cross-sectional design prevents causal inference, and longitudinal studies would be needed to track changes in biomarker expression over time or in response to treatment. The sample size, though adequate for initial analysis, could be expanded in future investigations to enhance the statistical power, particularly for grade-wise comparisons.

In addition, evaluating other members of the immunophilin family, and combining biomarker data with genetic, imaging, or histopathological information, may offer a more comprehensive diagnostic panel for OSMF.

In conclusion, this study provides the first evidence that salivary levels of Cyclophilin A and FKBP51 are significantly elevated in OSMF patients, suggesting their role in disease pathogenesis and their promise as non-invasive diagnostic biomarkers. Although grade-wise association should be checked, the consistent elevation across all OSMF cases and strong correlation with age highlight their potential utility in early detection and monitoring. Further research into immunophilin-targeted therapies and biomarker validation in larger cohorts is warranted to translate these findings into clinical practice.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

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