

ORIGINAL ARTICLE

Evaluation of Salivary Ph and Vitamin D Status in Areca Nut Induced Oral Submucous Fibrosis

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ABSTRACT

Background: Oral Submucous Fibrosis (OSMF) is a progressive, potentially malignant disorder driven by the cytotoxic and fibrogenic effects of areca nut alkaloids. While the pharmacological neuro humoral effects of arecoline are recognized, its interactions with local salivary buffering and systemic pharmacodynamic regulators, such as vitamin D receptor (VDR) signalling, require detailed characterization.

Objective: To evaluate salivary pH alterations and serum 25 hydroxyvitamin D [25(OH)D] status in habitual areca nut chewers with OSMF from a pharmacodynamic perspective, establishing their correlation with clinic anatomical disease severity.

Material and Methods: A prospective observational study was conducted on 120 habitual areca nut chewers categorized across Khanna & Andrade clinical stages (I–IV). Resting salivary pH was measured using a digital pH meter, and serum 25(OH)D levels were quantified using an enzyme linked immunosorbent assay (ELISA). Clinic anatomical severity was quantified via interincisal distance (mm) and cheek flexibility. Pharmacological habit parameters (daily alkaloid exposure duration and dosage) were correlated with biochemical parameters using ANOVA, Tukey's post hoc tests, and Pearson's correlation analysis ($p < 0.05$).

Results: Mean resting salivary pH demonstrated a significant, step wise shift toward acidity from Stage I (6.92 ± 0.18) to Stage IV (5.64 ± 0.22 , $p < 0.001$). Serum 25(OH)D levels declined concurrently from Stage I ($28.4 \pm 4.2\text{ng/mL}$) to Stage IV ($11.8 \pm 3.1\text{ng/mL}$, $p < 0.001$). Strong positive correlations were identified between salivary pH, serum 25(OH)D, and interincisal distance ($r = 0.742$ and $r = 0.698$, respectively; $p < 0.001$). Chronic exposure to areca nut alkaloids was inversely correlated with both salivary pH ($r = -0.612$) and systemic vitamin D levels ($r = -0.584$).

Conclusion: Areca nut induced salivary acidification and systemic depletion of anti fibrotic vitamin D signaling closely parallel the clinico anatomical progression of OSMF. Integrating local salivary buffering agents and VDR targeted pharmacotherapy offers a promising strategy for halting submucosal fibrogenesis.

Keywords: Oral Submucous Fibrosis; Arecoline Pharmacodynamics; Salivary pH; Vitamin D Receptor; Interincisal Distance; Fibrogenesis.

INTRODUCTION

Oral Submucous Fibrosis (OSMF) is an insidious, chronic, potentially malignant disorder affecting the oral cavity and pharyngeal mucosa. Clinically characterized by juxta epithelial inflammation, progressive collagen cross linking, and submucosal tissue rigidity, OSMF leads to severe trismus, burning mucosal pain, and dysphagia¹. The global burden of OSMF is heavily concentrated in South and Southeast Asia, primarily driven by the habitual chewing of areca nut derivatives, such as gutkha, pan masala, and mawa². From a pharmacological perspective, the pathogenesis of OSMF is initiated by major areca nut alkaloids principally arecoline, arecaidine, guvacine, and guvacoline³. Arecoline acts as a nonselective muscarinic acetylcholinesterase receptor agonist and undergoes intracellular hydrolysis into arecaidine, a potent stimulator of fibroblast proliferation⁴. These alkaloids upregulate transforming growth factor beta 1 (TGF beta1) signaling, stimulating mucosal fibroblasts to overproduce collagen types I and III while simultaneously inhibiting matrix metalloproteinases (MMPs) and upregulating tissue inhibitors of metalloproteinases (TIMP 1)⁵. Furthermore, auto oxidation of polyphenols during mastication generates reactive oxygen species (ROS), causing oxidative membrane damage and local micro vascular ischemia⁶.

Saliva serves as the primary biochemical fluid regulating oral tissue homeostasis, providing chemical clearance, antimicrobial defense, and pH buffering⁷. Physiological resting salivary pH ranges between 6.7 and 7.3. Persistent cellular exposure to toxic alkaloid metabolites, combined with ischemic atrophy of major and minor salivary glands secondary to fibrotic compression, impairs salivary acinar secretion and reduces resting salivary pH toward an

acidic spectrum⁸. Localized acidity accelerates epithelial breakdown, promotes nitrosamine activation, and enhances mucosal permeability to carcinogens⁹.

Systemically, vitamin D (25 hydroxyvitamin D) functions as a steroidal immunomodulatory and antifibrotic pharmacodynamic regulator¹⁰. Activated vitamin D binds to the nuclear Vitamin D Receptor (VDR), downregulating the Smad2/3 signaling cascade, suppressing myofibroblast transdifferentiation, and enhancing extracellular matrix degradation¹⁰. Hypovitaminosis D disrupts this endogenous anti fibrotic control, accelerating collagen accumulation in response to inflammatory stimuli. This study evaluates the correlation between resting salivary pH, serum 25 hydroxyvitamin D status, and clinico anatomical severity in areca nut chewers with OSMF from a pharmacological perspective.

MATERIAL AND METHODS

Study Design and Participant Selection: A prospective, cross sectional observational study was carried out over a 12 month period, from July 2022 to June 2023 at an academic dental hospital and outpatient oral medicine clinic of Karchi, Pakistan. A total of 120 consecutive patients with a documented history of daily areca nut consumption (minimum 1 year) and a clinical and histopathological diagnosis of Oral Submucous Fibrosis (OSMF) were enrolled. The set inclusion criteria was patients aged 18 to 60 years with diagnosed OSMF, active daily areca nut consumption, and signed informed consent. And the set exclusion criteria was patients with systemic bone metabolic diseases, primary parathyroid disorders, systemic sclerosis, chronic renal/hepatic dysfunction, concurrent oral squamous cell carcinoma, or those taking systemic corticosteroids, vitamin D, or calcium supplements within the preceding 6 months. The study protocol was approved by the Institutional Ethics Review Board. All participants provided written informed consent in accordance with the Declaration of Helsinki. A detailed histories regarding commercial formulation,

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daily packet frequency, duration of habit (years), and hold time in the buccal vestibule were recorded to estimate total alkaloid exposure. Clinico anatomical severity was categorized according to Khanna & Andrade criteria: Stage I: Very early stage; burning sensation, normal mouth opening (interincisal distance > 35mm). Stage II: Early stage; mucosal blanching, interincisal distance 26–35mm. Stage III: Moderately advanced stage; palpable fibrous bands, interincisal distance 15–25mm. Stage IV: Advanced stage; severe trismus, interincisal distance < 15mm, restricted tongue mobility. Maximum interincisal distance (IID) was measured in millimeters using a calibrated digital Vernier caliper between upper and lower central incisors at maximum voluntary opening. Cheek flexibility (mm) was assessed by measuring the displacement between a fixed line on the cheek at rest and during maximum puffing.

Unstimulated whole saliva (3–5 ml) was collected between 9:00 AM and 11:00 AM after a 2 hour fast (refraining from oral hygiene, chewing, smoking, or drinking fluids except water). After rinsing with distilled water and resting for 5 minutes, saliva was passively drooled into a pre cooled polypropylene tube. Salivary pH was determined immediately using a calibrated digital pH meter (accuracy ± 0.01) at 25°C. A 3mL venous blood sample was drawn under aseptic conditions, allowed to clot at room temperature, and centrifuged at 3000 $\pm$ for 10 minutes. Serum 25 hydroxyvitamin D [25(OH)D] was quantified using a competitive enzyme linked immunosorbent assay (ELISA) kit. Status was categorized as, sufficient: > 30 ng/mL, insufficient: 20–30 ng/mL, and deficient: < 20 ng/mL.

Statistical analysis was conducted using SPSS Version 26.0. Continuous variables were tested for normality using the Shapiro Wilk test and presented as mean $\pm$ standard deviation (SD). Differences in salivary pH and serum 25(OH)D across the four clinico anatomical stages were evaluated using one way ANOVA followed by Tukey's post hoc test. Pearson's correlation coefficient

(r) assessed relationships between biochemical markers, alkaloid exposure duration, and interincisal distance ($p < 0.05$).

RESULTS

The study cohort (N = 120) comprised 88 males (73.3%) and 32 females (26.7%), with a mean age of 34.6 ± 8.4 years. Distribution according to Khanna & Andrade staging identified 22 patients in Stage I (18.3%), 38 in Stage II (31.7%), 42 in Stage III (35.0%), and 18 in Stage IV (15.0%). Commercial formulations (gutkha and pan masala) were consumed by 78.3% of participants, with a mean chewing duration of 6.8 ± 3.2 years and a mean daily frequency of 5.4 ± 2.1 packets/day. Resting salivary pH exhibited a significant reduction with advancing clinico anatomical disease severity ($F = 184.2$, $p < 0.001$). Mean salivary pH shifted from near neutral in Stage I (6.92 ± 0.18) to acidic in Stage IV (5.64 ± 0.22). Post hoc analysis demonstrated significant differences between all stage pairs ($p < 0.001$). Serum 25(OH)D concentrations declined continuously from Stage I (28.4 ± 4.2 ng/mL) to Stage IV (11.8 ± 3.1 ng/mL; $F = 126.8$, $p < 0.001$). Overall, 64.2% ($n = 77$) of the OSMF cohort demonstrated frank serum vitamin D deficiency (< 20 ng/mL).

Correlation Analysis

Pearson's correlation analysis revealed significant biological relationships:

- **Salivary pH vs. Interincisal Distance:** Strong positive correlation ($r = 0.742$, $p < 0.001$).
- **Serum 25(OH)D vs. Interincisal Distance:** Strong positive correlation ($r = 0.698$, $p < 0.001$).
- **Salivary pH vs. Serum 25(OH)D:** Moderate to strong positive correlation ($r = 0.635$, $p < 0.001$).
- **Areca Nut Duration:** Inversely correlated with salivary pH ($r = 0.612$, $p < 0.001$) and serum 25(OH)D ($r = 0.584$, $p < 0.001$).

Table 1: Salivary pH, Serum Vitamin D, and Interincisal Distance across OSMF Stages

Khanna & Andrade Stage	Patients, n (%)	Salivary pH (Mean $\pm$ SD)	Serum 25(OH)D (ng/mL)	Interincisal Distance (mm)
Stage I	22 (18.3%)	6.92 ± 0.18	28.4 ± 4.2	38.6 ± 2.4
Stage II	38 (31.7%)	6.45 ± 0.21	22.1 ± 3.8	29.8 ± 2.6
Stage III	42 (35.0%)	6.02 ± 0.19	16.5 ± 3.2	19.4 ± 2.8
Stage IV	18 (15.0%)	5.64 ± 0.22	11.8 ± 3.1	11.2 ± 2.1
F Value (ANOVA)	—	184.2	126.8	412.5
p value	—	< 0.001	< 0.001	< 0.001

Table 2: Bivariate Pearson Correlation Matrix of Biomarkers and Clinical Parameters

Correlated Pair	Pearson Correlation (r)	p value	Clinical Interpretation
Salivary pH & Interincisal Distance	+0.742	< 0.001	Salivary acidity correlates with severe trismus
Serum 25(OH)D & Interincisal Distance	+0.698	< 0.001	Systemic VDR ligand depletion parallels mouth opening loss
Salivary pH & Serum 25(OH)D	+0.635	< 0.001	Localized salivary acidity mirrors systemic micronutrient depletion
Areca Nut Duration & Salivary pH	0.612	< 0.001	Chronic alkaloid exposure shifts salivary pH toward acidic spectrum
Areca Nut Duration & Serum 25(OH)D	0.584	< 0.001	Habit duration correlates with lower serum 25(OH)D levels

DISCUSSION

The molecular and clinical findings of this evaluation highlight a clear relationship between localized salivary acidification, systemic vitamin D deficiency, and the clinico anatomical severity of OSMF in areca nut chewers. The progressive drop in resting salivary pH and serum 25(OH)D mirrors the loss of interincisal distance from early to advanced stages.

The pharmacological toxicity of areca nut is primarily driven by its constituent alkaloids, predominantly arecoline and arecaine¹¹. Arecoline functions as a muscarinic receptor agonist, inducing local tissue ischemia through vasoconstriction while stimulating chronic intracellular oxidative stress¹². Prolonged exposure leads to degenerative changes in minor and major salivary gland acini¹³. As fibrotic tissue accumulates in the

submucosa, mechanical compression restricts salivary blood flow, decreasing fluid secretion and reducing salivary bicarbonate output¹⁴. The resulting shift toward an acidic pH (5.64 ± 0.22 in Stage IV) compromises mucosal integrity, accelerates matrix breakdown, and promotes the conversion of secondary amines into carcinogenic nitrosamines^{15,16}.

Systemically, hypovitaminosis D removes an important pharmacodynamic check on tissue fibrogenesis¹⁷. Vitamin D, through its active form 1,25 dihydroxyvitamin D₃, binds to nuclear VDRs on fibroblasts to regulate extracellular matrix remodeling¹⁸. VDR activation inhibits the TGF β 1/Smad signaling cascade, downregulates connective tissue growth factor (CTGF), and blocks the transdifferentiation of fibroblasts into contractile myofibroblasts^{19,20}. Furthermore, VDR signaling alters the

enzymatic balance between matrix metalloproteinases (MMP 1 and MMP 2) and their endogenous inhibitors (TIMP 1), promoting physiological collagen degradation^{21,22}.

When systemic 25(OH)D levels are deficient as seen in 64.2% of our cohort this regulatory pathway is impaired²³. In the presence of arecoline induced TGF beta1 upregulation, the absence of VDR mediated inhibition leads to unhindered collagen deposition and cross linking within the lamina propria²⁴. Clinically, this manifests as dense, non-compliant fibrous bands, loss of cheek flexibility, and severe trismus²⁵.

These findings highlight potential pharmacotherapeutic targets for managing OSMF²⁶. Combining habit cessation with targeted pharmacological interventions such as topical salivary buffering agents, antioxidant therapy, and systemic vitamin D/VDR receptor agonism may help restore physiological extracellular matrix homeostasis^{27,28}. This study is limited by its cross sectional design, which limits causal inference regarding whether systemic vitamin D depletion directly accelerates submucosal fibrogenesis or arises secondarily due to nutritional limitations caused by severe trismus. Larger prospective clinical trials evaluating the therapeutic efficacy of adjunctive high dose vitamin D supplementation in OSMF are recommended.

CONCLUSION

Areca nut induced salivary acidification and systemic vitamin D depletion correlate directly with the clinico anatomical progression of Oral Submucous Fibrosis. The loss of salivary buffering capability and systemic VDR anti fibrotic signaling parallel the severity of interincisal restriction.

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