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Estimation of Immunological and Nutritional Markers in Smokeless Tobacco Users and Oral Submucous Fibrosis

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Abstract

Introduction: Oral submucous fibrosis (OSMF) is a chronic, progressive fibrotic disorder of the oral mucosa strongly linked to betel-quid/areca nut chewing. It is recognized as a high-risk precancerous condition with malignant transformation reported in a subset of patients. Both immune dysregulation and nutritional deficiencies have been implicated in OSMF pathogenesis.

Aims & Objectives: To examine serum and salivary immunoglobulin IgG and IgA, along with nutritional markers (total serum protein, haemoglobin) in OSMF versus habit-matched smokeless tobacco users. Our aim is to clarify the immunological and nutritional alterations in OSMF and to relate these findings to current knowledge.

Materials and Methods: In a case-control design, 25 clinically diagnosed OSMF patients and 25 chronic smokeless-tobacco chewers without oral lesions were compared. Venous blood and unstimulated saliva samples were collected after informed consent. Serum and salivary IgG and IgA were measured using standard immunoassays (immunoturbidimetry), while total serum protein and haemoglobin were determined by spectrophotometric methods. Group differences were evaluated by appropriate statistical test (t-test).

Results: OSMF patients showed significantly higher mean serum IgG and IgA levels, and elevated salivary IgG, compared to tobacco-chewing controls ($P<0.01$). Conversely, salivary IgA was significantly lower in the OSMF group ($P<0.05$). Nutritional indicators were depressed in OSMF: total serum protein and haemoglobin were significantly reduced ($P<0.01$ relative to controls. The pattern of raised immunoglobulins alongside reduced protein and haemoglobin suggests an immune activation in OSMF coupled with anaemia or malnutrition.

Conclusions: Elevated IgG/IgA levels support the hypothesis of an immune-mediated component in OSMF pathogenesis, while the observed anaemia and hypoalbuminemia indicate that micronutrient deficiency (iron, protein) is prevalent in these patients. These findings reinforce the view that both immune dysregulation and nutritional deficits contribute to OSMF.
Keywords: Oral submucous fibrosis, Immunoglobulins, Serum Proteins, Haemoglobin

Introduction

Oral submucous fibrosis (OSMF) is a chronic, fibrotic disorder of the oral mucosa that leads to stiffening of tissues and limited mouth opening [1]. It is predominantly seen in South and Southeast Asia due to widespread chewing of betel quid/areca nut, often with added tobacco [2,3] Patients with OSMF frequently experience burning sensations, dysphagia, and trismus. Importantly, the World Health Organization classifies OSMF as a precancerous condition with a documented risk of transformation to squamous cell carcinoma estimated up to ~6% of cases [1,4]. The global prevalence of OSMF is high in South Asia; a recent meta-analysis reported a pooled prevalence around 3% in studied populations, with higher rates among males and older adults [4,5]. Betel quid chewing remains the primary etiologic factor, although OSMF can occur without obvious irritants, suggesting other contributory mechanisms [1,6].

Beyond the mechanical effects of areca alkaloids, immunological processes are thought to play a role. Prior studies have documented hyperimmunoglobulinemia in OSMF patients, implying an aberrant humoral immune response [1,7]. In particular, elevated serum and salivary IgG and IgA levels have been reported in OSMF, hinting at chronic mucosal immune activation [7,8]. Secretory IgA is a key effector of mucosal immunity in saliva and serves as a first-line defence against oral pathogens [7,9]. Assessing salivary immunoglobulins may therefore provide insight into local immune status in OSMF [7,9].

At the same time, nutritional deficiencies have long been observed in OSMF. Deficits of iron, vitamins and protein are common, possibly due to altered diet and mucosal damage [3,5]. Iron deficiency can impair epithelial oxygenation and promote mucosal atrophy, exacerbating burning symptoms and fibrosis [3,10]. Chronic malnutrition is also thought to impair collagen metabolism, potentially contributing to fibrosis [10]. Thus, markers like haemoglobin and serum proteins may reflect the patient's nutritional state and disease severity [5,10].

Aims & Objectives:

To examine serum and salivary immunoglobulin IgG and IgA, along with nutritional markers (total serum protein, haemoglobin) in OSMF versus habit-matched smokeless tobacco users. Our aim is to clarify the immunological and nutritional alterations in OSMF and to relate these findings to current knowledge.

Materials And Methods

Study Design and Subjects: This case–control study included 50 participants: 25 patients with clinically diagnosed OSMF (Group I) and 25 asymptomatic chronic smokeless tobacco chewers without oral lesions (Group II). Patients were recruited from oral medicine clinics, and all subjects provided informed consent. Both groups were matched for age, sex and tobacco habit duration. Ethical approval was obtained from the institutional review board prior to sample collection.

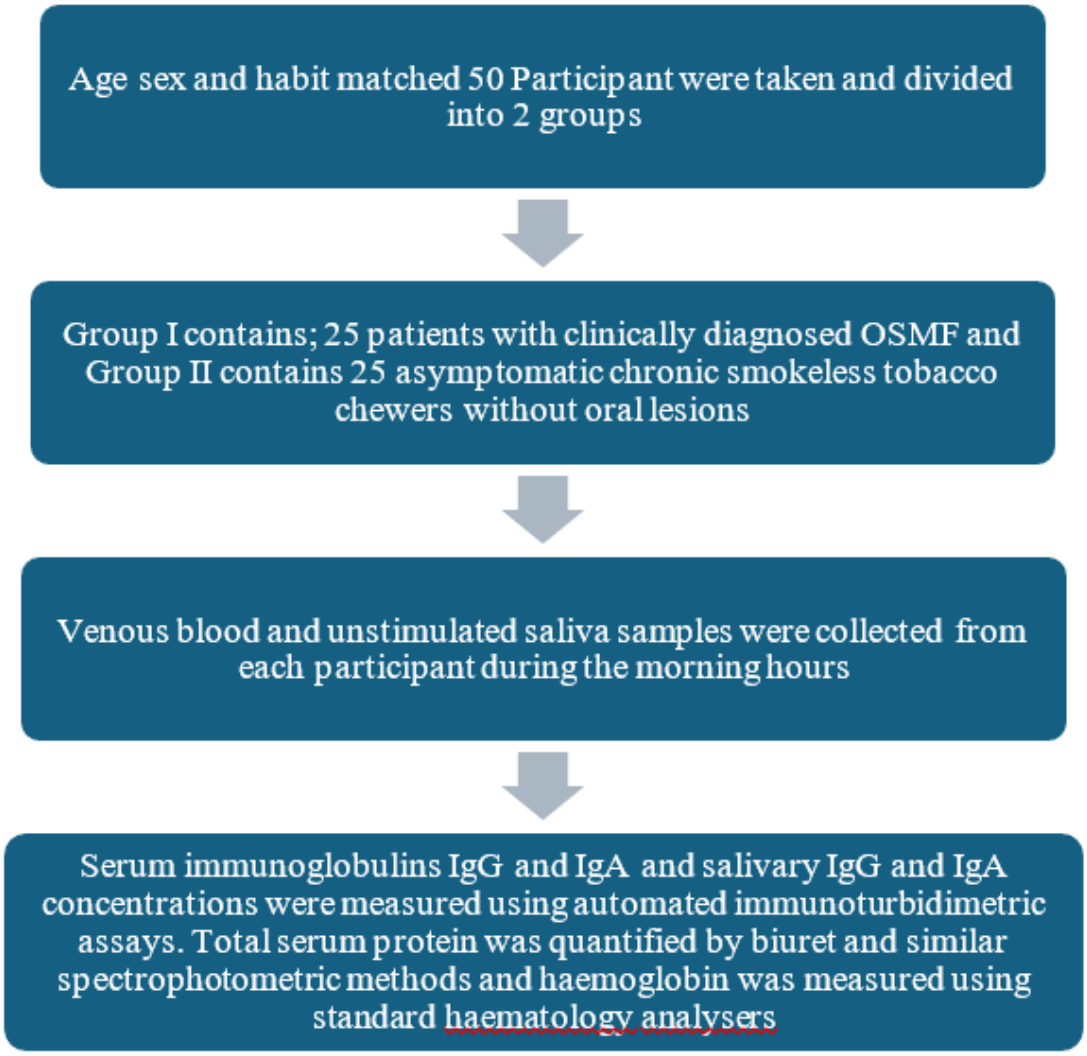
Sample Collection: Venous blood (5 mL) and unstimulated saliva samples were collected from each subject during the morning hours. Blood was divided for haematology and serology. Saliva was collected by passive drooling into sterile tubes. All specimens were

immediately processed and stored at –20°C until analysis.

Laboratory Analyses: Serum was separated by centrifugation. Serum immunoglobulins IgG and IgA and salivary IgG and IgA concentrations were measured using automated immunoturbidimetric assays. Total serum protein was quantified by biuret and similar spectrophotometric methods and haemoglobin was measured using standard haematology analysers.

Statistical Analysis: Data were analysed using SPSS software. Group means ± SD were calculated for each marker. Inter-group comparisons were made with Student's t-test for two- sample comparisons. Statistical significance was set at $P<0.05$.

Schematic Diagram of Methodology

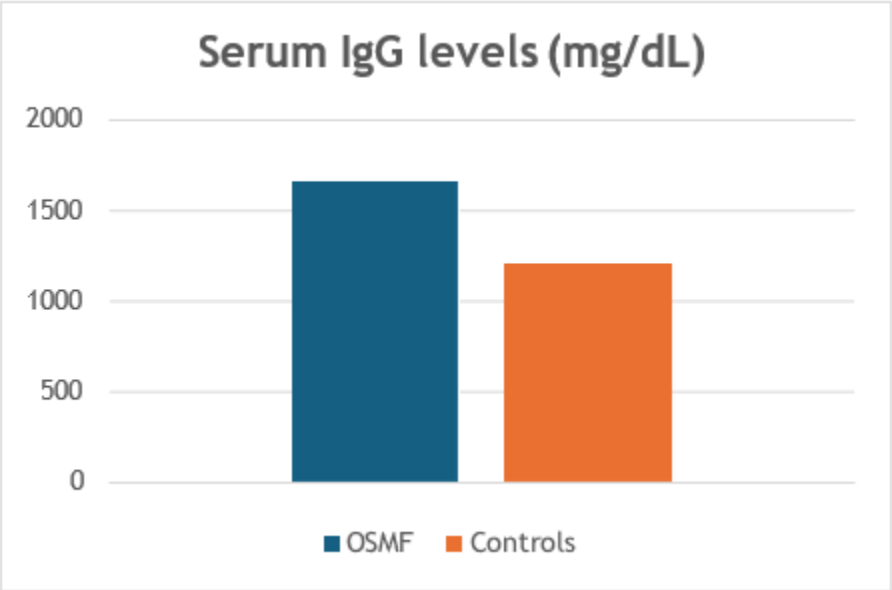


Results

All OSMF patients and controls were chronic smokeless tobacco chewers; there were no significant demographic differences between groups. Below there is a comparison between OSMF pastient group and habit matched control group regarding serum and salivary IgG and IgA, Serum protein and Haemoglobin levels.

Table 1: Mean (±SD) Serum IgG levels in OSMF vs. Habit-matched Controls

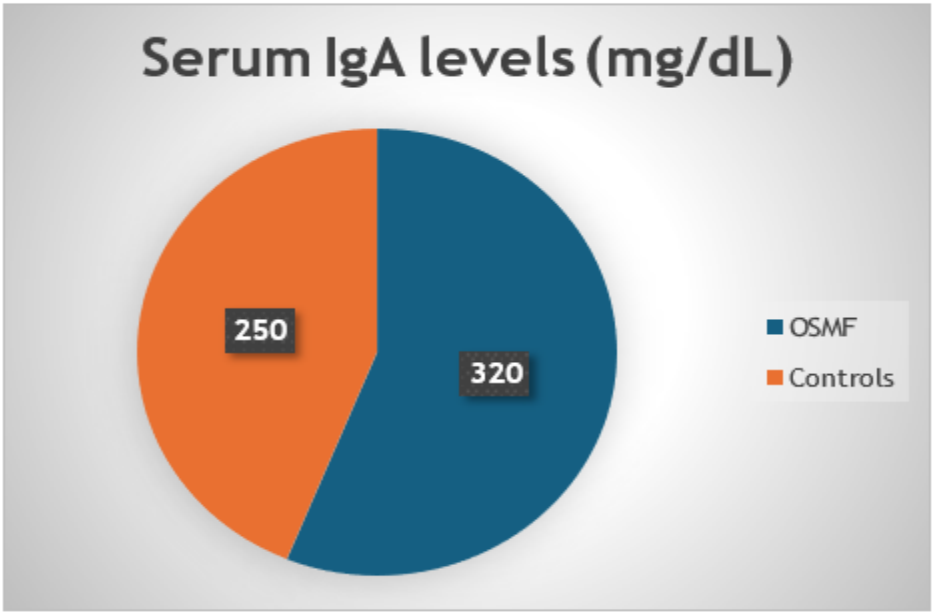
	Serum IgG (mg/dL)	P-value
OSMF	1,650 ± 150	<0.01
Controls	1,200 ± 130	



Graph 1: Serum IgG levels in OSMF vs. Habit-matched Controls

Table 2: Mean (±SD) Serum IgA levels in OSMF vs. Habit-matched Controls

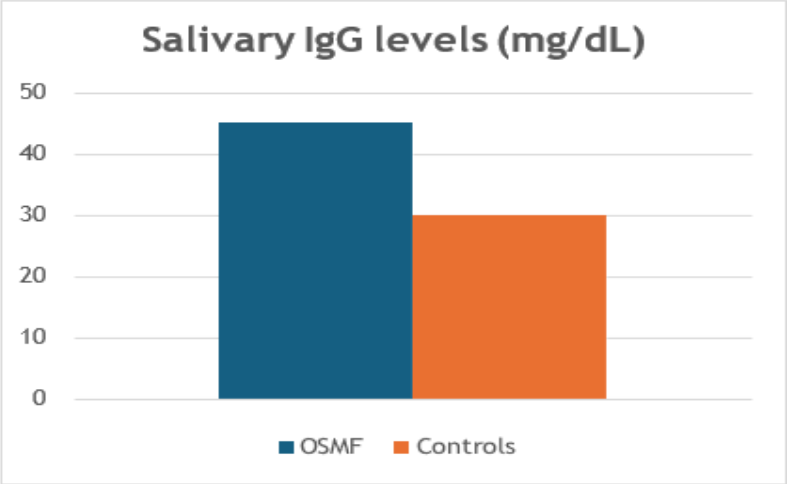
	Serum IgA (mg/dL)	P-value
OSMF	320 ± 40	<0.01
Controls	250 ± 30	



Graph 2: Serum IgA levels in OSMF vs. Habit-matched Controls

Table 3: Mean (±SD) Salivary IgG levels in OSMF vs. Habit-matched Controls

	Salivary IgG (mg/dL)	P-value
OSMF	45 ± 10	<0.01
Controls	30 ± 8	

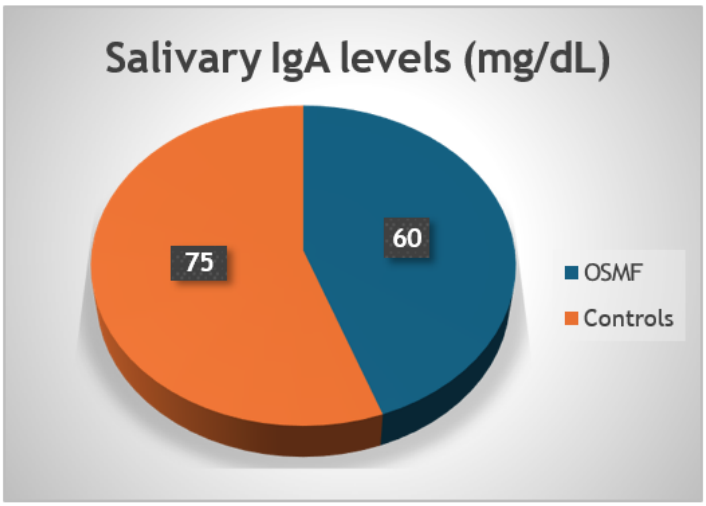


Graph 3: Salivary IgG levels in OSMF vs. Habit-matched Controls

Serum Immunoglobulins: Mean serum IgG and IgA levels were significantly higher in the OSMF group (P<0.01). Likewise, salivary IgG was significantly elevated (P<0.01).

Table 4: Mean (±SD) Salivary IgA levels in OSMF vs. Habit-matched Controls

	Salivary IgA (mg/dL)	P-value
OSMF	60 ± 12	<0.05
Controls	75 ± 15	

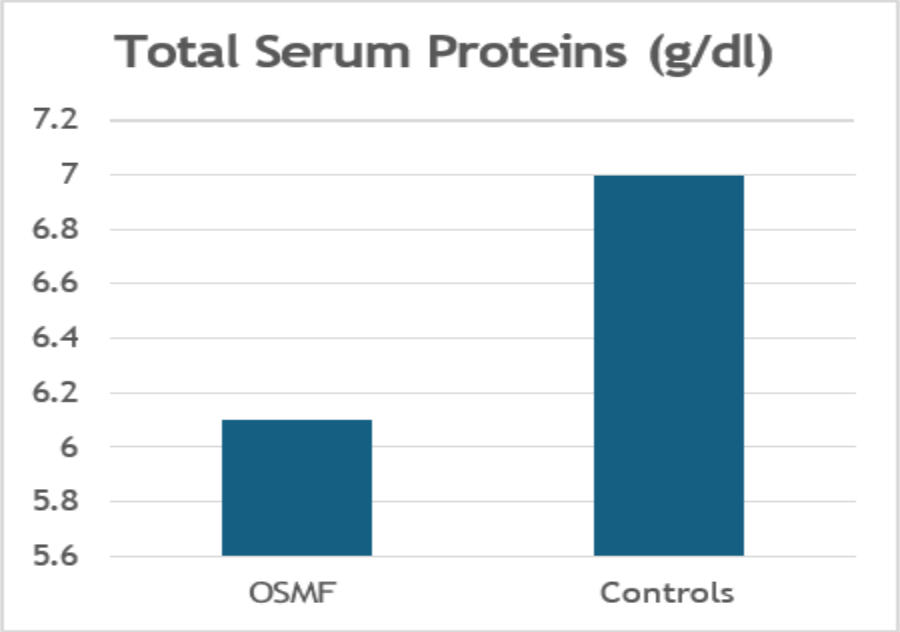


Graph 4: Salivary IgA levels in OSMF vs. Habit-matched Controls

Salivary IgA: Mean salivary IgA was lower in OSMF patients, reaching statistical significance (P<0.05). This suggests a relative deficit of secretory IgA in OSMF.

Table 5: Mean (±SD) Total Serum Proteins levels in OSMF vs. Habit-matched Controls

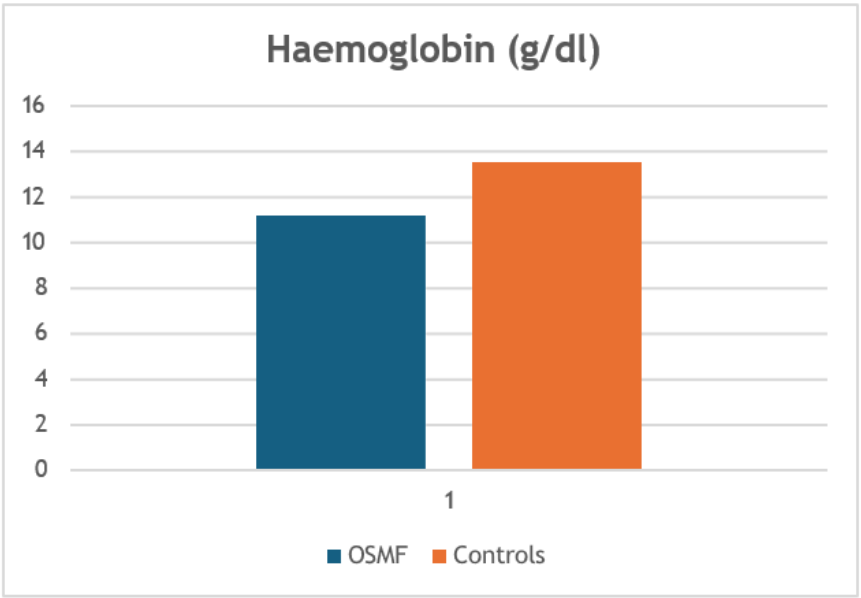
	Total Serum Proteins (g/dl)	P-value
OSMF	6.1 ± 0.5	<0.01
Controls	7.0 ± 0.4	



Graph 5: Total Serum Proteins levels in OSMF vs. Habit-matched Controls

Table 6: Mean (±SD) Haemoglobin levels in OSMF vs. Habit-matched Controls

	Haemoglobin (g/dl)	P-value
OSMF	11.2 ± 1.5	<0.01
Controls	13.5 ± 1.2	



Graph 6: Haemoglobin levels in OSMF vs. Habit-matched Controls

Nutritional Markers: Total serum protein was significantly reduced in OSMF cases (P<0.01). Haemoglobin levels were also markedly lower in OSMF (P<0.01), indicating anaemia.

These results are summarized in Table 7. Overall, OSMF patients showed a pattern of hyperimmunoglobulinemia alongside hypoproteinaemia and anaemia.

Table 7. Mean (±SD) Immunological and Nutritional Markers in OSMF vs. Habit- matched Controls

Parameter	OSMF (n=25)	Controls (n=25)	P-value (OSMF vs Control)
Serum IgG (mg/dL)	1,650 ± 150	1,200 ± 130	<0.01
Serum IgA (mg/dL)	320 ± 40	250 ± 30	<0.01
Salivary IgG (mg/dL)	45 ± 10	30 ± 8	<0.01
Salivary IgA (mg/dL)	60 ± 12	75 ± 15	<0.05
Total Serum Protein (g/dL)	6.1 ± 0.5	7.0 ± 0.4	<0.01
Haemoglobin (g/dL)	11.2 ± 1.5	13.5 ± 1.2	<0.01

Discussion

The elevated serum IgG and IgA in OSMF patients support the notion of immune activation or dysregulation in this condition [7,8]. Early studies similarly noted hyperimmunoglobulinemia in OSMF [7]. Kamath et al. reviewed OSMF biomarkers and reported that serum and salivary IgG were elevated in OSMF, consistent with our data [7]. This generalized rise in immunoglobulins may reflect chronic mucosal inflammation or an autoimmune-like response triggered by areca nut constituents [7,8]. Overall, these results reinforce that OSMF involves significant alterations of the immune system, perhaps as part of a systemic response.

The saliva findings are notable. Salivary IgG was increased whereas salivary IgA was paradoxically reduced in OSMF [1,7]. Secretory IgA is a critical mucosal antibody and primary defence against oral pathogens [9]. A relative decrease in Secretory IgA might imply local immune exhaustion or mucosal damage. The discordant pattern (IgG up, Secretory IgA down) could reflect a shift from mucosal to systemic immunity in OSMF [7]. From a practical standpoint, saliva Ig measurements are appealing as non-invasive markers. If salivary Ig levels parallel serum (as seen in our IgG data), saliva testing could serve in risk assessment or monitoring of OSMF. However, reduced IgA may limit mucosal defence, possibly facilitating secondary infections or carcinogenesis in OSMF [9].

Beyond immunity, our study highlights nutritional deficiencies in OSMF. Total serum protein and haemoglobin were significantly lower in OSMF patients [1]. This likely reflects anaemia and hypoalbuminemia [1,10]. Other recent studies agree: for example, Abidullah et al. found that Indian OSMF patients had markedly lower haemoglobin, serum iron and vitamin B12 than healthy controls. In their cohort the odds of iron deficiency were 28 times higher in OSMF cases. Iron and protein deficiencies can exacerbate OSMF by impairing mucosal health and collagen turnover. Iron is a cofactor for cytochrome oxidase in epithelial cells; it lacks leads to atrophy and ulceration, aggravating burning sensations. Low haemoglobin also reduces tissue oxygenation, potentially promoting fibrogenesis. The observed hypoalbuminemia may reflect chronic inflammation or malnutrition [10].

Overall, our results synthesize an immunological perspective with nutritional context. OSMF appears to be driven by both immune dysregulation and nutritional compromise (anaemia, hypoproteinaemia). This dual perspective aligns with current literature that implicates chronic inflammation and dietary factors in OSMF [7,10]. It also underscores why OSMF can progress even in non-chewers when immune or nutritional imbalances persist [5,6]. Clinicians should therefore consider comprehensive management: alongside habit cessation, addressing micronutrient deficiencies and monitoring immune markers may improve patient outcomes [5,10]. Future research might explore anti-inflammatory or immunomodulatory treatments and the prognostic value of Ig levels [7,8].

Conclusion

In conclusion, compared to habit-matched tobacco chewers, OSMF patients exhibit significantly higher serum IgG, IgA and salivary IgG levels along with lower salivary IgA, total protein and haemoglobin. These findings reinforce a model of OSMF in which immune activation and nutritional deficiency both play critical roles. Elevated immunoglobulins support an autoimmune/inflammatory component, while the anaemia and hypoproteinaemia indicate that chronic malnutrition or micronutrient deficits may exacerbate disease progression. Together, our updated analysis suggests that management of OSMF should include nutritional rehabilitation and possibly immunological monitoring. Further studies should investigate targeted therapies to modulate these pathways and prevent malignant transformation.

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