Original Article

Impact of Complete Denture Wearing on Salivary Uric Acid, pH, and *Candida albicans* Load: An *In Vivo* Study

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Abstract

Background: Complete denture prostheses are known to affect the oral environment, particularly salivary composition and microbial ecology. Salivary parameters such as pH and uric acid play a key role in maintaining oral homeostasis. Furthermore, complete dentures may serve as reservoirs for opportunistic fungal organisms such as *Candida albicans*, which may predispose individuals to oral candidiasis. Despite this, little comprehensive research has simultaneously explored the biochemical and microbial impact of denture wearing in completely edentulous patients. Complete denture prostheses are known to affect the oral environment, particularly salivary composition and microbial ecology. Salivary parameters such as pH and uric acid play a key role in maintaining oral homeostasis. Furthermore, complete dentures may serve as reservoirs for opportunistic fungal organisms such as *C. albicans*, which may predispose individuals to oral candidiasis. Despite this, little comprehensive research has simultaneously explored the biochemical and microbial impact of denture wearing in completely edentulous patients. **Aim:** The aim of this study was to evaluate the changes in salivary uric acid levels, pH, and *C. albicans* load in completely edentulous patients before and after wearing complete dentures, and to analyze the influence of age and smoking on these parameters. **Materials and Methods:** A total of 100 completely edentulous patients aged 40–80 years were included in the study. Participants were grouped by age and smoking habits. Unstimulated saliva samples were collected before denture insertion and again after 1 month. Salivary pH and uric acid were measured using a digital pH meter and a semi-automated biochemical analyzer, respectively. *C. albicans* colonization was assessed using Sabouraud Dextrose Agar culture methods and colony-forming unit counting. Statistical analysis was performed using SPSS 16.0. **Results:** There was a statistically significant decrease in salivary uric acid and pH after denture insertion. In addition, a significant increase in *C. albicans* load was noted, particularly among smokers and older participants. The changes in microbial colonization correlated inversely with salivary pH levels, suggesting a favorable environment for fungal growth post denture insertion. **Conclusion:** Complete denture wearing alters the salivary biochemical and microbiological profile, potentially increasing the risk of oral candidiasis. Monitoring salivary uric acid, pH, and *C. albicans* load can serve as a valuable diagnostic adjunct in edentulous patients undergoing prosthetic rehabilitation. Preventive measures, improved oral hygiene, and antifungal protocols should be considered in high-risk populations.

Keywords: *Candida albicans*, Complete denture, Oral candidiasis Oxidative stress, Salivary pH, Salivary uric acid.

Introduction

Saliva is a complex biological fluid essential for preserving the structural and functional integrity of oral tissues. It serves multiple roles – mechanical cleansing, antimicrobial action, digestion initiation, lubrication, and buffering of oral pH. In recent decades, saliva has emerged as a valuable diagnostic fluid due to its ease of collection and close correlation with systemic and oral health states.[1-5]

Among its many components, uric acid is a key antioxidant in saliva. Accounting for up to 85% of total salivary antioxidant capacity, uric acid reflects the redox status of the oral cavity and is integral to its defense against oxidative stress.[7,8] On the other hand, salivary pH is critical for maintaining enamel integrity, preventing microbial colonization, and preserving mucosal health. Any alteration in these parameters, especially in edentulous individuals, may disrupt oral homeostasis.

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Complete denture therapy, while essential in the rehabilitation of edentulous patients, can significantly influence the oral environment. The introduction of foreign acrylic materials changes in oral biomechanics and surface characteristics of the denture base can all impact saliva composition, flow, and microbial activity; the particular concern is the proliferation of opportunistic organisms like *Candida albicans*, a commensal yeast that becomes pathogenic under conducive conditions.[8]

C. albicans is a polymorphic fungus that can exist in yeast, pseudohyphal, and hyphal forms, enabling it to adhere, invade, and colonize mucosal surfaces and prosthetic appliances. In complete denture wearers, especially those with reduced salivary pH and compromised antioxidant defense, *C. albicans* can flourish, leading to denture stomatitis and systemic complications in immunocompromised patients.

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, plays a pivotal role in this scenario. ROS can be generated from environmental factors such as smoking, alcohol, and the leachables from denture base materials like methyl methacrylate. These compounds can initiate a cascade of events that alter salivary biochemistry, reduce uric acid levels, and predispose the oral cavity to inflammation and fungal overgrowth.[8,9]

While several studies have investigated changes in salivary uric acid and pH levels in response to prosthodontic rehabilitation, there is a distinct lack of *in vivo* studies that comprehensively evaluate both biochemical and microbial parameters – particularly focusing on *C. albicans* – in denture-wearing populations.

This study bridges that gap by assessing salivary uric acid, pH, and *C. albicans* load before and after complete denture insertion in edentulous patients. In addition, it explores the effects of age and smoking, two significant modifiers of oral health, on these salivary parameters.

Objectives of the study

1. To quantify salivary uric acid and pH levels in completely edentulous individuals before and after complete denture insertion
2. To evaluate the *C. albicans* load in these individuals before and after denture placement
3. To assess the influence of age (40–60 years vs. 61–80 years) on salivary uric acid, pH, and *C. albicans* colonization.
4. To determine the impact of smoking on these parameters and identify high-risk subgroups requiring preventive or adjunctive therapeutic interventions.

Materials and Methods

Study design and ethical approval

This was a prospective, *in vivo* clinical study conducted to evaluate the biochemical and microbiological changes in saliva following complete denture insertion. The study protocol was reviewed and approved by the Institutional Ethics Committee of Triveni Institute of Dental Sciences, Hospital and Research Center, Bilaspur, Chhattisgarh, India. Informed written consent was obtained from all participants before sample collection, in accordance with the Declaration of Helsinki.

Study population

A total of 100 completely edentulous patients aged between 40 and 80 years were selected from the outpatient department of prosthodontics. Subjects were stratified based on age and smoking status into two major age groups:

- Group A (40–60 years): A1 (smokers) and A2 (non-smokers)
- Group B (61–80 years): B1 (smokers) and B2 (non-smokers)

Each subgroup included 25 participants to ensure statistical validity.

Inclusion criteria

- Completely edentulous patients (no remaining natural teeth)
- Age range: 40–80 years
- Patients not previously wearing dentures
- Smokers and non-smokers (as self-reported)
- Patients willing to provide consent and comply with recall visits.

Exclusion criteria

- Oral mucosal lesions (e.g., leukoplakia and ulcerations)
- Recent systemic infections or febrile illness
- Systemic diseases affecting uric acid levels (e.g., gout and renal impairment)
- Use of medications influencing salivary composition (e.g., antidepressants and neuroleptics)
- Uncontrolled diabetes or immunocompromised conditions.

Denture fabrication and insertion

All patients received heat-cured acrylic resin complete dentures fabricated using standardized prosthodontic protocols. Dentures were fabricated using a long curing cycle, polished thoroughly to reduce surface roughness, and soaked in water for 48 h before insertion to minimize leachable monomers.[10,29]

Saliva collection protocol

Unstimulated whole saliva was collected from all subjects at 2 time points:

- T0 (Baseline): Just before denture insertion
- T1 (1 month): One month after continuous denture wearing

Subjects were instructed to avoid food, beverages (except water), and oral hygiene procedures for at least 2 h before sample collection.[13] Saliva was collected in the mid-morning (9–11 AM) to minimize circadian variation.

After rinsing the mouth with water, subjects were comfortably seated with head tilted forward. Unstimulated saliva was allowed to accumulate in the floor of the mouth and was

passively drooled into two sterile polypropylene vials, each collecting approximately 2 mL of saliva.

- Vial 1: For pH measurement
- Vial 2: For uric acid and *C. albicans* analysis.

Samples contaminated with blood were discarded. Samples for microbial testing were transported to the microbiology laboratory within 30 min using ice gel packs, and those for biochemical analysis were immediately stored at −20°C until analysis to prevent degradation.[8]

Salivary pH estimation

Salivary pH was measured immediately after collection using a calibrated digital pH meter (Lutron PH-222, Taiwan). The meter was standardized using buffer solutions at pH 4.0 and 7.0. Each measurement was taken in triplicate and the mean value was recorded.

Salivary uric acid estimation

Uric acid concentration was measured using the uricase-peroxidase enzymatic method, a standard colorimetric assay.[12] This was conducted using a semi-automated clinical chemistry analyzer (Micro Lab 300, Merck, Germany) with commercial kits (Erba Mannheim, India).

Procedure

1. Saliva samples were thawed and centrifuged at 3000 rpm for 15 min
2. Supernatant (20 µL) was pipetted into reaction tubes
3. 1000 µL of uric acid reagent was added and incubated at 37°C for 5 min
4. Absorbance was measured at 546 nm, and results were recorded in mg/dL.

The intensity of the red-colored compound formed was directly proportional to the concentration of uric acid present.

C. albicans estimation

To assess *C. albicans* colonization before and after denture wearing, saliva samples were processed for fungal culture.

Sample processing

- 100 µL of centrifuged saliva supernatant was inoculated onto Sabouraud Dextrose Agar plates supplemented with chloramphenicol (0.05%)
- Plates were incubated aerobically at 37°C for 48 h.

Identification and quantification

- Colonies suspected to be *Candida* were counted and expressed as colony forming units per mL (CFU/mL)
- Colonies were further identified using:
  - Gram staining (showing budding yeast cells)
  - Germ tube test (to confirm *C. albicans*)
  - CHROMagar Candida (optional for species differentiation).

A CFU count >400/mL was considered indicative of clinically significant colonization, as per previous prosthodontic mycology studies.[17,18]

Statistical analysis

Data were entered in Microsoft Excel and analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Paired *t*-tests were used to assess changes in salivary parameters between baseline and follow-up. Intergroup comparisons between smokers and non-smokers, and between different age groups, were performed using unpaired *t*-tests.

$P < 0.05$ was considered statistically significant, and <0.001 as highly significant.

Results

The present *in vivo* study evaluated changes in salivary uric acid, pH, and *C. albicans* load in completely edentulous patients before and after complete denture wearing. The influence of age and smoking was also analyzed.

Interpretation

There was a highly significant decrease in salivary pH after 1 month of denture insertion ($P < 0.001$). The acidic shift in pH may contribute to microbial imbalance and higher susceptibility to fungal overgrowth [Table 1].[8-11]

Interpretation

Uric acid levels dropped significantly after denture wearing. This reflects a reduction in salivary antioxidant capacity and a possible increase in oxidative stress [Table 2].[12-15]

Interpretation

There was a highly significant increase in *C. albicans* load post-denture insertion ($P < 0.001$). This dramatic rise indicates that the denture base provides a favorable niche for fungal colonization, especially under conditions of lowered salivary pH [Table 3].[16-20]

Interpretation

Older individuals (61–80 years) exhibited a more pronounced decline in uric acid levels and a greater increase in *C. albicans* colonization. This suggests age-related changes in immune response, mucosal defense, and oral microbial resistance [Table 4].[20-25]

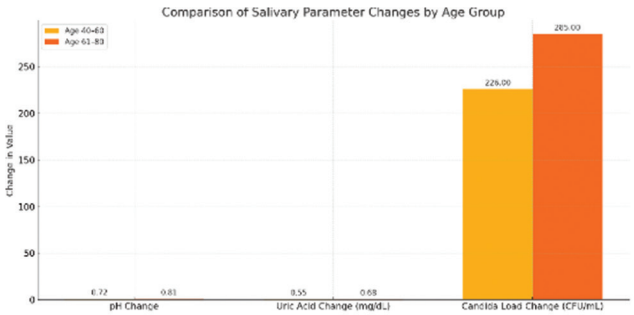
Interpretation

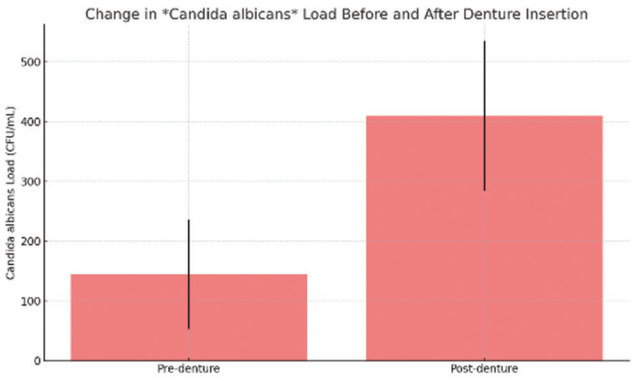
Smoking significantly amplified the decline in salivary pH and uric acid, and led to a higher *C. albicans* burden. Tobacco-induced oxidative stress and immune suppression are likely contributors [Table 5].[26-28]

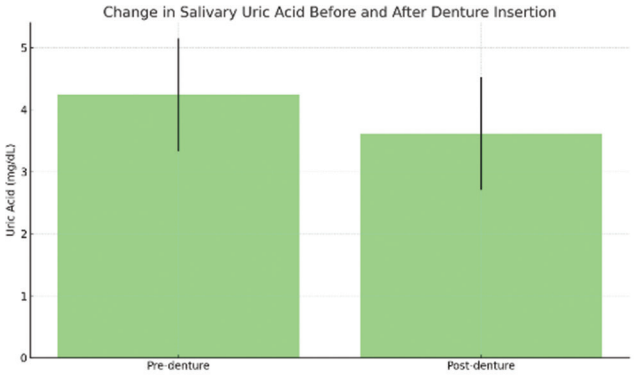
Table 1: Change in salivary pH before and after denture wearing

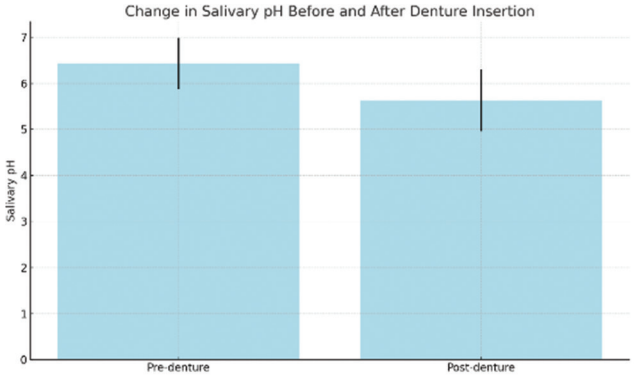
Time point	*n*	Mean pH	SD	% Change	*t*-value	*P*-value
Pre-denture insertion	100	6.43	0.56	–		
Post-denture insertion	100	5.63	0.67	↓12.48%	16.45	<0.001 (HS)

Consolidated Graphs









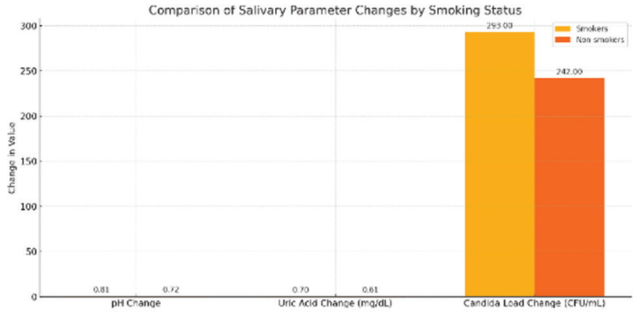


Table 2: Change in salivary uric acid before and after denture wearing

Time point	*n*	Mean uric acid (mg/dL)	SD	% change	*t*-value	*P*-value
Pre-denture insertion	100	4.24	0.91	–		
Post-denture insertion	100	3.62	0.91	↓14.48	10.95	<0.001 (HS)

Table 3: Change in C. albicans load before and after denture wearing

Time point	*n*	Mean CFU/mL	SD	% change	*t*-value	*P*-value
Pre-denture insertion	100	145	92	–		
Post-denture insertion	100	410	126	↑182.76	18.63	<0.001 (HS)

Table 4: Effect of age on salivary parameters

Parameter	Age group	Mean difference	% change	*t*-value	*P*-value
Salivary pH	40–60 years	0.72	11.3	1.18	0.23 (NS)
	61–80 years	0.81	12.46		
Uric acid	40–60 years	0.55	12.88	2.05	0.04 (Sig)
	61–80 years	0.68	16.44		
Candida albicans	40–60 years	226 CFU/mL	156	2.92	0.01 (Sig)
	61–80 years	285 CFU/mL	200		

Here's the comparative graph for smokers versus non-smokers, illustrating:

- Greater decrease in salivary pH and uric acid among smokers
- A significantly higher rise in *C. albicans* load in smokers compared to non-smokers.

Table 5: Effect of smoking on salivary parameters

Parameter	Group	Mean difference	% change	*t*-value	*P*-value
Salivary pH	Smokers	0.81	12.53	2.06	0.04 (Sig)
	Non-smokers	0.72	11.50		
Uric Acid	Smokers	0.70	16.22	2.07	0.04 (Sig)
	Non-smokers	0.61	14.52		
Candida albicans	Smokers	293 CFU/mL	203	3.31	0.002 (HS)
	Non-smokers	242 CFU/mL	173		

This supports the hypothesis that tobacco use exacerbates oral biochemical and microbial imbalances post denture insertion, likely through oxidative stress and immune suppression mechanisms.

Discussion

The oral cavity is a dynamic environment, where saliva plays a key role in maintaining homeostasis through its buffering capacity, antimicrobial activity, and antioxidant defense. Complete denture insertion alters this balance by affecting both the physical and biological landscape of the oral cavity. This study highlighted significant changes in salivary pH, uric acid, and *C. albicans* load following denture use.

Salivary pH reduction

Post-denture insertion, salivary pH dropped significantly (6.43–5.63), indicating acidification. An acidic oral environment promotes microbial overgrowth and mucosal inflammation.[14,15] The denture base, particularly heat-cured acrylic, reduces oxygen diffusion and fosters anaerobic conditions.[18] Incomplete polymerization can release methyl methacrylate monomers, further contributing to salivary acidification and cytotoxicity.[19,29,30]

Decline in salivary uric acid

Uric acid, the primary antioxidant in saliva, declined by 14.5%, reflecting increased oxidative stress. It contributes up to 85% of total salivary antioxidant capacity.[8,11] This reduction may stem from chronic irritation by denture materials, activation of inflammatory pathways, and consumption of antioxidant reserves.[10] Age, smoking, and systemic health can further lower antioxidant levels, compromising oral defense and increasing the risk of candidal infections and mucosal disorders.[6,12,24-26]

Increase in *C. albicans* load

A 182% increase in *C. albicans* post-denture use underscores the microbial impact of prosthetic rehabilitation. The denture surface supports fungal adhesion and biofilm formation, while acidic and anaerobic conditions beneath the denture enhance fungal proliferation.[17,22] Such colonization is associated with denture stomatitis, angular cheilitis, and systemic infections in immunocompromised patients.[20,21] The organism's ability to switch to a hyphal form under acidic conditions increases its pathogenicity.[16]

Influence of age

Older participants (61–80 years) showed more pronounced biochemical and microbial shifts, likely due to immunosenescence, mucosal thinning, and reduced salivary flow.[13,15] Polypharmacy in elderly patients may also affect oral pH and immunity, explaining the higher prevalence of denture-related stomatitis.[25]

Effect of smoking

Smokers experienced greater pH reduction, lower antioxidant levels, and higher fungal loads than non-smokers. Tobacco smoke contains thousands of chemicals, including ROS, that impair mucosal immunity.[27,28] Smoking suppresses salivary IgA, impairs neutrophil function, promotes biofilm formation, and disrupts epithelial healing. Nicotine itself enhances *Candida* adhesion and biofilm thickness.[28] These findings highlight the importance of smoking cessation counseling in prosthodontic care for at-risk patients.

Clinical implications and future considerations

The data from this study support a multi-factorial model where complete dentures interact with patient-specific variables (age, habits, and immune status) to influence oral biochemistry and microbiology. As such, dentists and prosthodontists should:

- Screen salivary pH and antioxidant levels before denture insertion
- Use advanced denture materials with antimicrobial surfaces or nanocoatings
- Educate patients on denture hygiene protocols, including overnight soaking and brushing with antifungal agents
- Consider routine oral mycological cultures in at-risk groups (elderly, smokers, and diabetics)
- Encourage dietary antioxidant intake and consider topical antioxidant gels or lozenges to restore oral redox balance.

Finally, future research should explore:

- Longitudinal studies beyond one month to assess long-term microbial shifts
- Interventions such as probiotics, phytochemicals, or photodynamic therapy for denture biofilm control
- Development of salivary diagnostics kits to monitor oxidative stress in real time.

Conclusion

This *in vivo* study provides compelling evidence that complete denture wearing has a significant impact on the salivary biochemistry and microbiological landscape of the oral cavity. The findings indicate that:

1. Salivary pH decreases significantly after 1 month of complete denture use, creating an environment more favorable for microbial colonization and reduced mucosal protection
2. Salivary uric acid levels decline, reflecting a decrease in antioxidant defense and a potential increase in oxidative

stress – especially concerning in older or medically compromised patients
3. *C. albicans* colonization increases dramatically, suggesting that complete dentures may serve as reservoirs for opportunistic pathogens, particularly under acidic and anaerobic conditions
4. Age and smoking are key modulators, with older individuals and smokers showing more pronounced biochemical and microbial changes, underscoring the need for targeted preventive strategies.

The results emphasize that prosthodontic care must go beyond mechanical replacement of teeth and consider the biological consequences of introducing prosthetic materials into the oral environment. Monitoring salivary parameters and microbial load should become part of standard denture follow-up protocols, particularly in vulnerable groups.

Clinical Recommendations

To mitigate the adverse effects observed, the following should be implemented in clinical practice:

- Prolonged curing cycles and proper denture processing techniques to minimize residual monomer leaching
- Presoaking dentures in water for 48 h before delivery
- Routine assessment of oral mucosa, salivary health, and microbial flora during denture maintenance visits
- Dietary and lifestyle counseling, especially smoking cessation and antioxidant-rich diets
- Antifungal and antimicrobial maintenance regimens, including cleansers or topical agents as required.

Final Remarks

- This study highlights the need for a holistic, biologically informed approach to denture therapy. As the global population ages, prosthodontic interventions must evolve to address not only esthetics and function but also the complex biochemical and microbiological dynamics of the oral cavity.
- Future research with longer follow-up periods, broader patient demographics, and interventional protocols will help optimize denture care and preserve the oral and systemic health of edentulous patients.

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