

EVALUATION OF URIC ACID & pH LEVEL IN SALIVA BEFORE AND AFTER COMPLETE DENTURE WEARING: AN IN VIVO STUDY

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ABSTRACT

An in-vivo study was conducted to evaluate uric acid & pH level in saliva before and after complete denture wearing. 100 completely edentulous patients (between 40-80-year ages) for the clinical study are taken. The test subject 100 in number was divided into two groups of 50 each and each group was further divided into two groups of 25 each. Group A (age between 40-60 years) contains two subgroups: group A₁ for smokers and group A₂ for nonsmokers. Group B (age between 61-80 years) contains two subgroups: group B₁ for smokers and group B₂ for nonsmokers. Saliva samples were collected from subjects immediately before the insertion of dentures. Subjects were recalled one month after the denture insertion for collecting their second set of saliva samples. Saliva of first container was used in pH estimation. Saliva of second container was used in uric acid estimation. pH estimation is carried out using digital pH meter & uric acid estimation is done by Semi-automated clinical chemistry analyzer (Micro Lab 300, Merck, Germany). It was found that salivary uric acid and pH was significantly decreased after one month of denture insertion. Effect of age on pH is not significantly decreased but effect of age on uric acid is significantly decreased. Effect of smoking on pH and uric acid is slightly higher significant as compared to nonsmokers. Some remedies are suggested to minimize this problem.

Key words: Potential of hydrogen (pH), Oxidative Stress (OS), Superoxide Dismutase (SOD), Reactive oxygen species (ROS)

INTRODUCTION

Saliva is a complex biological fluid that plays a very important role in maintaining the overall health of oral cavity¹. Oral fluid is often called the mirror of the body's health, is a perfect medium to be explored for health and disease surveillance². The use of saliva as a diagnostic fluid is a relatively recent trend to identify changes in the oral environment, which can influence treatment choices and strategies³. Research has revealed a link between the health of oral tissues and over all systemic health⁴. The presence of antioxidants and its relation to pollutants (alcohol, nicotine) can disturb the balance of oxidants in the oral cavity⁵.

Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Hence body maintains complex system of enzymatic antioxidants such as catalase, superoxide dismutase (SOD), peroxidases etc. and non-enzymatic antioxidants such as Vitamin C, Vitamin E & glutathione etc. Oxidative stress occurs as a result of increased oxidative metabolism. In normal condition, our cell can be capable of preventing free radical induced diseases by generating its own endogenous antioxidants or by taking them from food^{6,7}.

Antioxidants in saliva represent one of the defense mechanisms against free radical induced oxidative stress (OS). Uric acid is the dominant antioxidant present in saliva with clinical importance in monitoring oral oxidative stress⁸. OS represents the imbalance between the production of highly reactive molecular species such as reactive oxygen species (ROS) and the antioxidant defense systems⁹.

Monomer released from denture may be the cause of adverse biological effects such cytotoxicity and genotoxicity. Some of these methacrylates have been identified to cause gene mutation. Resin monomers may be able to alter the functions of the cells of the oral cavity. Pathways regulating cellular homeostasis or tissue repair may be modified

by monomers at concentrations well below those which cause acute cytotoxicity¹⁰.

New findings indicate that increased oxidative stress results in an impairment of the cellular pro- and antioxidant redox balance caused by monomers. It has been demonstrated that monomers reduced the levels of the natural radical scavenger glutathione (GSH), which protects cell structures from damage caused by reactive oxygen species (ROS). Depletion of the intracellular GSH pool may then significantly contribute to cytotoxicity, because a related increase in ROS levels can activate pathways leading to apoptosis¹⁰.

Saliva is rich in antioxidants, mainly uric acid, with lesser contributions from albumin, ascorbate, glutathione and salivary urate¹¹. Uric acid is an important salivary biomarker with clinical importance in monitoring the oxidative stress (OS). Uric acid contributes approximately 85% of the total salivary antioxidant capacity for these reasons research is now directed towards assays that evaluate the uric acid of saliva. Estimation of saliva uric acid levels of individuals before and after wearing complete dentures can detect changes in uric acid levels which would influence total antioxidant capacity in oral cavity^{8, 12}.

Salivary defense properties reside principally in saliva flow rate, pH, and buffer capacity¹³. The salivary pH normally varies from 5.3 to 7.8¹⁴. There are various sources of hydrogen ions in oral fluids, secretion by the salivary glands in the form of organic and inorganic acids, production by the oral microbiota, or acquisition through food. The oral cavity is protected against damage due to pH changes by the components of saliva which exhibit buffering capacity, such as bicarbonate, phosphate and proteins. Estimation of saliva pH in individuals after wearing complete dentures will allow detection of changes in the buffering capacity¹⁵.

Many work has been done on effect of smoking and aging on salivary uric acid and pH but changes in uric acid and pH level in completely edentulous individuals before and after wearing complete dentures of two different age group in smokers and nonsmokers is has not been done before. There is a need to understand the relationship of uric acid and pH level of saliva when mucosa tolerance is compromised.

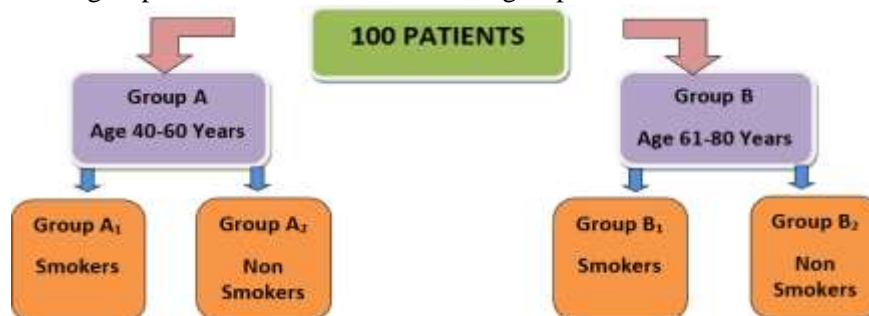
Keeping this in mind the present in vivo study is undertaken to evaluate uric acid & pH level in saliva before and after complete denture wearing. The main objectives of the study were as follows:

- To quantify salivary uric acid and pH level in completely edentulous individuals before and after wearing completedentures.
- To compare salivary uric acid and pH level in completely edentulous individuals before and after wearing complete dentures of two different age group (i.e. 40-60 years of age and 61-80 years of age) in smokers and non-smokers.

MATERIALS AND METHOD

Source of data:

100 completely edentulous patients (between 40-80-year age) for the clinical study are taken from the outpatient section of the Department of Prosthodontics of Triveni Institute of Dental Sciences Hospital & Research Centre, Bilaspur, Chhattisgarh. Informed consent was obtained from all the subjects. The subjects were examined intraorally following a complete medical history taking. The test subject 100 in number was divided into two groups of 50 each and each group was further divided into two groups of 25 each.



Criteria for subject selection:

| Inclusion Criteria | Exclusion Criteria |
|---|--|
| <ul style="list-style-type: none"> • Completely edentulous patients | <ul style="list-style-type: none"> • Oral lesion present |
| <ul style="list-style-type: none"> • Sex-both male and female | <ul style="list-style-type: none"> • Recent history of infections |
| <ul style="list-style-type: none"> • Patients having no history of denture wearing | <ul style="list-style-type: none"> • Systemic condition with increased uric acid levels |
| <ul style="list-style-type: none"> • Smokers | <ul style="list-style-type: none"> • Patients taking antidepressant & neuroleptic drugs |

Equipment & materials:

| EQUIPMENT & MATERILS | MANUFACTURER |
|--|----------------------|
| Polypropylene Vials | Astra Bioscience Ltd |
| Test Tube | Borosil |
| Gel Ice Pack (FRE-GEL) | Senso Medi systems |
| Digital pH Meter (pH -222) | Lutron, Taiwan |
| Pipette (Biohit proline) | Sartorius, Germany |
| Centrifugal Machine (R-8C BL) | Remi Laboratory |
| Semi-automated clinical chemistry analyzer (Micro Lab 300) | Merck, Germany |
| Deep Freezer (GS-40NA30) | Siemens |

Method:

Collection of saliva samples:

Saliva samples were collected from subjects immediately before the insertion of dentures. Subjects were recalled one month after the denture insertion for collecting their second set of saliva samples. All salivary samples were collected in sterile containers. Unstimulated whole saliva was collected by passive drooling at least 2 hours after any food intake. Subjects were informed in advance not to eat or drink (except for water) or chew gum for 2 hours before saliva collection. Samples visibly contaminated with blood were discarded. After rinsing mouth 3-4 times with water, the patient is comfortably seated with eyes open, head tilted forward.

Saliva was allowed to accumulate in the floor of the mouth for approximately 2 minutes and repeatedly expectorated into two ice-chilled polypropylene vials to collect about 2 ml each. The time and date of denture insertion and specimen collection were recorded. Saliva of first container was used in pH estimation. Other salivary samples were stored on gel ice pack and were carried immediately to the Dr. Menghani's Advanced Diagnostic Centre, Bilaspur where they kept frozen at the deep freezer (Siemens) at -20°C to avoid bacterial growth until their uric acid analyses.

Saliva pH estimation:

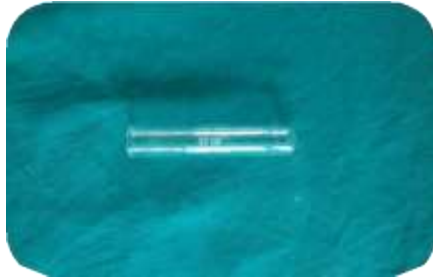
Saliva pH was recorded using digital pH meter.

Saliva uric acid estimation:

Analyses were performed using Erba Mannheim Chemistry Products Uric acid test kit on Semi-automated clinical chemistry analyzer (Micro Lab 300, Merck, Germany). This Analysis based on uricase principle. In this assay, uric acid was transformed by uricase into allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase oxidized the chromogen (4-ami-nophenazone/ N -ethyl- N -(2 hydroxy-3-sul-fopropyl)-3-methylaniline) to form a red compound whose intensity of colour was proportional to the amount of uric acid present in the sample.

Saliva samples were transferred from polypropylene container to sterile test tube then they were centrifuged at 3000 rpm for 15minute and the supernatants were used for uric acid analyses. 20 μl (0.02mL) aliquots of the supernatant was transferred to new, sterile micro centrifuge tubes with the help of pipette and 1000 μl (1mL) uric

acid reagent was added. Mixed it and incubated for 5 minutes at 37°C. Then analyzer nozzle dipped into tubes and results were obtained.



TEST TUBE



POLYPROPYLENE CONTAINER



ICE GEL PACK



DIGITAL pH METER



PIPETTE



CENTRIFUGAL MACHINE



SEMI-AUTOMATED CLINICAL CHEMISTRY ANALYZER



COLLECTION OF SALIVA SAMPLE

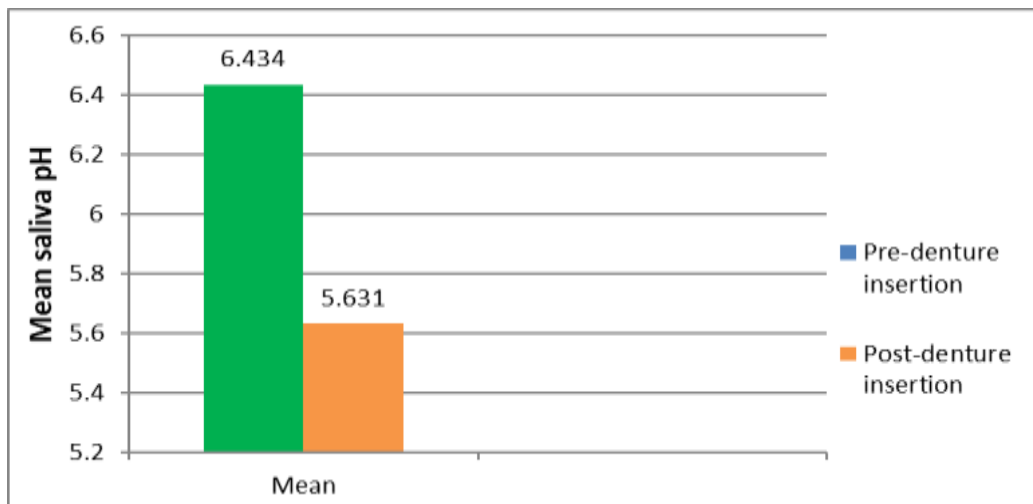
RESULTS

The present in vivo study was conducted to evaluate uric acid & pH level in saliva before and after complete denture wearing. The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS (Statistical Package for Social Sciences) version 16.0 (SPSS Inc; Chicago, Illinois, USA). The values were statistically analyzed. A tabulation of the results of the analysis is as under:

Table 1: Saliva pH values in completely edentulous individuals before and after wearing complete dentures

| pH | N | Mean | SD | % Change | 95% Confidence interval | | t value | P value |
|------------------------|-----|-------|-------|----------|-------------------------|-------------|---------|--------------|
| | | | | | Lower bound | Upper bound | | |
| Pre-denture insertion | 100 | 6.434 | 0.562 | 12.48% | 6.324 | 6.544 | 16.45 | <0.001 HS |
| Post-denture insertion | 100 | 5.631 | 0.669 | | 5.5 | 5.762 | | |

Table 1 demonstrates the mean saliva pH and standard deviation of all the groups and individual groups. The changes percent of post denture insertion as compared to pre denture insertion is 12.48%. The lower mean is showed in post denture insertion time (5.63) and higher mean is showed in pre denture insertion group (6.43).



Graph 1: Saliva pH

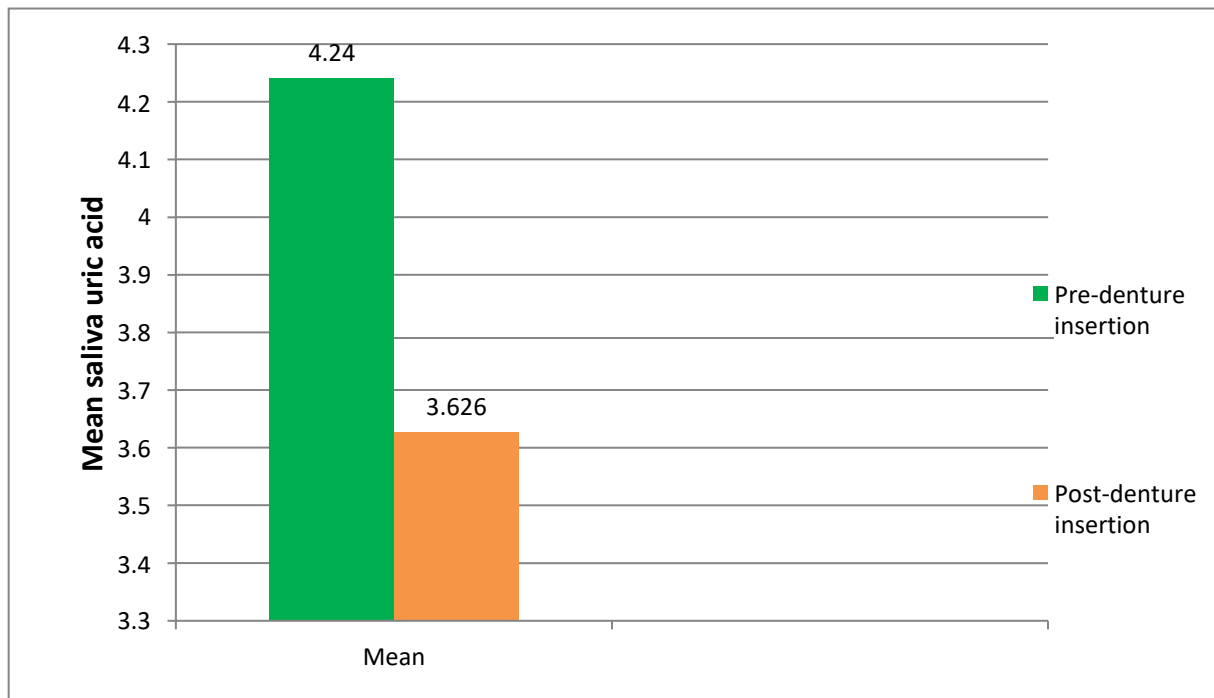
Graph 1 demonstrates the mean difference of saliva pH before and after denture insertion

Saliva pH significantly decreased with mean pH 5.63 in edentulous subjects one month after insertion of complete denture compared to their mean pH value of 6.43 before denture insertion (Table 1, Graph 1).

Table 2: Saliva uric acid levels in completely edentulous individuals before and after wearing complete dentures

| Uric Acid | N | Mean | SD | % Change | 95% Confidence interval | | t value | p value |
|------------------------|-----|-------|------|----------|-------------------------|-------------|---------|-----------|
| | | | | | Lower bound | Upper bound | | |
| Pre-denture insertion | 100 | 4.24 | 0.91 | 14.48 | 4.062 | 4.418 | 10.95 | <0.001 HS |
| Post-denture insertion | 100 | 3.626 | 0.91 | | 3.448 | 3.804 | | |

Table 2 demonstrates the mean saliva uric acid and standard deviation of all the groups and individual groups. The changes percent of post denture insertion as compared to pre denture insertion is 14.48%. The lower mean is showed in post denture insertion time (3.62) and higher mean is showed in pre denture insertion group (4.24).



Graph 2: Saliva uric acid

Graph 2 demonstrates the mean difference of saliva uric acid before and after denture insertion

Salivary uric acid was found to decrease significantly with mean value 3.62 mg/dL in edentulous subjects one month after insertion of complete denture compared to the mean value 4.24 mg/dL before denture insertion (Table 2, Graph 2).

Table 3: Effect of age group on the changes in saliva parameters

| Parameter | Age group | N | Mean difference | SD difference | Change % | .t test | p value |
|-----------|--|----|-----------------|---------------|----------|---------|-------------|
| pH | 40-60 years A ₁ & A ₂ | 50 | 0.721 | 0.214 | 11.3% | 1.18 | 0.23 NS |
| | 61-80 years B ₁ & B ₂ | 50 | 0.809 | 0.474 | 12.46% | | |
| Uric acid | 40-60 years A ₁ & A ₂ | 50 | 0.548 | 0.376 | 12.88% | 2.05 | 0.04 Sig |
| | 61-80 years B ₁ & B ₂ | 50 | 0.681 | 0.262 | 16.44% | | |

Table 3 demonstrates effect of age group on the changes in saliva parameters. The least mean difference showed for pH is group A₁ and A₂ (0.721). The least mean difference showed for uric acid is group A₁ and A₂ (0.548).

Table 4: Effect of smoking on the changes in saliva parameters

| Parameter | Group | N | Mean difference | SD difference | Change % | .t test | p value |
|-----------|---|----|-----------------|---------------|----------|---------|--------------|
| pH | Smokers A ₁ & B ₁ | 50 | 0.812 | 0.214 | 12.53% | 2.059 | 0.042 Sig |
| | Nonsmokers A ₂ & B ₂ | 50 | 0.722 | 0.223 | 11.50% | | |
| Uric acid | Smokers A ₁ & B ₁ | 50 | 0.698 | 0.231 | 16.22% | 2.07 | 0.04 Sig |
| | Nonsmokers A ₂ & B ₂ | 50 | 0.607 | 0.208 | 14.52% | | |

Table 4 demonstrates effect of smoking on the changes in saliva parameters. The least mean difference showed for pH is group A₂ and B₂ (non smokers group) 0.722. The least mean difference showed for uric acid is group A₂ and B₂ (non smokers group) 0.607

With limitation of this study the result of the present study revealed that saliva pH and uric acid is significantly decreases after one month of denture insertion and it is more significantly decreases in denture wearing smoker's patients.

DISCUSSION

Saliva is critical for the maintenance and function of all tissues in the mouth. Therefore, any situation that disturbs saliva production or its composition will probably have broad negative sequelae in the mouth and may result in systemic complications¹⁶. The presence of saliva is essential for the maintenance of healthy oral tissues. Severe reduction of salivary output not only results in a rapid deterioration in oral health but also has a detrimental impact on quality of life for the sufferer. Patients suffering from dry mouth experience difficulty in eating, swallowing, speech, retention of dentures, taste alteration, oral hygiene maintenance, trauma and ulceration of the oral mucosa, a burning sensation of the mucosa, candidal infections¹³.

Provision of acrylic resin dentures is believed to alter the makeup of oral microflora by encouraging the growth of certain microorganisms. This change is believed to occur as a result of the roughness of this material, even if highly polished and finished, and the ability of various microorganisms to adhere and colonize various surfaces of

this material¹⁷. It has been reported that salivary pH is associated with bacterial number and increase in salivary bacteria may reduce pH of saliva in complete denture wearers¹⁸.

A study on the influence of salivary acidity on leachability of denture base acrylic resin revealed that lower pH increased leachability of methyl methacrylate in artificial saliva and suggested that chemotoxic actions of autopolymerized resins are potentially ascribable to methylmethacrylate¹⁹. In another study it has been reported that wearing of complete dentures for one month altered the microbial flora of the oral cavity, significant increase in the total colony forming units and appearance of *Streptococcus mutans* (*S. mutans*)¹⁷. These results coincide with studies which found that samples of saliva from edentulous patients before wearing dentures contained no *S. mutans*, however, these started to appear after denture wearing²⁰. Also, it has been shown that streptococci contribute to a wide range of cultivable micro-flora of plaque on removable dentures in patients with healthy oral mucosa²¹. Alteration of microbial flora and appearance of *S. mutans* after denture wearing has been attributed to the presence of micro-porosities in the acrylic resin surface to which salivary pellicle is attached, colonization of the pellicle with microorganisms and denture plaque formation^{22, 23}.

A study on the genetic and cellular toxicology of dental resin monomer revealed that the organic matrix of dental resin materials has been recognized as a source of compounds that cause a wide variety of adverse biological reactions such as cytotoxicity and genotoxicity. Monomers and co-monomers have been identified as the cytotoxic compounds of the organic matrix of these complex materials, and a relationship between the structural and biological activities of monomers has been reported. Resin monomers were also identified as chemicals that are able to disrupt the stable cellular redox balance, resulting in an increase in the levels of reactive oxygen species (ROS) and subsequent cell death *via* apoptosis¹⁰.

In the present study it was found that salivary pH was significantly decreased with mean value 5.63 in edentulous subjects one month after insertion of complete denture compared to the mean value 6.43 before denture insertion (Table 1, Graph 1). The decrease in salivary pH observed in the present study could have been caused by increase in oral bacteria. Decrease in pH in turn favors microbial growth, alters leachability of denture resins which, can further disturb the oral environment.

Uric acid is the major antioxidant in saliva accounting for more than 85% of total antioxidant capacity of both unstimulated and stimulated saliva, with clinical importance in monitoring oxidative stress (OS)^{8, 24}. Saliva may contribute a first line of defense against oxidative stress caused by imbalance between production of highly reactive free radicals and antioxidant defense systems².

In the present study it was found that salivary uric acid was significantly decreased with mean value 3.62 mg/dL in edentulous subjects one month after insertion of complete denture compared to the mean value 4.24 mg/dL before denture insertion (Table 2, Graph 2). The decrease of salivary uric acid levels in edentulous subjects after complete denture insertion illustrates the presence of oral oxidative stress. Oxidative stress has been implicated in the pathology of several oral diseases such as oral lichen planus and oral cancer²⁵. Decrease in the levels of saliva antioxidants such as uric acid may be considered an important mechanism by which toxic effects of free radicals can initiate precancerous transformations, oral diseases and destroy the oral cavity homeostasis²⁶.

In present study both smokers and nonsmokers showed effect on saliva pH and uric acid. pH and uric acid both significantly decrease. Smokers were slightly more significant as compared to nonsmokers (Table 4). Cigarette smoke is a mixture of chemicals containing over 4000 constituents. Some of the compounds identified include nicotine, ammonia, acrolein, phenols, acetaldehyde, benzopyrine, nitrogen oxides, carbon monoxide, polonium, radium and thorium. In addition, cigarette smoke contains free radicals that can cause cellular damage. It was demonstrated that one cigarette puff contains 1014 free radicals in the tar phase and 1015 radicals in the gas phase, (the tar and gas phases are two major phases in cigarette smoke). Pryor and Stone showed the relationship between DNA damage and tar phase free radicals after smoking. These radicals are mostly quinone-hydroquinone and they are not highly reactive. On the other hand, gas phase free radicals are generally more reactive. Tobacco smoke can alter the antioxidative capability of saliva^{27, 28}.

CONCLUSION

The size and life span of elderly population is increasing, but we have not heard that the life span of teeth is increasing. As a result, the period for wearing complete denture is increasing. The increased period of denture wearing is causing changes in the oral homeostasis and tissues around the dentures, which we have never previously experienced.

In light of the results of this investigation the following conclusion can be derived:

1. Salivary pH was significantly decreased after one month of denture insertion.
2. Salivary uric acid was significantly decreased after one month of denture insertion.

3. Age has not statistically significant on salivary pH.
4. Age has statistically significant on salivary uric acid.
5. Effect of smoking on pH and uric acid is slightly higher significant as compared to non smokers.

Wearing of acrylic complete dentures for one month lead to significant decreases in levels of salivary pH and the antioxidant uric acid. Hence to minimize these problems the result of this study suggests:

1. Long curing cycle is preferred for denture fabrication.
2. The amount of the monomer in the mixture ratio should be correct.
3. Soak the acrylic resin prostheses in water for at least 48 hours before placing them in the patient's mouth.
4. Adoption of better oral hygiene and denture cleaning practices.
5. Adequate intake of Vitamin C known to increase saliva uric acid levels may prevent oxidative damage caused by decrease in uric acid.

Saliva is most valuable oral fluid that is taken for granted. The presence of optimal salivary flow, consistency and composition is even more critical in the completely edentulous patients. Knowledge of salivary system and saliva is essential for evaluating problems and for educating patients in what to expect in this phase of denture use. Complete dentures constitute one of the most important treatment modalities in Prosthodontics. It is imperative for the prosthodontist to give due attention to these salivary characteristics before, during and after denture fabrication.

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