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A Study to Compare Salivary Ph, Uric Acid, C Reactive Protein and IgA Levels before and after Wearing Removable Complete Dentures.

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**Conflicts of Interest:** Nil

## Abstract

**Context:** Limited literature is present that have correlated salivary IgA levels with the parameters of oxidative stress, buffering capacity and inflammation in saliva of new complete denture wearers.

**Aims:** To compare salivary pH, Uric Acid, C reactive protein and IgA immediately before and after wearing complete dentures and correlate the change in IgA with above mentioned parameters.

**Methods:** Unstimulated saliva was collected from 30 systemically healthy completely edentulous subjects immediately before, three days after and one month after denture insertion.

- Ph was evaluated using pH meter.
- CRP and IgA were evaluated by Sandwich ELISA.
- Uric Acid was quantified with Modified Trinder method.

Data collected was analysed by Paired t test and scattered plot between salivary IgA and the other biomarkers was used to calculate Pearson's correlation coefficient.

**Results:** Mean pH levels significantly increased from  $7.21\pm 0.25$  to  $7.78\pm 0.27$  one month after denture insertion. Mean Uric Acid levels significantly reduced from  $3.38\pm 1.57$  to  $1.00\pm 0.52$  mg/dl. Mean CRP levels significantly increased from  $1.12\pm 0.54$  to  $1.58\pm 0.39$  and  $2.11\pm 0.35\mu$ g/ml immediately before, three days and after one month after insertion of denture, whereas mean IgA level reduced from  $1.84\pm 0.89$  to  $0.69\pm 0.36$  and  $1.69\pm 0.36\mu$ g/ml. Pearson's Correlation Coefficient value between IgA and Uric Acid, pH and CRP were statistically insignificant, and were -0.10, -0.01 and 0.18 respectively.

**Conclusions:** Results suggested that salivary components have diurnal variations but, saliva is an

excellent alternative to serum as a diagnostic/ prognostic biomarker for detection of oral diseases. It was found that new dentures increased salivary flow leading to increase in ph. Foreign materials like heat cure acrylic resin, leached residual monomer, psychological stress and denture plaque led to oral oxidative stress causing reduction in antioxidant Uric Acid. They create oral immune response lowering IgA and increasing inflammatory marker CRP.

**Keywords:** Complete Denture, CRP, ELISA, IgA, Ph, Saliva, Uric Acid

## Introduction

Edentulism is an irreversible and debilitating global public health dilemma and causes physical impairment, functional limitation, psychological and social disability, which have an impact on the general health of the individual.<sup>1</sup>

Considering the significant role played by saliva in denture rehabilitation, saliva is an ideal medium to be explored for oral health and disease surveillance in denture wearers. Saliva can be considered as a good alternative over other diagnostic biofluids and major advantages of using saliva is that the sample collection is non-invasive, painless when compared with venous blood sampling, can be collected without any training several times during the day and good correlation has been found with blood concentration of various substances, making saliva a quintessential diagnostic and prognostic tool for immunochemical assays.<sup>2,3</sup>

Conventional complete dentures are the most typical treatment for edentulous patients and most of them are well adapted to their dentures with no significant complaints. But, even when all technical and clinical prosthetic objectives are achieved, new patients are unable to adapt. Phosphate and bicarbonate buffer system maintain the oral resting pH between 6-7.

There is an increase in salivary flow following new dentures and increased flow increases the bicarbonate concentration; hence, estimation of salivary pH will help in assessing reasons for change in pH levels due to new complete dentures.<sup>4,5</sup>

Oxidative stress (OS) represents the imbalance between Reactive Oxygen Species and Total Antioxidant Capacity (TAC). Saliva acts as the first defense system against OS and Uric Acid accounts for 85% of the Total Antioxidant Capacity. Studies have shown that polymorphonuclear leukocytes in saliva produce reactive oxygen species during phagocytosis of bacteria and cause primary host response during inflammation and infection, hence reducing the total antoxidant capacity.<sup>6</sup> Recent research has also observed a close relationship between OS and anxiety. Hence, changes in level of salivary Uric Acid post new dentures can be assessed to prevent alteration of oral homeostasis.<sup>7</sup>

C - reactive protein (CRP), an acute-phase protein, is a prognostic bio marker of inflammation and increased level is known to represent underlying inflammatory processes. Psychological stress is known to activate the sympathetic nervous system resulting in the release of catecholamines and glucocorticoids and initiates an acute phase inflammatory response. This relates stress in first time denture wearers with changes in saliva CRP.<sup>8,9</sup> Similarly, IgA is associated with inflammation and is the main mucosal immuno globulin.<sup>10</sup> Studies have shown that psychological stress can alter the levels of IgA. However, limited literature is present that have correlated salivary IgA levels with the parameters of oxidative stress, buffering capacity and inflammation in saliva of complete denture wearers. Thus, this study was aimed to compare salivary pH, uric acid, C- reactive

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protein and IgA immediately before and 1 month after wearing removable complete denture and to correlate the change in IgA levels with the above-mentioned parameters.

#### Methodology

This prospective in-vivo study was conducted on thirty new complete denture wearers with ASA (American society of Anaesthesiology) score 1. Subjects with recent history of chronic systemic in flammatory diseases or intake of immuno suppressive drugs, those with diseases that interfere with immuno competence (Human Immuno deficiency Virus, Asplenia, chronic renal disease, hematopoietic malignancies), history of recent chemo/ radiation therapy, existing salivary gland infections, salivary stones or systemic diseases having associated salivary gland infection were excluded from the study.<sup>8</sup> Also excluded were those with existing oral lesions, smoking habit, history of chewing tobacco products and those past recent history of surgical trauma. The methodology adopted here was granted ethical clearance by the Ethical Committee of the Institution prior to the commencement of the study [BDC/ Exam/ 467/2018 - 19]. The participants of the study had the protocols explained to them in their own mother tongue. They underwent removable complete denture treatment after detailed medical and dental history of each patient was recorded and signed informed consent was taken from them. The sample size was determined by convenient sampling technique which was numbered up to 30.11

For the analyses, unstimulated saliva was collected thrice in five ml polypropylene vials by spitting method from each subject after one hour fasting.<sup>12</sup>

- 1. One hour prior to complete denture insertion.
- 2. Three days after complete denture insertion.
- 3. One month after complete denture insertion.

Ph was recorded chairside immediately after collection using PH- 80 Ph meter (HM Digital) (fig. 1).<sup>4</sup>

For the other biomarker analyses, saliva samples were stored at 4°C and transported to the laboratory on the same day. They were centrifuged at 2000-3000 rpm for twenty minutes (Bio Bee Tech Mini Centrifuge). The clear supernatant was then transferred to a sterile Eppendorf tube and stored at -80 °C until subjected to ELISA analysis using ELISA kit (Kinesis Dx) (fig. 2,3, 4, 5, 6).

A linear standard curve was generated by plotting the mean absorbance/ optical density (OD) value for each reference standard against its concentration in  $\mu$ g/ml, with OD value on the vertical (y) axis and concentration on the horizontal (x) axis using Spectra Max 190 Absorbance Microplate reader able to measure absorbance at 450 nm (Seeding Labs) (fig 7). The mean absorbance (OD) value of each sample was used to determine the corresponding concentration of CRP and IgA from the standard curve (fig 8).<sup>8,10</sup>

Salivary uric acid was quantitatively determined by Uric acid DES (Dynamic Extended Stability) with lipid clearing agent using modified Trinder method (Erba V2 Mannheim) (fig. 9). The intensity of chromogen (Quinonei mine) formed was proportional to the Uric Acid concentration in the sample when measured at 505 nm. URIC ACID (mg/ dl) = absorbance of test/ absorbance of Standard X concentration of Standard (mg/dl).<sup>13</sup> The data obtained after microbiological tests were subjected to statistical analysis using Paired-t test. Pearson's Correlation Coefficient between salivary IgA and salivary pH, Uric Acid and CRP levels one month post denture insertion were calculated using Minitab (Version16) software.

## Results

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[Table I] shows mean Uric acid levels of 3.38±

1.57mg/dl and 1.00±0.52 mg/dl immediately before and 1 month after denture insertion. Using Paired t test there was statistically significant difference (p value <0.001).

[Table II] summarizes the pH values with mean pH levels of  $7.21\pm0.25$  and  $7.78\pm0.27$  immediately before and 1 month after insertion with statistically significant difference (p value <0.001).

[Table III] shows mean CRP levels of  $1.12 \pm 0.54$ ,  $1.58 \pm 0.39$  and  $2.11 \pm 0.35$  µg/ ml immediately before, 3 days after and 1 month after insertion of complete denture. Using Paired t test there was statistically significant difference against the baseline values. [Table IV] depicts the mean IgA levels  $1.84\pm0.89$ ,  $0.69\pm0.36$ of and  $1.69\pm0.36$  µg/ml immediately before, 3 days after and 1 month after insertion of complete denture. Using Paired t test there was statistically significant difference (p value <0.001) after 3 days and of wearing complete dentures against the baseline values. The mean difference in IgA values between baseline and 1 month after denture usage was 0.15, which was statistically non-significant (p value>0.001).

[Table V] Pearson's Correlation Coefficient (r) value between IgA and Uric Acid was -0.10, between IgA and pH was -0.01 and between IgA and CRP was 0.18. Negative sign indicates inverse relationship between the parameters. Statistically significant correlation was not obtained between IgA and the other three parameters (p > 0.05). With the increase in level of IgA there was a decrease in the level of Uric Acid and ph. but, level of IgA was directly proportional to CRP levels.

#### Discussion

The ability to monitor health status, disease onset, progression and treatment outcome through

non-invasive means like saliva is a highly desirable goal in Prosthodontic treatment.<sup>14</sup>

Mohamed R. and others from their study, pointed out that acid stimulated saliva gave lesser values of CRP, Myoglobin and Immunoglobulin E when compared with resting and mechanically stimulated samples. When, saliva was mechanically stimulated, large volume of water is present in this type of saliva and it has less protein concentration as compared with resting saliva. Also, Uric acid, due to its smaller molecular volume like myoglobin, will show significantly lower concentration in mechanically stimulated saliva. Hence, resting or unstimulated saliva was collected.<sup>15</sup>

In a study conducted by Yurdokuru, it was found that new complete dentures acted as a stimulant and increased salivary flow rate, thereby increasing the pH.<sup>5</sup> Another study by Nikolopoulou and Tzortzopoulou measured salivary pH using electropH meter CONSORT P903 and found similar results after patients wore complete dentures for 15 days. Hence, new complete dentures act as mechanical stimulant/ foreign body and increase the salivary flow. Bicarbonate concentration increases with increasing flow rate so, the pH level was elevated at high flow rates.<sup>4</sup> The other probable reason was the stimulation of the mucous glands in the posterior third of the palate because of denture coverage, causing increase in salivary secretion. Matsuda stated that replacement of edentulous jaws with complete dentures improved maximal occlusal force, hence improving the masticatory performance. This stimulates the mechanoreceptors of the oral mucosa and salivary glands and leads to increased stimulated/ unstimulated salivary flow rate which could be another reason for increase in pH in our study.<sup>16</sup> The decrease in salivary Uric Acid in the subjects after complete denture insertion illustrated

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the presence of oral oxidative stress following Prosthodontic treatment.

In a similar study done by Bhat and others, decrease in levels of antioxidants like Uric Acid was considered to be an important reason for initiation of precancerous transformations, oral diseases and destruction of oral homeostatis by free radicals.<sup>8</sup> Another study was done by Daniel A. to estimate the psychological and oxidative salivary stress markers in edentulous patients before and 1 week after denture insertion. Oxidative stress markers, Thiobarbituric Acid Reactive Substances and Nitric Oxide (NO) significantly increased one week post denture insertion. The mean levels of the salivary stress marker, Alpha Amylase was 8.26+/- 1.32 min/ml during denture insertion and it decreased to 3.44+/- 1.25 min/ml 1 week after insertion. This suggested increase in oxidative stress or reduction in salivary antioxidant levels and no reduction in psychologic stress up to 1 week due to adjustments for a new denture.<sup>17</sup> Similarly, in our study decrease in uric acid levels proves presence of oxidative stress due to inflammation caused by foreign materials like dentures.

Ajwani, in his study, stated that elevated CRP levels were found in completely edentulous denture wearers.<sup>19</sup> The present study also showed elevated CRP 3 days and 1 month post denture insertion hence, stating the possibility that inflammation is due to initial wear of new denture and leads to elevated CRP. The high levels of pro in flammatory cytokines that were found in saliva of auto polymerised and heat cure acrylic denture material may be linked to their protective effect against tissue inflammation caused by release of residual monomer. The individual components leach from the dentures and diffuse into saliva thus influencing the cytokine and antibody levels of the oral tissues. A member of the pentraxin family, CRP is a major acute phase protein in humans. Lu J found that major IgA receptor; Fc $\alpha$ RI is a ligand for pentraxin. Fc $\alpha$ RI and its activation by IgA immune complexes lead to phagocytosis, antigen presentation and release of cytokines, superoxide and other in flammatory mediators like CRP. CRP also binds to the same receptor hence, directly correlating IgA and CRP.<sup>20</sup>

Kugler, Hess and Hake determined the salivary IgA and salivary flow in 84 medical students by radial immuno diffusion technique and weighing of test tubes respectively. They found a significant negative correlation between saliva flow and concentration of salivary IgA. Similarly, insertion of new dentures causes an increase in salivary flow as stated by previous studies and this is a possible reason for decrease in IgA three days after insertion of dentures as seen in our present study.<sup>21</sup> A study was done by Deinzer on 27 participants in a major medical exam and 27 controls, where saliva samples were collected 6th day prior to their last exam until 14th day afterwards. Results showed reduction in salivary IgA during a period of academic stress in all subjects as compared to controls not giving any exam.<sup>22</sup> Dehis further designed a study to use SIgA as a parameter to evaluate the response of oral immuno competence to prosthetic appliances like complete dentures. Significant decrease in SIgA levels 3 to 7 days post denture insertion similar to our present study concludes that wearing complete denture fabricated from heat cure acrylic resins can be immuno suppressive.<sup>10</sup>

Statistically significant correlation was not obtained between IgA and the other three parameters (p > 0.05).

With the changes in levels of IgA there was release of inflammatory cytokines like CRP, which also bound to the same receptor, hence, playing a major role in oral immune response in first time denture wearers. In our study this additional parameter was used to correlate in

flammation and psychological stress to changes in levels of salivary pH, uric acid and CRP and use IgA in saliva as a useful and easy tool for diagnosing and monitoring denture related disease initiation and progression.

#### Conclusion

Within the limitations of the study, it was found that the initial period of denture usage is a relevant source of dissatisfaction due to alteration in inflammatory and psychological components of saliva in the denture wearers. Changes in the oral environment in completely edentulous individuals after wearing complete denture was verified by estimating markers of oxidative stress, immunity, in flammation and buffering capacity in their saliva and levels of these parameters would serve as biological markers for diagnosing and tracking the progression of various denture induced diseases in the future that alter the mucosal immunity of the denture wearer, reinforcing the fact that values of these parameters should be easy to evaluate and must be calculated to prevent future oral diseases.

The limitation of this study includes the current obstacles to the use of saliva as a diagnostic tool and is due to the reasons that:

1. Concentration of analytes may be influenced by diurnal/ circadian cycles of salivary secretion.

2. Denture biofilm index needs to be clinically calculated to see if increase in inflammatory mediators and alteration in antibody level is due to formation of biofilm on the intaglio surface of denture.

3. Questionnaire for assessing acute psychological stress to be included in further studies.

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Abbreviation	Definition
4-AAP	4-Aminoantipyrine
- ve	Negative
%	Percentage
°C	Degree Celsius
μg	Microgram
μL	Microlitre
ASA	American Society of Anaesthesiologists
CRP	C-Reactive Protein
DES	Dynamic Extended Stability
dl	Decilitre
ELISA	Enzyme Linked immuno sorbent Assay
HS	Highly significant
IgA	Immunoglobulin A
mg	Milligram
ml	Millilitre
Na	Not applicable
Nm	Nanometre
NO	Nitric oxide
NS	Non-significant
OD	Optical density

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OS	Oxidative Stress
p value	Probability value
r value	Pearson's Correlation Coefficient
rpm	Revolutions per minute
SD	Standard Deviation
TAC	Total Antioxidant Capacity
TBHB	2,4,6-Tribromo-3 hydroxy benzoic acid
TMB	3,3',5,5'-Tetramethylbenzidine
UA	Uric acid

# **Legend Figures**

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Figure 1: Armamentarium Used for Evaluation of Salivary pH



Figure 2: Armamentarium used for ELISA analysis of salivary CRP and IgA.



Figure 3: Armamentarium used for ELISA analysis of

salivary CRP and IgA.

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Figure 4: Armamentarium used for ELISA analysis of saliva.



Figure 5: Armamentarium used for ELISA analysis of salivary CRP and IgA.



Figure 6: ELISA micro titre plate after adding stop reagent



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Figure 7: Microplate reader for ELISA test. Figure 9: Uric acid DES



Figure 8: optical density vs concentration of substrate.



Figure 9: Uric acid DES kit.



Table 1: salivary uri	c acid values in com	pletely edentulous indivi	iduals before and after w	vearing complete denture.
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	Before	After1 Month	Difference	t value	P value
Mean	7.21	7.78	0.57	13.90	P < 0.001, HS
SD	0.25	0.27	0.22	-	
Range	6.8 - 7.7	7.1 - 8.4	0.20 - 1.0		

Table 2: salivary Ph values in completely edentulous individuals before and after wearing complete denture

	Before	After 1 Month	Difference	t value	P value
Mean	7.21	7.78	0.57	13.90	P<0.001, HS
SD	0.25	0.27	0.22		
Range	6.8 - 7.7	7.1 - 8.4	0.20 - 1.0		-

Table 3: Salivary CRP values in completely edentulous individuals before and after wearing complete denture.

	Before	After 3 Days	After 1 Month	Before-3 Day diff.	Before- 1Month diff.
Mean	1.12	1.58	2.11	0.44	0.99
SD	0.54	0.39	0.35	0.33	0.49

Range	0.21 - 1.98	0.82 - 2.10	1.38 - 2.85	0.05 - 1.42	0.25 - 2.47
			t value	7.73	11.00
			P value	P < 0.001, HS	P < 0.001, HS

Table 4: Salivary iga values in completely edentulous individuals before and after wearing complete denture.

	Before	After 3 Days	After 1 Month	Before-3 Day diff.	Before-1 Month diff.
Mean	1.84	0.69	1.69	1.15	0.15
SD	0.89	0.36	0.36	0.73	0.9
Range	0.58 - 3.86	0.22 - 1.81	0.95 - 2.80	0.06 - 2.73	(-)1.3 - 2.0
			t value	8.72	0.93
			P value	P < 0.001, HS	0.36, NS

Table 5: correlation analysis

Relationship between	r value	P value
IgA & Uric Acid	-0.10	0.64, NS
Iga & pH	-0.01	0.98, NS
IgA & CRP	0.18	0.34, NS



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Graph 3





