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# Assessment of Salivary Changes in Patients on Poly-pharmacy Medications: A Pilot Study

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author LMA has made substantial contributions towards study design, acquiring an analysis of data, drafting the final paper and revising it critically. Author MA has made substantial contributions towards study design, acquiring an analysis of data, drafting the final paper and revising it critically. Both authors read and approved the final manuscript.*

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## ABSTRACT

Saliva is a biological fluid with multifactorial functional applications with regards to the maintenance of general health. Collection of saliva is non-invasive, easy and cost-effective. Xerostomia, a subjective sensation of dry mouth is one of the most common complaints in elderly and patients on polypharmacy as it is proven as a risk factor. Polypharmacy is the simultaneous use of multiple drugs by a patient for more than one systemic disease. The aim of the study was to assess the salivary changes in patients on polypharmacy. Unstimulated saliva samples were collected from 20 individuals who reported to the Department of Special Care Dentistry at Saveetha Dental College between November to December 2019. The collected samples were then processed for analysis of three salivary parameters-Salivary glucose, amylase and pH. The data were recorded and statistically analyzed. Out of the 20 samples,12 male (60%) and 8 female(40%) with mean age group as 41.2 years. Independent t test showed statistically significant ($p<0.05$) values for salivary parameters in patients on polypharmacy medication. This study has shown a significant increase in salivary parameters and further studies relating the salivary changes, systemic disease and the medication involved salivary analysis can be suggested as one of the investigations in patients on polypharmacy.

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*Keywords: Unstimulated saliva; polypharmacy; pH estimation; salivary glucose; salivary amylase.*

## 1. INTRODUCTION

Saliva is a clear, slightly acidic mucinous-serous secretion and salivary composition includes various electrolytes, organic substances, proteins, peptides, and polynucleotides. Whole saliva (WS) is a body fluid constantly cleansing the mucous membranes of the mouth, throat, and larynx. The minor salivary glands and major salivary glands are the contributions to the whole saliva. About 10% of saliva is produced from minor salivary glands and 90% from the three major salivary glands. About 65% of unstimulated (resting) saliva comes from the submandibular gland, 25% from the parotid gland, 4% from the sublingual gland, and 8% from other salivary glands [1]. Saliva constitutes an important factor in maintenance of homeostasis in the mouth due to the presence of organic and inorganic components and aids in articulation, digestion and swallowing. It also acts as a protective surface of the teeth and mucous membranes against biological, mechanical, and chemical factors and perception of taste stimuli, temperature, and touch. Saliva contains 99.5% water, 0.3% protein, and 0.2% inorganic and organic substances [2]. Sodium, potassium, calcium, magnesium, chlorides, and carbonates constitute the inorganic substances whereas organic components include amylases, peroxidases, lipases, mucins, lysozyme, lactoferrin, hormones, and growth factors. Secretion of saliva on a daily basis is estimated at 0.5 to 2 litres [3]. There are different methods of saliva collection (stimulated/unstimulated) which includes passive drooling and spitting, of which the spitting method is commonly used as it is comparatively easier than other methods but has the drawback of contamination of bacteria while spitting directly into a container. Saliva samples should be stored preferably on ice and frozen as soon as possible to maintain the sample integrity. Saliva has significant advantages as it is an unique non invasive diagnostic tool. The collection is fast, inexpensive to the patients and safe. Saliva is often termed as “body mirror” and reflects physiological and pathological state of the oral cavity. National Institute of Dental and Craniofacial Research (NIDCR) in the year 2002, has approved body fluids as a diagnostic tool to assess the state of health and disease [4]. Medically compromised patients are those patients who are diagnosed with systemic diseases and are under medications for the same. Polypharmacy is the concurrent use of multiple medications by an individual. Hence, medically compromised patients who are diagnosed with multiple systemic diseases are invariably under polymedications. Drugs, especially antimuscarinic agents, some sympathomimetic agents, and agents affecting serotonin and noradrenaline uptake, as well as a miscellany of other drugs such as protease inhibitors and appetite suppressants, may produce subjective dry mouth. Drugs with anticholinergic activity against the M3 muscarinic receptor are the most common reported cause of reduced salivation and efforts are in hand to reduce this activity in newer drugs. Elderly people are at a greater risk for adverse drug reactions due to the metabolic changes along with decreased drug clearance with aging and is furthermore increased on polypharmacy. Polypharmacy has also led to “Prescribing cascades”. The aim of the study was to assess the alterations in the levels of Salivary glucose, Salivary amylase and Salivary pH in patients on polypharmacy medications. Our recent research portfolio in slides numerous articles in reputed journals [5–9]. Based on this experience we planned to pursue to assess the salivary changes in patients on polypharmacy medications.

## 2. MATERIALS AND METHODS

A total of 20 patients were involved in the study. These patients were divided into two groups of 10 patients each. Group A being the control group and Group B being the patients who were under polymedications and visited the Department of Special Care Dentistry at Saveetha Dental College during the period of November 2019 to December 2019.

The patients are conveyed that their names and initials will not be published and efforts will be made in concealing their identities, but anonymity cannot be guaranteed. Participants from Group A were examined and the patients who were devoid of being under any medications and not diagnosed with any systemic diseases were selected. Group B participants were patients who were under medication for hypertension, diabetes mellitus, psychotic disorders and for renal disorders. The drugs consumed by the patients included calcium channel blockers, beta blockers, ACE inhibitors, metformin, sulfonylureas and chlorpromazine. All

the selected patients were subjected to a detailed general and intra oral examinations and the collected saliva underwent analysis for Salivary glucose, amylase and pH examination. The data was retrieved, recorded and was statistically analysed using IBM SPSS software for frequency distribution analysis, independent t test; association between the groups and the salivary parameters; gender and salivary parameters.

## 2.1 Method of Collection of Saliva

After the patient was in a comfortable and relaxed state, the patient was asked to spit(unstimulated saliva) into a sterile container given by pooling of the saliva in their floor of mouth at an interval of five minutes for fifteen minutes [10]. Patients were asked to rinse their mouth prior to spitting to be devoid of any food particles contamination and were collected during the hours 9am-11pm. The salivary container was placed in a cooling box and taken for analysis immediately [Fig. 1].

## 2.2 Estimation of Salivary Ph

The collected saliva was allowed to settle for 5-10 minutes. Then with the help of a digital pH (Pen type pH meter) meter each sample and the value were noted. This was determined as the pH of saliva for the particular individuall [Fig. 2].

## 2.3 Estimation of Salivary Glucose

Salivary glucose estimation was done using the GOD-POD method. It is based on the principle that glucose is oxidized by the enzyme Glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide in the presence of the enzyme Peroxidase (POD) to produce a red colored quinoneimine dye. The intensity of the color developed is proportional to the glucose concentration in the sample. The sample was pipetted and added to the reagents [Fig. 3]. This sample was then placed into a digital photo colorimeter (Alpha-03) and the values were noted [Fig. 4].

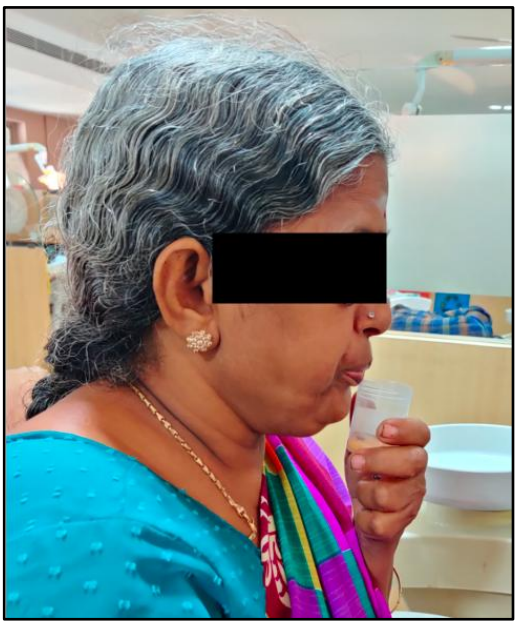


**Fig. 1. This image shows the method of saliva collection. The study patient has collected the saliva using the spitting method into the sterile container**


**Fig. 2. This image shows the estimation of salivary pH. (A) - Digital pH meter (Pen type pH meter). (B) - pH meter used for estimation of pH from the sample**


**Fig. 3. (A) - This image shows the Amylase reagent used in the study (DIATEK). (B) - This image shows the Glucose reagent used in the study (GLUCOSE-EGD)**

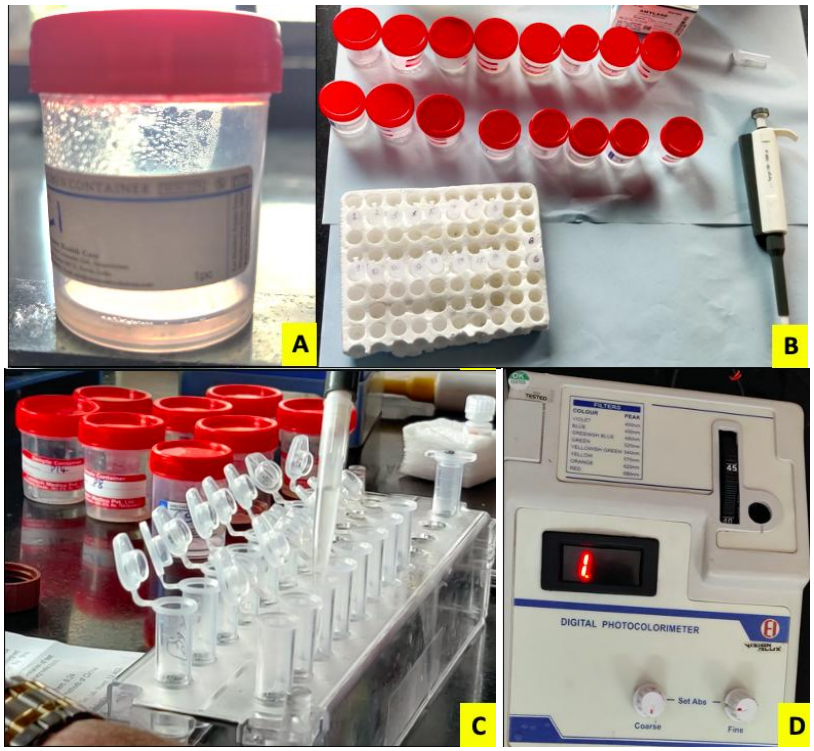



**Fig. 4. (A) - Image shows the collected saliva sample in the container, (B) - The armamentarium used for the analysis in the study, (C) - Image shows the micropipetting of the saliva into the vials for testing, (D) - Image shows the Digital Photocolorimeter (Alpha-03) used in the study analysis**

### 2.4 Estimation of Salivary Amylase

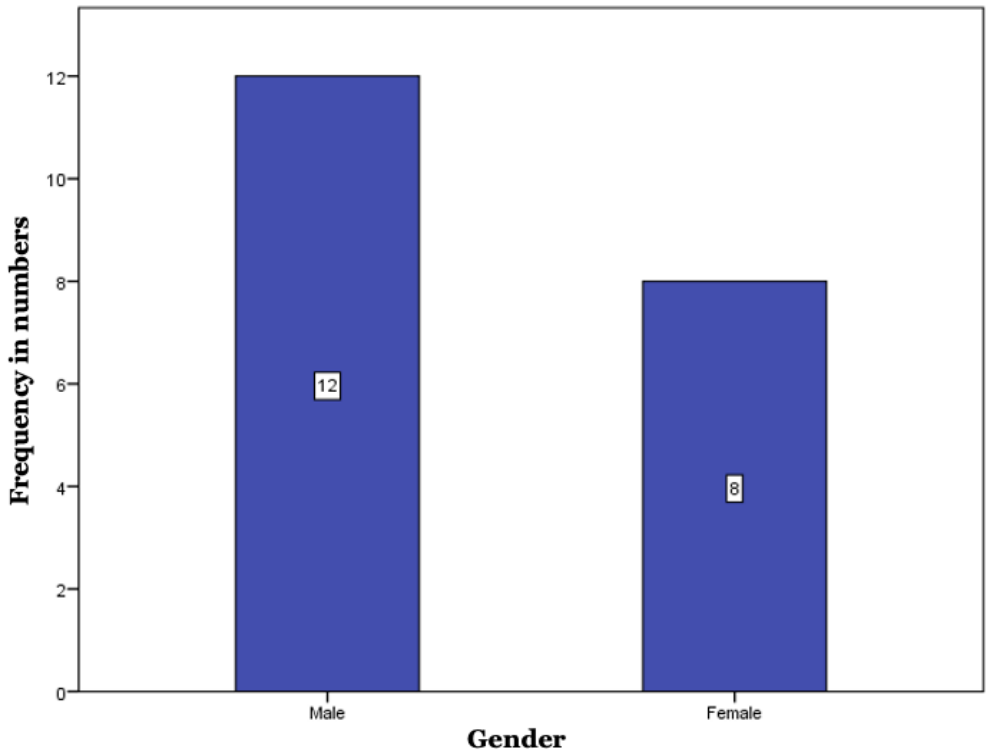
Salivary amylase estimation was done using the CNPG3 method. It is based on the principle that alpha amylase hydrolyzes 2-chloro-p-nitrophenyl-alpha-D-maltotrioside (CNPG3) to release CNPG2, maltotriose (G3) and glucose (G). The rate of increase in absorbance is measured and is proportional to the enzyme alpha amylase in the sample. The sample was pipetted and added to the reagents [Fig. 3]. This sample was then placed into a digital photo colorimeter (Alpha-03) and the values were noted [Fig. 4].

## 3. RESULTS AND DISCUSSION

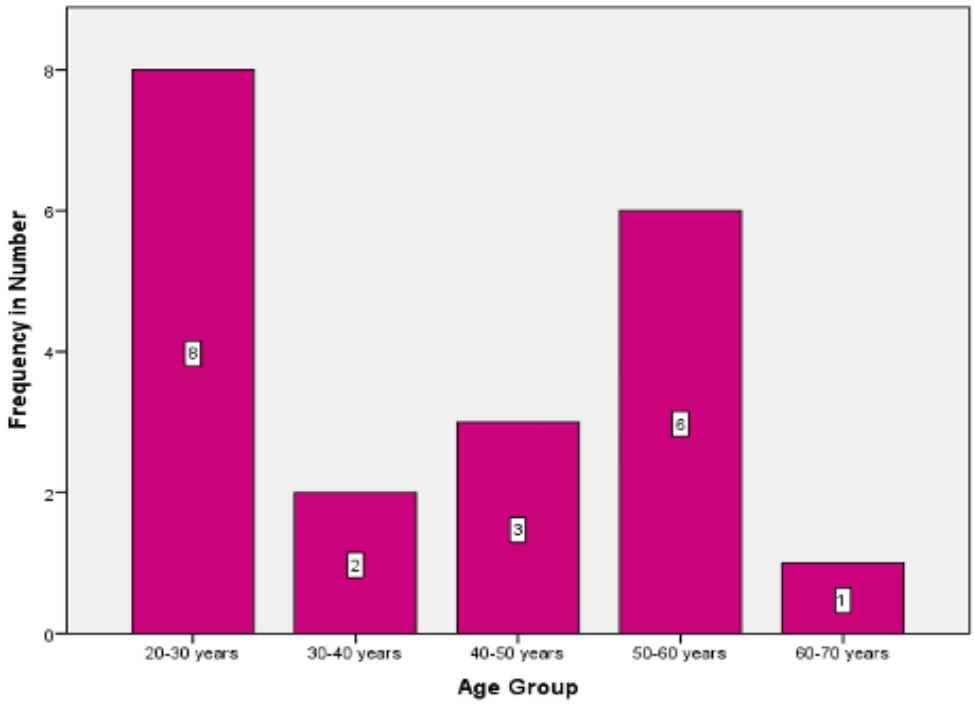
In this study, the 20 patients were grouped into two groups named Group A and Group B with 10 samples in each. Group A consisted of patients with no systemic disease and were not under any kind of medications were taken as the control group and Group B were the patients who were under antihypertensives, hypoglycemic drugs, antipsychotic drugs and those under medication for renal disorders. Out of the 20 patients in the study,12 were male (60%) and 8 of them were female (40%) [Graph 1]. The age groups of the patients were grouped and there were 8 patients in 20-30 years (40%), 2 patients under 30-40 years (10%), 3 patients in 40-50 years (15%), 5 patients in 50-60 years (25%) and 2 patients in 60-70 years (10%) age group with mean age of 41.2 years [Graph 2].

Table 1 shows the group statistics for mean and standard deviation between the salivary parameters (Glucose, Amylase and pH) in between Group A and Group B.

Table 2 shows the independent t test done between the Group A and Group B for the parameters, glucose, amylase and pH. The values of Salivary glucose were t (18) = -6.087,p=0.000; Salivary amylase t(18)=16.528,p=0.000; Salivary pH t(18)=16.213,p=0.000. There was a statistically significant difference ($p<0.05$) for all the three parameters (p=0.000).




**Graph 1. This graph depicts the gender distribution among the study population. X-axis depicts the gender and Y-axis depicts the frequency of population in number. There were 12 males (60%) and 8 females (40%) in the study**




**Graph 2. This graph depicts the age distribution in the study population. X-axis depicts the age groups and Y-axis is frequency of population in number. The age groups of the patients were grouped and there were 8 patients in 20-30 years (40%), 2 patients under 30-40 years (10%), 3 patients in 40-50 years (15%), 5 patients in 50-60 years (25%) and 2 patients in 60-70 years (10%) age group**

**Table 1. This table shows the mean and standard deviation between the two groups (A and B) for Salivary glucose, Salivary amylase and Salivary pH. The mean value for Salivary glucose in Group A was 0.46 mg/dL and in Group B was 1.26 mg/dL. The mean value for Salivary amylase in Group A was 16.32 U/L and in Group B was 35.37 U/L. The mean value for Salivary pH in Group A was 7.45 and in Group B was 6.76.This infers that all three parameters presented with an increased value in Group B when compared to Group A**

| Group statistics | | | | |
|---|---|---|---|---|
| | **Group** | **N** | **Mean** | **Std. Deviation** |
| **Salivary Glucose** | Group A | 10 | .4620 | .14250 |
| | Group B | 10 | 1.2610 | .38986 |
| **Salivary Amylase** | Group A | 10 | 16.3220 | 5.71122 |
| | Group B | 10 | 35.3790 | 7.76929 |
| **Salivary pH** | Group A | 10 | 7.4500 | .32745 |
| | Group B | 10 | 6.7600 | .23190 |

Graph 3 shows the difference in the mean values between Group A and Group B for the salivary parameters. Group A shows mean value of Salivary Glucose as 0.46 mg/dl; salivary amylase as 16.32U/L; and Salivary pH as 7.45 but when compared to Group B there is a significant increase in all the three parameters. Salivary glucose is 1.26 mg/dl; Salivary amylase is 35.38U/L and Salivary pH is 6.76.

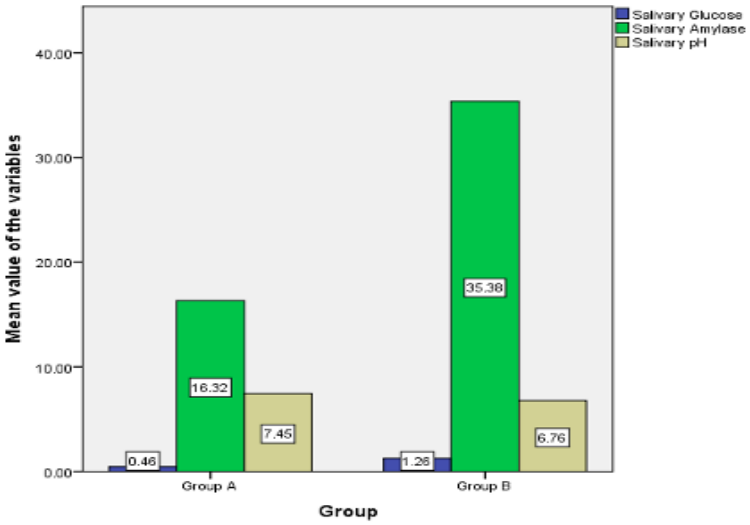
Graph 4 shows the association between gender and the salivary parameters between the two groups. The mean values in males for Salivary glucose, amylase and pH was 0.89 mg/dl; 25.79 U/L; 7.04 and in females the values were 0.81mg/dl; 25.94U/L; 7.20. This graph shows that there is no significant relationship between gender and the salivary parameters.

**Table 2. This table shows the independent t test done for the salivary parameters between the two groups. The values of Salivary glucose were t (18)=-6.087, p=0.000; Salivary amylase t (18)=16.528, p=0.000; Salivary pH t (18)=16.213,p=0.000. There was a statistically significant difference (p<0.05) for all the three parameters (p=0.000)**

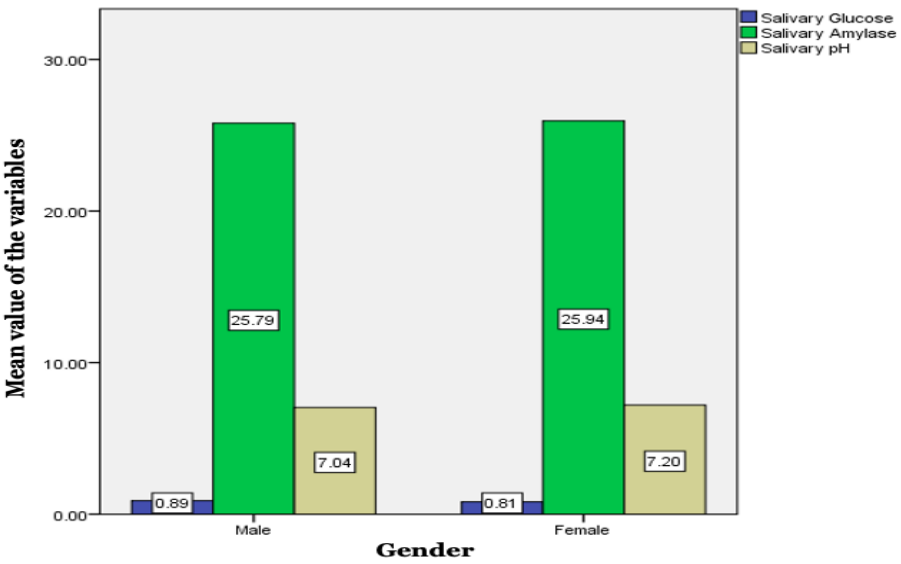
| Independent samples test | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig.(2 tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Salivary Glucose | Equal variances assumed | 3.559 | .075 | -6.087 | 18 | .000 | -.79900 | .13126 | -1.07477 | -.52323 |
| | Equal variances not assumed | | | -6.087 | 11.363 | .000 | -.79900 | .13126 | -1.08678 | -.51122 |
| Salivary Amylase | Equal variances assumed | 1.453 | .244 | -6.250 | 18 | .000 | -19.05700 | 3.04926 | -25.46326 | -12.65074 |
| | Equal variances not assumed | | | -6.250 | 16.528 | .000 | -19.05700 | 3.04926 | -25.50439 | -12.60961 |
| Salivary pH | Equal variances assumed | 1.037 | .322 | 5.438 | 18 | .000 | .69000 | .12689 | .42342 | .95658 |
| | Equal variances not assumed | | | 5.438 | 16.213 | .000 | .69000 | .12689 | .42130 | .95870 |

Saliva consists of two components that are secreted by independent mechanisms, one being the fluid component which is produced mainly by parasympathetic stimulation and secondly a protein component mainly in response to sympathetic stimulation. A study done by Strekfus et al says that the drugs that are most commonly implicated in dry mouth are the tricyclic antidepressants, antipsychotics, beta blockers and antihistamines [11]. Medication induced hyposalivation is among the most common finding in the patients on polypharmacy. Our study reported a male prevalence (80%) and mean age being 41.2 years which was in concordance to the study done by Heft et al. [12]. The mean value for Salivary glucose in Group A was 0.4620 mg/dL was lower when compared to Group B was 1.26 mg/dL. For Salivary amylase, Group A was 16.32U/L was lower to that when compared with Group B was 35.3U/L similar to the study done by Indira et al [13]. The mean value for Salivary pH in Group A was 7.45 and in Group B was 6.76.This inferred that all three parameters presented with an increased value in the dependent group when compared to that of control group. The results of the independent t test done showed that there was a significant difference with salivary glucose, amylase and pH when compared between both the groups. Studies done by Indira et al. [13] and Raghunathan et al. [14] have compared in patients only under hypoglycemic drugs and have represented a significant correlation.

Amylase is a protein and is responsible for glycoprotein complex formation within the pellicle formed on the surface of the teeth. It also has a higher affinity for bacteria in the mouth. On comparison of mean values between the two groups, there was a significant increase in all of the three salivary parameters with a two fold increase in Salivary amylase values 16.32U/L to 35.38U/L. The increase in salivary amylase could be because of greater expression of amylase and cyclic amp receptors in the parotid gland was demonstrated by Piras et al especially in patients on hypoglycemic drugs [15]. On association between gender and salivary parameters, the mean values in males for Salivary glucose, amylase and pH was 0.89 mg/dl; 25.79 U/L; 7.04 and in females the values were 0.81 mg/dl; 25.94U/L; 7.20. There was no significant difference noted between males and females. In a study done by Rutherfurd-Markwick et al has shown that salivary amylase levels were significantly higher in a group of women than men [16]. There has not been any association between gender to that of Salivary glucose and pH reported. The limitations of this study include a lesser sample size and storage and transportation of saliva as the salivary compounds tend to denature and in turn affecting the analysis results.




**Graph 3. This graph depicts the relationship between the salivary parameters in the two groups.X-axis depicts the two groups (study and control) and Y-axis depicts the mean values of the variables. Purple depicts for Salivary Glucose, Green depicts for Salivary amylase and yellow depicts for Salivary pH. Group A shows mean value of Salivary Glucose as 0.46 mg/dl; salivary amylase as 16.32U/L; and Salivary pH as 7.45. In Group B, Salivary glucose is 1.26mg/dl; Salivary amylase is 35.38U/L and Salivary pH is 6.76. There is a significant difference in all the three parameters in the Group B (patients under polypharmacy)**




**Graph 4. This graph depicts the relationship between the gender and salivary parameters. X-axis depicts the gender and Y-axis depicts the mean values of the variables. Purple depicts for Salivary Glucose, Green depicts for Salivary amylase and yellow depicts for Salivary pH. The mean values in males for Salivary glucose, amylase and pH was 0.89 mg/dl; 25.79 U/L; 7.04 and in females the values were 0.81 mg/dl; 25.94U/L; 7.20. This graph shows that there is no significant relationship between gender and the salivary parameters**

## 4. CONCLUSION

Salivary composition and salivary flow are altered directly in systemic diseases or as a result of medications for the underlying systemic disorders. Alterations in salivary flow and salivary composition affects the normal oral ecology and architecture. With increased life expectancy and geriatric patients on polypharmacy, salivary alterations aggravate or initiate the already existing underlying disorders. Common changes are Xerostomia, hyper salivation, dysgeusia and atypical facial pain. This pilot study confirms alterations in salivary glucose, amylase and pH in patients on polypharmacy.

## CONSENT AND ETHICAL APPROVAL

The study was approved by the scientific review board (SRB) and institutional ethical committee and have obtained consent forms from all the patients which provides their consent in usage of his/her/their images and other clinical information to be reported in a journal.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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