Sustenance of Salivary Response in Human Population and Suggestions for Elderly Denture Wearer: An in vivo Study

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Aim: The purpose of this study was to confirm whether psychic stimulation of salivation occurs in human also and to formulate certain guidelines for complete denture wearers. Material and Methods: Armamentarium used for this study includes preweighed cotton packets, two glass beakers, stopwatch, electronic digital balance, cotton tweezers, and hot fresh snacks. After this, the mopping protocol for the participants was made, and each participant was told to follow the instructions for all the 6 days of observations. The collected data of salivary flow as per the protocol were tabulated in terms of increase in cotton weight for all the steps. Similar tables were prepared for all the forty participants. As the sample size was small, a nonparametric analytical plan was adopted. Wilcoxon signed-rank test (nonparametric variant of paired t-test) and Mann–Whitney U-test (nonparametric variant of Student’s t-test) were used. Results: For baseline secretions (day 1 without thought of food) values for all steps were overall higher in males, mean value is 2.99 ± 1.14 g as compared to 2.08 ± 0.82 g for females for Step 1 (0 – 3) mts, in males as compared to in females for Step 5 (12 – 15) mts. Overall, a significant difference between two genders was observed for baseline flow during all the steps except for Step 5 (P < 0.05) with males showing significantly higher flow as compared to females. It was observed that there was a significant difference between baseline and stimulated salivary flow in all the participants on all the days of observation, except for the Step 4 and 5 where it was near to baseline. Conclusions: The findings generally suggest that yes there is an enhancement of salivary flow with the thought of food, but it lasts for 6–9 min after which it turns to base level.

Keywords: Conditioned reflex, salivary flow, salivary response

Introduction

With the thought, sight, or smell of food, it is natural for a human being to salivate, which is due to the stimulation of the special sensory organs in the human body that triggers a complete chain of chemical reactions in the brain which initiates the salivary secretion from the salivary glands. Researchers have demonstrated the stimulation of salivary secretion “salivary response” due to factors other than food in animals as well as human beings and termed them as “conditioned reflex.”[1-3] However, there are other studies which show that the thought in itself was not a stimulus. There are studies which say that after a certain period of excessive secretion, there was decrease in salivary flow.

Such changes in the salivary flow, if any, with the thought of meal, may alter the salivary volume and the due retention of the prostheses. It may also affect the chewing, pregastri digestion due to saliva, and deglutition. As per many studies, the salivary secretion enhances with thought, smell, or sight of food.[4,5] If we could utilize this period of salivary stimulation properly, we might not have the problems as big as they appear in the denture patients with inadequate salivary flow or it may prove worse with the thought of food, if the thought is sustained for a long period of time but without meals. Hence, there appeared a need to assess and quantify this enhanced salivary flow as compared to baseline flow and its correlation with duration, with thought of food and to assess its sustenance. The aims of this study were to confirm whether this psychic stimulation...
occurs in humans also including male and females and for how long it sustains.

Material and Methods

This “in vivo” study was conducted in the Department of Prosthodontics, Babu Banarasi Das College of Dental Sciences, Lucknow. For the study, a total of 40 participants were taken aged 50–70 years of age which were divided into a group of 20 dentulous and 20 edentulous participants and into subgroup of males and females (10 each) [Table 1]. They were selected randomly from regular outpatient department of prosthodontics. It was ensured that none of the participant was having any type of regular medication at least during the period of the study and 6 months before the project. Signed informed consent has been taken by all the participants. Ethical committee approval for this study had been taken from the dental school. For this study, we prepared one chamber [Figure 1] of the postgraduate prosthodontic clinic and prepared it like a usual household room. Armamentarium used for this study include preweighed cotton packets [Figure 2], mineral water bottle, two glass beakers (1 liter each) [Figure 3], stopwatch, wall clock (facing the subject), digital balance (Anamed Model M300) [Figure 4], cotton tweezers, and hot fresh snacks. The mopping protocol had been decided for the collection of saliva and examiners were calibrated for collection methods. These calibrated examiners were used for all the phases of the study.

Protocol for actual procedure of salivary flow estimation

When the patient arrived, he/she was made seated on the dental chair first and then asked to drink 50 ml water 10 min before the start of the estimation, and after that, the patient was instructed not to spit or swallow unless the estimation gets over. At each step, the fresh preweighed cotton packet (5 g) was taken out from the bulk and saliva was being mopped from the maxillary and mandibular region. For every 3-min interval, the saliva was mopped by the cotton balls and weight of saliva was measured on the digital balance. The total salivary estimation was divided into five steps of 3 min interval each.

The salivary flow estimation was done on 6 consecutive days. On day 1, the patient was not informed about the snacks to be given, and baseline flow was estimated for 15 min baseline flow. From day 2, participants were informed that the snacks would be provided after 6 min and the snacks were actually delivered at the said time. The estimation was carried for two steps (i.e., for initial 6 min). On day 3, the same protocol was followed by the patient and the aroma of the food was there inside the chamber during the estimation. The snacks were delivered to the patient at the same time (i.e., after 6 min) for the conditioning effect. For day 4, 5, and 6, after intimating about foodstuffs, the same protocol was followed to be delivered after 6 min by each patient and the salivary flow estimations were performed for 3, 4, and 5 steps (i.e., for 9, 12, and 15 min), respectively, for all the patients and the snacks were delivered at the end. A total of 40 patients, 20 dentulous and 20 edentulous with equal proportion of males and females in each group were included in the study. The collected data of salivary flow as per the protocol were tabulated (in terms of increase in cotton weight for all steps) and the actual salivary flow on all days was later on computed for all steps [Table 2]. Similar tables were prepared for all 40 patients. Compilation of observed salivary flow on day 1 (Control = without thought of food) and day 2, 3, 4, 5, and 6 (with thought of food).

Total weight of cotton for:

- Step 1 (5 g cotton + wiped out saliva) = A1
- Step 2 (A1 + 5 g cotton + added saliva) 3–6 min = A2
- Step 3 (A2 + 5 g cotton + added saliva) 6–9 min = A3
- Step 4 (A3 + 5 g cotton + added saliva) 9–12 min = A4
- Step 5 (A4 + 5 g cotton + added saliva) 12–15 min = A5

| Table 1: Distribution of patients |
|----------------------------------|
| Group    | Males (n = 20), n (%) | Females (n = 20), n (%) |
|---------|----------------------|------------------------|
| Dentulous | 10 (50)              | 10 (50)                |
| Edentulous | 10 (50)             | 10 (50)               |

Figure 1: Prepared chamber

Figure 2: Preweighed cotton packets
Five hypotheses tested were as follows:

1. Is there any difference between the salivary flow in males and females?
   - H₀: There is no difference between the salivary flow in males and females
   - H₁: There is difference between the salivary flow in males and females

2. Is there any difference between the salivary flow in dentulous and edentulous?
   - H₀: There is no difference between the salivary flow in dentulous and edentulous
   - H₁: There is difference between the salivary flow in dentulous and edentulous

3. Is there any stimulation of salivary flow with the thought of food?
   - H₀: There is no stimulation of salivary flow with the thought of food
   - H₁: There is stimulation of salivary flow with the thought of food

4. Is there any difference between this stimulated salivary flow in males and females?
   - H₀: There is no difference between this stimulated salivary flow in males and females

5. Is there any difference between this stimulated salivary flow in dentulous and edentulous?
   - H₀: There is no difference between this stimulated salivary flow in dentulous and edentulous
   - H₁: There is difference between this stimulated salivary flow in dentulous and edentulous

Overall, a significant difference between two genders was observed for baseline flow during all the steps except for Step 5 ($P < 0.05$). Table 3 shows the mean unstimulated salivary flow on Day 1 for the five steps as detailed above. The null hypothesis was rejected, male participants exhibited higher flow rates compared to females. For baseline flow, during all the steps, the null hypothesis again was rejected, dentulous patients exhibited higher flow rates compared to edentulous.

For Step 1 and Step 2, changes were monitored on day 4, 5, and 6. Step 4 changes were monitored on day 5 and 6, while Step 5 changes were monitored on day 6 only. All the changes shown in [Table 4] are significant statistically ($P < 0.001$).

On all the 5 days, the mean percentage change in salivary flow for Step 1 was significantly higher in edentulous group as compared to dentulous group [Table 5]. No significant
Table 3: Mean salivary flow on day 1 at different steps - gender-wise comparison

| Step | Total | Males | Females | Significance of difference (males vs. females) |
|------|-------|-------|---------|-----------------------------------------------|
|      | Mean±SD | n=40 | n=20 | n=20 | Z   | P   |
| Overall | 2.54±1.08 | 2.99±1.14 | 2.08±0.82 | -2.584 | 0.009 |
| Step 1 | 2.13±0.78 | 2.47±0.84 | 1.79±0.55 | -2.680 | 0.007 |
| Step 2 | 1.65±0.70 | 1.96±0.83 | 1.35±0.35 | -2.615 | 0.008 |
| Step 3 | 1.39±0.52 | 1.58±0.62 | 1.21±0.31 | -2.369 | 0.018 |
| Step 4 | 1.33±0.52 | 1.49±0.64 | 1.17±0.30 | -1.563 | 0.127 |
| Dentulous | 2.76±1.24 | 3.21±1.33 | 2.31±1.01 | -1.399 | 0.165 |
| Step 1 | 2.27±0.91 | 2.63±1.01 | 1.91±0.66 | -1.626 | 0.105 |
| Step 2 | 1.84±0.85 | 2.19±1.03 | 1.48±0.46 | -1.746 | 0.089 |
| Step 3 | 1.54±0.65 | 1.76±0.77 | 1.32±0.42 | -1.252 | 0.218 |
| Step 4 | 1.48±0.65 | 1.70±0.78 | 1.26±0.41 | -0.998 | 0.353 |
| Edentulous | 2.31±0.88 | 2.78±0.94 | 1.85±0.52 | -2.269 | 0.023 |
| Step 1 | 1.99±0.63 | 2.31±0.66 | 1.66±0.40 | -2.308 | 0.019 |
| Step 2 | 1.47±0.45 | 1.72±0.53 | 1.22±0.12 | -2.196 | 0.029 |
| Step 3 | 1.24±0.29 | 1.39±0.36 | 1.10±0.04 | -2.295 | 0.023 |
| Step 4 | 1.17±0.28 | 1.27±0.38 | 1.07±0.04 | -1.496 | 0.143 |

Mann-Whitney U-test. SD: Standard deviation

Table 4: Step-wise mean salivary flow at different time intervals - overall (n=40)

| Step | Total (n=40) | Mean±SD | Change from baseline (day 1) | Percentage change from baseline | Significance of change |
|------|-------------|---------|--------------------------------|-------------------------------|------------------------|
|      |             | %       | %                             | Z    | P         |
| Day 1 (baseline) | 2.54±1.08 | 2.99±1.14 | 2.08±0.82 | 35.61 | 18.41 | -5.512 | <0.001 |
| Step 1 | 2.13±0.78 | 2.47±0.84 | 1.79±0.55 | 35.35 | 23.85 | -5.512 | <0.001 |
| Step 2 | 1.65±0.70 | 1.96±0.83 | 1.35±0.35 | 34.59 | 24.34 | -5.511 | <0.001 |
| Step 3 | 1.39±0.52 | 1.49±0.64 | 1.17±0.30 | 34.28 | 21.15 | -4.974 | <0.001 |
| Step 4 | 1.33±0.52 | 1.49±0.64 | 1.17±0.30 | 34.28 | 21.15 | -4.974 | <0.001 |
| Day 2 | 3.32±1.19 | 0.79±0.28 | 18.41 | -5.512 | <0.001 |
| Step 1 | 2.79±0.91 | 0.66±0.40 | 35.61 | 18.41 | -5.512 | <0.001 |
| Step 2 | 1.65±0.70 | 0.39±0.36 | 21.15 | -4.974 | <0.001 |
| Step 3 | 1.39±0.52 | 0.30±0.32 | 18.41 | -5.512 | <0.001 |
| Day 3 | 3.32±1.18 | 0.79±0.26 | 35.72 | 17.99 | -5.511 | <0.001 |
| Step 1 | 2.79±0.87 | 0.66±0.36 | 35.72 | 17.99 | -5.511 | <0.001 |
| Step 2 | 1.96±0.76 | 0.30±0.32 | 21.15 | -4.974 | <0.001 |
| Step 3 | 1.32±1.16 | 0.79±0.24 | 35.95 | 18.14 | -5.511 | <0.001 |
| Step 4 | 1.54±0.60 | 0.15±0.18 | 21.15 | -4.974 | <0.001 |
| Day 4 | 3.32±1.16 | 0.79±0.24 | 35.95 | 18.14 | -5.511 | <0.001 |
| Step 1 | 2.79±0.85 | 0.65±0.35 | 35.95 | 18.14 | -5.511 | <0.001 |
| Step 2 | 1.94±0.78 | 0.29±0.32 | 21.15 | -4.974 | <0.001 |
| Step 3 | 1.48±0.60 | 0.11±0.16 | 21.15 | -4.974 | <0.001 |
| Step 4 | 1.39±0.58 | 0.09±0.16 | 21.15 | -4.974 | <0.001 |

Wilcoxon signed rank test. SD: Standard deviation

The difference in mean percentage change in salivary flow for Step 2 was observed between two groups throughout all the days, the mean percentage change in salivary flow in edentulous group was higher as compared to that of dentulous group.
Table 5: Comparison of percentage change in conditioned salivary flow between edentulous and dentulous patients

| Step   | Mean ± SD Dentulous (n=20) | Mean ± SD Edentulous (n=20) | Significance of change Z | P    |
|--------|-----------------------------|----------------------------|--------------------------|------|
| Day 2  |                             |                            |                          |      |
| Step 1 | 28.39±15.23                 | 42.84±18.81                | -2.854                   | 0.004|
| Step 2 | 32.30±21.94                 | 38.39±25.81                | -0.595                   | 0.565|
| Day 3  |                             |                            |                          |      |
| Step 1 | 29.50±16.24                 | 39.59±23.21                | -2.259                   | 0.023|
| Step 2 | 30.92±23.09                 | 38.26±25.58                | -0.866                   | 0.398|
| Day 4  |                             |                            |                          |      |
| Step 1 | 29.71±14.90                 | 41.73±19.13                | -2.489                   | 0.013|
| Step 2 | 33.80±22.20                 | 38.02±26.11                | -0.433                   | 0.678|
| Step 3 | 24.87±28.21                 | 17.75±20.38                | -0.379                   | 0.718|
| Day 5  |                             |                            |                          |      |
| Step 1 | 29.74±15.39                 | 42.16±18.90                | -2.489                   | 0.012|
| Step 2 | 33.05±21.66                 | 38.69±26.34                | -0.379                   | 0.718|
| Step 3 | 26.02±27.69                 | 13.09±16.90                | -1.474                   | 0.142|
| Step 4 | 11.12±11.78                 | 9.59±9.48                  | -0.041                   | 0.968|
| Day 6  |                             |                            |                          |      |
| Step 1 | 27.01±21.07                 | 41.55±19.04                | -2.583                   | 0.009|
| Step 2 | 34.36±23.70                 | 39.00±24.79                | -0.688                   | 0.496|
| Step 3 | 27.96±29.74                 | 12.78±16.89                | -1.756                   | 0.079|
| Step 4 | 7.23±9.44                   | 7.06±7.14                  | -0.691                   | 0.496|
| Step 5 | 6.15±9.45                   | 5.53±10.44                 | 0.000                    | 1.000|

Mann-Whitney U-test. SD: Standard deviation

No significant difference between two groups was observed for Steps 3, 4, and 5 on any observation. However, for initial stages of stimulation, that is, Step 1 and 2, the edentulous participants had shown higher stimulation of salivary flow, the null hypothesis was rejected.

No significant statistical difference between two groups was observed for any of the steps on any of the days under observation except for day 3 [Table 6], when the mean percentage change in salivary flow among females was observed to be significantly higher as compared to males for step 1 (P = 0.026). The salivary stimulation was found to be higher in females during initial 6 min of observation than males. The null hypothesis was rejected, female patients exhibited higher values of salivary flow stimulation than males. It was observed that there was a significant difference between baseline and stimulated (with the thought of food) salivary flow in all the participants on all the days of observation, so the null hypothesis was rejected for the stimulation of salivary flow. For initial stages of stimulation, that is, in Step 1 and 2, the edentulous participants had shown higher stimulation of salivary flow than dentulous patients, the null hypothesis was rejected.

### Discussion

Saliva, as a precious body secretion has many functions, for example, facilitation of taste perception, protection, and lubrication; buffer capacity; dilution and cleaning; promotes repair; integrity to tooth enamel; enhances remineralization; oral homeostasis; and pregastric digestion by comminution and amalgamation of food bolus; and swallowing and maintaining oral ecologic balance. Normal average salivary flow (widely accepted) is 1.0–3.0 ml/min and 0.7–1.0 ml/min is considered to be low and below 0.7 ml/min is considered to be hyposalivation as investigated in the study by Tenovuo and Langerlof. The individual basal salivary secretion shows a large range of variation in response to olfactory, gustatory, and masticatory stimulation which varies with diet as quoted in a study by Bourdiol et al., Chambers et al., and Panu showed that the secretion is modified in relation to body hydration level.[10,11]

In his classical work, The Nobel Laureate Pavlov demonstrated the role of brain, instead of foodstuff in oral cavity, for enhancing the salivary secretions, to which he termed justifiably as “Psychic Secretion” which is also claimed by some other studies.[12-14] Guyton’s textbook of physiology also confirms the stimulation/inhibition of saliva by nervous signals to salivary nucleus. Winsor has demonstrated enhancement of salivation quoted as “Salivary response” in human beings by mastication, thought, smell, sight, seeing someone else eating, deprivation, appetite, and palatability of food. Winsor in 1930 also found a reduction in baseline salivary flow when the brain was involved in some other activity; the reduction in flow was proportional to intensity of involvement.[15] However, some
other studies by Winsor and Kerr et al. have questioned the failure of thought, smell, appetite, etc., as a stimulus?

**Effect of age**

Mioche et al. quoted that the secretion appeared to be stable with age, particularly parotid secretion. The parotid reflex has been observed in edentulous individuals through afferent nerve endings of mucosa which takes over the sensory role. The lack of salivary flow from the major glands in xerostomic patients does not a priori preclude secretions from minor salivary glands. Therefore, it is possible that a proportion of xerostomic patients have sufficient saliva secretion. Percival et al. demonstrated that the whole saliva secretion varied significantly between males and females both in oldest age, that is, 80 years or more. Sreenby in his study evaluated the salivary secretion percentage wise and found there was no difference with age in unstimulated/stimulated saliva in both males and females which was also claimed by Cathey and Goldenberg. Arhakis et al. mentioned in their study that the relationship between flow rate and age changes through lifespan; in newborns, the salivary activity is detected very low and climbs to the adult levels within the first 3 years. Over the adulthood until the older ages, the salivary activity remains to be unchanged which is being claimed by Ben-Aryeh and Aguirre et al. Studies by Ghezzi and Ship have shown that with increasing age morphological change of oral mucosa occurs, but only marginal alterations of salivary gland functions are observed. In view of the above contradictions, this clinical project was planned to reassess the confusion looming over this issue and to check for how long this salivary response made the salivary flow increased.

**Criterion for exclusion of subjects**

Navazesh et al. observed in there study that patients who were on some prolonged medication and any visible pathology affects salivary flow. This is in agreement with the studies by Turner and others. The diet regime of selected individual from morning till the time of observation was noted down. Individuals having heavy diet before the period of observation were also excluded because decreased salivary response has been demonstrated by Wooley and Wooley after having heavy meals. Now, finally, selected patients were requested to follow clock time (xx: xx am) in early hours of day between 10 and 12 am for observation was selected with every patient consent (because more saliva secretion is reported in early hours of day) and it was fixed for everyday (because salivary secretion varies with daytime) as Winsor has stated that almost any stimulus that had previously preceded the actual ingestion food might act as a natural conditioned stimulus. The observations were kept on 6 consecutive days in sequence like in a study by Winsor and no variations were allowed, to assess the development of a “conditioned reflex” and a memory pattern in reference to the fixed time of food delivery. Many authors have reported in the literature that thinking of food had considerable effect on salivary secretions. The feeling of getting the food at the particular time has stimulated the salivary response. In the following study, it has been found that salivary flow levels were found raised in both the dentulous and edentulous individuals, but after due course of time, it has come to baseline level. Hence, it is important for the family members of the geriatric individuals to take good care of them while providing them meals at the time scheduled. According to this study, it can be said that salivary response of any older individual plays a key role in the actual ingestion of the food, so one should not get totally dependent on the artificial salivary substitutes to fulfil his needs of swallowing of food. This in vivo study was performed on healthy individuals for the assessment of increase salivary flow. Findings from this study can be utilized and open a future scope for this type of evaluation on xerostomic individuals as well.

**Conclusions**

The findings generally suggest that yes there is an enhancement of salivary flow with the thought of food, but it lasts for 6–9 min after which it turns to base level. It is interesting to note that the patient in whom the baseline salivary flow is minimum, that is, edentulous females, the salivary stimulation/salivary response is more among edentulous individual. The reason being for this is females are more foodies than males. The response lasts for 6 min and in some up to 9 min. If these individuals keep waiting for the food (in the presence of expectation and aroma), the enhance salivary secretion for this group of patient may not be utilized if the individual has hyposalivation.

**Suggestions for complete denture wearer**

1. The edentulous patient should try to eat at different times on different day so that fixed time does not start enhanced secretion, as a conditioned reflex
2. The denture wearer, especially edentulous females should try to minimize the gap between call of food and actual ingress of food in the mouth. Family members should be instructed to call the elderly when everybody else to accompany and the food is ready on the table
3. The patient should be instructed to wear maxillary denture as little as possible, to avoid undue compression of ducts of salivary glands
4. During eating, the discussions involving the thought mechanism should be avoided
5. Try not to be in stress and gel the mood of the individual to avoid hyposalivation.

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