Original Research

Evaluation of antioxidant activity in saliva among young adults having diverging food habits and its relation to oral health: A pilot study

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Abstract

Introduction: Saliva enables the maintenance of oral and systemic health. Evaluation of saliva is very valuable for multiple parameters to be evaluated as they are easy to collect, allow easy and safe sample collection, are non-traumatic, can be repeated with ease, and are non-invasive in nature. Salivary enzyme systems have antimicrobial, antioxidant, and similar functions which aid in the maintenance of homeostasis in the oral cavity. Antioxidants scavenge free radicals from cells and prevent or reduce the damage caused by oxidation.

Materials and Methods: In the present study, the pH and antioxidant capacity of the saliva were evaluated. Subjects were categorized as GROUP A: Vegetarians: Diets were entirely devoid of eggs or meat of any type (for more than 20 years). GROUP B: Non-vegetarians: Diets included both red and white meat, consumed either daily or frequently. GROUP C: Eggetarians: Otherwise vegetarian diets which includes eggs, consumed frequently. Ten samples of each group were collected. The pH profile and antioxidant activity of the samples were analysed. Each of the individuals was subjected to oral examination for grading of the status of oral hygiene, caries teeth, missing and extracted teeth, and the health of gingiva. For the same Oral Hygiene Index Simplified (OHIS), Decayed, Missing, and Filled Teeth (DMFT) and Gingival Status indices were used and the observations were noted.

Observations and Results: The average salivary pH for the vegetarians was 7 ± 0.5, that for eggetarians was 7.1 ± 0.5, and in the non-vegetarian group, the average pH was equal to 7.3 ± 0.5. Using the DPPH method, the percentage antioxidant activity of saliva in vegetarians was 20.9 ± 2.1%, while those of eggetarians and non-vegetarians were equal to 5 ± 0.6% and 11.4 ± 2%, respectively. Each individual was subjected to oral examination for grading of the status of oral hygiene (OHIS); decayed, missing, extracted teeth, filled teeth index (DMFT); and the health of gingiva (gingival status index). Overall, eggetarians had a high OHIS index (mean 1.08). The DMFT index was high in non-vegetarians with values ranging from 1 to 8. Statistical analysis using the T-test revealed that the antioxidant potential of the vegetarian group was significantly higher than those of the eggetarian and non-vegetarian dietary groups ($P < 0.001$). However,

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the eggetarian and non-vegetarian dietary groups did not significantly differ from each other with respect to this parameter.

Conclusion: The antioxidant capacity is markedly high in vegetarians, 20.9+/- 2.1%, as compared to non-vegetarians, 11.4+/- 2.1%, and was the lowest in eggetarians, 5+/- 0.6%.

Keywords: Ascorbic acid, DPPH assay, DMFT, gingival status, OHIS, oral health, pH profiles, salivary antioxidant level, salivary flow, vegetarian/eggetarian/non-vegetarian

INTRODUCTION

Saliva is a valuable body fluid, secreted in the oral cavity, which is exposed to varying environmental conditions. The oral cavity is exposed to various food substances and air, along with a variety of constituents. It is also a house to a variety of normal and pathogenic microorganisms. This makes saliva a fluid which is easily accessible, in required amounts for a variety of tests associated to oral and systemic health.[1,2]

It is more than proven that good oral health is reflected almost entirely in saliva. It plays a crucial role in maintenance of health and homeostasis in the oral cavity. Evaluation of antioxidant capacity, production of oxidative stress, and imbalances in the same are responsible for many periodontal and oral pathologies.[2]

It also acts as a potential mediator in many diseases including heart diseases (myocardial infraction), neurogenous pathologies (Alzheimer's diseases and Parkinson's diseases), and different types of cancer. It is the fast-growing ground for a evaluation in pathological state in pregnancy and different muscular and skeletal pathologies. Saliva houses a set of enzyme systems which has a rich functional, antimicrobial, antioxidant, and similar functions. Antioxidant activity or the ability of saliva is very important as it impairs or alters the regenerative potential of gingiva and oral status. The level of antioxidant activity is reflected in the saliva of oral cavity, and the oral cavity reflects the systemic health and oral health depending on the diet consumed by an individual.[2-4]

In our study, we focus on the antioxidant levels created in saliva upon consumption of vegetarians', eggetarians', and non-vegetarians' diets. We also extend the study for the evaluation of oral health in these subjects.

AIMS AND OBJECTIVES

Comparison of biochemical properties of saliva between vegetarians, eggetarians, and non-vegetarians with respect to

- Salivary pH profiles among vegetarian, eggetarian, and non-vegetarian samples.
- Evaluation of antioxidant activity in young adults whose diets are vegetarian, eggetarian, or non-vegetarian.
- Evaluation of oral health and hygiene in young adults whose diets are vegetarian, eggetarian, or non-vegetarian.
- Comparison of antioxidant properties of saliva with oral health status.

MATERIALS AND METHODS

This study was carried out at the Department of Biotechnology and the Department of Oral Pathology. The study was approved by institutional ethical committee on 11th May 2023. The reference number for the same is KCDSHEC/IP/2023/V/OP3. A total of 30 subjects were evaluated for their salivary antioxidant levels with variable diets.

The subjects were categorized as follows (N=10):

- **GROUP A: Vegetarians:** Persons whose diets were entirely devoid of eggs or meat of of any type for a period of more than 20 years
- **GROUP B: Non-vegetarians:** Persons whose diets included both red and white meat, consumed either daily or frequently.
- **GROUP C: Eggetarians:** Persons whose otherwise vegetarian diets included eggs, consumed frequently.

The inclusion criteria considered were adults of both genders belonging to the age group of 20–25 years without any systemic or oral pathology. The exclusion criteria included pediatric and geriatric individuals; patients with deficient salivary flow or dry mouth; patients with local oral pathologies; and systemic diseases like tuberculosis, human immunodeficiency virus (HIV) disease, diabetes, bleeding disorders, liver disorders, auto-immune disease, or allergic disorders; and subjects undergoing radiotherapy or chemotherapy for malignancies of any type.

Saliva Collection: The unstimulated saliva was collected using the syringe-hub method. Briefly, the subjects were made to sit comfortably on a dental chair, 2 hours after their last meal (during which time no further food/

water consumption was allowed). Thereafter, they were asked to recline backwards in order to allow pooling of unstimulated saliva on the floor of the mouth for a period of time ranging from 5 to 15 minutes, after which the salivary fluid (1.0–2.0 ml) was collected from each of the subjects, using a sterile syringe hub of 2.0 ml capacity, as shown in Figure 1. The time of collection was diurnal, and collection was carried out between 12:00 noon and 1:00 pm in all cases.

After collection of the salivary samples, they were stored in a dry safe place and were used for the biochemical tests mentioned below.

Biochemical parameters

pH profiles: The pH of all samples was measured using a standardised pH meter after performing the routine calibration for neutral (pH 7), acidic (pH 4), and basic (pH 10) pH as specified. The pH meter (microprocessor pH meter) was cleaned with distilled water. The electrode bulb was then dipped in the beaker containing the sample. The pH reading for the samples was noted down.

Antioxidant activity: Using the DPPH assay method with ascorbic acid as the standard, the antioxidant activity of all the saliva samples was estimated and expressed in terms of ascorbic acid equivalents. Controls containing distilled water in place of salivary fluid were set up. Antioxidant activity was calculated using the formula

$$\text{Antioxidant Activity}(\%) = \frac{\text{Absorption of control} - \text{Absorption of sample X 100}}{\text{Absorption of control}}$$

Figure 1: Saliva collection using sterile syringe hub

Ascorbic acid: About 10 mg of ascorbic acid was dissolved in 100 ml of distilled water (stock). Working stock = Stock diluted 1:2 with distilled water (final concentration = 50 μg/ml)

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay: Around 5.91 mg of DPPH was dissolved in 30 ml methanol to a final concentration of 0.5 mM. (Note: DPPH is highly light-sensitive and reactive if exposed to the skin. Handle with care). Results were expressed as the mean of 10 replicates ± standard error in all cases.

Oral hygiene status: Each of the individuals was subjected to oral examination for grading of the status of oral hygiene; caries teeth, missing and extracted teeth, and the health of gingiva. For the same Oral Hygiene Index Simplified (OHIS) that measures debris and calculus index (good score 0.1 to 2, fair 1.3 to 3, and poor 3.1 to 6], Decayed, Missing, Filled Teeth (DMFT) and Gingival Status Index were used and the observations were noted. The maximum DMFT score is 32, and lesser the score, less is the potential to develop caries.[1,5]

OBSERVATION AND RESULTS

The observations made in the study were compiled based on clinical and experimental bases. All the 30 subjects in our study belonged to the age group of 19 to 26 years (average 23 years). It consists of 22 females and 8 males with a female: male ratio of 11:4. The subjects selected were of good systemic health. About 2 ml of saliva was collected for complete evaluation with the salivary secretion rate being consistent in all the subjects of all the groups. The salivary secretion rate ranged from 6 to 8 minutes with an average of 7 minutes.

Antioxidant activity

Antioxidant activity in the saliva was evaluated using DPPH assay using 200 to 500 ml of saliva. The percentage antioxidant activity in vegetarians was found to be equal to 20.9 ± 2.1%, while those of eggetarians and non-vegetarians were equal to 5 ± 0.6% and 11.4 ± 2%, respectively [Graph 1 and Figure 2]. The percentage of antioxidant activity recorded in the vegetarian category ranged from 9.34 to 32.93. Similarly, in the eggetarians and non-vegetarians, they were between 0.35 and 11.49 and between -1.59 and 21.11, respectively.[6]

DPPH assay was done as indicated in materials and methods (N = 10), and the average percentage of antioxidant activity ± S.E of each group was plotted. Statistical analysis using the T-test revealed that the

antioxidant potential of the vegetarian group was significantly higher than those of the eggetarian and non-vegetarian dietary groups ($P < 0.001$). However, the eggetarian and non-vegetarian dietary groups did not significantly differ from each other with respect to this parameter.

pH analysis

The pH of saliva was analysed using a pH meter (King lab KLPHM-119 PH Meter PH Meter). A set of two readings of the same was taken for consistency, and the final pH reading was analysed. The pH was measured for all the samples under the three groups as indicated under materials and methods. The average pH for the vegetarians was equal to 7 ± 0.5, and that for eggetarian groups was equal to 7.1 ± 0.5. In the case of the non-vegetarian group, the average pH was equal to 7.3 ± 0.5. The pH recorded in the vegetarian category ranged from 6.25 to 7.66. Similarly, in the eggetarians and non-vegetarians, they were between 6.36 and 7.86 and between 6.46 and 8.12, respectively.

Graph 2 represents the pH profiles measured and the average pH ± standard error plotted in the case of each of the dietary groups (N = 10). Statistical analysis using the *t*-test indicated that these differences were not statistically significant ($P > 0.05$).

Oral hygiene status

The vegetarians showed an average OHIS value of 0.98, which is good, and DMFT values ranging from 1 to 3, whereas the gingival status and salivary flow status were normal. The eggetarians show an average OHIS index of 1.08, which is good, and DMFT values ranging from 0 to 5. One of the patients showed gingival recession, whereas the rest showed normal gingival and salivary flow status. The non-vegetarians showed an average OHIS index of 0.83, which is also good, and the DMFT index ranged from 0 to 8.

The overall mean values of parameters assessed are tabulated in Table 1.

DISCUSSION

The use of saliva as a diagnostic medium in evaluation of multiple parameters in health and disease is highly accepted. The advantages include: easy to collect, bloodless procedure, atraumatic, acceptable to many, easy reproduction, and repetition of the test sample.[2,7] Saliva can be used as a body fluid for the evaluation of local/oral health or systemic health.

In case of oral health, saliva is used for assessing the caries index or in forensic evaluation. In a systemic disease background, it can be used for determination of certain enzyme levels, drug levels, and specific proteins associated with cancers and other diseases.[7,8]

Salivary evaluation of multiple enzyme systems, chemicals, immunoglobulins, bacterial components, and medications is one of the latest and effective diagnostic methods. Salivary proteomics and genomics are gaining a significant credit

Table 1: Overall average analysis of the parameters

Category	OH index	DMFT	Gingival status	Salivary flow	pH	AA%
Vegetarian	0.98	2	N	N	6.955	21.135
Eggetarian	1.08	2.5	N	N	7.11	5.92
Non-vegetarian	0.83	4	N	N	7.29	9.79

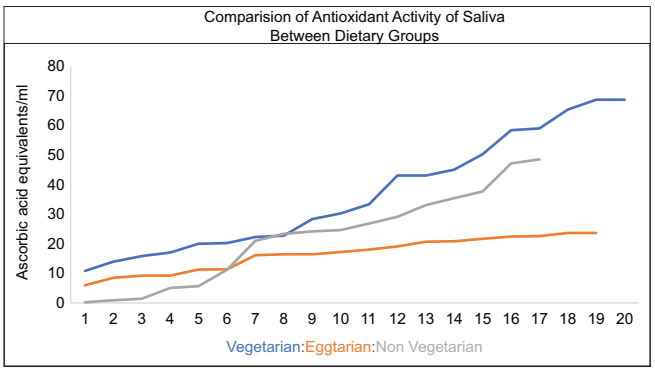
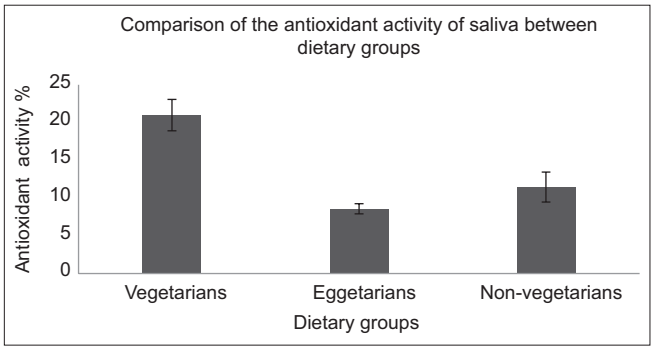
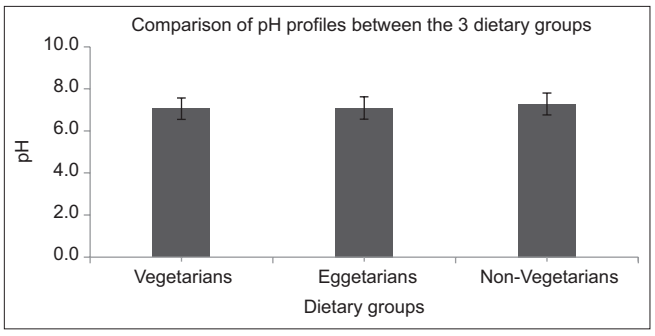


Figure 2: Antioxidant profiles of the three groups



Graph 1: Antioxidant profiles of the three groups



Graph 2: Comparison of pH profiles between the three dietary groups

and are the center stages of application in various states of health and disease. One among all these is evaluation of the antioxidant property of saliva which acts in stabilizing oral and systemic health.[7]

The present study aims at the evaluation of salivary pH and antioxidants in young individuals having a dietary habit which is variable. A detailed evaluation was also done to assess the oral health and general health in the subjects.

Multiple studies are available for the evaluation of salivary pH. In healthy individuals, the range varies from 6.2 to 7.4.[2] Very few studies are available, where dietary variations and assessment of salivary pH have been observed. In our study, we observed that the salivary pH was in the range from 6 to 8 in all the three categories considered. There was very little variation in all the three groups, and the data had no statistical significance. The average pH for the vegetarian was equal to 7.1 ± 0.5, and that for eggetarian groups was equal to 7.1 ± 0.5. In the case of the non-vegetarian group, the average pH was equal to 7.3 ± 0.5.

The antioxidant capacity of saliva depicts the capability of salivary components to reduce reactive oxygen species (ROS). The loss of balance between the production of free radicals and antioxidant capacity of saliva is termed as oxidative stress. Oxidative stress (OS), which is the cause for many oral and systemic illnesses, is implicated in multiple diseases and cancers. The antioxidants are of two categories, enzymatic oxidants like peroxide, superoxide dismutase, and glutathione. The other category is the non-enzymatic oxidants like ascorbic acid, flavonoids, tocopherol, and carotene. Among these, ascorbic acids and glutathione are found frequently in saliva in higher concentrations than in serum. Ascorbic acid is found to function as an endogenous regulator for nitric oxide metabolites; hence, ascorbic acid is a valuable meter to predict oral and systemic health.[2,9-11]

Saliva as a biological fluid in the unique anatomical site, like the oral cavity because of its location and association with other body spaces, offers a new perspective for evaluation of antioxidative activity. There is exposure of the oral cavity to numerous environmental factors like air pollutants, dental materials, diet, medications, and other products which contact the oral cavity. All these induce formation of free radicals and antioxidant activity.[1]

There are very few studies which implicate the dietary habits to alter the salivary antioxidant levels. Amirmozafari *et al*.[12] studied the antioxidant levels of saliva in vegetarians and non-vegetarians by assessing the peroxidase levels and found that vegetarians have less anti-oxidant activity as compared to non-vegetarians.

According to the study done by Ramamurthy S *et al*.,[13] the salivary antioxidant levels assessed using spectrophotometry do not alter in vegetarians and non-vegetarians.

In our study, we noted that the antioxidant levels in vegetarians are at the peak with an average of 21.13%. The range of activity in these subjects varied from 8.69 to 32.93. A high activity of antioxidants in vegetarians might be associated with the high amounts of antioxidants in fresh fruits and vegetables consumed.[14] Hence, the antioxidant activity is good, which acts against ROS overproduction and oxidative stress. It also adds a perspective that a local mechanism and systemic mechanism might be involved in maintaining high antioxidant levels in the saliva of vegetarians.

The antioxidant activity in eggetarians and non-vegetarians is significantly lower at 5.9 and 9.7, respectively. The values in these two categories range from 0.35 to 18.59 with a few readings in negative. This shows that consumption of eggs and meat products as major diet constituents alters the local and systemic activity of antioxidation levels finally in the saliva.

It is a proven fact that food rich in polyphenols, vitamin C, and vitamin supplements are rich in antioxidants. The polyphenols are richly present in plant and plant-based foods. More so in a variety of tea, coffee, and berries. They act by scavenging of ROS and RNS and also by ion chelation.[14]

The salivary proteins interact with these polyphenols. Some of the mechanisms might allow the polyphenols to remain in oral cavity for long periods of time. Their mechanism not only helps in the systemic antioxidant activity but also allows protection of oral mucosa. In humans and mammals, an indirect effect mechanism associated with tannin-rich diet in herbivores or vegetarians allows protection to the oral mucosa.[1]

Lastly, the amount of mastication plays a big role in the masticatory-parotid reflex and thus in the parotid salivary antioxidant capacity. Probably, uncooked vegetables and food in the form of fruits, berries, and salads demand a higher masticatory efficiency, leading to a better antioxidant capacity of saliva in vegetarians, which is curbed in very well cooked non-vegetarian diets.

Considering the similar pH but dissimilar antioxidant values in salivary evaluation of vegetarians and non-vegetarian

subjects, a detailed evaluation of caries index, periodontal status, and oral hygiene index was done. The observations did not show any strong variation among the three groups. The caries (DMFT) index varied between 0 and 8 in these groups. The oral hygiene status (OHIS) showed mean values of 0.98, 1.08, and 0.83 in vegetarians, eggetarians, and non-vegetarians, respectively. So, eggetarians showed the highest OHIS status compared to vegetarians or non-vegetarians. Gingival and periodontal statuses were found to be satisfactory in all the subjects. No statistically significantly result was found between the different groups. A larger group might throw better light on these parameters.

CONCLUSION

Saliva is a very potent biofluid for predictions and diagnostic and prognostic evaluation in many stages and states of health and disease. The non-invasive, atraumatic, simple, and safe method of collecting samples allows it to be more popular and a frequently used diagnostic method. In the present study, it is suggestive, beyond doubt that the antioxidant capacity of saliva is better in individuals with vegetarian dietary habit compared with eggetarian and non-vegetarian dietary habits in young individuals. A larger sample-size study will be needed to affirm the alone findings.

Author's contribution

All the authors have contributed equally in conducting the project and study. They have contributed to make, correct and approve the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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