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Age correlation study of DNA, RNA and protein concentration in human saliva of Amravati city (M.S.) India

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ABSTRACT

This study reveals that the age correlated study of DNA, RNA and Protein concentration in human saliva from Amravati city to analyses the DNA, RNA and protein concentration of different age groups and different nutritional classes of people and found differences in concentration therefore this study suggests that there is much significant difference in overall DNA, RNA and protein concentration with age, gender, nutrition and health. This study suggested that the DNA, RNA and proteins from saliva of an individual may provide vast information about his health status, immune response, genetic makeup, enzymatic activity etc.

Keywords: Saliva, DNA, RNA, Protein

INTRODUCTION

At present, saliva represents an increasingly useful auxiliary means of diagnosis (Malamud D. 2006). Many researchers have made use of sialometry and sialochemistry to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health (González *et al*., 2003).However, since several factors can influence salivary secretion and composition a strictly standardized collection must be made so the above-mentioned exams are able to reflect the real functioning of the salivary glands and serve as an efficient means for monitoring health.

Salivary fluid is an exocrine secretion (Berkovitz *et al*., 2002) consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health. There is also glucose and nitrogenous products, such as urea and ammonia. The components interact and are responsible for the various functions. Total or whole saliva refers to the complex mixture of fluids from the salivary

glands, the gingival fold, oral mucosa transuded, in addition to mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells, as well as traces of medications or chemical products (Humphrey *et al*., 2001).

Despite numerous studies on salivary secretion the effect of aging on salivary flow remains obscure due to conflicting observations in the literature leaving little information available regarding salivary flow in healthy elderly persons.

The total unstimulated salivary flow is significantly lower in healthy patients between the ages of 65 and 83 years, in comparison with patients between the ages of 18 and 35 years. However, total stimulated salivary flow was significantly higher in the elderly in comparison with the younger persons (Navazesh *et al.,* 1992).

The total unstimulated salivary flow is related to age, being significantly reduced in healthy non-medicated elderly persons aged 80 years or older. However, no age-related reductions in stimulated SF from the parotid were detected. It is suggested the elderly do not present dysfunctions in the ability to respond to sialogogues. However, the reduction in unstimulated SF could contribute to the appearance of diseases in the oral mucosa (Percival *et al*., 1994).

MATERIALS AND METHODS

Saliva collection

The saliva samples were collected from the locality around the Amravati city at Rajkamal area, preferably in the morning 7:00 am to 10:00 am. Prior to the sample collection we take subsequent visits to gather the information regarding their food habit, health and hygiene status. Before a day of sample collection we inform them and take their appointment without disturbing their daily routine. In the current study, three volunteers were recruited (age range, 20–70years), and demographic information, including age, health condition, sex, *etc*.,

Two common, well-documented methods of saliva collection are:

(1) The passive drool technique, and

(2) The absorbent device technique.

In order to maintain consistency in the type of sample collected, some researchers prefer to use the unstimulated, whole saliva that pools on the floor of the mouth, collected by the passive drool technique. On the other hand, use of an absorbent device that can be placed in the mouth often allows for studies with small children or other individuals that have difficulty with the passive drool technique, however the mouth location placement of the absorbent devices may collect localized saliva rather than whole saliva, which may affect results for many analytes.

On the final day of collection, unstimulated saliva was collected according to a modification of the method described by Navazesh (1992). Subjects were asked to avoid oral hygiene measures (i.e., flossing, brushing, and mouth rinsing), eating, drinking, or gum chewing for 1 hour before collection. Subjects rinsed their mouth with tap water, after which they expectorated 5ml saliva into sterile tubes containing a protease inhibitor solution. Samples were immediately placed on ice and transferred to the laboratory.

1. Total proteins were estimated by Biurette Method. Similarly, the DNA, RNA was estimated by DPA method and Orcinol method respectively.
2. Calculate total protein content in the sample by using standard graph.
3. Method for Extraction of RNA and DNA from the Saliva sample:
4. Estimation of DNA by Diphenylamine reaction method:
5. Estimation of RNA by Orcinol Method

RESULTS AND DISCUSSION

The aim of this study was to estimate the concentration of DNA, RNA and proteins in human saliva of tribal people of different age groups.

Table 1 : Shows concentration of RNA in different saliva samples

S.NO.	Age of volunteers	CONC. OF CONTROL	CONC. OF EXPERIMENT
1	20 YEARS	5.5 ug/ml	5 ug/ml
2	40 YEARS	9 ug/ml	7 ug/ml
3	70 YEARS	7.5 ug/ml	6 ug/ml

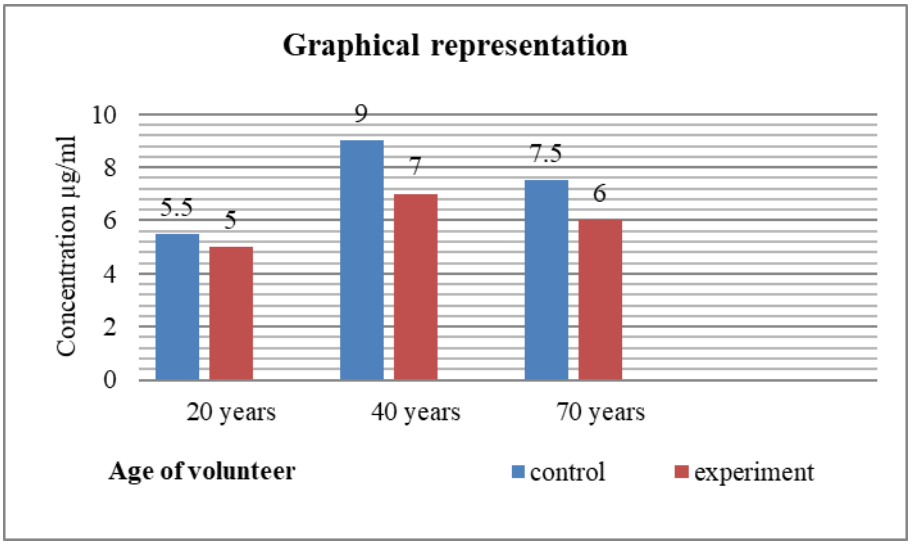

Figure 1: Shows concentration of RNA in different saliva samples

Table 2 : Shows concentration of DNA in different saliva samples

S.NO.	AGE OF SUBJECT	Conc. Of control	Con.of experiment
1	20 YEARS	17.5 ug/ml	5 ug/ml
2	40 YEARS	20 ug/ml	10. ug/ml
3	70 YEARS	15 ug/ml	7.5 ug/ml

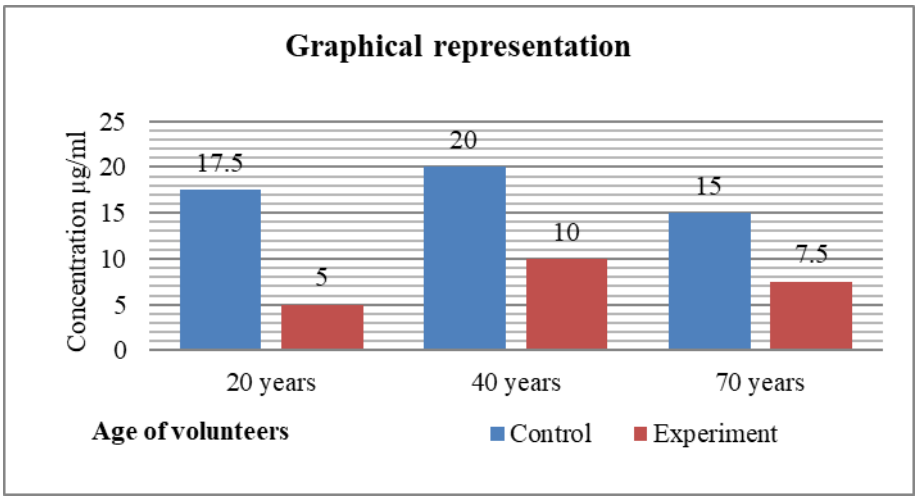

Figure 2: Shows concentration of DNA in different saliva samples

Table 3:- Shows concentration of proteins in different saliva samples

S.NO.	AGE OF SUBJECT	Conc. In control	Conc. In experiment
1	20 YEARS	0.2 mg/ml	3.1 mg/ml
2	40 YEARS	0.5 mg/ml	2.4 mg/ml
3	70 YEARS	0.3 mg/ml	1.6 mg/ml

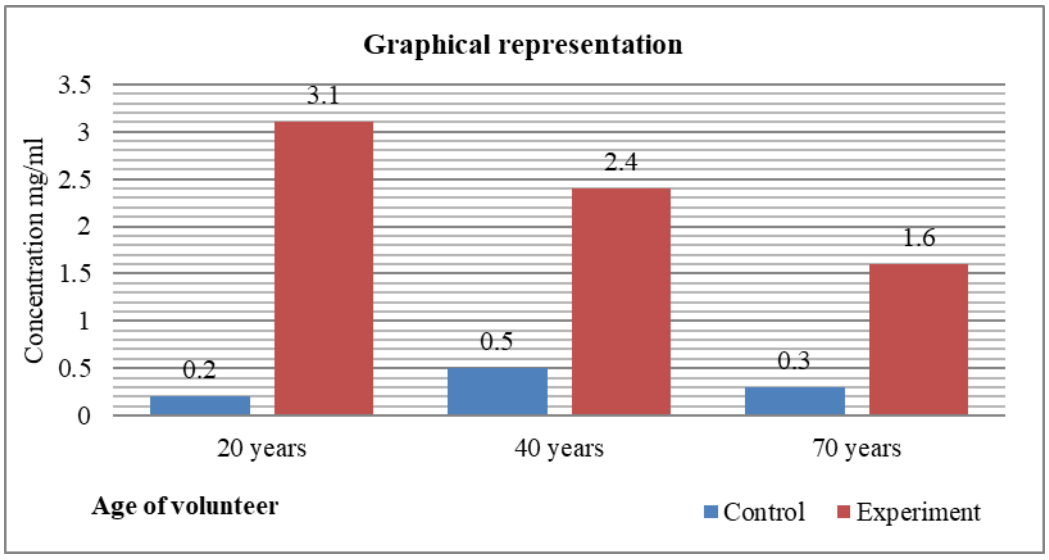

Figure 3: Shows concentration of proteins in different saliva samples

Thomas *et al.,* (2007) estimated amount of total DNA extracted from 2 mL blood samples varied between 11.3 and 59.6 µg with a mean of 28.4 µg, from 0.5 mL Oragene saliva samples between 0.9 and 64.2 µg with a mean of 10.8 µg, from mouth swabs between 9.1 and 194.9 µg with a mean of 64.4 µg, and from FTA cards (one punch) between 0.09 and 1.33 µg with a mean of 0.36 µg.

Rudqvist *et al.,* (2006) extracted DNA from 90 randomly selected saliva samples and roughly estimated total DNA yield by UV absorption. The mean total yield was 135.9µg/2.5ml saliva. They also measured the total DNA yield by using the PicoGreen method. The total DNA yield ranged from 1.2 to 169.7 µg, witha mean of 40.3 µg/2.5ml. By real-time PCR of the human prothrombin gene, they estimated that the yield of human DNA ranged from 11% to 100% of total DNA, with a median of 68% .The human DNA yield ranged from 0.8 to 85.6 µg, with a mean of 25.4 µg/2.5ml.

In this study the concentration of DNA was found to be high in middle age (40 years) in both tribal people saliva and upper class people saliva which was found to be 10µg/ml and 20µg/ml respectively, as compared to early age (20years) and late age (70 years). The concentration of DNA in tribal people was found to be 5 µg/ml, 10 µg/ml and 7.5 µg/ml for 20 year, 40 year and 70 year age group respectively. The concentration of DNA in upper class people was found to be 17.5 µg/ml, 20 ug/ml and 15 ug/ml for 20 year, 40 year and 70 year age group respectively.

The concentration of RNA was found to be more in middle age group (40 years) which was 7 µg/ml as compared to early age (20 years) and old age (70 years) which was found to be 5 µg/ml and 6 ug/ml respectively. This was also correlated with upper class people of same age groups and it was found that the same pattern of concentration was there i.e, 9 µg/ml for middle age (40 years), 5.5 µg/ml for early age (20 years) and 7.5 µg/ml for old age (70 years). When we compared the tribal people RNA concentration and upper class people RNA concentration, it was observed that the RNA concentration is more in saliva of upper class people in each age group as compared to tribal people saliva of same age group.

Alaama *et al.,* (2011) observed the protein at A280 that has been measured for different leech saliva extracts ranges from 0.159 to 0.521 with a mean of 0.311. The protein concentration measured by Bradford method ranges from 39 to 105 µg/ml with a mean of 67.9182 µg/ml and SD=29.14 µg/ml.

The concentration of proteins in saliva sample of tribal people was found to be 0.2 mg/ml, 0.5 mg/ml and 0.3 mg/ml for 20 year, 40 year and 70 year age group respectively. The concentration of proteins in saliva sample of upper class people was found to be 3.1 mg/ml, 2.4 mg/ml and 1.6 mg/ml for 20 year, 40 year and 70 year age group respectively.

CONCLUSION:

Saliva is an important body fluid. It is composed of a variety of important constituents like buccal cells,

Proteins, enzymes etc. Saliva exhibits our genetic blueprint. In this study we estimated DNA, RNA and proteins from different saliva samples and from the results we conclude that the saliva is a significant body fluid of the DNA, RNA and protein source.

This study suggested that the DNA, RNA and proteins from saliva of an individual may provide vast information about his health status, immune response, genetic makeup, enzymatic activity etc.

The RNA provides information about one's transcriptional and translational efficiency along with proteins. Proteins may also provide diagnostic information about a variety of disorders.DNA recovered from saliva can be sequenced and can be processed for different purposes like genetic information etc.

In this study we compares the DNA, RNA and protein concentration of different age groups and different nutritional classes of people and found differences in concentration therefore this study suggests that there is much significant difference in overall DNA, RNA and protein concentration with age, gender, nutrition and health.

Observations and results of this study indicated that standardised procedures for saliva collection and estimation of DNA, RNA and proteins can be very useful in:-

- Solving criminal cases in forensic science.
- Identification of genetic disease.
- Trace the evolutionary history of organisms.
- Diagnosis of many diseases.
- Determining immunological responses.

Determining the oral microbial activity.

Conflict of Interest

The author declares that there is no conflict of interest.

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