Salivary Changes with the age and their effect on plaque related disease

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Abstract

Background: Saliva is an exocrine clear oral fluid predominantly secreted by salivary glands both major and minor. It consists of many constituents, mainly water and others are electrolytes, enzymes, immunoglobulins and other antimicrobial factors.

Saliva plays an important role in the oral health, the level of its components change with age, and that has direct effect on teeth and periodontal tissue, because saliva plays a critical role in the development of dental caries and periodontal diseases.

The objective of this study to estimate salivary changes by investigation the level of alpha- amylase and MMP-8 enzymes, sIgA, and salivary minerals (Calcium, Magnesium and Phosphorus) in relation to age.

Materials and Methods: Ninety persons were chosen from different age groups (60) males and (30) females, the age ranged between 20 up to 50, and divided into three subgroups.

ELISA technique was used to evaluate the level of salivary alpha-amylase, MMP-8, and sIgA. Salivary electrolytes were evaluated according to their kits.

Results: Results showed that the level of sIgA significantly decreased with the age, salivary alpha –amylase decreased with the age but not significant statistically, and MMP-8 increased with age but not significant statistically. Regarding salivary minerals (Ca, P, and Mg) results showed that calcium and magnesium levels significantly increased with age, while phosphorus level increased with age also but not significant statistically.

Conclusion: Decrease in the level of sIgA and alpha-amylase with age, while increase in the level of MMP-8 and salivary minerals (Ca, P and Mg) with age, may indicate decrease in the incidence of dental caries and increase in the incidence of periodontal disease with age.

Key words: *sIgA; salivary immunoglobulin A, MMP-8; matrix metalloproteinase-8, Alpha amylase enzyme.*

Introduction

Saliva is a clear, slightly acidic oral fluid, mostly produced by parotid, submandibular, and sublingual glands and the minor glands. [(1)] Saliva is an exocrine secretion containing many constituents. It consists mainly of water about 99%, which contains many electrolytes like; calcium, potassium, sodium, magnesium, chloride, bicarbonate, phosphate, and proteins, characterized by immunoglobulins, enzymes, and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides which are of importance to oral health. [(2, 3)] Saliva plays an important role in the oral health, the level of its components change with age, and that has direct effect on teeth and periodontal tissue, because saliva plays a critical role in the development of tooth decay and periodontal diseases.

[2] Histological analysis; with the advancing age the parenchyma of the salivary glands is replaced gradually by adipose and fibro-vascular tissue, and the capacity of the acini is reduced. [4, 5] Salivary immunoglobulin A (sIgA) is characterized as a part of the immune system "first line of defense" against pathogenic microbes. Restricting the adhesion of microbes, these antibodies respond to biofilm development and thus interfere with the defense of plaque related diseases; dental caries and periodontal diseases. [6, 7, 8] Alpha amylase enzyme considered as one of the main components of saliva that play a role in oral health. In solution, this enzyme, contributing to the bacterial clearance by binding to bacteria. In comparison, it initiates the digestion of starch in enamel pellicle thus give substrates for colonization of bacteria and enhance their adhesion to tooth surface. [9, 10]. MMP-8 (matrix metalloproteinase-8) is the most proficient proteinase to initiate degradation of type I collagen and extracellular matrix that associated with the destruction of periodontal and peri-implant tissue, leading to the loss of tooth and dental implant. MMP-8 act as a central mediator in chronic infection, encouraged inflammatory conditions and can exert, anti-inflammatory and defensive properties in addition to the classical surrogate tissue destructive properties. [11, 12, 13]. Calcium contributes about 1.9% of the body weight and considered as the most abundant mineral in the body. About (99%) of this percentage in the skeletal system, and the remaining; in the teeth (0.6%), the extracellular fluid (0.6%), the soft tissues (0.6%), and the plasma (0.3%) Calcium provides a structural role in providing rigidity (structure and strength) to the skeleton. [14]

Phosphorus considered as the next most abundant mineral in the body after Calcium. These two minerals work together to form strong teeth and bones. Nearby (85%) of the phosphorus in the body is in the teeth and bones. [15]

Magnesium regarded as the fourth more abundant mineral in the body. It act as a cofactor for more than 300 enzymatic reactions, where it is essential for the metabolism of adenosine triphosphate (ATP). Magnesium is necessary for the synthesis of DNA and RNA, with protein synthesis. [16]

Aim of study:

To study salivary changes according to age by evaluation the level of Alpha- amylase enzyme, MMP-8 enzyme, salivary IgA, and the level of salivary minerals (Phosphorus, Calcium and Magnesium) according to the aging process.

Material and Methods:

This study was conducted in the center of Hilla city carried out from December 2017 to January 2018. The samples were collected from different age groups from general population. The data were analyzed in University of Babylon \ College of Dentistry

Ninety persons from different ages were chosen: Males (60) and females (30), the age of the persons ranged from 20- up to 50 years. This group of persons are divided into three different age group, as shown in the table below.

	Age group	Male	Female	Total
Group I	(20-35) years	24	9	33
Group II	(36-50) years	22	10	32
Group III	(above 50)	14	11	25

The saliva was allowed to accumulate in patient's mouth for two minutes, then the patient was asked to spit the accumulated saliva into the receiving vessel [17]. Two ml of un stimulated saliva samples were centrifuged at 4000 rpm for 15 minutes; the clear supernatant was separated by micropipette and pouted in plane tubes then stored at (-20 ˚C) in a deep freeze for subsequent analysis which was carried out in maximum period of three weeks. The swab was rotated to remove saliva from the oral cavity, inoculated in media then plate incubated

at (37 ˚C) for 48 hours anaerobic condition. [18]

ELISA technique used to evaluate salivary IgA, Alpha- amylase and MMP-8.

Phosphorus according to Phosphorus kit (Phosphomolybdate method) by Mindrary, Calcium according to Calcium kit (Arsenazo III method) by using Mindray. Magnesium according to Magnesium Kit (Xylidyl Blue Method) by using Mindray

Statistical Analysis

Data were processed and analyzed with independent Anova- test using statistical package of social science SPSS 19 and the results were expressed as (Mean±SD).P-values < 0.05 were considered statistically significant. [19]

Results

The study group consists of three different age groups; group I (33) person, group II (32) persons and group III (25) persons, as shown in table (1).

Table (1): Distribution of study samples.

Study	NO.	Percentage%
Group I	33	36.66
Group II	32	35.55
Group III	25	27.77
Total	90	100%

Evaluation of salivary IgA

The results showed that Mean ± SD of salivary IgA level for age group I (20-35years) is (492.63±18.30) which is higher than Mean ± SD of age group II (36-50 years) (290.34±22.10) and higher than Mean ± SD of age group III (51 years and above) and the difference among the study groups is significantly decreased with age (P≤0.05). As shown in table (2).

Table (2): Significance of salivary IgA among different age groups

Characteristic	Age Group	No. of patients in each group	Mean ± S.D	P. value	Anova Test Significance
Salivary IgA	20-35 years 36-50 years 51-years and above	33 32 25	492.63±18,30 290.34±22.10 333.62±36.41	≤0.05	*S

*P. value of Anova test≤0.05 was significant.

Evaluation of salivary amylase alpha I enzyme and salivary MMP-8:

Results show that Mean±SD of amylase alpha I enzyme of the age group I is

(110.35±66.42), while that of age group II is (71.19±58.53) and of age group III is (85.87±56.62). The difference among the three study groups was decreasing with the age, but not significant statistically (P≥0.05). As shown in table (3).

For the level of MMP-8; in age group I is (0.48±0.50), while in age group II is (0.66±0.85) and in age group III is (0.70±0.80), that shows increasing with age, but not significant statistically (P≥0.05). As shown in table (3).

Table (3) Significance of Amylase Alpha I, enzyme and MMP8, enzyme among different age groups.

Characteristic	Age group	Number of patient in each group	Mean ± S.D	P. value	Anova test Significance
Amylase Alpha I	20-35 Years 36-50 Years 51 Years and above	33 32 25	110.35±66.42 71.19±58.53 85.87±56.62	≥0.05	*NS
MMP-8	20-35 Years 36-50 Years 51 years and above	33 32 25	0.48±0.50 0.66±0.85 0.70±0.80	≥0.05	*NS

*P. value of Anova test ≥ 0.05 was no significant.

Evaluation of salivary minerals (Calcium, Phosphorus and Magnesium) among different age group:

Table (4) shows that Mean±SD of salivary Calcium for age group I is (6.04±1.87), and for age group II is (5.67±0.79), while for age group III is (8.07±0.56), which shows significant increasing with age (P≤0.05).

For salivary Phosphorus, Mean±SD for age group I is (88.38±16.5) and for age group II is (90.38±5.21), while for age group III is (90.56±17.30) that showed increasing with age but not significant statistically (≥0.05). As shown in table (4).

While Mean±SD of salivary Magnesium level in age group I is (1.94±0.26), for age group II is (1.96±0.38) and for age group III is (2.70±0.44), which shows significant increasing with age (P≤0.05), as shown in table (4).

Table (4) Significance of salivary minerals (calcium, phosphorus and magnesium) among different age group.

Characteristic	Age Group	Number of patient in each group	Mean ± S.D	P. value	Anova Test Significance
Calcium	20-35 Years 36-50 years 51(Y) and above	33 32 25	6.04±1.87 5.67±0.79 8.07±0.56	≤0.05	*S
Phosphorus	20-35 years 36-50 years 51 Years and above	33 32 25	88.38±16.55 90.38±5.21 90.56±17.30	≥0.05	*NS
Magnesium	20-35 years 36-50 years 51 Years and above	33 32 25	1.94±0.26 1.96±0.38 2.70±0.44	≤0.05	*S 0.000

*P. value of Anova test ≤0.05 was significant.

Discussion

Saliva plays an important role in the maintenance of oral health (dental caries and periodontitis). The data of the present study showed that all the participants had dental caries. However, current data expressed a variation in the saliva among different age group. With the age there are some changes occur in salivary flow, amount and composition.

Regarding salivary IgA is a part of the immune system "first-line of defense" against pathogenic microbes, by restricting the adhesion of microorganism.

These antibodies counter to the formation of dental biofilm and therefore interfere with the defense of plaque related pathologies "caries and periodontal diseases" [20, 21, 22]. In addition, they act to neutralize enzymes, toxins, and viruses; or by working in cooperation with other factors like lactoferrin and lysozyme. [23]

The results of this study showed that the level of salivary IgA in age group I, is higher than the level of age group II, and higher the level of age group III, and the differences among the study groups show significant decrease with age.

Jafarzadeh , *et al* ., in (2009) and Jafarzadeh , *et al* ., in (2010) found that decline in the salivary IgA levels after the age of sixty years could be attributed to the higher risk of oral infections in the elderly.[24,25]

While Eliasson, *et al*., in (2006) investigated IgA concentrations in secretions of palatal, buccal, and labial salivary glands in individuals aged 18-72 years. The saliva samples of individuals beyond the age of 65 have shown to have higher salivary IgA levels than other individuals. Increased whole salivary IgA concentrations in older ages have been attributed partly to positive age-related effects on IgA concentrations in the buccal gland secretions. [26]

Childers, *et al*., in (2003) determined the concentrations of IgA in the parotid saliva of healthy children (age 6-12) and healthy adults (age 22-51) and found that IgA levels increased with the age. [27]

Regarding the concentration of salivary enzyme, the results showed that the concentration of α- amylase I, among different age groups is decreasing with age, but the difference is not significant. These findings are in agreement with that of other studies who establish lesser

α- amylase levels in the elderly [28, 29], while others demonstrated no significant difference, or even

Variations in the results among studies may be due to differences in the methods used, alteration in age groups, and method of saliva collection and saliva used, stimulated or resting type. [30]

There is positive correlation between the level of Ca and α-amylase, because the micro molar levels of Ca2+ are required to stabilize the structure of barley alpha-amylases in the endoplasmic reticulum of the aleurone layer where these enzymes are synthesized [31]. The stabilization mechanism includes an interaction between some negatively charged amino-acid residues and cations. The benefit of this interaction is to keep the three-dimensional structure of protein, which is necessary for the activity of this enzyme. [32, 33]

Elimination of Ca from the genus bacillus, result in reducing thermal stability and enzymatic activity of α- amylase [34], or even increased the susceptibility proteolytic degradation of this enzyme. [35]

Salivary amylase has the ability of metal- binding. Its structure has two sites for metal ion binding, and has one site is selective for Ca binding. It has been found that copper or zinc cannot replace Ca in salivary α-amylase. Therefore, the stability of Ca-amylase binding is a unique interaction. [36]

The presence of Ca in enamel pellicle is necessary for the process of remineralization. While the presence of α-amylase in the enamel pellicle provides the essential substrates for bacterial colonization and enhancing their adhesion to the structure of tooth, and lead to demineralization; therefore, studies about the interaction between Ca and α-amylase in enamel pellicle can provide better explanation for process of remineralization. [36]

Matrix metalloproteinases (MMPs) are family of (24) proteases that play a role in both physiological and pathological conditions. They almost degrade all the components of extracellular matrix and regulate inflammatory processes. They are inhibited by metalloproteinases tissue inhibitors. The main collagenolytic MMP identified in oral fluids is MMP-8, like saliva, oral mouth rinse, gingival crevicular fluid, and peri-implant fluid. MMP-8, that present in oral fluids considered as a strong biomarker that associated with the diagnosis of periodontal disease, their severity, progression, and in fellow-up process. [37]

High level of MMP-8 indicate the loss of supporting periodontal tissues relatively than inflammation. The main collagenolytic MMP detected in the gingival tissue and oral fluids is MMP-8, about (80%) of collagenases found in the gingival crevicular fluid and considered as a periodontal biomarker. [37]

MMP-8 defined as one of the most salivary biomarkers used for detection of alveolar bone destruction that associated with different clinical and radiological parameters, like deepening of periodontal pockets, progression loss of attachment, bleeding on probing and alveolar bone loss. [38]

That explaining the finding of this study, which demonstrated that the level of MMP-8 decreased with age.

Nassar *et al*., in 2014 found positive correlation between increasing age and salivary MMP-8 levels. These findings were in agreement with the findings of the present study. [39]

A plausible explanation for increased periodontal disease severity with increasing age is prolonged exposure to risk factors over a longer duration and possible influence of undiagnosed concurrent systemic diseases predisposing periodontal breakdown. [39]

Regarding the concentration of salivary minerals, the results showed significant increasing in the level of salivary calcium among different age groups with the age. These findings were in agreement with Sevon *et.al,* in 2008, who found that salivary Ca levels increased with age [40], while Salvolini*, et al*., in 1999 and Chauncey, *et al.,* in 1981, found reduced Ca level in old males.[41, 42]

Phosphorus is the most abundant mineral in the body. Calcium and phosphorus act together to form strong bones and teeth. About (85%) of phosphorus of the body present in the bones and teeth [43]. Phosphorus also found in little amounts in tissues and cells all over the body. [43]

The present study showed increasing in the level of salivary Phosphorus with the age. These findings were

in agreement with Sevón, *et. al*., 2008 who showed that concentration of salivary phosphorus increased with age. (40)

In this study when compared the level of salivary calcium and phosphate in patients with dental caries, periodontitis, and control group, the results showed highly significant statistically, which show high level of calcium and phosphate in patients with periodontitis when compared with controls and dental caries group.

That in agreement with a study done by Sewon *et. al.,* in 1990 who found positive correlation between periodontitis and high level of salivary calcium (44). Others demonstrated higher concentration of calcium in plaque is associated with low caries incidence. (45, 46)

On the other hand, results showed significant increasing of magnesium level with age.

Both Calcium and Magnesium identified as important elements for the function of different systems in the organisms of human and animal. High level of salivary magnesium, decrease the colonization of streptococcus mutans and therefore, reduced caries possibility. (46)

Gutman and Ben in 1974 demonstrated an elevation in the mean of electrolyte content (Na, K, Ca, and Mg) and a reduction in salivary flow with age; these results indicate probable correlation between the salivary properties and aging process. (47)

The above results explaining the relationship between increased incidence of periodontal disease and decreased incidence of dental caries with the age.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry and all experiments were carried out in accordance with approved guidelines.

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