# Effect of ageing on selected salivary chemical compositions and dental caries experience among group of adults

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**Background**: Ageing is a continuous accumulative ordinary physiological phenomena occurs upon all organs and body structures including saliva by its constituents which can influence the caries process, for that this study was conducted to determine the impact of ageing on selected chemical composition of saliva and their effects on dental caries experience characteristics at different age groups among apparently healthy men.

**Materials and method:** A descriptive comparative study was conducted to compare between three study samples (young, middle and old age adults), thirty male in each study insert, aged (20 years, 40 years and 60 years) respectively. They were selected from private health center in Baghdad - Iraq, from the mid of November 2017until the end of February 2018, where they subjected to complete body investigations to confirm their health status, followed by thorough general body history and oral examination. Caries experience was recorded according to the criteria of WHO in1987. Un-stimulated saliva was collected according to Navazesh and Kumer and analyzed chemically to determine the level of calcium ions and alkaline phosphatase. All data then statistically analyzed by using SPSS version 22.

**Results**: The current study, showed that the caries experience represented by DMFs increased with increasing age, by which the highest mean value of DMFs was established in the oldest age ( $60.37\pm31.39$ ) and the lowest mean value was noted among the youngest age ( $7.27\pm8.02$ ). A significant differences existed between all study samples ( $p\leq0.05$ ). However, the missing surfaces represented the largest proportion of DMFs value when compared to Ds and Fs among all ages lin the current study, with significant differences ( $p\leq0.05$ ) was followed by the decay fraction that found to be increased with age but with no significant difference. Salivary Calcium ions showed decreasing with increasing age with statically significant differences. Concerning the caries experience among the study samples, the salivary Ca<sup>2+</sup> ions inversely correlated while alkaline phosphates correlated positively with caries experience.

**Conclusion:** there is a significant age related changes on the selected chemical constituents of saliva that could affect the caries experience

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# INTRODUCTION

1)

Aging is a time-dependent biological process involved changes in organ structure after maturity and the functions of organs can be regularly changed. Ageing happens over all organs and overall individuals so no one can escape <sup>(1)</sup>. Age -related topics gain particular attention in aging societies, where adult people represent the growing target groups <sup>(2)</sup>. Some age-related changes affect the structures of oral cavity (both of hard and soft tissues) which experience continuous changes throughout the life <sup>(3)</sup>.

Saliva has been well-defined as the mirror of the body through its ingredients such as enzymes, immunoglobulins and ions which have profound effects on oral homeostasis and any change in their equilibrium may lead to oral diseases including dental caries or periodontal problems <sup>(4)</sup>.

Alkaline Phosphatase includes a group of enzymes that catalyze the hydrolysis of phosphate esters in a

M. Sc. Student, Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad. basic surroundings. This enzyme shows a vital role in the bone metabolism and bone homeostasis by probably accumulating calcium ions and matrix vesicles during calcification process <sup>(5)</sup>. Along with Alkaline Phosphatase, calcium also acting a vital role in the hard tissue establishment and it upturns the confined concentration of inorganic phosphate and supports the mineralization, in this manner decreasing the meditation of extracellular pyrophosphate(an inhibitor of mineral formation) <sup>(6)</sup>. There was a controversy regarding the levels of calcium and alkaline phosphatase with age after maturity of salivary glands. Nasser et al. (7) who studied two different age groups (20-30) (60-80) to estimate the age related changes on un-stimulated salivary biomarkers found elderly groups revealed lower concentration of calcium when compared with young adults, while other studies found that salivary calcium was increased with age <sup>(8,9)</sup>.

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Punteeth *et al* <sup>(5)</sup> measured Ca<sup>+2</sup> and ALP in saliva and urine to estimate the rate of bone resorption and age related changes among adults aged (30-60), they found that as the age increase the ALP increased and Ca decreased. Thus releasing the fact that saliva can acts as a mirror of blood, so saliva can also be used with equivalent efficacy as that of the serum for the estimation of ALP and calcium levels <sup>(6, 10)</sup>.

In spite of a knowledge explosion in the science of cariology, dental caries process still a misinterpreted phenomenon by the researchers. In order to practice the wide-ranging of the preventive approaches efficiently, it is imperious to look beyond those black and white spots that are obvious on the tooth surfaces. Dental caries, as a common complex chronic infectious disease of the oral cavity, is a multi-factorial miracle elaborate

Ninety healthy males, were incorporated in this comparative study in three study samples; young, middle and old adults aged (20, 40 and 60 years) respectively. According to the World Health Organization, every person's age was estimated regarding his last birthday (14). Each convenient sample entailed of thirty subjects, they were attending the International Medical Center (private medical center) at Baghdad-Iraq for general health check-up and treatment if there is any need. An approved permission was achieved from the International Medical Center before data collection in order to examine the selected persons with no obligation. The purpose of study was effusively clarified to the participants in order to achieve their full responsiveness and cooperation. In addition, a special consent was arranged and specified to the selected participants to get the permission for their participation in the study. The present study was approved by the Committee of pedodontics and preventive department at Baghdad College of Dentistry

#### The inclusion criteria

.Didn't have any systemic disease and apparently healthy according to the findings of full body investigations they did.

. Non- smokers

. Did not receive any medicaments in the last three months before examination.

• Did not have any oral lesion and they didn't wear any removable or fixed appliance.

 $\cdot$  Whom agreed to contribute in this study, and were existing in the time of collection.

Un-stimulated saliva was collected between 9, and 11 am to reduce any circadian rhythm. A total

with salivary constituents <sup>(11)</sup>. Different biochemical characteristics of saliva, like salivary ALP and Ca ions may affect the development of dental caries with wide range of controversy <sup>(12,13)</sup>.

Very little information is available, concerning the effect of age on the salivary calcium and alkaline phosphatase and caries experience among apparently healthy adults, however, unfortunately no previous Iraqi study is present at hand to investigate the impact of ageing on this salivary chemical consequents and dental caries experience, so this study was done to determine the effect of age on salivary calcium and alkaline phosphatase and caries experience among apparently healthy adults participants.

# SUBJECTS, MATERIALS AND METHODS

of 5 mL of unstimulated saliva were collected in centrifuge tubes one hours after subjects were instructed to do not eat, drink or brush their teeth, according to the Navazesh & Kumer method <sup>(15)</sup>. After the collection of saliva, samples were centrifuged at 3000 rpm for 5 minutes, the clear supernatants were separated and stored frozen at -20 °C in plastic polyethylene tubes until the time of biochemical analysis in the Center of Toxicology/ Specialized Surgeries Hospital/ Baghdad.

In the present study, dental caries experience was diagnosed and recorded according to (DMFs) index following the criteria of WHO as it is more sensitive index for caries intensity since it was measured in the term of surfaces rather than teeth <sup>(16)</sup>. Dental caries experience was carried out by using dental explorer and plane mouth mirror. Assessment of caries experience was carried out by calculating the DMFs for each participant. Calcium level (mg/dl) determined using air - acetylene atomic absorption spectrophotometer (Buck Scientific, 210VGP, USA). Instrumental settings were performed according to instrumental manufacturer specifications <sup>(17)</sup>. Salivary alkaline phosphatase (UI/L) was estimated by using alkaline phosphatase kit, which functions on the basis of modified Kind and King's method, using alkaline phosphatase-kit by BIOLABO SAS, France. Colorimetric determination of alkaline phosphatase activity, then all data were collected statistically analyzed by using computer software program as Statistical Package for social Sciences (SPSS version 22). Statistical analysis can be classified into two categories:

#### **1-Descriptive Analysis:**

Frequency, Percentage for nominal variables while mean, and Standard deviation for numeric variables (Quantitative).

#### 2- Inferential analysis:

A- Levene test: test the equality of variances between groups. When the P value of levean test is significant, Dunnet T3 was used while if the P value of levean te is not significant, the Least Significant Differences (LSD) was used.

#### RESULTS

Results of the present study revealed that, the highest value of caries free was found among the youngest age, who recorded 20% caries free and decreased with age to be 0% in the oldest adult, these results were demonstrated in Table 1.

Dental caries experience presented by DMFs index and its fractions was illustrated in the table 3 .It was clear that caries experience (DMFs) was increased with age, in which it was found to be higher mean among old adults ( $60.37\pm 31.39$ ) when compared to the mean of DMFs among middle age ( $22.07\pm 20.33$ ) and young adults ( $7.27\pm8.02$ ). Regarding DMFs fractions, the decayed surface (Ds) found to be increased with age where the lowest mean value was found among the (20 years) followed by 40 years and highest mean value was recorded among the old adults as listed in Table 3. However, no significant difference was found between the three ages.

B- One Way Analysis of Variance (ANOVA): test the hypothesis for a quantitative dependent variable by a single factor (independent) variable.

C- Pearson Correlation is a bivariate analysis that used to measure the strengths of association between two quantitative variables.

Level of significance: Not Significant at P>0.05, Significant at P $\leq$ 0.05.

According to DunnetsT3, a significant difference was existed between DMFs among all ages of the current study (p≤0.05) as in Table 2. Concerning the missing fraction (Ms), it was found to be the largest proportion of DMFs value when compared to Ds and Fs among all age groups. Moreover, the old age group had the highest Ms (52,  $60 \pm 31.36$ ) when compared with the other groups that demonstrated in Table 3. DunnetsT3 with Ms revealed a statically significant difference ( $p \le 0.05$ ) among all ages of the present study Table 4. The smallest fraction of DMFs index in the current study was found to be the Fs fraction, when compared with Ds and Ms fractions, among different study samples of the current study. The results revealed a highest mean value of filling surface among middle age  $(4.27 \pm 7.84)$  compared with young and old adults  $(0.83 \pm 1.72)$   $(2.47 \pm$ 7.07) respectively as listed in Table 4.

	Table 1	l: ]	Distribı	ition of	f caries	free among	subjects	s by	ages
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Age years	No. of subjects	No. of caries free	%
20	30	6	20
40	30	5	16.7
60	30	0	0

Table 2	2: I	Dunnett's	<b>T3</b>	between	DMFs	fraction	among	different	ages



Variables	Аде	Mean	+ SD	ANOVA		
v ar rables	(Years)		- 52	F	p-value	
	20	3.50	4.10		0.452	
Ds	40	3.93	7.21	0.800	0.452	
	60	5.30	5.52			
	20	2.93	6.09			
Ms	40	13.97	15.23	48.884	0.000*	
	60	52.60	31.36			
	20	0.83	1.72			
Fs	40	4.27	7.84	2.317	0.105	
	60	2.47	7.07			
	20	7.27	8.02			
DMFs	40	22.07	20.33	46.208	0.000*	
	60	60.37	31.39			
* P≤0.05						

Table 3: Caries experience by DMFs index and its fractions among different ages

Table 4: Dunnett's T3 between Ms fraction among different ages



Concerning the means and standard deviation of salivary  $Ca^{+2}$  measured in mg/dl, the results showed the mean value of salivary calcium decreased gradually with increasing age with significant difference among the different study samples. While for alkaline phosphatase (ALP), the results of the current study showed that the mean value was higher in both middle and old adults when compared with young adults, however statistically there was no significant differences, as illustrated in the Table 5.

Regarding the correlations coefficient of salivary  $Ca^{+2}$  and caries experience that showed in

the table 4, among young adults, the table revealed that, a weak positive correlations coefficient between salivary  $Ca^{+2}$  with DMFs and its fractions, and statistically no significant correlations were recorded.

In another hand, among middle age group, a negative correlations were found between salivary  $Ca^{+2}$  with DMFs, Fs fractions, a significant correlation was found between salivary  $Ca^{+2}$  with Fs fractions among the same age.

While among old adults, a negative correlations was existed between salivary Ca<sup>+2</sup> with Ds, Ms and

DMFs, but statically failed to reach the significant differences.

Regarding the correlation coefficients between the salivary ALP and caries experience among different study samples that illustrated in the Table 6, the current investigation found a positive non-significant correlation between salivary ALP with Ds, Ms fraction and DMFs among young age, while a negative non-significant correlation was found between salivary ALP and Fs fraction. Among 40 years, the same table illustrate a positive significant correlation was found between ALP with Ds fraction. Whilst among 60 years, the current findings revealed, a negative significant correlation between alkaline phosphatase with Ds fraction. In addition a strong positive significant correlation between salivary ALP and Fs fraction while a negative non-significant correlation was found between ALP with DMFs fraction.

				ANG	OVA
Age (Years)	Variables	Mean	±SD	F	p-value
20		4.34	0.43		
40	$Ca^{+2}(ma/d1)$	3.47	0.59	56 86*	0.000
60	Ca (ilig/ul)	3.25	0.58	30.80	0.000
20		0.51	0.31		
40	ALP (UI/L)	0.56	0.26		
60		0.55	0.28	1.000	0.372
* P≤0.05					

# Table 5: Selected Salivary Chemical Component among the three ages

Table 6: (	Correlation	coefficients	between	salivarv	calcium	with	caries	comp	onents	among
		councients		Sanvary	carcium	** 1 UII	cartes	comp	JICHUS	amone

Salivary variable	Age	D	S	Μ	IS	FS	5	DM	FS
	(years)	r	р	r	р	r	р	r	р
Ca <sup>+2</sup>	20	0.310	0.096	0.138	0.468	0.018	0.926	0.267	0.154
	40	0.094	0.620	0.090	0.636	-0.381*	0.038	-0.042	0.826
	60	-0.336	0.070	-0.275	0.142	0.082	0.665	-0.315	0.090
* P≤0.05									

# Table 7: Correlation coefficients between salivary alkaline phosphatase with caries components among ages

Salivary	Age	Age Ds		5	Ms		Fs		DMFs	
variable	(year)	r	р	r	р	r	р	r	р	
ALP	20	0.268	0.152	0.107	0.573	-0.138	0.468	0.189	0.137	
	40	0.414*	0.023	0.028	0.882	0.013	0.948	0.168	0.375	
	60	-0.398*	0.029	-0.316	0.089	0.551*	0.002	-0.261	0.163	
* P≤0.05										

# **DISCUSSION:**

The current study revealed a decreasing in the percentage of caries free subjects with age till it reached 0% among the old adults, the observed finding was close to that found by other studies (18,20). The current study recorded a significant differences in caries experience represented by DMFs: this result came in accordance with the finding of Farsi (20). However this was in contrast with the other study (21). The variations between the findings of the studies may be related to many factors such as the sample size and the method by which dental caries was examined, in addition to the environmental and the genetic factor (22). In addition the Ms fraction represented the largest fraction of DMFs among all the study samples, especially among the oldest adult who recorded the highest Ms which could be due to the fact that dental caries is an irreversible process and accumulative in nature with ageing on one hand and to the insufficiency of the planed preventive programs especially among the old adults who had poor dental knowledge as well as oral health, didn't receive an optimal dental care and had complicated past dental history (23), In addition to that, they were prefered tooth extraction over restorations because there were several barriers to self-care and professional care. <sup>(24)</sup>. These findings came in agreement with the others <sup>(25-27)</sup>, however other studies recorded the same result but with no significant differences (17, 28). Concerning Ds fraction, which was increased with age and this result came accordance with other study (19). the current study came discordance with other study that recorded also an increase of Ds with age, as the finding of the recent study, but the dissimilarity was with Ds fraction that was not-represented the largest fraction of DMFs of current study with no significant difference (29). The variations in the caries experience between studies may be related to the variations in the methodology of each study, the absence of bitewing radiographs to diagnose the interproximal caries in the recent study could leading to the underestimation of Ds component, in addition to that, the participants of present study preferring tooth extraction. While the other causes of increasing the Ds mean value among old age groups may be related to the most cariogenic risk with ageing which could be the dysfunction of the salivary glands, less effective oral hygiene habits, decreased motor function and variation in dietary habit, hygiene measures, and consumption the diet rich in cariogenic food <sup>(30)</sup>.

The highest mean value of Fs fraction, which represented the smallest fraction of DMFs index in the current study, was found within the middle age group. This finding came with agreement with the result of other study (31) which may be related to the utilization of dental health services among the middle age group and that reflect their knowledge. attitude and behavior about the importance of preserving teeth even after they affected by decay <sup>(20)</sup>. However, this finding was in disagreement with that of other studies <sup>(17, 19)</sup>. The differences in these findings between the studies may be related to the variation in the dental health services provided. subjects health knowledge and preferences, geographical location and racial factors. In addition to the cultural, social and economic differences found between the studies (31).

Concerning the mean value and standard deviation of salivary Ca<sup>+2</sup>, The present investigation recorded decrease in the mean value of salivary Ca<sup>+2</sup> with age which could be attributed to the lower intestinal absorption rate, lesser Vitamin D absorption and metabolism with age. decreased muscular proteins and flow of blood to bone (29). This result came with agreement with other studies <sup>(5,7)</sup> However the current study was in discordance with the findings of other studies which found the salivary calcium level was increased with age this may be related to different age groups, genders included in both studies <sup>(8,9)</sup>. Regarding the correlations coefficient of salivary Ca<sup>+2</sup> with caries experience, the current study revealed inverse but not significant correlation of salivary calcium with the dental caries indicated by Ds, Ms and DMFs with ageing, this showed the importance of Ca<sup>+2</sup> in increasing the resistance of the outer enamel surface to acid dissolution and enhancing remineralization of the initial carious lesion <sup>(32)</sup>. The present result was in consistent with the finding of Hasan and Diab in 2010 (33), but inconsistent with the findings of others (34, 35) who found that the salivary Ca<sup>+2</sup> had no relation to the caries experience. In addition, the present study recording inverse relation between age and levels of ALP and Ca<sup>+2</sup> indicating that as age increases, ALP increased and Ca+2 levels decreased. The explanation for these results which could be related to the known fact that, the levels of calcium depletes with age, resulting in the reduction of bone strength. Thus the increase in the level of salivary ALP with ageing can represented as

compensatory mechanism to overcome the depletion that occurs to calcium ions with ageing <sup>(36)</sup>. The result of current study was in agreement with the study of other <sup>(5)</sup>. Concerning the correlations coefficient of salivary ALP and caries experience, the result was recorded a negative significant correlation with (Ds) fraction among old adults who recorded highly caries experience, this result could be due to the variations in ALP levels caused changes in phosphate levels which leading to initiation and progression of caries <sup>(37)</sup>. This result came with agreement of the results of other studies (38, 39), while disagreement with the results of others (6, 40), who found a positive correlation between salivary alkaline phosphatase with dental caries. The variations between the results of current study and other previous study may be related to different age groups, method of measurement.

# CONCLUSION

The thoughtful elaboration of the age related changes in the saliva and its constituents is an imperative manner, as well increasing in the mindfulness and consideration to enhance the knowledge of the community about the importance to identify oral health damage at early time as it linked to general health, that oral substance involving teeth and gum which can significantly affect over well-being and capability to oldness positivity.

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### الخلاصة

ا**لخلفية** : تعد الشيخوخة من الظواهرالفزيولوجية الطبيعية التراكمية المستمرة التي تطرأ على جميع اعضاء وهياكل الجسم متضمنة اللعاب ومكوناته والتي يمكن ان تؤثر على عملية التسوس لذلك اجريت هذه الدراسة لتحديد تاثير تقدم العمر على مكونات كيميانية مختارة من اللعاب ودراسة تاثيرها على خصائص عملية التسوس في مجموعات عمرية مختلفة بين البالغين الاصحاء.

المواد والطرق: SPSS أجريت دراسة مقارنة وصفية للمقارنة بين ثلاث عينات دراسية (الشباب ، متوسطي العمر وكبار السن) ، ثلاثون نكراً في كل عينة دراسة (20 سنة ، 40 سنة ، 60 سنة ) على التوالي ، تم اختبار هم من مركز صحي خاص في بعداد. - العراق ، من منتصف نوفمبر 2017 حتى نهاية فبراير 2018 ، حيث خضعوا لفحوصات مختبرية شاملة لتأكيد وضعهم الصحي ، تلاه اخذ التاريخ الصحي العام والفحص الفموي. تم تسجيل تجربة التسوس وفقاً لمعايير منظمة الصحة العالمية (1987) مة جمع اللعاب غير المحفّز وفقاً ل Navazesh and Kumer (2008) وتم تحليله كيميائياً لتحديد مستوى أيونات الكالسيوم والفوسفاتيز القلوية ، تمم تحليل الألبات من التقليب المحفّز وفقاً ل جميع البيانات إحصائياً باستخدام SPSS الإصدار اصدار 22.

النتائج : اظهرت الدراسة الحالية أن تجربة التسوس التي تم تمثيلها بواسطة DMFs تزداد مع تقدم العمر ، حيث تم العثور على أعلى متوسط قيمة DMFs في الكبار البالغين (60.37 ± 60.31) وأدنى متوسط للقيمة وجد بين الأصغر سنا (7.27) ± 8.02). وجدت فروق ذات دلالة إحصائية بين جميع عينات الدراسة (0.05). ومع ذلك ، مثلث الأسطح المفقودة (Ms) أكبر نسبة من قيمة DMFs بالمقارنة مع DS و Fs بين جميع اعمار الدراسة الحالية ، مع وجود فروق ذات دلالة (25.0=p) وتلا ذلك جزء التسوس(Ds) الذي وجد أنه يزداد مع التقدم في السن ولكن بدون دلالة احصائية . وأظهرت أيونات الكالسيوم اللعابية تتاقصماً مع زيادة العمر مع وجود فروق ذات دلالة إحصائية بين عينات الدراسة المختلفة في حين أن الفوسفاتاز القلوي اللعابي زاد بزيادة العمر مع عدم وجود فروق ذات دلالة إحصائية. فيما يتعلق بتجربة التسوس بين عينات الدراسة ، ارتبطت اليونات الكالسيوم عكسيا في حين ارتبطت الفوسفات القلوية إيجابيا مع تجربة تسوس. الاستنتاج: هذاك اختلافات واضحة في المكونات الكيميائية المختارة من للعاب والتي اثرت بدورها على عملية التسوس.

كلمات مفتاحية: الشيخوخة ، تسوس الأسنان ولعاب غير محفز .