Taste Detection Thresholds in Relation to Salivary and Serum Zinc in Patients on Simvastatin Treatment

Mohammed J. Mohammed, B.D.S. (1)
Taghreed F. Zaidan, B.D.S., M.Sc., Ph.D. (2)

ABSTRACT

Background: Hyperlipidemia is an elevated fat (lipids), mostly cholesterol and triglycerides, in the blood. These lipids usually bind to proteins to remain circulated so-called lipoprotein.

Aims of the study: To determine taste detection threshold and estimate the trace elements (zinc) in serum and saliva of those patients and compare all of these with healthy control subjects.

Methods: Eighty subjects were incorporated in this study, thy were divided into two groups: forty patients on simvastatin treatment age between (35-60) years, and forty healthy control of age range between (35-60) years. Saliva was collected by non-stimulated technique within 10 minutes. Serum was obtained from each subject. Zinc was estimated in serum and saliva by flame atomic absorption assay. Taste detection threshold was estimated by using 15 different concentrations of the four basic tastes solutions, the test use sip and spit with deionized water as mouth wash interval. Diabetics, thyroid and parathyroid disease, autoimmune disease, chemotherapy, smoking, alcoholics, neoplastic diseases were excluded.

Results: The study showed that the taste detection threshold of sour and bitter were highly significantly higher in those patients than that in control subjects, sweet detection threshold were significantly high in patient on simvastatin. The salt detection threshold showed no significant differences between study groups. Salivary flow rate was significantly decreased in patients on simvastatin treatment than that in control subjects. Salivary and serum zinc were highly significantly decreased in control subjects than those in patients. There was highly significantly positive linear correlation between salivary flow rate and the mean of detection threshold of sweetness and sourness of both study groups, and highly significantly negative linear correlation with the mean of detection threshold of saltiness and bitterness in both study groups.

Key words: Hyperlipidemia, simvastatin, zinc, salivary flow rate, taste. **(Received: 15/1/2018; Accepted: 4/3/2018)**

INTRODUCTION

Hyperlipidemia is a medical situation in which level of fat called lipids, mostly cholesterol and triglycerides, increase in the blood (1). Statins is a lipid-lowering agent that is derived synthetically from fermentation end products of *Aspergillus terreus*. After oral ingestion, simvastatin, which is an inactive lactone, is hydrolyzed to the corresponding β-hydroxyacid form. This is an inhibitor of 3-hydroxy-3-methylglutarylcoenzyme A (2). The interactions between HMG-CoA and statins reductase inhibit the opposing of HMG-CoA to L-mevalonate that end in the inhibition of the circulating cholesterol biosynthesis (3). Statins in proposed studies, which are well-established lipid-lowering drugs, impact bone turnover by encouraging bone formation (4). Simvastatin therapy resulted in a considerable decrease of serum lipid profile but to have no effect in decreasing parotid gland weight (5). Zinc is a mandatory dietary nutrient and episodic intake of zinc is necessary for survival. Zinc is needed for the suitable functioning of important enzymes and metabolic systems (6). Taste detection threshold means the ability of the subjects to be clearly differentiated taste solution from deionized water (7).

(1) Master Student, Department of Oral Diagnosis University of Baghdad, College of Dentistry, Iraq.
(2) Professor, Department of Oral Diagnosis University of Baghdad, College of Dentistry, Iraq.

MATERIALS AND METHODS

The study samples consisted of 80 subjects, samples were collected from Al-Samawa teaching hospital in Samawa city, Iraq. The laboratory works was done in the poison's management centre at Baghdad hospital. The subjects were divided into two groups:

Group 1: Forty patients on simvastatin treatment (20mg/day) age between (35-60) years.

Group 2: Forty healthy non- smoker, non-alcoholic subjects age between (35-60) years. Consent and fulfilled a case sheet were done for each subject.

Any subject demonstrates the following conditions` was not included in the study; diabetics, thyroid and parathyroid disease, autoimmune disease, chemotherapy, smoking, alcoholics, and neoplastic diseases (8). Trace element solution for flame atomic absorption assay with standard solution cupric nitrate BDH Chemicals Ltd Poole England was used to detect serum and saliva zinc, taste detection threshold was determined for each subject. Venous blood sample (5ml) was drawn from all subjects (fasting 12 hr.), The blood was allowed to clot at 37^0 C for 15-20 minutes and centrifuged at 3000 rpm for 15 minutes, the serum was obtained, then divided in parts in sterile eppendroffs and was stored at -40^0 C in deep freezer until analysis. The tubes were labelled subject's name by water resistant marker.

Unstimulated whole saliva was collected under standardized condition by using spitting method [9].

Collection is done at the morning from (9-11 a.m.), the subjects were fasting 12 hr before collection and were asked to rinse their mouths well to remove debris, spitting into graduated tubes for about 10 minutes. The subjects were asked to sit on a chair so their heads bent foreword to prevent stimulation by tongue movement and prevent swallowing of saliva. Then, stop watch was used, and the saliva was collected into graduated tube, closed immediately by plastic stopper. Salivary flow rate was calculated as volume in ml of the sample divide by time in minutes required for collection [8] (Salivary flow rate =salivary volume /time = ml/min).

The collected saliva was centrifuged at 3000 rpm for 10 minutes the clear supernatant is separated, divided in parts and was stored at -40^0 C in deep freeze until analysis.

The taste solutions were prepared as follow: for sweet: 15 solutions of sucrose from (1.5 -15 mmol /L) in 1mmol increment. Salt: 15 solutions of sodium chloride from (1-78 mmol /L) in 5.5 mmol increment. Sour: 15 solutions of citric acid from (48- 720 micro mol /L) in 48 micro mol increment. Bitter: 15 solutions of urea from (89-117 mmol /L) in 2 mmol increment (7). Deionized water was used as solvent. Taste solutions were prepared freshly in regular intervals. A volume of 10 ml of each taste gradient solution, previously brought to room temperature (22-25 C), was offered to the participants in disposable cups coded in random numbers, ordered progressively higher concentration starting from deionized water as blank [8].

A computerized program, the statistical package for the social sciences (SPSS) was used for data analysis. Both descriptive and inferential statistics will be used: .1-descriptive statistics Statistical tables, Mean, Standard deviation (SD), Range. 2-inferential statistics, Student t-test (paired) Pearson correlation. The results considered significant, when the level of significance is 0.05% or less.

RESULTS

Clinical Findings: -

1-Age: - The mean age of patients on simvastatin treatment was 47.65 ± 7.63 years and 41.12±7.20years for control subjects and age range (35-60) years for both study groups. The study showed that there were no significant differences between the age of patients on simvastatin treatment and ages of controls subjects ($p > 0.05$) (Table 1).

Table 1: - The range and mean of ages of patients on simvastatin treatment and control subjects.

Groups	No	Age range (years)	Mean (year)	SD	p-value
Patients on simvastatin	40	35-60	47.65	7.63	0.88 (NS)*
Control	40	35-60	41.12	7.20	

***NS: - None significant**

2-Gender: -

The results showed that the number of male patients was 23 (57.5%) and the number of female patients was 17 (42.5%). Also, for the control subjects the number of male subjects was 23 (57.5%) and the number of female subjects was 13 (42.5%) (Table 2).

1-Taste detection thresholds: -

The study showed that the mean of the detection threshold for sucrose (sweetness) of patients on simvastatin treatment was 14.90 ±0.63 mmol/l, salty taste was 17.63 ± 5.21 mmol/l , sour taste was 330.40 ±44.75 µmol/l, , for the bitter taste was 104.15±1.72 mmol/l ,while for control subjects, sweet was 9.40 ±0.74 mmol/l,

Table 2: - The number and percentage of male and female subjects in the study groups

Group	No. of M.	%	No. of F.	%	Total	
Patients on simvastatin	23	57.5%	17	42.5%	40	100%
Control	23	57.5%	17	42.5%	40	100%

M: males, F: females

3- Duration of therapy:

The mean of duration of treatment was 2.36 ± 1.36 years, the range was (1-6) years. The number of patients with (1-3) years treatment duration was 25 (62.5%), from (3-5) years was 11 (27.5%), and patients on simvastatin treatment ≥ 5 years were 4 (10%) (Table 3).

Table 3: - The number and percentage of patients on simvastatin according to treatment duration.

Duration of treatment (years)	No. of patients	%
1-3	25	62.5
3-5	11	27.5
5 ≥	4	10

Salty taste was 16.55 ±4.88 mmol/l , sour taste was 172.80±30.35 µmol/l, bitter taste was 97.1±10.75mmol/l. Statistical analysis showed that the detection threshold of sour and bitter tastes in those patients were highly significantly higher (p<0.001) than that in the control subjects , the detection threshold of sweet in patients on simvastatin treatment was significantly higher (p< 0.05) than that in the control subjects, while the detection threshold of salt taste in patients on simvastatin treatment showed no significant differences than that in the control subjects (p>0.05) (Table 4).

Table (4): - The mean and standard deviation of detection threshold of the four tastes of patients on simvastatin treatment and control subjects.

Group	Sweet		Salt		Sour		Bitter	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Patients on simvastatin	14.90	.63	17.63	5.21	330.40	44.75	104.15	1.72
Control	9.40	.74	16.55	4.88	172.80	30.35	97.10	1.75
p- values	*P< 0.05		p> 0.05 NS		P< 0.001**		**P< 0.001	

All units in mmol/L except for sourness in µmol/l, ** highly significant p<0.001,
*** Significant p< 0.05, NS: - None significant**

2-Salivary flow rate:
This study showed that the mean of salivary flow rate of patients on simvastatin was 0.33±0.08 ml/min, while for the control subjects was 0.59± 0.04 ml/min. Statistical analysis showed that salivary flow rate was significantly decreased in patients on simvastatin treatment than that in control subjects (p < 0.05) (Table 5).

Table 5: - The mean and standard deviation of salivary flow rate of patient on simvastatin treatment and control subjects.

	Patients on simvastatin treatment (mean ± SD)	Control (mean ± SD)	P - value
Salivary flow rate(ml/min)	0.33±0.08	0.59± 0.04	* 0.0001

*** P<0.001**

Laboratory Findings: -
Salivary and serum zinc: -
The results showed that the mean and standard deviation of salivary zinc in patients on simvastatin treatment was 2.03±0.58 mcg/dl, while in the control subjects was 4.96 ±0.38 mcg/dl. Serum zinc of patients on simvastatin treatment was 58.85 ± 6.83 mcg/dl, while in the control subjects was 90.32 ±7.81 mcg/dl. Statistical analysis using t- test showed that salivary and serum zinc were highly significantly decreased in patients on simvastatin treatment than that in control subjects (p<0.001) (Table 6)

Table 6: - The mean and standard deviation of salivary and serum zinc in study groups with (t-test).

Group	Salivary zinc mcg/dl		Serum zinc mcg/dl	
	Mean	SD	Mean	SD
Patients on simvastatin	2.03	0.58	58.85	6.83
Control	4.96	0.38	90.32	7.81
t-test	23.5		19.75	
p-value	**0.001		**0.001	

**** P ≤0.001**

The correlation of parameters: -
I-Duration of treatment
It has been shown that there was a significantly positive strong linear correlation between duration of treatment and age (p= 0.005), a significantly negative linear correlation was found between duration of treatment and salivary flow rate and between duration of treatment and serum zinc (Table 7).

Table 7: - The correlation between duration of treatment and age, taste detection threshold (sweetness, saltiness, sourness, bitterness), salivary flow rate, salivary and serum zinc of patient on simvastatin treatment.

Groups	Duration of treatment	
	Correlation coefficient (r)	P value
Age	0.615	0.005*
Sweet	0.212	0.190
Salt	0.042	0.795
Sour	-0.058	0.722
Bitter	0.033	0.840
Salivary flow rate ml/min	-0.585	0.005*
Salivary zinc mcg/dl	-0.164	0.312
Serum zinc mcg/dl	-0.352	*0.025

*** Significant p < 0.05**

II- Salivary and serum zinc: -

Statistical analysis using the correlation coefficient (r) showed no significant correlation between serum and salivary zinc in patients on simvastatin and control subjects (Table 8).

Table 8: - The correlation coefficient (r) between serum and salivary zinc in patients on simvastatin treatment and control subjects in both groups.

	Salivary zinc	Serum zinc	(r)	p-value
Patients on simvastatin treatment	2.03±0.58	58.85±6.83	0.04	0.76 NS
Control	4.96±0.38	90.32±7.81	-0.03	0.84 NS

3- Salivary and serum zinc and taste detection thresholds: -

The results showed that no significant correlation was found between all the four basic tastes and serum and salivary zinc in patients on simvastatin and in control subjects (p>0.05) (Table 9).

Table 9: - The correlation and p-value between salivary and serum zinc with the four basic tastes in patients on simvastatin treatment and control subjects.

Sample / Taste	Salivary zinc				Serum zinc			
	Patient on simvastatin		control		Patient on simvastatin		Control	
	(r)	P	(r)	P	(r)	P	(r)	P
Sweet	-0.06	0.71	-0.05	0.72	0.16	0.30	0.06	0.69
Salt	-0.02	0.89	-0.01	0.91	0.08	0.59	-0.08	0.58
Sour	-0.10	0.51	0.12	0.44	0.11	0.49	0.10	0.52
Bitter	-0.10	0.50	-0.04	0.76	0.18	0.29	0.06	0.69

4- The correlation with salivary flow rate

The study showed that there was a highly significant positive linear correlation between salivary flow rate and the detection threshold of sweet and sourness tastes (p≤ 0.001) and a highly significant negative linear correlation between salivary flow rate and the detection threshold of saltiness and bitterness tastes (p≤ 0.001) in both study groups, also there was a highly significant positive linear correlation between saliva flow rate and salivary and serum zinc(p< 0.001) (table 10).

Table 10: - The correlation coefficient (r) and p-value between salivary flow rate and other study parameters in both study groups.

S.F.R / Parameters		Patients on simvastatin			Control		
		(r)	p	Sig.	(r)	p	Sig.
Age	**35-60 years**	-0.32	*0.03	S	0.03	0.83	NS
Duration of treatment	**1-6 years**	-0.585	*0.005	S			
Taste detection threshold	**Sweetness**	0.005	**0.0001	HS	0.64	**0.0001	HS
	Saltiness	-0.06	**0.0001	HS	-0.20	**0.0001	HS
	Sourness	0.21	**0.0001	HS	0.25	**0.0001	HS
	Bitterness	-0.21	**0.0001	HS	-0.17	**0.0001	HS
Saliva	**Zinc**	0.16	**0.0001	HS	0.23	**0.0001	HS
Serum	**Zinc**	0.25	**0.0001	HS	0.20	**0.0001	HS

****P ≤0.001 (HS) Highly significant, *P< 0.05 (S) Significant, (NS) None significant p>0.05**

S.F.R: Salivary flow rate

DISCUSSION

Age and gender: - The mean age and standard deviation of patients with hyperlipidemia on simvastatin treatment was 47.65 ± 7.63 years with age range (35 -60) years and was found to occur in male more than females which was agreed with other studies who found that the hypercholesterolemia was high among people of Spain age 35-64 and was higher in males [(10)].

Oral Findings: -

Salivary flow rate: - Salivary flow rate was 0.33±0.08 ml/min in patients on simvastatin treatment while for control subjects was 0.59± 0.04 ml/min, showed highly significantly decreased in patients on simvastatin than that in control subjects. A frequent association between oral symptoms and treatment with statins might be an indication of parotid gland's microstructural changes, functional impairment. It is obvious that statins, reducing the serum lipid profile, may play a protective role in organs injuries caused by hyperlipidemia by repairing the occurred alterations [(13) (14) (15) (16)17].

Taste detection threshold: -

This study showed that sweet detection threshold of patient on simvastatin treatment was significantly higher than that of control subjects , this may be due to drug-induced xerostomia , zinc deficiency [(18)(19)], or progress age [(20)] .Salt detection threshold in patients on simvastatin treatment had little or no differences between those patients and control subjects due to reduction of appetite which was not accompanied by any other symptoms and was fixed to a certain types of food, particularly those with a salty flavor [(21)] .Sour detection threshold in patients on simvastatin treatment was highly significantly higher than those of control subjects, this due to that patients on simvastatin were subjected to a dose-dependent drop in intracellular pH, specify a reduction in Na+/H+ exchange, with a rounding of cell shape [(22)], and because sour taste, has been famous , comes from acidic media [(23)] .Bitter detection threshold , in patients on simvastatin treatment was highly significantly higher than those of control subjects, this due to that possibility of simvastatin mechanism could damage peripheral nerves through the function of mitochondria and inhibit energy utilization by neuron [(24)], or functional changing in the nerve membranes [(25)], or statins may cause chemosensory disorders [(26)(27)].

Salivary flow rate and taste detection thresholds: -

There were highly significant positive linear correlation between salivary flow rate and the detection thresholds of sweet and sour taste and highly significant negative linear correlation for saltiness and bitterness in both study groups, these results agreed with study of Matsuo (2000) who found that various effects of saliva on the taste perception differ depending on the anatomical relationship between the taste buds and oral openings of the ducts of the salivary glands [(16)(17)].

Salivary and serum zinc in study groups: -

This study showed salivary and serum zinc were highly significantly decreased in patients on simvastatin treatment than that in control subjects, this agreed with similar study performed by Ghayour-Mobarhan [(11).]

Serum zinc was significantly decreases ($p<0.05$) in patients on simvastatin treatment in comparison with those control subjects , this result was with agreement with study by Ghayour [(11)] who found that simvastatin treatment may attribute the propagated serum depletion and alteration may be linked to the known anti-inflammatory properties of the statins class of medications , in addition , down regulate matrix metalloproteinases by simvastatin after a year of simvastatin treatment parallel to levels estimated[(28)],or antioxidant properties of statins [(29)] and decreasing in zinc-copper superoxide dismutase dependent activity and recovered endothelial response, this may be due to reduced production of superoxide anion [(30)] . There was consequent lowering serum zinc and following low salivary zinc as an outcome of their capability to minimize cholesterol biosynthesis, fundamentally in the liver, where they are selectively allocated, as well as to lipid metabolism [(31)].

This study showed no significant correlation between serum and salivary zinc and this was agreed with Hiroshi who found there was no distinct correlation between zinc in parotid saliva and serum zinc [(32)].

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المستخلص:

الخلفية: ارتفاع الدهون بالدم هو من الامراض التي تتميز بارتفاع مستوى الكوليسترول وادهون الثلاثية في الدم والتي بمجموعها تمثل الدهون في جهاز الدوران
ان الغرض من الدراسة هو مقياس عتبه التذوق وقياس مستوى العناصر النادرة (الزنك) في اللعاب ومصل الدم لمرضى ارتفاع دهون الدم والذين يتناولون علاج السمفاستاتين ومقارنتها بالاشخاص الاصحاء.

طريقة العمل: تمثلت باخذ عينات من 80 عينه قسمت الى 40 عينه من المرضى على علاج السمفاستاتين و40 من الاشخاص الاصحاء والذين تتروح اعمارهم بين (35-60) سنه. تم جمع عينات اللعاب بطريقة الغير محفزة وخلال عشر دقائق وتم جمع عينات مصل الدم من كل المشمولين بالدراسة بواسطة السحب الوريدي المباشر وتم قياس الزنك بواسطة طريقة الطيف الذري اللهبي.
قياس عتبه التذوق قد تمت بواسطة استعمال 15 محلول تذوق لكافة انواع التذوق الرئيسية الاربعة (الحلو، المالح، الحامض، والمر).
تم استخدام طريقة السكب والبصق مع الماء اللايوني كغسول فموي بين فترات فحص التذوق لكل تركيز من المحاليل.
تم استبعاد اي عينه مشاركة في هذه الدراسة والمصابه بالسكري الغذه الدرقية، جنيب الدرقية،امراض المناعة , العلاج الكيمياوي , المدخنين , الكحوليون , والمصابين بالاورام .
النتائج: اظهرت الدراسة ان علاج السمفاستاتين يقوم بتقليل مستوى جريان اللعاب في مرضى السمفاستاتين بصورة واضحة عما في عينات الاصحاء.
واظهرت الدراسة ان هناك علاقة واضحة الاهمية في ان مرضى السمفاستاتين يتذوقون الحلو والحامض والمر بمحلول اعلى تركيز ممافي عينات الاصحاء ماعدا فحص الملوحة حيث اظهرت الدراسة بان لا يوجد هناك فرق بين عينات المرضى وعينات الاصحاء.
واظهرت الدراسة ايضاً بأن عينات المرضى السمفاستاتين لديهم مستوى اقل للزنك في اللعاب الدم عما في لعاب ومصل دم عينات الاصحاء.

وقد اظهرت الراسة ان هناك علاقة خطية موجبة ذات اهمية عالية في تحسس التذوق لكل من الحلاوة والحموضة وعلاقة خطية سالبة ذات اهمية عالية في تحسس التذوق لكل من الملوحة والمرارة مع معدل جريان اللعاب.
الاستنتاج: اظهر علاج السمفاستاتين انه يقلل مستوى جريان العابويرفع عتبة التذوق للحلو والحامض والمر عدا المالح ويقلل مستوى الزنك في اللعاب والدم لمرضى ارتفاع الدهون بالدم والذين يتناولون علاج السمفاستاتين.