### Dental caries and salivary physiochemical characteristics among osteoporotic old adult women

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### ABSTRACT

Background: Osteoporosis is a systemic skeletal disorder affects general health in addition to effect on salivary physical and chemical composition which lead to an adverse effect on oral health status. This study was conducted to evaluate the concentration of glycoprotein (osteonectin) in saliva and its effect on dental caries in relation to salivary flow rate and viscosity among osteoporotic women and compared to control group.

Materials and Methods: The total sample composed of 60 females aged 60-65 years attending Al-Yarmook Teaching Hospital, 30 females diagnosed with osteoporosis by measuring bone density at the spine and femur with a dualenergy X-ray absorptiometry at T-score of >2.5, and 30 women without osteoporosis with T-score of -1 (control group). The diagnosis and recording of dental caries was through the application of  $D_{1-4}MFS$  index according to criteria of Mühlemman (1976). Stimulated salivary samples were collected under standardized condition, according to Tenovuo and Lagerlöf, (1994). The flow rate and viscosity were estimated and then the saliva was analyzed for estimation of glycoprotein (osteonectin) by using Enzyme-linked immunosorbent assay (ELISA).

Results: The percentage of dental caries occurrence was 100% among osteoporotic group and control group. Results revealed that DMFS value was higher but statistically not significant among osteoporotic women, Concerning DMFS components, the data of the present study showed that the DS was lower but statistically not significant among osteoporotic women, while MS value was significantly higher among them (t=2.044, P<0.05, df =58), on the other hand the opposite figure was found concerning filling component FS however the difference was not significant.

Correlation coefficients of caries experience with salivary flow rate revealed a weak negative not significant correlation with  $D_{1.4}$ MFS and its component.On the other hand, the data of the present study showed that salivary viscosity correlate weakly not significant in negative direction with DS and its severity and in positive direction with MS, FS, DMFS.. The correlation coefficient between salivary osteonectin and dental caries were weak significant in positive directions, the correlation were in negative direction were also found between salivary osteonectin and MS,DMFS. Concerning the relation between salivary osteonectin and physical properties of saliva, the data of the present study revealed a non-significant relation in negative direction with salivary flow rate and in positive direction for salivary viscosity.

Conclusions: Dental caries revealed lower percentage of occurrence among osteoporotic group.

Key words: Osteoporosis, Dental caries, Enzyme-linked immunosorbent assay (ELISA), flow rate, viscosity. (J Bagh Coll Dentistry 2014; 26(3):122-128).

### INTRODUCTION

Osteoporosis is a major public health concern that affects millions of women around the world. Osteoporosis is generally discovered in older age, but it is the result of many factors-modifiable and non- modifiable <sup>(1)</sup>.Bone quality is not readily quantifiable <sup>(2)</sup>. The mechanism of osteoporosis is such that the delicate balance of bone formation and bone reabsorption is disrupted, which results in long-term and uninterrupted bone loss <sup>(3)</sup>. Older women experience the onset of menopause and increased vulnerability to osteoporosis, as sex hormone deficiency (estrogen in women, testosterone in men) is similarly associated with bone loss <sup>(4)</sup>. Oral tissues and other parts of the human body are affected by aging; age-related systemic diseases and functional changes predisposed elderly patients to oral conditions <sup>(5).</sup>

Osteoporosis affect general health also has an effect on oral health. The health of a woman's body and oral cavity are bidirectional.

Saliva plays an important role in the maintenance of a healthy oral environment through its physical properties, including secretion rate and viscosity<sup>(6)</sup>. Because saliva is a fluids easily collected and contain locally and systemically derived markers of many systemic disease, they may offer the basis for the assessment and several biomarkers were quantitated in saliva collected by immunoassay <sup>(7,8)</sup>. Salivary Osteonectin is a matricellular protein regulating matrix assembly, osteoblast differentiation, and survival. The researchers indicated that osteonectin is essential for normal bone mass <sup>(9)</sup>. Osteonectin binds both collagen and hydroxyapatite and regulates collagen fibril assembly (10). It inhibits the growth of hydroxyapatite crystals leading to development of dental caries <sup>(11)</sup>.

Dental caries is the process of dynamic interaction between the tooth surface and the plaque biofilm. The balance between mineral loss

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and gain can shift to favor either re-or demineralization  $^{(12, 13)}$ .

The aims of this study were to determine thedental caries experience and their relationwith the salivary physiochemical characteristic include salivary floe rate, viscosity and osteonectin concentration among osteoporotic group andcompared with control group.

### **MATERIALS AND METHODS**

The studied sample consisted of 60 adult women (aged 60\_65 years) (30 females with osteoporosis and 30 with a presumably healthy bones). The age was recorded according to the last birthday (14). They were patients attending Al-Teaching Yarmook Hospital. Subjects demonstrating the medical disorders that were not included in the study and use of medications such as corticosteroids, thyroid hormone, insulin dependent therapy, anti-Seizure drugs and other supplements <sup>(15,16)</sup>. This study was approved by the scientific committee in the institute and a verbal consent form was obtained from each participant enrolled in this study. The patients diagnosed with osteoporosis by measuring bone density at the spine and femur with a dual-energy X-ray absorptiometry <sup>(17)</sup>.

The assessment of bone mineral density was obtained from dual energy x-ray absorptiometry (DEXA- scan) by using T-score. Osteoporosis was defined by classifying the T-score measurements according to World Health Organization (WHO) <sup>(18)</sup>: T score: < -1 is considered normal, -1 to -2.5 is considered osteoporosis.

The referral cases to the DXA investigation by consultants were randomly allocated to admit in this study. Specific information was obtained from each patients taking in consideration; the age, medical history and then, the saliva samples were obtained from patients for biochemical test.

The assessment and recording of caries experiences were done through the application of decayed (D), missing (M) and filled (F) surface index. In this study, the decayed fraction of the index was recorded according to the lesion severity using the criteria described by Mühlemann <sup>(19)</sup>. The collection of the stimulated salivary samples from the children was performed under standardized conditions according to the instructions cited by Tenovuo and Lagerlöf <sup>(20)</sup>. Straightaway after saliva collection, salivary flow rate was measured freshly and without centrifuged (after foam had all disappeared) by dividing the volume of the collected stimulated saliva in milliliter (ml) on the collection time in minute (min) <sup>(21)</sup>. Salivary viscosity was determined by

measuring the volume rate of flow through a tube of known dimensions. This was done by Using Ostwald's viscometer which is simple device for measuring the viscosity of liquid <sup>(22)</sup>. In this study, salivary viscosity was measured at room temperature and before centrifuging of salivary samples <sup>(23)</sup>. The Ostwald method is a simple and available method for the measurement of viscosity, in which viscosity of liquid is measured by comparing the viscosity of an unknown liquid with that of liquid whose viscosity is flow times of two liquids of equal volumes using same viscometer, and the coefficient of the viscosity of the saliva is determined <sup>(24)</sup>. The salivary samples were then taken to the laboratory for biochemical analysis and centrifuged at 10,000 g at 2-8° C for 15 minutes. The clear supernatant was separated by micropipette and was stored at (-20°C) in a deep freeze and further assessment for osteonectin level in saliva was done by special osteonectin kit (CUSABIO) using ELISA technique which is a quantitative sandwich enzyme immunoassay technique (Enzyme-linked immunosorbent assay). At the end of the assay, results are automatically obtained by the reading machine and then calculated in relation to the calibration curve. These results were obtained in traditional unit (ng/ml).

Intra- and inter calibration were performed to overcome any problem that could be faced during the research, and to ensure proper application of diagnostic criteria used in recording dental status through inter calibration. Statistical Analysis and processing of the data were carried out using SPSS version 18.

After exploring the data, it had been found that they were not normally distributed. The nonparametric Mann-Whitney U test was utilized for the parameters of the data which were not normally distributed and in this test the median and mean rank were used to analyze and determine the differences between the study and control groups. The correlation coefficient tests between the variables were done by using Spearman's correlation. The confidence level was accepted at the level of less than or equal to 5%. The highly confidence level was accepted at the level of less than or equal to 1%.

### RESULTS

The percentage of dental caries occurrence was found to be 100% for both groups.Results revealed that DMFS value was higher but statistically not significant among osteoporotic women, Concerning the data of the present study showed that caries-experience represented by DS was lower but statistically not significant among

osteoporotic women (Table 1), while for missing component, data analysis of the present study showed that the MS value was significantly higher among osteoporotic women (t=2.044, P<0.05, df =58), while the opposite figure was found concerning filling component FS however the difference was not significant. On the other hand the severity of dental caries represented by grades of decayed fraction among osteoporoticare seen in Table (2), the higher mean rank value was D3 than other grades of Ds, however the mean rank value of both  $D_4$ ,  $D_3$  grades were found to be higher among osteoporotic women than control group, while the opposite figure was found for both  $D_1$  and  $D_2$ , however the differences were not significant. Correlation coefficients of caries experience with salivary flow rate for both osteoporotic women and control groups are seen in Table (3).

Analysis among osteoporotic groups revealed a weak negative not significant correlation between salivary flow rate and DS, DMFS and weak negative not significant with MS, FS. While among control groups, data analysis showed weak positive not significant correlation between salivary flow rate and DS, and weak positive not significant correlation with FS, while the correlation was weak negative not significant with MS, and weak negative not significant correlation with DMFS. Correlation coefficients of caries severity with salivary flow rate are seen in (Table **4**). As the mean value of  $D_1$  is constant (all equal to 0), there is no statistical correlation between flow rate and D<sub>1</sub> among osteoporotic groups, but very weak negative not significant correlation between salivary flow rate and D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, concerning control group, the data showed weak positive not significant correlation with all grades of DS  $(D_1, D_2, D_3, D_4)$ . Table (5) illustrates the correlation coefficient of caries-experience in relation to salivary viscosity among osteoporotic women and control groups.

Analysis among osteoporotic group revealed that the salivary viscosity correlate weakly not significantly in negative direction with DS and in positive direction with MS, FS, DMFS, While among control group salivary viscosity correlate weakly not significantly in positive direction concerning DMFS, Ms and in negative direction concerning DS, FS. In addition correlation coefficients of caries severity with salivary viscosity are seen in Table 6 that shows the mean value of  $D_1$  is constant (all equal to 0), there is no statistical correlation between viscosity and D<sub>1</sub> among osteoporotic group, and weak negative not significant correlation with D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, while among control group both  $D_1$  and  $D_3$  revealed a weak positive not significant correlation with salivary viscosity as well as it correlate negatively weakly not significantly with  $D_2$  and very weakly with D<sub>4</sub>.The correlation coefficient between salivary osteonectin and dental caries represented by DMFS and its components DS, MS, FS are shown in Table (7), this table illustrate that these relations among osteoporotic groups were weak significant in positive directions concerning Ds (r=0.373, p<0.05, df=58), and weak not significant correlation in positive direction with MS, DMFS, while with FS the correlation was the same but in negative direction. While among the control groups, the figure is different as all correlation were weak negative not significant between salivary osteonectin and DS, MS, DMFS, but very weak with FS. While Concerning the correlation of salivary osteonectin with grades of dental caries, Table (8) shows that as the mean value of  $D_1$  is constant (all equal to 0), there is no statistical correlation between osteonectin and D<sub>1</sub> among osteoporotic women and very weak positive not significant correlation with D<sub>2</sub>, but weak with D<sub>4</sub>, in concerning to D<sub>3</sub> grade, data analysis showed that this relation was very weak negative not significant among both osteoporotic women and control groups. Also all remaining grades (D<sub>1</sub>, D<sub>2</sub>, and D<sub>4</sub>) in control groups revealed same weak negative not significant the correlation. In the present study data analysis showed that the relation between salivary osteonectin and salivary flow rate were weak not significant in negative direction for osteoporotic and for control group, concerning the correlation between salivary osteonectin and viscosity, the data of present study showed a weak positive not significant correlation among both osteoporotic and control groups, as shown in Table (9).

Table 1: Dental caries experience (mean± S.D.) among osteoporotic women and control groups.

<b>Dental caries</b>	Osteoporotic women		<b>Control group</b>		Statistical difference		
experience	Mean	<u>+</u> SD	Mean	<u>+</u> SD	t. test	(p-value)	
DMFS	67.17	33.85	54.70	25.95	1.60	0.12	
MS	46.80	32.30	31.47	25.40	2.04*	0.04	
FS	14.20	20.68	18.97	21.10	0.87	0.39	
DS	6.17	8.72	4.60	3.81	_	_	

	mean rank) among osteoporotic women and control groups									
Dental Caries	Oste	eoporotic zomen	Control group		Test statistics					
experience and Severity	Median	Mean Rank	Median	Mean Rank	Mann-Whitney U	Z. test	p-value			
<b>D</b> <sub>1</sub>	0.00	29.00	0.00	32.00	405.00	-1.76	0.08			
$D_2$	1.00	29.52	1.00	31.48	420.50	-0.45	0.65			
$D_3$	0.00	32.65	0.00	28.35	385.50	-1.15	0.25			
$\mathbf{D}_4$	0.00	29.07	2.00	31.93	407.00	-0.68	0.50			
DS	3.00	30.07	3.50	30.93	437.00	-0.19	0.85			

# Table 2: Dental caries experience (Ds) and Severity represented by grades of D1-D4 (median&mean rank) among osteoporotic women and control groups

Table 3: Correlation coefficients of salivary flow rate with caries	experience (DS, MS, FS, and
DMFS) among osteoporotic women and contr	ol groups

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Dontol comica	Osteop	orotic w	Con	Control group			
Dental carles	Salivary flow rate			Salivary flow rate			
experience	r	р	Sig.	r	р	Sig.	
DS	-0.32	0.08	NS	0.29	0.11	NS	
MS	-0.12	0.52	NS	-0.29	0.13	NS	
FS	-0.17	0.38	NS	0.15	0.43	NS	
DMFS	-0.26	0.17	NS	-0.12	0.51	NS	

## Table 4: Correlation coefficients of salivary flow rate with severity of dental caries (D<sub>1</sub>\_D<sub>4</sub>) among osteoporotic women and control groups

Severity of dental cariesOsteoporotic womenControl groupSeverity of dental cariesOsteoporotic womenControl groupSalivary flow rateSalivary flow rateD1pSig.rpSig.D1O.060.76NS							
Concentary of	Osteoporotic women			Control group			
Severity of	Saliva	Salivary flow rate			ary flov	v rate	
dental carles	r	р	Sig.	r	р	Sig.	
D1				0.06	0.76	NS	
D2	-0.25	0.19	NS	0.24	0.20	NS	
D3	-0.21	0.27	NS	0.06	0.77	NS	
D4	-0.05	0.78	NS	0.08	0.66	NS	

## Table 5: Correlation coefficients of salivary viscosity with caries experience (DS, MS, FS, and DMFS) among osteoporotic women and control groups

Destal	Osteoporotic women Contro				trol gro	rol group	
Dental carles	Viscosity			Viscosity			
experience	r	р	Sig.	r	р	Sig.	
DS	-0.03	0.89	NS	-0.06	0.75	NS	
MS	0.04	0.85	NS	0.10	0.60	NS	
FS	0.07	0.71	NS	-0.04	0.85	NS	
DMFS	0.06	0.74	NS	0.05	0.79	NS	

Table 6: Correlation coefficients of salivary viscosity with severity of dental caries (D <sub>1</sub> -I	<b>D</b> 4)
among osteonorotic women and control groups	

Comoritan of	Osteop	orotic w	Control group			
Severity of	V	iscosity	Viscosity			
uentai caries	r	р	Sig.	r	р	Sig.
$\mathbf{D}_1$				0.14	0.48	NS
$D_2$	-0.05	0.78	NS	-0.31	0.10	NS
$D_3$	-0.13	0.51	NS	0.04	0.83	NS
$D_4$	-0.06	0.74	NS	-0.04	0.82	NS

	Osteon	orotic w	omen	Control groun		
Dental caries	Osteop	teonecti	n	Ost	in	
experience	r	р	Sig.	r	р	Sig.
Ds	0.37*	0.04	Sig	-0.22	0.24	NS
Ms	0.23	0.23	NS	-0.26	0.17	NS
Fs	-0.14	0.48	NS	-0.05	0.80	NS
DMFS	0.21	0.28	NS	-0.33	0.08	NS

 Table 7: Correlation coefficients of salivary osteonectin with caries experience (DS, MS, FS, and DMFS) among osteoporotic women and control groups

Table 8: Correlation coefficients of salivary osteonectin with severity of dental caries (D1-	<b>D</b> 4)
among osteoporotic women and control groups	

among oscoporotic women and control groups								
Severity of	Osteop	orotic w	vomen	Control group				
	Os	teonecti	Osteonectin					
uentai caries	r	р	Sig.	r	р	Sig.		
<b>D</b> <sub>1</sub>	_			-0.08	0.69	NS		
$D_2$	0.05	0.79	NS	-0.14	0.47	NS		
$D_3$	-0.10	0.60	NS	-0.08	0.66	NS		
$\mathbf{D}_4$	0.31	0.10	NS	-0.19	0.31	NS		

 Table 9: Correlation coefficients of salivary osteonectin with physicochemical characteristics

 among osteonorotic women and control groups

among osteoporotie women and control groups								
Dhaai oo ah ami oo l	Osteop	orotic w	omen	Control group				
Physicochemical	Os	teonecti	n	Osteonectin				
characteristics	r	р	Sig.	r	р	Sig.		
Salivary flow rate	-0.22	0.24	NS	-0.11	0.56	NS		
Salivary viscosity	0.36	0.05	NS	0.25	0.19	NS		

### DISCUSSION

Aging is the accumulation of changes in a person over time; it is an important part of all human societies reflecting the biological changes that occur <sup>(25,26)</sup>. Osteoporosis is a major public health problem all over the world: it remains the major epidemiological burdens of postmenopausal women<sup>(27)</sup>, and could affect oral health. Thus, the elderly could help live a healthier and more meaningful life with the prevention of these diseases <sup>(28)</sup>. In terms of caries experience among osteoporotic women group, the present study revealed that in spite of no significant differences between both groups but the mean rank level of decade surfaces in osteoporotic groups is slightly lower than control one, also there is a weak negative not significant correlation between salivary flow rate and caries experience. The salivary flow rate plays an important role in relation to dental caries because the washing action of saliva as well as its protective constituents increased with increase flow rate. This result was in agreement with the results reported by many previous studies <sup>(29-30)</sup>, and was in disagreement with the results that concluded by others <sup>(31-35)</sup>. Another explanation could be given to the salivary viscosity, as the result of present study showed an inverse but not significant relation between salivary viscosity and dental caries, this result agree with many studies that positive correlation found between а concentration of mucins and salivary viscosity that are known to have a protective role toward oral surfaces and their absence has been associated with an increased prevalence of dental caries in adults <sup>(36-41)</sup>, but in contrast result shown by others  $^{(42,43)}$  reported a weak non-significant positive correlation, in addition other explanation could be given to the salivary osteonectinthat showed positive correlation with statistical significance with DS, this is due to physiological role of osteonectin as a protein associated with the formation of collagen containing mineralizing tissues like human bone, as well as human dentin and cement, it inhibits the growth of hydroxyapatite crystals leading to development of dental caries. This result was in agreement with the results reported by many previous studies (44- $^{47)}$ , and was in disagreement with the results that concluded by others  $^{(48-50)}$ . Concerning the correlation of the salivary osteonectin with flow rate, data analysis of the present study reported a weak not significant in negative direction for osteoporotic group. This is in agreement with some previous studies <sup>(35, 51)</sup>. This may be explained by the fact that slight increase in salivary flow rate among them leadto decrease protein concentration (osteonectin)<sup>(10, 52-54)</sup>.

The correlation of the salivary osteonectinwith viscosity was found to be a positive relation in both osteoporotic and control groups. The explanation for this can be attributed to the fact that the salivary osteonectin is one of the important protein components of human saliva, as the main contributor to salivary viscosity is the mucous glycoprotein <sup>(30,55,56)</sup>. In addition the concentrations of proteins, increased in subjects with moderate and severe inflamatory disease while flow rate decreased, this is showed by others <sup>(57-59)</sup>.

In conclusion, Osteoporosis associated with decreased bone mineral density had an effect on oral health status. This was affected by changes in physiochemical characteristic especially osteonectin. Therefore old adult women may need special oral health preventive and educational programs.

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