

Research Article

Oral health status of salivary vitamin D and estradiol in osteoporotic postmenopausal Iraqi women

Ameera Yaseen Alamiry¹, Areej Ahmed Najm^{2*}, Nurulhuda Mohd

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1 Al karkh Health Department / Specialized Dental Center in Baghdad, Iraq.

2 Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Iraq.

3 Department of Restorative Dentistry, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur 50300, Malaysia.

*Corresponding author: areejah2004@yahoo.com

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Abstract: Background: This study aimed to investigate the association between oral health with salivary vitamin D and estradiol in postmenopausal women in Iraq. Materials and Methods: in this cross-sectional comparative study, 90 participants were divided into three groups based on dual-energy X-ray absorptiometry data either (i) osteoporosis (ii) osteopenia; or (iii) healthy control. The questionnaire was used to measure level of xerostomia. Clinical parameters, including clinical attachment loss and missing teeth were recorded. Salivary flow rate was measured; and the salivary vitamin D and estradiol levels were evaluated using the enzyme-linked immunosorbent assay. Result: In the osteoporosis group, the average clinical attachment loss was significantly greater and statistically significant, while the missing teeth was considerably higher in the osteopenia group. Meanwhile, the control group showed the highest levels of salivary flow rate, vitamin D, and estradiol. A negative association was found between estradiol and tooth loss in the osteoporosis group. On the contrary, a moderately positive correlation was found between vitamin D and salivary flow rate in the control group. Conclusion: Osteoporosis negatively impacted oral health by increasing the number of missing teeth and clinical attachment loss and decreasing the flow rate of saliva. Salivary vitamin D and estradiol correlate with tooth loss and salivary flow rate, among other oral health conditions. Thus, a person's level of dental health can be an early warning sign of osteoporosis.

Keywords: DEXA , Osteoporosis, Vitamin D, Estradiol, Xerostomia.

Introduction

Osteoporosis is a major public health problem worldwide and it remains a significant epidemiological burden of postmenopausal women and could affect oral health [1]. Periodontal disease is an inflammatory disorder that affects the periodontium, which includes the alveolar bone, gingiva, cementum, and periodontal ligament. Periodontal disease affects between 10% and 15% of the global population [2,3]; the association between osteoporosis and the postmenopausal state is driven by a decrease in estrogen levels forming bone-resorbing cytokines, which can contribute to the onset of a series of diseases, including osteopenia and osteoporosis [4]. Thus, women's menopause stage is a very high-risk time for this disease [5]. Postmenopausal osteoporotic women are more prone to periodontal diseases [6]. The increase in osteoporosis and periodontal diseases augments with the rise in women's age. The body's bone mineral density decreases by 1% annually in postmenopausal women because of the rapid decline in estrogen levels [7,8].

Hyposalivation is an objective, quantifiable measure of reduced saliva flow rate (SFR), whereas xerostomia is a subjective measure when the patient reports a daily feeling of dry mouth [9]. Age plays a role in xerostomia prevalence. The functioning of the remaining salivary glands compensates for the absence of acinar

tissue. Dental erosion, demineralization, dental caries, periodontitis, and intraoral infections such as oral candidiasis, halitosis, burning sensation, difficulty chewing, vocal dysfunction, dysgeusia, and dysphagia are all made worse by insufficient saliva production [10,11].

Estradiol (E2) is the primary female sex hormone responsible for developing and regulating the female reproductive system and secondary sex characteristics [12,8]. It can affect oral mucosa directly or through neural mechanisms, thereby altering the periodontal health in menopausal women [13].

Vitamin D (vit. D) is a secosteroid hormone produced by human skin cells in response to UV light or obtained through food [14]. In addition, vit. D is crucial for calcium, bone metabolism, and homeostasis [15]. Mineralization and maintenance of tissue integrity [16] are the major purpose of vit. D and are vital for maintaining oral health..

This study aimed to evaluate the oral health status, salivary vit. D and E2 between osteoporotic, osteopenic, and control postmenopausal Iraqi women.

Materials and Methods

The research protocol was approved by the Iraqi Ministry of Health and the Ethical Committee at the University of Baghdad/College of Dentistry (433/2021/12/27) for the research. Convenience sampling was used in this study involving post-menopausal women in Dexa-Scan Center, Baghdad Medical City, Iraq. The study was conducted from January until May 2022. Only postmenopausal women with amenorrhea lasting more than one year were included in the study. However, those with a history of diabetes, thyroid, or autoimmune disease; cancer treatment; history of periodontal surgery; or the use of corticosteroids or supplements were excluded. Written consent was obtained from all the participants.

Dual-energy X-ray absorptiometry (DEXA) (DEXXUM3, Osteo Sys Co. Ltd., Seoul, Korea) was used to assess bone mineral density (BMD) of the spine and femur bones. Participants were categorized into three groups based on Dualenergy X-ray absorptiometry (DEXA) findings:

- A. Osteoporotic group: T-Score -2.5 .
- B. Osteopenic group: T-Score -1.0 .
- C. Control group: T-Score <-1.0 .

A self-administered questionnaire was distributed to participants in order to assess the level of xerostomia (XI) Table (1) [17]. Following to that, the collection of unstimulated whole saliva was carried out according to the protocol by Tenovuo and Lagerlöf [18]. The participant was advised to refrain from intake of any food or beverage one hour prior to saliva collection. Smoking and chewing gum also were prohibited during this hour. Whole unstimulated saliva was taken by spitting for 10 min in a sterilized graduated test tube between 9 and 11 a.m. The salivary flow rate was calculated by dividing the collected saliva in ml by the time in minutes [19]. The collected saliva samples were centrifuged at 3000 rpm for 15 min and stored at -20°C until further used for biochemical analysis. Vitamin D and estradiol ELISA kit (YL biotech, Shanghai Yehua, China) was used to assess the vitamin D and estradiol (E2) levels using the Enzyme Linked Immunosorbent Assay (ELISA). All participants underwent oral examinations under standardized conditions [20]. The number of missing teeth (MT) was calculated, and then all teeth present in the mouth were examined by CPI probe to record loss of attachment from the index teeth. As shown in Figure 1, the difference between the distance from the cemento-enamel junction to the free gingival margin minus the pocket depth measurement at the same place was used to compute clinical attachment loss (CAL).

Using the G power program with a power of study 80%, an alpha error of 0.05, and an effect size of 0.035 for study with three groups, the required sample size is 84 subjects, therefore, having 90 subjects is sufficient for this study.

Statistical analysis

The Statistical Package for Social Research was used to conduct the statistical analysis version 22 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was employed and data with normal distribution were analyzed using ANOVA and the relationship was determined using Pearson’s Correlation were used. Means with standard deviations were used to summarize the data. Statistical significance was set at the 95% confidence level ($\alpha = 0.05$), in which a p-value of < 0.05 was considered statistically significant.

Table 1. Questionnaires for Xerostomia Inventory [17].

Complaint	Never	Hardly Ever	Occasionally	Fairly Often	Very Often
1. I sip liquids to help swallow food	1	2	3	4	5
2. My mouth feels dry when eating a meal.	1	2	3	4	5
3. I get up at night to drink.	1	2	3	4	5
4. My mouth feels dry.	1	2	3	4	5
5. I have difficulty eating dry foods.	1	2	3	4	5
6. I suck sweet or cough lollies to relieve dry mouth.	1	2	3	4	5
7. I have difficulties swallowing certain foods.	1	2	3	4	5
8. The skin of my face feels dry.	1	2	3	4	5
9. My eyes feel dry.	1	2	3	4	5
10. My lips feel dry	1	2	3	4	5
11. The inside of my nose feels dry	1	2	3	4	5

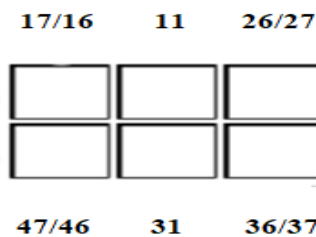


Figure 1. Index teeth for CAL (clinical attachment loss).

Results

The present study sample included a total of 90 postmenopausal women divided into three groups (30 osteoporosis, 30 osteopenia, and 30 healthy control). Table 2 shows the difference in mean of (SFR, CAL, XI, and several missing teeth) among the studied groups, the average CAL was substantially higher and statistically significant in the osteoporosis group, while a statistically significant higher rate of tooth loss was in the osteopenia group. In contrast, the control group had the greatest SFR. The difference in mean salivary biomarkers among the groups, vitamin D, and estradiol showed the highest level in the control group with a significant difference, as shown in Table 3. Regarding the correlations between the variables with oral salivary vit. D and E2, the osteoporosis group showed a moderate negative correlation

between estradiol and tooth loss, whereas, a moderate positive correlation between vit. D and SFR was found in the control group, as shown in Table 4.

Table 2. Comparison of study variables between groups.

Variable:	Groups	Mean	±SD	±SE	Mini.	Maxi.	F	P value
SFR	Osteoporosis	0.594	0.122	0.022	0.400	0.900	18.269	0.000 S
	Osteopenia	0.603	0.107	0.019	0.400	0.800		
	Control	0.743	0.090	0.016	0.600	0.900		
XI	Osteoporosis	31.367	10.17607		16.0	47.0	2.452	0.092 N
	Osteopenia	32.867	9.1792	1.675	15.0	46.0		
	Control	27.733	8.2417	1.504	15.0	40.0		
MT	Osteoporosis	8.567	6.146	1.122	.0	23.0	4.053	0.021 S
	Osteopenia	8.867	6.611	1.207	.0	22.0		
	Control	4.933	4.996	0.912	.0	17.0		
CAL	Osteoporosis	3.421	2.360	.431	.000	6.500	6.904	0.002 S
	Osteopenia	1.472	2.129	.389	.000	5.000		
	Control	1.650	2.240	.409	.000	6.000		

*NS-non-significant, *S-significant

Table 3. Difference in mean biochemical markers between the groups.

Variables	Groups	Mean	P value
Vitamin D	Control	27.95	0.00
	Osteopenia	16.05	
	Osteoporosis	16.05	
Estradiol	Control	161.71	0.00
	Osteopenia	161.71	
	Osteoporosis	8.02	

Discussion

Although osteoporosis is a well-known serious public health problem linked to higher morbidity, mortality, and medical expenses among the elderly, there is insufficient information about oral health and its relationship to salivary vit. D and E2 in postmenopausal osteoporotic women in Iraq. Osteoporosis and control groups were seen with different salivary flow rates. The control group has a higher salivary flow rate than osteopenia and osteoporosis, which is consistent with Mirzaii-Dizgah & Agha-Hosseini [21]. Reduced salivary flow reduces saliva quantity and changes the quality [22], thereby accelerating Candida albicans infection, tooth decay, and periodontal disease. Low salivary flow also affects chewing, swallowing, and speech [22,23].

Table 4. Correlation between oral health variables and salivary biomarkers.

Groups		Estrogen		Vit D	
		r	p	r	p
Osteoporosis	MT	-0.36	0.04	0.01	0.92
	Flow rate	-0.10	0.57	-0.15	0.42
	CAL	0.05	0.79	0.09	0.61
	(XI) score	-0.02	0.88	-0.29	0.11
osteopenia	MT	-0.01	0.92	-0.22	0.23
	Flow rate	0.19	0.29	0.19	0.31
	CAL	-0.13	0.47	0.00	0.99
	(XI) score	0.04	0.80	-0.07	0.69
control	MT	0.18	0.31	0.13	0.49
	Flow rate	-0.02	0.91	0.37	0.04
	CAL	0.19	0.31	-0.19	0.30
	(XI) score	0.06	0.75	0.03	0.85

This study indicated that missing teeth were much greater in patients with osteoporosis and osteopenia than in control groups, thereby implying that bone problems can cause tooth loss through periodontal infections or dental caries. The osteoporotic group had more toothless participants [24-26]. Famili et al. observed no link between edentulism, periodontal disease, and systemic bone loss [27]. This variation may be due to study methodologies, osteoporosis periods, educational levels, oral hygiene practices, and dental care practices of patients.

The osteoporosis group's CAL was much higher than the control group's. These findings matched with Penoni et al. [28] but are not consistent with the study done by Sultan et al. [29]. These contradicting study findings may be due to variances in periodontal disease, duration after menopause, and clinical procedures used to evaluate osteoporosis and periodontal health.

Menopause is associated with the symptom of dry mouth, which is a common complaint among the elderly. However, what exactly triggers this feeling in women remains up for debate, but it seems to be linked to the gradual loss of estrogen that occurs with aging [30]. This study confirmed the findings of Arbabi-Kalati et al., who found that the mean xerostomia inventory score was more significant in the osteopenia and osteoporosis groups than in the control group. The differences were not statistically significant [31]. Postmenopausal women who have bone mineral density loss may sense an increase in the sensation of mouth dryness according to Mirzaii-Dizgah and Agha-Hosseini [21]. However, the current investigation did not confirm this connection. This discrepancy could be due to some reasons, such as the study's design or the patient's age. The current study found lower levels of E2 in the osteoporosis and osteopenia groups than in the control group, which was consistent with Jabber et al., who found E2 deficiency accelerates bone turnover [32], thereby interfering with calcium uptake by the intestines and increasing calcium excretion [30].

Given that estrogen receptors may be found in salivary glands, oral mucosa, fibroblasts, and osteoblasts, a variation in estrogen concentration may impact the oral cavity [33]. The current study discovered a

moderate negative association between E2 and tooth loss in the osteoporosis group. However, no link was found in the other two groups, and no connection was established between E2 and CAL, SFR, and XI scores in any of the analyzed groups. Women who utilized hormone replacement treatment had a much-reduced incidence of tooth loss than those who did not [34]. Norderyd et al. found no discernible variations in attachment loss or alveolar bone loss in the estrogen supplement group compared to the control group [35].

The current investigation showed lower salivary vit. D levels in osteoporosis and osteopenia than in the healthy group. The health of the oral cavity can be strongly impacted by vit. D control over calcium phosphate metabolism, bone remodeling, and anti-inflammatory and immunomodulatory actions [36]. There was a connection between low vit. D levels and the development of recurrent aphthous stomatitis, development and response to therapy of oral squamous cell carcinoma, and the severity of periodontal disease [37,38,39].

Salivary vit. D in this study showed a moderate positive association with SFR in the control group but did not substantially connect with CAL, missing teeth, and XI, thus agreeing with the findings of Pavlesen et al., who found no association between vit. D status in postmenopausal women with tooth loss [40].

Conclusion

Oral health status is greatly affected by low bone mineral density. The SFR was directly proportional to bone mineral density. XI, CAL, and MT were higher in the osteoporotic group. Salivary estradiol hormone had a significant inverse relationship with tooth loss, while vit. D was positively correlated with SFR. Salivary vit. D and E2 biomarkers can be used as a simple, non-invasive method for the diagnosis of osteoporosis.

Conflict of interest

The authors have no conflict of interest to declare

Author contributions

AYA and AAN; study conception and design. AYA; data collection. AYA and AAN; Methodology, statistical analysis and interpretation of results. AYA; original draft manuscript preparation. AYA and AAN; Writing - review & editing. Supervision; AAN. Both authors reviewed the results and approved the final version of the manuscript to be published. As for NM, some modifications and notes have been added.

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حالة صحة الفم و علاقتها بفيتامين د والإستروجين اللعابي في النساء العراقيات المصابات بهشاشة العظام اميرة ياسين العامري , واريح احمد نجم , نورالهدى محمد المستخلص

هدفت هذه الدراسة إلى تقييم حالة صحة الفم فيما يتعلق بفيتامين د اللعابي والإسترايول ومقارنتها بين النساء العراقيات المصابات بهشاشة العظام بعد سن اليأس. الطريقة: تم استخدام تصميم دراسة مقارنة واجريت الدراسة في مدينة بغداد الطبية ، في قسم اشعة هشاشة العظام. وفقاً لنتائج الاشعة، تم تقسيم 90 امرأة بعد سن اليأس إلى ثلاث مجموعات (30 حالة هشاشة العظام ، و 30 ضعف العظام، و 30 مجموعة الضابطة صحية). تم جمع عينات اللعاب غير المحفزة ثم عدداً الأسنان المفقودة وفقدان التعلق ومعدل تدفق اللعاب وشدة جفاف الفم سيتم قياسها سريريًا. تم إجراء تقديرات لفيتامين د اللعابي وهرمون الاسترايول باستخدام تقنية الاليزا. النتائج: هناك علاقة سلبية بين استرايول وفقدان الأسنان ، بينما يوجد ارتباط إيجابي بين فيتامين دي و معدل تدفق اللعاب. الخلاصة: هشاشة العظام أثرت سلباً على صحة الفم عن طريق زيادة عدد الأسنان المفقودة ، فقدان التعلق وتقليل تدفق اللعاب. لذا فإن حالة صحة الفم ستكون تنبئها لاحتمال وجود هشاشة العظام.