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The Association between Saliva and Serum Vitamin D with Knee Osteoarthritis

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ABSTRACT

Background: The incidence of osteoarthritis (OA) is high in patients suffering from 25-hydroxyvitamin D3 (25(OH)D) deficiency. The goal of this study is to examine the association between saliva and serum 25(OH)D and knee OA. **Methods:** Serum and saliva 25(OH)D levels of 30 patients with knee OA and 30 matched healthy people in a control group were measured by ELISA. Knee pain was assessed by Western Ontario and McMaster Universities Arthritis Index (WOMAC). Data were analyzed through student's *t-test*, Pearson correlation test and receiver operating characteristic (ROC). **Results:** The mean serum and saliva 25(OH)D levels were lower in knee OA group than the healthy group. WOMAC negatively correlated with serum (r = -0.37; *P* = 0.02) and with unstimulated (r = -0.30; *P* = 0.04) saliva 25(OH)D. The unstimulated saliva 25(OH)D cutoff value was 27.8 pg/m1 regarding the diagnosis of knee OA. **Conclusion:** Serum 25(OH)D levels were positively associated with saliva 25(OH)D, and 25(OH)D level in saliva, as in serum, was low in knee OA.

Keyword: *Knee osteoarthritis; 25- hydroxycholecalciferol; Saliva*

Introduction

Osteoarthritis (OA) is the most common chronic joint disease and the main cause of disability in individuals. It also has a significant financial burden on the health system (Cross *et al.*, 2014, Litwic *et al.*, 2013). Knee OA is a common musculoskeletal disorder accounting for 83% of all types of osteoarthritis. It is a common, progressive and degenerative musculoskeletal disorder which causes about 83% of all type of osteoarthritis (Hussain *et al.*, 2017). The occurrence of knee OA is higher in women (13%) than men (10%) at the age of above 60 (Johnson and Hunter, 2014). With increasing life expectancy and aging in total population, it is projected to rise further (Zhang and Jordan, 2010). It is estimated that more than 250 million people worldwide are affected by knee OA (Hussain *et al.*, 2017). By 2050, 130 million people over 60 will have OA worldwide (Zheng *et al.*, 2019). It mostly occurs after the age of 50, but, it can also happen among young people (March *et al.*, 2014).

Knee OA is one of the most important causes of physical disability. In fact, many patients with knee OA undergo complete knee surgery which is

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costly for the community (Weinstein *et al.*, 2013). Because of the lack of treatment for OA, there is a need for cost-effective solutions to prevent this disease (Zhang and Jordan, 2010). Among the emerging risk factors regarding knee OA, there is a great focus on hypovitaminosis D, which is commonly reported as low serum levels of 25-hydroxyvitamin D (25(OH)D). Lack or insufficiency of cholecalciferol is a global health problem affecting approximately one billion people worldwide (Holick, 2007). A pathophysiological function of cholecalciferol metabolites in OA is confirmed by the existence of their receptor in cartilage, bone, and muscle (Cao *et al.*, 2013). In addition, cholecalciferol can decrease bone turnover and cartilage destruction, inhibiting the progression and development of knee OA (McAlindon *et al.*, 2013). Accordingly, a possible relationship between low serum cholecalciferol levels and OA may arise through effects on cartilage metabolism, bone metabolism, or both (Bergink *et al.*, 2016).

Currently, synovial fluid (SF) is an option for evaluating OA. However, because of invasive sampling technique, assessing disease activity and drug response through SF tests becomes impossible. Saliva has been shown to provide useful data. It contains a good analytical fluid which can be accumulated pleasantly, and reserved easily. When it is equated to other bodily fluids used in clinical laboratories, it is economic as well (Agha-Hosseini *et al.*, 2011, Agha-Hosseini *et al.*, 2012, Agha-Hosseini *et al.*, 2015, Agha-Hosseini *et al.*, 2011, Agha-Hosseini *et al.*, 2012, Mirzaii-Dizgah *et al.*, 2016, Mirzaii-Dizgah and Riahi, 2013).

There is no information on salivary levels of 25(OH)D in patients with knee OA. The purpose of the present study is to evaluate the salivary level of 25(OH)D in patients with knee OA and in a matched healthy control group, as a potential biological marker of the disease.

Materials and Methods

Study protocol and participants: Based on the Kellgren and Lawrence system (Altman *et al.*, 1986), 30 knee OA patients (19 male/11 female) admitted to Firozgar Hospital in 2019 participated in the study. Furthermore, 30 healthy individuals (19 males/11 females) from the university staff and patient companions were also recruited. All of the patients had grades 2 or 3 of knee OA.

Measurements: Knee pain was assessed by a self-administered questionnaire which included the Western Ontario and McMaster Universities Arthritis Index (WOMAC) (Ebrahimzadeh *et al.*, 2014). The WOMAC consists of 24 items, each scored from 0 to 4, yielding a total score from zero to 96. Higher scores indicate the higher severity of the disease.

Venous blood and saliva were obtained from all the participants in the morning at the same time. For saliva sampling, the participants washed their mouths, and then, swallowed the fluid in their mouths. After that, 2–3 ml of the whole unstimulated saliva was collected in a microtube. They were then asked to chew a piece of natural gum with a certain size. Two minutes after chewing, people either threw out or swallowed their saliva. They continued chewing gum, and collected the whole saliva in the microtubule. Immediately after saliva collection, the authors drew venous blood. The samples were centrifuged at 5000 rpm for 10 minutes; then, serum and saliva supernatant were stored at -80 °C for subsequent measurement of 25(OH)D.

Human 25(OH)D ELISA kits were provided from Padtan Gostar Isar Co. (Tehran, Iran). The ELISA kit used in this study was designed for a total of 25(OH)D. 25(OH)D level was measured according to the manufacturers' instruction.

Ethical considerations: The study was approved by the ethics committee of National Institute for Medical Research Development, Deputy of Research and Technology, Ministry of Health and Medical Education of Iran (Ethic Code: IR.NIMAD.REC.1396.206). Moreover, a written informed consent was obtained from all participants.

Data analysis: Data were expressed as mean ± SD. Student's *t-test* was used to compare mean

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scores between groups. Pearson correlation test was used to determine the relationship between parameters. ROC was used to detect the cutoff point for saliva 25(OH)D between knee OA and healthy participants. P-value < 0.05 was considered statistically significant. Analyses were performed using SPSS 16 software.

Results

The mean (±SD) age regarding the knee OA group and control group was 55.3 ± 3.4 and 54.5 ± 3.2, respectively. The mean of serum as well as stimulated and unstimulated saliva 25(OH)D levels were lower in knee OA patients than the healthy group (**Table 1**).

There was a moderate correlation between the unstimulated salivary 25(OH)D and its serum level (r = 0.48; *P* =0.02). But, serum and stimulated salivary 25(OH)D level were not significantly correlated (r = 0.10; *P*=0.68).

The mean (±SD) WOMAC was 40.7 ± 4.9 in the knee OA group. WOMAC negatively correlates with serum 25(OH)D (r = -0.37; *P* = 0.02) and with unstimulated saliva 25(OH)D (r = -0.30; *P* = 0.04). There was no significant correlation between WOMAC and stimulated saliva 25(OH)D (r = -0.29; *P* = 0.06).

Regarding the sensitivity of 81%, the specificity of 85%, and the area under the ROC curve of 0.88, the cutoff value of unstimulated saliva 25(OH)D was 27.8 pg/m1 for the diagnosis of knee OA.

Table 1. Concentrations of 25-hydroxyvitamin D in serum, unstimulated, and stimulated saliva of patients suffer knee OA and control individuals.

Variables	Healthy	Knee OA	P-value[a]
Serum 25-hydroxyvitamin D (ng/ml)	65.1 ± 4.6[b]	48.2 ± 6.7	0.041
Unstimulated saliva 25-hydroxyvitamin D (pg/ml)	36.1 ± 3.1	25.9 ± 0.7	0.016
Stimulated saliva 25-hydroxyvitamin D (pg/ml)	28.1 ± 0.9	24.2 ± 1.5	0.022

[a]: Student t-test, [b]: Mean ± SEM, OA: Osteoarthritis.

Discussion

Osteoarthritis and cholecalciferol deficiency are common health problems among the elderly. Cholecalciferol levels have been associated with progression of OA, decreased cartilage thickness, knee osteophyte formation, poor radiological grading, and poor functional status. Cholecalciferol can decrease bone turnover and cartilage destruction, possibly preventing the development and progression of knee OA (McAlindon *et al.*, 2013). Epidemiological studies demonstrated that low serum 25(OH)D levels were associated with greater knee pain, a higher prevalence of radiographic knee osteoarthritis, and a higher risk of progression (Bergink *et al.*, 2009).

Magnetic resonance imaging (MRI and evaluation of some biomarkers in SF can help diagnose and track OA (Veronese *et al.*, 2018). Since MRI is expensive and taking SF is an invasive procedure, evaluation of salivary biomarkers is considered an alternative means of SF and serum. In this study, the salivary and serum values of 25(OH)D among knee OA group and healthy group were investigated.

The results indicated that the mean score of 25(OH)D in the serum of patients was less than healthy people. It is inconsistent with many previous studies (Arya and Agarwal, 2014, Heidari *et al.*, 2011, Malas *et al.*, 2014, Zhang *et al.*, 2014). As in previous studies, the results support the involvement of 25(OH)D in the incidence of OA. Accordingly, the results of this study suggested an inverse significant association between serum 25(OH)D and WOMAC (Heidari *et al.*, 2011).

Saliva is regarded as a diagnostic fluid in future due to rapid, simple, and non-invasive access to it (Mirzaii-Dizgah *et al.*, 2019, Mirzaii-Dizgah *et al.*, 2020, Mominzadeh *et al.*, 2014). In order for saliva to be a plasma substitute for various biological assays, there must be a high correlation between plasma levels and salivary parameters. The results of this study demonstrated that stimulated and

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unstimulated saliva as well as serum level of 25(OH)D in patients with knee OA were significantly lower than healthy participants. Besides, there was a correlation between the level of serum and unstimulated saliva 25(OH)D. It can be concluded that the salivary level of 25(OH)D approximately reflects its serum concentration; therefore, salivary-based assays of 25(OH)D may have the potential to be used in the diagnosis of knee OA.

Today, the measurement of 25(OH)D is often used for its total concentration. This overlooks the possible importance of free 25(OH)D in the blood. In this regard, the measurement of free 25(OH)D can be particularly addressed. It is difficult to directly measure its free amount in the blood. This is because 25(OH)D binds strongly to its transfer protein and also due to technical problems. The measurement of steroid hormones in saliva indicates its free plasma concentration (Fairney and Saphier, 1987). 25(OH)D appears to enter saliva by diffusion through salivary gland cells, but conjugated 25(OH)D enters saliva by ultrafiltration. Since the salivary level of 25(OH)D may reflect the free level of 25(OH)D in plasma and most of the 25(OH)D binds to the protein in the plasma, the concentration of 25(OH)D in saliva is very low.

There were some limitations regarding the current study. For example, the authors did not assay 25(OH)D in SF due to ethical limitations, especially in the control group. So, they could not analyze the correlation of 25(OH)D with serum, saliva, and SF.

Conclusion

25(OH)D level in saliva as in serum was low in knee OA patients. Serum 25(OH)D levels correlated positively with unstimulated whole saliva 25(OH)D in knee OA. Thus, salivary 25(OH)D levels are associated with knee OA.

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Authors' Contributions

The corresponding author was involved in all aspects of conceptualizing and designing the study, the acquisition of data, and the analysis and interpretation of data. All authors made substantial contributions to designing this research and were involved in writing the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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