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Reduction of Saliva and Serum 25-Hydroxycholecalciferol in Multiple Sclerosis

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Abstract

Methods: The prevalence of multiple sclerosis (MS) is high in patients suffering from 25-hydroxyvitamin D3 [25(OH)D] deficiency. The aim of this study was to investigate the possible association of saliva and serum 25(OH)D concentrations with MS in women.

Methods: Serum and saliva 25(OH)D levels of 30 MS women and 30 matched healthy controls in this case-control study were measured by ELISA. Data were analyzed by unpaired two-tailed student's t-test, Pearson correlation test and Receiver operating characteristic (ROC).

Results: The mean levels of 25(OH)D in serum and in both stimulated and unstimulated saliva were significantly lower in patients with MS. Serum levels of 25(OH) significantly correlated with stimulated (r = 0.575; P=0.003) and unstimulated saliva (r = 0.548; P=0.004). The mean (±SD) EDSS was 3.6±1.9 in the MS group. EDSS was not significantly correlated with 25(OH)D in serum or in stimulated and unstimulated saliva. The cut-off points of 25(OH)D in serum and saliva were 18 ng/ml and 65 pg/ml, respectively.

Conclusions: 25(OH)D level in saliva like in serum was low in MS women. Serum 25(OH)D levels correlated positively with saliva 25(OH)D in women suffering from MS.

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Introduction

Multiple Sclerosis (MS) is the most usual inflammatory demyelinating defect of the central nervous system (CNS) causing axonal destructions (1). The incidence of MS is increasing in Asia as well as Iran (2). The incidence of MS in females, especially in young individuals, is high (2). The origin of MS is not clearly implied, but it is highly considered to be an autoimmune disease happening in inherently vulnerable persons who subsequently experience environmental agents (3).

Databased surveys have revealed that vitamin D has a strong immunomodulatory function (4), remarkably affecting the adjustment of immune reactions, with the overall outcome of decreasing inflammatory reactions (5). Moreover, vitamin D supplementation increases IL-10 (6), reduces IL-6, IL-12, IL-17, TNF-α and PGE2 (7,8) and attenuates B-cell immunoreactivity (9).

Today, several studies are to confirm a relationship between low levels of 25-hydroxyvitamin D3 [25(OH)D] -as a metabolite of vitamin D- and the beginning and progress of MS and also disease vulnerability (10,11). It has been shown that low level of 25(OH)D is correlated with an augmented MS risk (12). On the other hand, the risk of MS reduced with increasing 25(OH)D intake (13).

Up to the present time, a specific recognitional test of MS is not available, and recognition depends on disease background, clinical assessment, MRI, and accessory tests, which mostly comprise the examination of cerebrospinal fluid (CSF) (14). Therefore, new biomarkers, which can be checked in combination with the present clinical findings, are essential for a more precise and early diagnosis. They are also essential to assume prognoses, to check the disease and to recognize the effectiveness of treatment. Today, CSF is an option for evaluation of MS and other CNS disorders (15). However, because taking CSF is a very invasive procedure and may damage the spinal cord, routine use of CSF in the diagnosis and monitoring of disease and medication is not appropriate. As saliva getting is a non-invasive procedure, it makes sense to examine biomarkers in saliva to diagnose and monitor disease. There are few studies about biomarkers in saliva of MS (15,16).

It has been revealed that saliva, as a reflection of oral and systemic health, offers useful data. Saliva presents a good analytical fluid, which can be accumulated pleasantly, is easy to reserve, and is also economic when equated to other bodily fluids used in clinical laboratories (17).

There is no information about salivary 25(OH)D levels in MS patients, thus, the objective of the present study was to evaluate the levels of salivary 25(OH)D in women with MS and in healthy matched controls.

Materials and Methods

Study protocol and subjects

The study was agreed 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.1397.477) and written informed consent was got from all participants. Based upon McDonald 2010 criteria (13), 30 definitive MS patients who were hospitalized in the MS unit of Imam Reza and Sina Hospitals in 2018 participated in the study. Furthermore, 30 demographically and ethnically matched healthy females from our university staff were also recruited. Participants with known diseases related to vitamin D deficiency like rickets or parathyroid diseases and those taking drug or supplements containing vitamin D or calcium were excluded. Two neurologists assessed expanded disability status scale (EDSS) in MS patients (18).

Serum and Sample collection

Venous blood and saliva were obtained from each participant at the same time in the morning. For saliva sampling, the subjects cleaned their mouth and then swallowed all their oral fluid. Thereafter, they collected 2-3 ml of their resting whole saliva in a plastic tube by spitting method with no dynamic movement of mouth wall, sucking the oral cavity, or mastication. Then, the participants were asked to chew a piece of neutral gum with a given size. Two minutes after the start of chewing, subjects either spat all the oral fluid out or entirely swallowed it and thereafter, they began to collect the stimulated whole saliva into another tube while continuing chewing the gum. Immediately after the saliva collection, venous blood was drawn. Upon completing sample collection, the specimens

were centrifuged at 5000 g for 10 minutes and then, the serum and saliva supernatants were stored at -80°C for later measurement of 25(OH)D.

Laboratory assays

Human 25(OH)D ELISA kit was provided from the PadtanGostarIsar Company (Tehran, Iran). The ELISA kit which we used was designed for 25(OH)D. Measurement of 25(OH)D level was performed based on the manufacturers' instruction.

Statistical analysis

The data were offered as mean ± s.e.m. The means of the groups were compared using an unpaired two-tailed student's t-test. The Pearson correlation test was used to determine the association between the parameters. Receiver operating characteristic (ROC) analysis was used to detect cut-off point for salivary 25(OH)D between MS patients and healthy individuals. The results were considered statistically significant if $P<0.05$. The analysis was performed using SPSS software version 16.

Results

This case-control study assessed 30 MS women as case group and 30 healthy women as control. The mean age in case and control groups was 34.6±8.6 and 36.5±2.3 years, respectively. There was no significant difference in mean age between two groups (P= 0.554).

The mean serum concentration of 25(OH)D level was lower in patients than that of controls (Table 1). Stimulated and unstimulated salivary concentrations were significantly lower in patients with MS (Table 1).

Table 1. Concentrations of 25(OH)D in serum, unstimulated and stimulated saliva of patients suffering from multiple sclerosis (MS) and control individuals.

Group Parameter	Healthy	MS	P-value
serum 25(OH)D (ng/ml)	37.2±4.3	20.4±3.1	0.003
Unstimulated saliva 25(OH)D (pg/ml)	22.9±4.4	7.5±1.3	0.002
Stimulated saliva 25(OH)D (pg/ml)	28.1±6.1	3.1±0.6	0.000

Data has been presented as mean ± s.e.m

Statistical evaluation of the data using Pearson analysis indicated a moderate correlation between the unstimulated salivary concentration of 25(OH)D and its serum concentration (0.548; P=0.004); and also, between the stimulated salivary concentration of 25(OH)D and its serum concentration (r = 0.575; P=0.003).

The mean (±SD) EDSS was 3.6±1.9 in the MS group. EDSS was not significantly correlated with 25(OH)D in serum or in stimulated and unstimulated saliva.

The cut-off points of 25(OH)D in serum and saliva were 18 ng/ml and 65 pg/ml, respectively (Table 2).

Table 2. The cut-off points of 25(OH)D in serum and saliva for the diagnosis of MS

	Cut off	Sensitivity %	Specificity%	AUC	P-value
Serum	18 ng/ml	82	67	0.803	0.014
Unstimulated saliva	65 pg/ml	91	67	0.807	0.013
stimulated saliva	65 pg/ml	92	95	0.883	0.002

Discussion

The serum level of 25(OH)D reflects the consumption of vitamin D in food, its synthesis from cholesterol in the skin under the impact of UV light and also serum 1,25 (OH)2D levels in individuals with normal kidney function (19). The receptors of 1,25 are expressed on a widespread variety of cell kinds, such as immune system and neuronal and glial cells in the human CNS (20). It has been shown that vitamin D deficiency results in an augmented occurrence of autoimmune diseases such as MS (3). The mechanism is probably related to the development of self-tolerance as 25(OH)D adjusts T helper (Th) and dendritic cells function and induces regulatory T cells resulting in a reduction in Th-1 (5).

MS may affect all aspects of CNS functions. It affects the quality of life of patients. MRI and evaluation of some biomarkers in CSF can help the diagnosis of MS and its follow up (14). As MRI is expensive and CSF is an invasive procedure, evaluation of biomarkers in the saliva as an alternative medium for CSF and serum is considered. In this study, the salivary and serum values of 25(OH)D in MS and healthy women were evaluated.

The results showed that the mean 25(OH)D in the serum of patients was less than in healthy people. It is inconsistent with many previous studies (21-24), hence, the results like previous studies support the involvement of 25(OH)D in the incidence of MS. In contrast to most previous studies (10,21,25), which showed an inverse significant association between serum 25(OH)D and EDSS; such an association was not identified in the present study. However, this study is in accordance with some studies(24,26) most of which have been conducted in Iran.

Saliva is being accepted as a diagnostic fluid of the future. Much of the consideration saliva receives as a biological sample is owing to the rapid, simple and noninvasive nature of sample get(27,28). To establish saliva as a substitute for plasma for various biological assessments, there must be a high association between plasma and saliva levels of assayed parameters (29,30). Our results showed that stimulated and unstimulated saliva level of 25(OH)D in patients suffering from MS is significantly lower than that in healthy individuals and there was a moderate positive correlation between serum and salivary levels of 25(OH)D. Therefore, one can conclude that the salivary levels of 25(OH)D reflect approximately its serum concentration and based on this observation, we can put forward this idea that saliva-based assays may have the potential to be used as point-of-care testing to detect MS by measuring salivary 25(OH)D.

Today, the assays of 25(OH)D have often been prepared for its total concentration and have neglected the probable importance of free 25(OH)D in the blood. Accordingly, a precise measurement of free 25(OH)D would be of great interest in this state. As 25(OH)D binds to its transport protein

very strongly and also for technical problems, direct detection of the free fraction of it in the blood is difficult. It has been shown that the measurement of steroid hormones in saliva reflects the unbound plasma concentration (31). It seems free 25(OH)D enters saliva by diffusing through the cells of the salivary glands but conjugated 25(OH)D enters saliva via ultrafiltration through the junction between the acinar cells. As the salivary level of 25(OH)D may reflect the free level of 25(OH)D in serum and most of 25(OH)D bind to the protein in serum, the concentrations in saliva are very low. Salivary level of 25(OH)D in saliva was about 0.07% of its serologic level in this study.

Conclusion

25(OH)D level in saliva, like in serum, was low in MS women. Serum 25(OH)D levels correlated positively with unstimulated and also with stimulated whole saliva 25(OH)D in women suffering from MS. Thus, salivary 25(OH)D levels appear to be associated with MS.

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Conflict of interest

None to declare.

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