

COMPARISON OF SERUM LEVELS OF VITAMIN D IN GRAVES' PATIENTS AND HEALTHY INDIVIDUALS

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POREĐENJE SERUMSKIH NIVOVA VITAMINA D KOD PACIJENATA SA GREJVSOVOM BOLEŠĆU I KOD ZDRAVIH POJEDINACA

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ABSTRACT

Objective. Relationship between Graves' disease and vitamin D deficiency is a challenging matter and some of researchers had suggested the role of vitamin D deficiency in the pathogenesis of Graves' disease. We designed this study to evaluate serum level of vitamin D in Graves' disease.

Methods. During a cross-sectional study in 2014, 27 patients with Graves' disease and 29 healthy subjects were enrolled. From all of the patients and healthy group intravenous blood samples for assessment of serum vitamin D levels were taken. Serum level of vitamin D of all of patients and healthy group were compared with each other. Serum vitamin D level lower than 15 ng/ml was considered as vitamin D deficiency. Serum vitamin D levels between 15-30 and 30-50 ng/ml were considered as vitamin D insufficiency and normal vitamin D levels respectively.

Results. The mean age in Graves' patients and healthy group were 41.23 ± 2.58 and 34.40 ± 1.44 years respectively ($P=0.02$) and there was a significant difference between the age in the two groups. 77.8 and 58.6% of Graves' patients and healthy groups were females respectively ($P=0.12$). Serum level of vitamin D in Graves' patient was 18.04 ± 1.92 ng/ml and in healthy controls was 17.15 ± 2.06 ng/ml and there was not a statistically significant difference in vitamin D levels between the two groups ($p=0.75$). Vitamin D status did not differ between the two groups of the study (p value=0.92).

Conclusion. According to our study, there is no association between serum vitamin D levels and Graves' disease.

Key words: vitamin D; Graves' disease; vitamin D deficiency.

INTRODUCTION

Graves' disease was reported by Robert Graves in 1835 by constellation of thyromegaly, proptosis and palpitation. It was found later that the disease is mediated by IgG antibodies that stimulate thyroid activity (1). A defect in T cells with unknown mechanism leads intolerance to thyroid stimulating hormone receptor

SAŽETAK

Cilj. Odnos između Grejvsove bolesti i nedostatka vitamina D veliki je izazov, a nekim istraživačima je predloženo da ispitaju ulogu nedostatka vitamina D u patogenezi Grejvsove bolesti. Ovu studiju dizajnirali smo kako bismo procenili nivo seruma vitamina D u Grejvsovoj bolesti.

Metode. U studiju preseka u 2014. godini uključeno je 27 bolesnika sa Grejvsovom bolešću i 29 zdravih osoba. Od svih pacijenata i zdravih osoba uzeti su uzorci intravenske krvi za procenu nivoa vitamina D u serumu. Serumski nivo vitamina D svih pacijenata i zdrave grupe poreden je međusobno. Serumski nivo vitamina D manji od 15 ng/ml smatran je nedostatkom vitamina D. Serumski nivoi vitamina D između 15–30 i 30–50 ng/ml smatrani su insuficijencijom vitamina D i normalnim nivoima vitamina D, respektivno.

Rezultati. Prosečna starost u grupi pacijenata sa Grejvsovom bolešću i u zdravoj grupi bila je 41.23 ± 2.58 i 34.40 ± 1.44 godine respektivno ($p = 0.02$) i postojala je značajna razlika između uzrasta u dve grupe. Žene su činile 77.8 u grupi pacijenata sa Grejvsovom bolešću, a 58.6% u zdravoj grupi ($p= 0.12$). Nivo serumskog vitamina D kod bolesnih pacijenata iznosio je 18.04 ± 1.92 ng/ml, a kod zdravih 17.15 ± 2.06 ng/ml; nema statistički značajne razlike u nivoima vitamina D između dve grupe ($p = 0.75$). Status vitamina D nije se razlikovao između dve grupe ($p = 0.92$).

Zaključak. Prema našoj studiji, nema povezanosti između nivoa vitamina D u serumu i Grejvsove bolesti.

KLJUČNE REČI: vitamin D; Grejvsova bolest; nedostatak vitamina D.

(TSHR) and induces autoimmunity against this receptor and creates a cycle that ultimately causes the differentiation of B cells into plasma cells. Differentiated B cells produce anti-TSHR antibodies that cause thyroid hyperplasia and increase the secretion of its hormones (1).

Vitamin D is an important hormone in the body that is fat-soluble and can be synthesized in the skin from 7-dehydrocholesterol following exposure to ultraviolet B rays of the sun initially in the form of previtamin D3. The liver

converts the previtamin D3 to 25 hydroxyvitamin D3 [25(OH)D3] and finally its active form (1 and 25 dihydroxyvitamin D) is synthesized in the kidney. Other sources of vitamin D include natural food sources, and vitamin D supplements (2). Dark skin, use of sunscreens, old age, kidney and hepatic diseases, malabsorption, obesity, use of some medications such as anticonvulsants and corticosteroids and air pollution are all predisposing factors for vitamin D deficiency (3).

It has been reported that monocytes and activate lymphocytes have Vitamin D receptors and active metabolites of vitamin D, such as 1,25-dihydroxyvitamin D coordinate the response of T cells (4). At present, there is a growing evidence of the impact of this vitamin on autoimmune disorders, infections, cardiovascular disease and cancer risk (5-7). The association of vitamin D deficiency with some autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis (MS), type 1 diabetes, systemic lupus erythematosus and Graves' disease have been reported (2,5,8).

In diabetic rats high dose vitamin D therapy could effectively protect against insulinitis and further pancreatic damage (9). Also in some studies, treatment with vitamin D had a positive effect on the autoimmune process such as multiple sclerosis, type 1 diabetes, rheumatoid arthritis and psoriatic arthritis (6, 10-12). However, the role of vitamin D in autoimmune thyroid disease is still in debate (13). In a study of Yasuda et al. in 26 Graves' patients and 46 control group, the frequency rate of vitamin D deficiency in women with Graves' disease was significantly greater than in the control group. They also found significant reverse correlation between thyroid size and vitamin D levels, but there was no association between vitamin D levels and TSH receptor antibodies, thyroid hormones and thyrotropin levels (8). Kivity et al. found higher frequency of vitamin D deficiency in patients with autoimmune thyroid disorders especially Hashimoto's disease compared to control group and nonautoimmune thyroid disorders (6). They also found relationship between vitamin D deficiency and anti-thyroid antibodies in patients with autoimmune thyroid disorders. Due to different results in previous studies regarding serum vitamin D levels and Graves' disease, in

this study we compare the serum levels of vitamin D in patients with Graves' disease and healthy subjects.

MATERIAL AND METHODS

After approval by the Ethic Committee of Urmia University of Medical Sciences, a cross-sectional study was conducted from April to September 2014, in 27 patients with Graves' disease. They were 21-65 years old and all of them were referred to the Endocrinology clinic of Imam Khomeini hospital of Urmia city. Twenty-nine healthy subjects who had no history of thyroid disease were selected as controls. Subjects who received a vitamin D supplement over the past three months or with a history of liver or kidney disease, as well as subjects with history of corticosteroid use were excluded from study. We received a written consent from all patients and healthy subjects. Graves' diagnosis was made based on the clinical and laboratory findings of hyperthyroidism in the presence of anti-TPO Antibodies and/or ophthalmopathy. Venous blood samples were taken from all study participants and serum 25(OH)D3 level was measured by Electrochemiluminescence method (ECL) (Roche kite, elecsys 2010). Roche vitamin D calibrator was used for results quality control.

Serum 25(OH)D3 levels were compared in two groups of Graves' patients and healthy subjects. Serum vitamin D level, lower than 15 ng/ml, was considered as vitamin D deficiency. Serum vitamin D levels between 15-30 and 30-50 ng/ml were considered as vitamin D insufficiency and normal vitamin D levels, respectively. We used an independent t-test for analysis of quantitative variables between the two groups. Chi-Square and Fisher's exact tests were used to investigate the relationship between qualitative variables in the study groups. SPSS20 package was used for data statistical analysis. The level of significance was considered p value <0.05.

RESULTS

The majority of study population were female (77.8% and 58.6% in the patients and control group respectively), and there was no significant difference in sex distribution

Table 1. Comparison of Frequency rate of Vitamin D status in Graves' patients and control group

Variable	Category	Patients (n=27)	Controls (n=29)	p-value
Frequency rate of Vitamin D status *		n (%)	n (%)	
	Normal	3 (11.1)	2 (6.9)	
	Inadequate	10 (37.0)	12 (41.4)	0.92
	Deficiency	14 (51.9)	15 (51.7)	

*Fisher exact test

between the two groups. The mean age in Graves' patients and healthy group were 41.23 ± 2.58 and 34.40 ± 1.44 years respectively ($P=0/02$) and there was significant difference between age in the two groups. Serum level of vitamin D in Graves' patient was 18.04 ± 1.92 ng/ml and in healthy controls was 17.15 ± 2.06 ng/ml and there was not statistically significant difference in vitamin D levels between the two groups ($p=0.75$).

As shown in Table 1, in the majority of Graves' patients and controls, vitamin D levels were lower than normal (88.9% and 93.1% respectively) and only 11.1% of patients and 6.9% of healthy subjects had normal levels of serum vitamin D. The difference between serum vitamin D status in Graves' patients and healthy individuals was not statistically significant ($P=0.92$).

Four patients with Graves' disease (14.8%) had radioiodine treatment history. Of the 27 Graves' patients, 21 patients were taking medication (18 methimazole, 2 Levothyroxin and 1 Propylthiouracil).

DISCUSSION

Many studies have shown the association of vitamin D deficiency with malignancies, mental illness, respiratory, cardiovascular and autoimmune diseases (5). These autoimmune diseases include: type 1 diabetes mellitus, multiple sclerosis, asthma, rheumatoid arthritis and autoimmune thyroid disorders (5).

In the study of Yasuda and colleagues in 2012, serum 25(OH) D₃ levels in newly diagnosed women with Graves' disease were significantly lower compared to healthy women (14.4 ± 4.9 and 17.1 ± 4.1 ng/ml respectively, p -value < 0.05), but there was no significant difference in age and body mass index between the two groups (8). Our study results did not agree with Yasuda et al. study results and we did not observe any significant difference regarding 25(OH)D₃ levels between the two study groups.

In Yamashita et al. study in Japan on 208 Graves' patients, the prevalence of vitamin D deficiency in women with Graves' disease was significantly higher than in men (40% and 18% respectively ($p < 0.05$)), and also mean of 25(OH) D concentration in women patients with Graves' disease was significantly lower than in men (14). They were advised to measure vitamin D levels and treat its deficiency in Graves' patients who received antithyroid drugs. In our study only 11.1% of Graves' patients and 6.9% of healthy subjects had normal serum vitamin D levels and 88.9% of Graves' patients and 93.1% of healthy women had lower serum vitamin D. Just like Yamashita et al. study, a significant percentage of our Graves' patients were women.

In our study, there was no statistically significant difference in the percentage of vitamin deficiency among the two groups. Vitamin D deficiency existed in 51.9% of patients with Graves's disease and 51.7% of healthy subjects. We did not find any significant difference in serum levels of vitamin D in both study groups.

On the other hand, D'Aurizio and colleagues conducted their own case- control study that included 52 patients with Hashimoto's thyroiditis, 48 Graves' cases and 126 healthy subjects and concluded that there was no association between hypovitaminosis D and Hashimoto's thyroiditis or Graves' disease. Also in their own case control study they observed higher serum vitamin D levels in patients with autoimmune thyroid disease compared to healthy subjects, but the difference between the study groups was not significant in this regard. In addition, they found similar frequency rates of hypovitaminosis D between their study groups. Our findings are consistent with the results of the D'Aurizio et al. study. Although our study population was limited to Graves' patients and healthy subjects (15)

Rotini et al. noted the role of vitamin D in calcium metabolism, bone homeostasis and immune system, and highlighted the high prevalence of vitamin D deficiency in autoimmune thyroiditis and Graves' disease, and concluded that the role of vitamin D in Graves' disease is still challenging and they suggested further studies in this regard (13). Kriegel et al. in their systematic review have shown the role of vitamin D in the prevention of autoimmune diseases in cross-sectional, experimental and observational studies, but concluded that there is a need of prospective human interventional studies and double blind randomized controlled trials for investigating relationship between vitamin D supplementation and autoimmune disease protection (16). Agmon-Levin and colleagues explained that there was a strong correlation between serum vitamin D levels and some of the autoimmune diseases, not all of them. They also pointed to the possible role of vitamin D receptor gene polymorphisms in the development of some autoimmune disorders (17).

Some patients with Graves' disease in our study had a history of radioactive iodine treatment, and some were medically treated, and both of these factors could affect our study results. Due to the high prevalence of vitamin D deficiency in Iran, and in particular in women (18), as well as considering that most of our patients were women, the sample size of this study seems to be low and it can influence the results of our study. Regarding the results of our study and the absence of a significant association between serum vitamin D status and Graves' disease, it may be that the role of other genetic, innate immunity and environmental factors in the pathogenesis of this disease are much more important. Given the nature of Graves'

disease, which is a rare disease, and because of the high cost of preparing vitamin D kits and financial limitations, this research was performed with a small sample size, and this matter is the main limitation of our study. Of course, for definitive statement, large-sample-sized and prospective studies are needed.

In conclusion, according to our study results, there is no association between serum vitamin D status and Graves' disease. Because of higher frequency of vitamin D deficiency and insufficiency status in our study participants, we also recommend routine measurement of vitamin D level in all healthy and Graves' individuals for earlier diagnosis and treatment.

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ABBREVIATIONS:

TSHR - Thyroid stimulating hormone receptor

ECL - Electrochemiluminescence method

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