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Original Article

Internal Medicine Section

Effect of Vitamin D Replacement on Serum TSH in Women with Anti-TPO Positive Sub-clinical Hypothyroidism

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ABSTRACT

Introduction: The coexistence of Sub-Clinical Hypothyroidism (SCH) and vitamin D deficiency is a common problem and may lead to various clinical disorders. Several genetic studies have shown an association between gene polymorphism of Vitamin D receptor and of 1α hydroxylase with autoimmune thyroid diseases.

Aim: To evaluate the effects of vitamin D_3 replacement on Thyroid-Stimulating Hormone (TSH) and anti-TPO levels in women with SCH.

Materials and Methods: This clinical trial was performed on 58 Sub-Clinical Hypothyroidism vitamin D deficient women. Patients were treated with 50,000 unit/week vitamin D_3 capsule for eight weeks and TSH, Free thyroxine (FT4) and anti-TPO levels were measured before and after drug administration, using an electrochemiluminescence system.

Results: The mean age of the population was 38.02±12.68 years. Vitamin D_3 replacement caused serum 25(OH) D_3 level correction in SCH patients (p<0.001). Additionally, a significant decrease in anti-TPO and TSH levels were also observed in anti-TPO positive patients (n=29) (p=0.002 and p=0.02, respectively). No significant differences in anti-TPO and TSH levels were seen in anti-TPO negative group (p=0.507 and p=0.447, respectively).

Conclusion: The study showed that vitamin D_3 replacement may be considered as an effective therapy to reduce TSH and anti-TPO levels in vitamin D deficient SCH patients.

Keywords: Anti-thyroid peroxidase, Autoimmune thyroiditis, Free thyroxine, Sub-clinical hypothyroidism, Thyroid stimulating hormone, Vitamin D deficiency

INTRODUCTION

Sub-Clinical Hypothyroidism (SCH) is a common laboratory finding in clinical practice and is characterised by elevated serum TSH and normal FT4 levels compared to population-based normal ranges [1]. Patients having SCH are usually asymptomatic, however, hypothyroidism signs and symptoms including skin dryness, obesity, cold sensitivity, muscle cramps and constipation are sometimes observed. This disease may or not progress to hypothyroidism and is mostly caused by Hashimoto's thyroiditis (HT) [2,3].

Vitamin D is a fat-soluble vitamin which is mainly synthesised in sun-exposed skin and in lower levels is obtained from diet [4]. 1α, 25-hydroxyvitamin D_3 [$25(OH)_2D_3$] is the active hormonal form of vitamin D_3 [5]. Genomic mechanism action involves its binding to nuclear vitamin D receptor which is a dimer counterpart of retinoid X receptor [5].

Different studies have investigated the correlation between vitamin D deficiency and the prevalence of Autoimmune Thyroid Disease Autoimmune thyroiditis (AITD) including HT and Grave's Disease (GD) [6-8]. Kim D et al., in a study on 776 patients reported decreased levels of 25(OH) D_3 in patients having AITD compared with non-AITD individuals [9]. Additionally, Metwalley K et al., reported significantly lower levels of vitamin D in AITD patients compared to normal controls [10].

Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors [7]. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid disease including GD and HT. For these reasons, it is important for patients with thyroid problems to understand how the vitamin D system works [7].

However, in Iran, no study on the relationship between vitamin D and SCH has been conducted. Therefore, the aim of the present study was to investigate the effects of vitamin D replacement on improvement of SCH features in Vitamin D deficient women.

MATERIALS AND METHODS

This clinical study (IRCT2016062813612N8) was performed on 58 vitamin D deficient female patients having SCH from March 2016 until March 2017 and approved by Ethics and Research Committee (code: IR.TBZMED.REC.1395.416) of Tabriz University of Medical Sciences. Patients were selected on the basis of negative or positive anti-TPO levels (29 Anti-TPO positive and 29 Anti-TPO negative patients).

Sample size was based on "hypothesis testing for paired sample means" formula, keeping 'Z' as 1.96 and 'S' as 2.355. According to the pilot study, 29 patients were selected for each group.

Inclusion criteria were age >18 years, 25(OH) D_3 <30 ng/mL and 4.5 <TSH ≤10 mLU/L. Age >65, systemic kidney, lung, gastrointestinal and blood diseases, autoimmune and inflammatory disorders, drugs affecting 25(OH) D_3 level including vitamin D, multivitamins, calcium or vitamin D/mineral fortified food during the previous three months and pregnancy were considered as exclusion criteria.

Patients were treated with vitamin D_3 pearls, 50000 unit/week for eight weeks [11] and biochemical parameters were measured before and after drug administration. Written informed consent was obtained from all participants before sampling.

Laboratory Measurements

Serums were collected before and after vitamin D_3 replacement and TSH, FT4, Anti-thyroid peroxidase (Anti-TPO) and 25(OH) D_3 levels were measured using an electrochemiluminescence instrument (IMMULITE 2000, Germany). 0.5-4.5 mIU/L, 0.8-1.8 ng/dL, 1-75 IU/mL and 30-100 ng/mL were considered as normal ranges for TSH, FT4, Anti-TPO and 25(OH) D_3, respectively. Patients were

divided into anti-TPO positive and negative groups based on anti-TPO levels. Serum anti-thyroid peroxidase (anti-TPO Ab) was measured by Rapid enzyme-linked immunosorbent assay (Genesis Diagnostics, Littleport, UK). Anti-TPO Ab concentrations more than 75 IU/mL was considered positive [12]. SCH was also defined as serum TSH concentration above the upper limit of the reference range when serum FT4 concentration is within its reference range.

STATISTICAL ANALYSIS

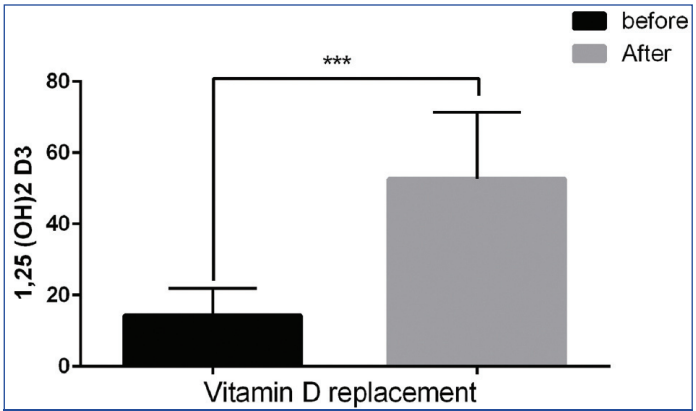
Data are presented as mean±standard deviation (SD). The non-parametric test (Wilcoxon) was used for comparing means between groups by SPSS 23.0 software. $p<0.05$ was considered as statistically significant.

RESULTS

The [Table/Fig-1] shows general characteristics of SCH patients. The mean age and BMI were 38.02±12.68 years and 25.5±3.13 kg/m^2, respectively. The average levels of serum TSH, FT4, anti-TPO and 25(OH) D_3 were also 7.41±1.63 mIU/L, 1.03±0.11 ng/dL, 541.89±698.46 IU/mL and 14.32±7.60 ng/mL, respectively, before vitamin D replacement. A significant increase in serum 25(OH)D_3 level was observed after vitamin D_3 replacement in SCH patients indicating the effectiveness of chosen protocol (52.72±18.64, $p<0.001$) [Table/Fig-2].

Version	Mean±SD (n=58)	Range
Age (Years)	38.02±12.68	20-73
Body mass index (kg/m^2)	25.5±3.13	20-34
TSH (mIU/L)	7.41±1.63	4.55-10.30
FT4 (ng/dL)	1.03±0.11	0.8-1.30
Anti-TPO (IU/mL)	541.89±698.46	51-2000
25(OH) D3 (ng/mL)	14.32±7.60	2.50-28

[Table/Fig-1]: General characteristics of SCH patients.
Data are presented as mean±SD.



[Table/Fig-2]: 1,25(OH)2 D3 status before and after replacement in SCH patients. (***$p<0.001$)

As shown in [Table/Fig-3], significant decrease in anti-TPO and TSH levels were observed in anti-TPO positive patients after vitamin D_3 replacement ($p=0.002$ and $p=0.02$, respectively). No significant differences in anti-TPO and TSH levels were seen in anti-TPO negative group ($p=0.507$ and $p=0.447$, respectively). Additionally, a decrease in FT4 level was also observed in two groups. However, this decrease was not statistically significant ($p=0.34$ and $p=0.31$, respectively).

Varibles	Anti-TPO (+) (n=29)		p-value	Anti-TPO (-) (n=29)		p-value
	Before	After		Before	After	
Anti-TPO (IU/mL)	755.57± 749.36	535.37± 491.23	0.002	65.23± 7.14	72.23± 35.92	0.507
TSH (mIU/L)	7.96±1.64	7.51±1.81	0.02	6.86±1.44	6.69±1.71	0.447
FT4 (ng/dL)	1.04	1.01	0.34	1.02	0.99	0.31

[Table/Fig-3]: The effect of vitamin D replacement on anti-TPO and TSH levels in positive and negative anti-TPO groups.
Data are presented as mean±SD. p-value was adjusted for age and BMI.

DISCUSSION

In this study, SCH women with vitamin D deficiency were divided into anti-TPO positive and negative groups regarding to serum anti-TPO levels. Vitamin D replacement resulted serum 25(OH) D_3 level correction in patients. Present data also showed a significant decrease in TSH and anti-TPO levels after vitamin D replacement in anti-TPO positive group. The study found vitamin D levels of all patients was <30 ng/mL, and about 75% of patients had below 20 ng/mL. This study showed a clear relationship between vitamin D and anti-TPO levels and TSH in AITD, and confirmed vitamin D to be an independent factor related to the presence of anti-TPO. The study showed that vitamin D_3 replacement may be considered as an effective therapy to reduce TSH and anti-TPO levels in vitamin D deficient SCH patients.

Several studies have been performed to survey the effects of Vitamin D replacement on thyroid diseases [11,12]. Kivity S et al., found that the prevalence of vitamin D deficiency was observed in 72% of patients with AITD and 30.6% of healthy subjects [13]. Mackawy AM et al., found that vitamin D levels in patients with hypothyroidism were significantly lower than healthy subjects and were lower in women than in men [14]. Agmon-Levin N et al., suggested that the administration of vitamin D supplements could improve the autoimmune status of patients with autoimmune diseases, including HT [15]. Ucan B et al., study on patients with HT, which was carried out for eight weeks in patients with 50,000 units of vitamin D weekly administration led to a reduction in the progression of the disease [16]. Mazokopakis EE et al., found that administering 1200 to 4000 units of vitamin D daily for four months resulted in a significant reduction of the anti-TPO level (20.3%) and this could indicate that vitamin D deficiency could be one of the pathogenic factors of the AITD [17]. In experimental animal studies, the beneficial effects of Vitamin D replacement on autoimmune thyroid diseases are well demonstrated [18,19]. Fournier C et al., in an induced autoimmune thyroiditis mouse model showed that simultaneous low dose treatment with 25(OH) D_3 and cyclosporine leads to a 26% decrease in tissue injuries without any changes in thyroid antibody status [18]. Chen W et al., also reported that the incidences of experimental autoimmune thyroiditis in the immune prevention group and treatment group, with administration of low dose of cyclosporine and 25(OH)$_2$$D_3$, were decreased by 44.44% and 37.50% respectively [19].

Some studies did not find any association between vitamin D deficiency and AITD. Chailurkit LO et al., did not find any relationship between 25(OH) D_3 levels and thyroglobulin Ab or anti-TPO status in their study population [20]. Effraimidis G et al., found that in patients with AITD with normal thyroid hormone levels, vitamin D levels were 21 and in healthy subjects, 18 ng/mL [21]. Mansournia N et al., shown vitamin D levels in patients with Hashimoto thyroiditis was significantly higher than healthy patients [22]. Musa IR et al., stated in their study that there is no significant relationship between vitamin D and hypothyroidism in women with these and healthy subjects [23].

Vitamin D replacement has also exerted beneficial effects on HT and GD and a significant decrease in thyroid antibodies is achieved following this treatment [24]. Non-skeletal actions of this vitamin such as involvement in autoimmune diseases, metabolic syndrome, cardiovascular diseases and cancer are explained in recent decades [25]. Additionally, the correlation between low levels and impaired vitamin D signalling with AITD and thyroid cancer are also demonstrated [13].

Ethical approval: The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz Iran (code: IR.TBZMED.REC.1395.416). The current study was performed according to the Institutional Committee for the Protection of Human Subjects, which was adopted by the 18th World Medical Assembly, Helsinki, Finland and its later amendments.

LIMITATION

The limitations of present study include the relatively small number of cases, the complex nature of SCH pathogenesis and the possible genetic variations among patients, which may affect the association of vitamin D deficiency and SCH progression. Therefore, further studies with a larger number of patients regarding Vitamin D receptor (VDR) genetic polymorphism analysis are needed before any conclusion about the relation of vitamin D and SCH in adult women can be established.

CONCLUSION

The present data show that vitamin D replacement may be considered as an effective therapy to reduce TSH and anti-TPO levels in vitamin D deficient SCH patients. However, more studies with larger number of individuals regarding genetic variation of genes included in vitamin D hemostasis are needed to further clarify the exact mechanism of vitamin D deficiency on SCH.

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