Calcifediol Concentration *vs* Circulating Thyrotropin and Free Thyroxine in Human Blood of Postmenopausal Women

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A total of 55 patients were: G1 of 9 patients, G2 of 23 subjects, and G3 of 23 patients. Similar age and years since menopause was found between the groups. There was no statistical significant difference between the groups regarding these aspects: p-value G1-G2 of 0.67, p-value G1-G3 of 0.72, p-value G2-G3 of 0.47. Similar results were obtained when TSH, FT4 and anti-thyroperoxidase antibodies were analysed between the groups. No TSH-25-OHD correlation reached the statistical significance, neither FT4-25-OHD, TPO-25-OD. P-values between G1 and G2 for TSH, FT4, TPO were 0.23, 0.38, respective 0.7, between G2 and G3 were 0.19, 0.1, 0.35, between G1 and G3 were 0.48, 0.84, respective 0.75. Bone profile analyze based on 25-OHD levels did not identify any statistical significance difference between the mentioned groups (including bone turnover markers).

Keywords: calcifediol, thyrotropin, thyroxine

Calcifediol ($C_{27}H_{44}O_2$ with molar mass of 400.64 g/mol) or (*6R*)-6-[(*1R,3aR,4E,7aR*)-4-[(*2Z*)-2-[(*5S*)-5-Hydroxy-2-methylidene-cyclohexylidene]ethylidene]-7a-methyl-2,3,3a,5,6,7-hexahydro-*1H*-inden-1-yl]-2-methyl-heptan-2-ol, also known as 25-hydroxyvitamin D_3 (25-OHD) or calcidiol is produced at liver lever by 25-hydroxylation and then it is 1α-hydroxylated at kidney and it becomes the activated form of vitamin D. [1,2] Vitamin D is synthesised through parathormone (PTH) stimulation and through circulating levels based on a negative feedback. [3,4] Vitamin D has been associated with multiple pathways as skeletal integrity and function, insulin resistance, inflammation, infections control, oxidative stress, and potential regulation of endocrine glands like thyroid [5-9]. Thyroid produces thyroxine, also named T4 or 3,5,3',5'-tetraiodothyronine as well as triiodothyronine, also named T3 (both are tyrosine based hormones) [10]. Thyroid activity is regulated by pituitary thyrotropin or TSH (Thyroid Stimulating Hormone) [10] (fig. 1).

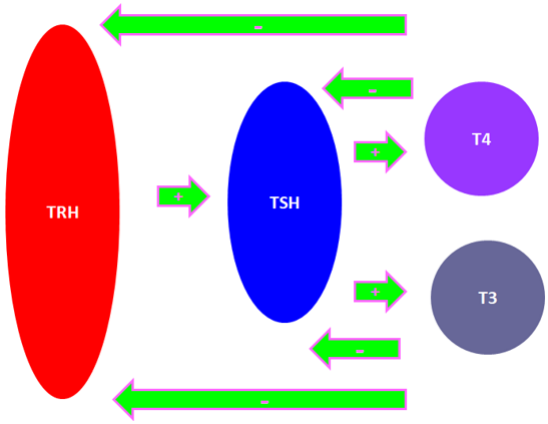

Fig. 1. Thyroid gland produces T4 and T3 hormones (thyroxine, respective triiodothyronine). T4 and T4 production is controlled by TSH (Thyroid Stimulating Hormone) which is stimulated by hypothalamic TRH (Thyroid Releasing Hormone). T4 and T4 exert a negative feedback on TSH and TRH release

Purpose

Our purpose is to introduce a clinical study on adult apparently healthy women in menopause but with low levels of 25-OHD and to analyze these levels in relationship with blood TSH and T4.

Experimental part

Method

This is a transversal non-interventional study on Romanian subjects. The study was conducted between 2016 and 2018.

The collected data include age, age of menopause and calculated years since menopause. The clinical exam included thyroid examination and the calculation of Body Mass Index or BMI (in kg/sqm). Blood tests included TSH (ECLIA method or electro-chemiluminescence), FT4 (immune detection based on ECLIA assay), anti-thyroid antibodies, for instance, anti-thyroperoxidase antibodies (immune method of detection based on CMIA, electrochemiluminescence immune assay). Calcium metabolism was assessed based on blood assays, also. 25-OHD was tested based on chemiluminescence method

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and PTH used an electro-chemiluminescence method. Moreover, bone turnover markers are assessed: bone formation markers alkaline phosphatase (colorimetry VITROS), osteocalcin (electrochemiluminescence), P1NP (ECLIA method), and bone resorption marker CrossLaps (electrocemiluminescence).

Statistical analysis: database was introduce using Excel program; then the database was exported in SPSS program; parameters were described using mean and standard deviation (SD) level of statistical significance was considered at $p<0.05$; student ttest and simple regression were used.

Subjects

The patients met the inclusion which introduced in table 1. Also the exclusion criteria are in table 2. Three groups were formed based o 25-OHD: group 1 (G1) with 25-OHD of 0 ng/mL and 9.99 ng/mL (normal less than 30 ng/mL), group 2 (G2) with 25-OHD between 10 and 19.99 ng/mL, and group 3 (G3) with 25-OHD between 20 and 29.99 ng/mL.

Results and discussions

A total of 55 patients were: G1 of 9 patients, G2 of 23 subjects, and G3 of 23 patients. The parameters regarding age, age of menopause, respective years since menopause are included in table 3. All these parameters were similar speaking of statistical aspects of significance. There was no statistical significant difference between the groups regarding these aspects: p-value G1-G2 of 0.67, p-value G1-G3 of 0.72, p-value G2-G3 of 0.47. Similar results were obtained when TSH, FT4 and anti-thyroperoxidase antibodies were analysed between the groups (table 4) No TSH-25-OHD correlation reached the statistical significance, neither FT4-25-OHD, TPO-25-OD. P-values between G1 and G2 for TSH, FT4, TPO were 0.23, 0.38, respective 0.7, between G2 and G3 were 0.19, 0.1, 0.35, between G1 and G3 were 0.48, 0.84, respective 0.75. Bone profile analyze based on 25-OHD levels did not identify any statistical significance difference between the mentioned groups.

As limits of the study we mention the lack of data correlation between 25-OHD and ultrasound features of thyroid, also the small sample size. 25-OHD was considered as part of epigenetic mechanisms involved in thyroid regulation and multiple studies found a tide correlation between vitamin D status and thyroid function and immune profile [11, 12]. Also, we mention the fact that a high level of TPO in G1/G2/G3 suggested chronic autoimmune thyroiditis which might intersperse as confounding factor in 25-OHD interpretation in relationship with thyroid features (table 5).

Table 1
THE INCLUSION CRITERIA

Inclusion criteria
menopause
age above 40 years
25-OHD less than normal level of 30 ng/mL
apparently healthy thyroid background

Table 2
THE EXCLUSION CRITERIA

Exclusion criteria
thyroid cancer of any type
active cancers
bone metastases
prior/current specific anti-osteoporotic drugs

Group	Number of patients	Age (mean±SD) years	Age of menopause (mean±SD) years	Years since menopause (mean±SD)
G1	9	60.22±9.73	46.33±4.00	15.88±8.13
G2	23	60.82±7.76	46.22±4.30	15.00±9.08
G3	23	60.08±6.38	48.00±6.02	12.08±9.54

Table 3
PARAMETERS OF THE STUDIED POPULATION (N=55)

Group	Number of patients	TSH (mean±SD) µUI/mL Normal: 0.5-4.5 µUI/mL	FT4 (mean±SD) (pmol/L) Normal:10.3-24.4 pmol/L	TPO (mean±SD) Normal: 0-35 UI/mL
G1	9	5.96±6.77	13.60±2.59	152.75±373.61
G2	23	1.43±0.97	14.61±2.67	100.56±245.13
G3	23	3.50±2.74	13.83±2.89	200.66±334.98

Table 4
THYROID PROFILE FEATURES BETWEEN THE THREE GROUPS

	P alk	CL	OC	P1NP	25OHD	PTH
G1-mean	86.15889	0.438111	23.2575	47.28167	6.51889	65.49556
G1-SD	20.85257	0.202622	10.2614	24.34471	1.51338	37.62329
G2-mean	78.125	0.4646	24.4495	53.51143	14.9009	50.63474
G2-SD	15.40509	0.176903	11.54	14.23706	2.24897	21.8874
G3-mean	85.68421	0.502591	26.5536	59.68714	25.7291	45.39053
G3-SD	43.7087	0.256491	13.04	41.46888	3.03791	13.35254
p G1-G2	0.281771	0.723925	0.80135	0.478407	2.5E-11	0.196407
p G2-G3	0.515759	0.583188	0.5844	0.602642	1.6E-17	0.37854
p G1-G3	0.975713	0.507477	0.52504	0.506471	1.3E-17	0.045439

Table 5
BONE PARAMETERS ANALYZE

Conclusions

The groups of suboptimal 25-OHD levels in menopause do not reflect TSH and FT4 anomalies.

Abbreviations

BMI = Body Mass Index
25-OHD = 25-hydroxyvitamin D_3
PTH = parathormone
T4 = thyroxine
T3 = triiodothyronine
TSH = Thyroid Stimulating Hormone
TRH = Thyroid Releasing Hormone
TPO = anti-thyroperoxidase antibodies
SD = standard deviation

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Manuscript received: 7.02.2018