

# Relationship between Serum 25-Hydroxyvitamin D and Thyroid Hormones during Pregnancy in the North of Rome

Gabriella Lavalle<sup>1</sup>, Maria Elisabetta Onori<sup>2</sup>

<sup>1</sup>MD, Clinical Pathology Laboratory, Bracciano Hospital, Via delle Coste snc, Bracciano, Rome, Italy

<sup>2</sup>Researcher in Biological Science, University of Rome Tor Vergata, Italy

**Abstract:** *It is vital to ensure that during pregnancy the necessary vitamins and minerals are obtained. Vitamin D and thyroid hormones are essential for the correct health and proper development of a fetus. Vitamin D is a lipophilic substance linked to bone growth and it promotes the normal development of the brain of a fetus. Thyroid hormones are also fundamental for the good growth. The aim of this project was to investigate, during the summer period, the relationship between 25(OH) D (25-hydroxy vitamin D) status and circulating TSH (thyroid-stimulating hormone) and how the vitamin D is correlated with FT4 (free thyroxine) levels in pregnant women. The study was conducted from July to September 2014 at the Hospital Clinical Pathology Laboratory in Bracciano, in the north of Rome (Italy). For the purpose of this study, we selected 120 pregnant women aged between 17 and 40 years (mean age 31.08), who declared themselves in good health. For each subject, we tested Thyroid Peroxidase Antibody (TPOAb) and Thyroglobulin Antibody (TgAb), excluding 22 patients because having positive levels. 25-Hydroxyvitamin D and thyroid parameters were measured by electrochemiluminescence methods Architect 2000 (Abbott, IL, USA) and Immulite 2000 (Siemens Healthcare, UK), respectively. The results of our study reported that the mean serum 25(OH)D of the pregnant women was 24.64 (SD 7.92) ng/mL. According to the European average, we observed the prevalence of vitamin D insufficiency for 52% of subjects, deficiency for 28% and sufficiency just for 20% of women. Regarding thyroid, the mean TSH level was 1.74 (SD 1.16)  $\mu$ IU/mL and the mean FT4 level was 0.92 (SD 0.25) ng/dL, as the correct levels in pregnancy. Vitamin D deficiency is a common condition throughout world. During pregnancy, due to fetus demands, hypovitaminosis D and thyroid parameters drops are frequent. The high levels of sunshine in Italy do not necessarily guarantee a right vitamin D concentration, and although there are many studies about the role of vitamin D in the human body, there are few experiments about the correlation of this analysis and thyroid hormones during pregnancy, especially during the summer period and in a touristic sunny Italian place. The results of this study showed an association between higher vitamin D and lower TSH in pregnant women with sufficient level of 25-hydroxyvitamin D (>30ng/mL). However, we found no correlation between 25-hydroxyvitamin D and thyroid-stimulating hormone and FT4 hormone in pregnant women with 25(OH)D serum <30 ng/mL.*

**Keywords:** 25-hydroxy vitamin D, thyroid stimulating hormone, pregnancy, hypovitaminosis D.

## 1. Introduction

Correct levels of Vitamin D and thyroid hormones are essential in pregnancy. During pregnancy, calcium metabolism is stressed and sufficient vitamin D concentration is important for the demand of calcium from the fetus, as this is required in particular for growth and bone development [1]. Vitamin D is also required to ensure healthy pregnancy, for fetusskeletal development and to prevent fetal immune and neurological disorders. In addition to, hypovitaminosis D may cause pre-eclampsia [2-3]. The right bone development of the fetus is linked to a form of the 25-hydroxyvitamin D(25(OH)D) hormone. It is a steroidal hormone and it is introduced in the body through food, but the major source is the skin's synthesis of the vitamin D through exposure to solar ultraviolet light [4]. Ultraviolet B (UVB) radiation in the range of 280–320 nm penetrates through the skin causing the conversion of 7-dehydrocholesterol to provitamin D3 and it undergoes rapid conversion to cholecalciferol (vitamin D3). Vitamin D, derived from the skin or from the diet, is converted by the liver to 25-hydroxyvitamin D form which is used to determine the status of circulating vitamin D in the body. The 25(OH)D is metabolized in the kidneys by the enzyme 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase (CYP27B1) to its active form 1,25-dihydroxyvitamin D. The renal production of 1,25-dihydroxyvitamin D is tightly regulated

by plasma levels parathyroid hormone and the levels of calcium and phosphorus [5].

The level of 25(OH)D is considered as sufficient when the concentration is > 30 ng/mL (> 75 nmol/L), insufficient if the concentration is between 20 and 30 ng/mL (50-75 nmol/L) and deficient if it is < 20 ng/mL (< 50 nmol/L)[6-7]. However, the 25(OH) vitamin D status is influenced by factors which include, but are not limited to, geographical zones, seasons, dietary patterns, physical environmental surroundings, darker skin pigmentation and obesity [8-9].

The thyroid plays an essential role: proper maternal thyroid function is fundamental to ensure the development and health of a fetus [10]. Placental estrogen on the thyroid exercises a strong stimulus of TBG (Thyroxine Binding Globulin) synthesis. High TBG levels and the consequent reduction in the free fraction, metabolically active, increases the TSH (thyroid-stimulating hormone) levels, the hypothalamic hormone stimulating the synthesis of the two thyroid hormones T3 (triiodothyronine) and T4 (thyroxine). Thyroid hormones stimulate intrauterine growth, especially during the second half of gestation and they have an impact on fetal metabolism, for example through the consumption of fetal oxygen and glucose.

Many studies have shown the probable role of vitamin D in autoimmune thyroiditis and in other autoimmune diseases such as diabetes, atopic dermatitis and nephrological pathologies. Only a few studies have examined the relationship between vitamin D and no pathogenesis of autoimmune thyroid disease (AITDs) in non-pregnant women. There is also a little evidence concerning the role of 25 (OH)D in association with thyroid hormones in pregnancy.

The aim of our study was to explore the relationship between 25-hydroxyvitamin D status and circulating TSH and the correlation of vitamin D with FT4 levels in pregnant women in the North of Rome (Italy) during summer period and in a touristic place.

## 2. Materials and methods

The study was conducted from July to September 2014 at Clinical Pathology Laboratory in the Bracciano Hospital in the north of Rome (Latitude: 42°06'00" N, Longitude 12° 10' 00" E).

The project was carried out using 120 blood samples of pregnant Italian women undergoing prenatal examination. All subjects were in self proclaimed good health. All blood samples were centrifuged at 3000 rpm for 10 minutes and they were analyzed within two hours.

Firstly, we excluded subjects with dietary restrictions, consuming vitamin D supplementation and those on rehabilitative drugs therapy. Then, for every patient, we tested Thyroid Peroxidase Antibody (TPOAb) and Thyroglobulin Antibody (TgAb) to exclude the presence of autoimmune thyroid disease (AITD) and the elevated antibody peroxidase or thyroglobulin.

We detected all samples for TPOAb (threshold values: 0-35 UI/mL) and TGAb (threshold values: 0-40 UI/mL) using the Immulite 2000 system (Siemens Healthcare, UK) based on a solid phase, enzyme labelled, chemiluminescent immunometric assay.

After removal of 22 thyroid antibody positive women, we tested 98 blood samples collected from women in the morning after overnight fasting for the measurement of FT4, TSH and 25(OH) vitamin D.

Serum FT4 and TSH were detected by a chemiluminescent immunoassay diagnostic kit (Immulite 2000, Siemens Healthcare, UK). Reference values are between 0.4 and 4.0  $\mu$ IU/mL for TSH and from 0.89 to 1.76 ng/dL for FT4. Serum quantitative determination of 25-hydroxyvitamin D was measured using the chemiluminescent microparticle immunoassay Architect (Abbott, IL, USA). A deficient assessment was considered when 25(OH)D status was < 20 ng/mL (<50 nmol/L), an assessment was considered insufficient with levels ranging from 20 to 30 ng/mL (50-75 nmol/L) and considered normal when levels were >30 ng/mL (>75 nmol/L).

It is important to note that to uphold the ethics of the study, written informed consent was taken from each subject before they could be included in the study.

## 3. Statistical Analysis

The statistical program SPSS 13.0 for Windows (Chicago, USA) was used for all analysis. Continuous variables are expressed as media  $\pm$  standard deviation (SD). Categorical variables were presented as a percentage.

We assessed the correlation between 25-hydroxyvitamin D and thyroid hormones with the Pearson correlation coefficient. All data were considered statistically significant for *p*-value less than 0.01.

## 4. Results

The study included 120 pregnant women with mean age  $31.08 \pm 5.84$  (from 17 to 40) years. 18% of women were excluded because they presented positive levels of thyroid antibodies. The mean 25(OH)D status of the cohort was  $24.64 \pm 7.9$  (range 7.4 to 43.5). The mean level of Free T4 was  $0.92 \pm 0.25$  ng/dL and the mean concentration of TSH was  $1.72 \pm 1.16$   $\mu$ IU/mL.

From the results we obtained that 28% of women presented a vitamin D deficiency with a mean concentration of 15.74 ng/mL, while vitamin D insufficiency was reported in 52% of the subjects (mean 25(OH)D serum = 25.19 ng/mL). The remaining 20% of women showed a vitamin D sufficient level of 36.40 ng/mL. After this division in three categories, deficiency-insufficiency-sufficiency, we calculated the correlation between the 25(OH)D and TSH and between the vitamin D and FT4 as shown in Table 1. We found that there was no correlation between 25-hydroxyvitamin D and FT4 level ( $r = -0.08$ ,  $p < 0.01$ ) in pregnant women, while there was a mild ( $r = 0.54$ ) but significant ( $p < 0.01$ ), correlation between the mean 25(OH)D and TSH level, only in the group of women with sufficient vitamin D levels (>30 ng/mL). However, there was no significant relationship ( $p < 0.01$ ) between 25 (OH)D deficient and insufficient levels, and TSH status.

## 5. Discussion

Vitamin D deficiency is a common condition throughout world [11]. Recent studies have shown an increase of vitamin D insufficiency in the Mediterranean region. Considering this, our study indicated a prevalence of 25-hydroxyvitamin D insufficiency (52%) in the analyzed pregnant subjects who lived in a mainly sunny zone. High levels of sunshine in Italy do not necessarily guarantee a right vitamin D concentration. During pregnancy, hypovitaminosis D is caused by calcium loss. Fetal demands and a rise in urinary calcium excretion may cause serious maternal and offspring calcium disorders, such as preterm birth, low birth weight, pre-eclampsia and gestational diabetes.

Unfortunately, vitamin D deficiency [12] is so widespread in pregnant women that vitamin D supplementation is often advised [13-15]. Many studies have shown that this hor-

mone, seemingly simple, is pleiotropic and affects many aspects of the human body. This particular analyte influences the optimal function of cardiac and skeletal muscles and it is involved in the modulation of the immune system. Furthermore, it regulates cell proliferation, cell differentiation, and apoptosis. As mentioned previously, the 25(OH)D status is influenced by many factors. The vitamin D deficiency could be explained by the more skin pigmentation diffused in southern Europe and the reduced sun exposure of pregnant women. As shown by previously conducted studies, dermal synthesis of vitamin D through exposure to ultraviolet compounds from natural sunlight, for a set period of time per day, can provide 80-100% of human vitamin D requirements [16].

Similarly to the changes in levels of vitamin D, during pregnancy, thyroid hormones may also undergo alteration by fetus action. The correct functioning of the maternal thyroid helps to achieve successful pregnant outcomes. The lost increase of a mother's TSH, FT4 and FT3 levels is associated with placental abruption, miscarriage, fetal growth retardation, gestational hypertension and neuropsychological retardation of a fetus [17].

Vitamin D promotes cell growth, it facilitates the activity of the thyroid gland and strengthens the immune system, allowing the construction of a viable defence against potential infections.

Reports have proved a correlation between Vitamin D and TSH, especially in patients with thyroid diseases [18]. For instance a Chinese study, showed an association of high 25(OH)D status and low TSH in middle-aged and elderly Chinese males with serum thyroid autoantibody [19]. Moreover an Indian study reported the presence of a significant inverse correlation between vitamin D levels and thyroid autoimmunity [20]. Another project conducted in Thailand found the prevalence of vitamin D insufficiency in TgAb-positive subjects, and a high vitamin D status and low circulating thyrotropin in younger individuals [21].

Although there are many studies on the relationship between vitamin D and the thyroid parameters, there are very few researches, such as our, that seek to identify the relationship between 25-(OH)D and Thyroid hormones during pregnancy in women without AITD.

## 6. Conclusion

Our experiment was conducted in a touristic Italian place in the north of Rome. This place is close to the Bracciano's lake and 20 km far from the Tirreno see. Due to we conducted the study in the summer period, we can hypothesize that the vitamin D levels could be higher than the diffused hypovitaminosis D in Italy. Results showed a slight, but significant, correlation between high 25(OH)D status and low TSH in pregnant women with a sufficient level of Vitamin D ( $> 30$  ng/mL). Our observation on pregnant women does not provide any evidence of causative effect of vitamin D on thyroid hormones such as FT4. However, the correlation we found between TSH levels with 25-hydroxyvitamin D may reflect a specific vitamin D

influence on pituitary TSH production as shown in previous studies.

To further enhance knowledge in this particular subject, this research could be taken a step by including but not be limited to, dividing the women according to weeks of pregnancy and age or evaluating how a supplementation of vitamin D may change the 25(OH)D status in pregnancy.

## 7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## References

- [1] F.R. Pérez-Lopéz, "Vitamin D: the secosteroid hormone and human reproduction", *Gynecological Endocrinology*, vol. 23, n. 1, pp. 13-24, 2007.
- [2] L.M. Bodnar, J.M. Catov, H.N. Simhan, M.F. Holick, R.W. Powers et al. "Maternal vitamin D deficiency increases the risk of preeclampsia", *The Journal of Clinical Endocrinology and Metabolism* 92: 3517-3522, 2007.
- [3] C.W. Levenson, S.M. Figueirôa, "Gestational vitamin D deficiency: long-term effect on the brain", *Nutrition Reviews*, vol. 66, pp. 726-729, 2008.
- [4] B. Lehmann, K. Querings, J. Reichrath, "Vitamin D and skin: new aspects for dermatology", *Experimental Dermatology*, vol. 13, n. 5, pp. 4-11, 2004.
- [5] P. Lips, "Vitamin D and physiology", *Progress in Biophysics and Molecular Biology*, vol. 92, n. 1. Pp. 4-8, 2006.
- [6] L.O. Chailurkit, W. Aekplakorn, and B. Ongphiphadhanakul, "Regional variation and determinants of vitamin D status in sunshine-abundant Thailand", *BMC Public Health*, vol. 11, 853, 2011.
- [7] M.F. Holick, "Vitamin D deficiency", *New England Journal of Medicine*, vol. 357, pp. 266-281, 2007.
- [8] S.N. Karras, P. Anagnostis, C. Annweiler, D.P. Naughton, A. Petroczi, V. Harizopoulou, B.C. Tarlatzis, A. Persinaki, F. Papadopoulou, D.G. Goulis, "Maternal vitamin D status during pregnancy: the Mediterranean reality", *European Journal of Clinical Nutrition*, doi: 10.1038, 2014, 80.
- [9] W.G. Tsiaras, M.A. Weinstock, "Factors influencing vitamin D status", *Acta Dermato-Venereologica*, vol. 91, pp. 115-124, 2011.
- [10] A. Lapillonne, "Vitamin D deficiency during pregnancy may impair maternal and fetal outcomes", *Medical Hypotheses*, 74: 71-75, 2010.
- [11] G. Lippi, M. Montagnana, T. Meschi and L. Borghi, "Vitamin D concentration and deficiency across different ages and genders", *Aging Clinical and Experimental Research*, vol. 24, n. 5, pp. 548-551.
- [12] S.A. Hamilton, R. McNeil, B.W. Hollis, D.J. Davis, J. Winkler, C. Cook, G. Warner, B. Bivens, P. McShane, C.L. Wagner, "Profound Vitamin D deficiency in a diverse group of women during pregnancy living in a sun-rich environment at latitude 32 N", *International Journal of Endocrinology*, doi: 10.1155, 2010, 917428.
- [13] Y. Zhao, W. Miao, C. Li, X. Yu, Z. Shan, H. Guan, W. Teng, "Dynamic changes in serum 25-hydroxyvitamin

D during pregnancy and lack of effect on thyroid parameters”, *PLOS ONE*, doi: 10.1371, 2014, 0090161.

[14] S.Vandevijvere, S. Amsalkhir, H.Van Oyen, R.Moreno-Reyes, “High prevalence of vitamin D deficiency in pregnant women: a national cross-sectional survey”, *PLOS ONE*, vol. 7, 2012.

[15] T. McAree, B. Jacobs, T. Manickavasagar, S. Sivalokanathan, L. Brennan et al., “Vitamin D deficiency in pregnancy – still a public health issue”, *Maternal and Child Nutrition*, vol. 9, pp. 23-30, 2013.

[16] P. Mahon, N. Harvey, S. Crozier, H. Inskip, S. Robinson, N. Arden, R. Swaminathan, C. Cooper, K. Godfrey and SWS Study group, “Low maternal Vitamin D status and fetal bone development: cohort study”, *Journal of Bone and Mineral Research*, vol. 40, n. 1, pp. 14-19, 2010.

[17] F. Azizi, A. Amouzegar, L. Mehran, S. Alamdari, I. Subekti, B. Vaidya, K. Poppe, T. San Luis Jr and T. Akamizu, “Screening and management of hypothyroidism in pregnancy: Results of an Asian survey”, *Endocrine Journal*, vol. 61, n. 7, pp. 697-704, 2014.

[18] G. Tamer, S. Arik, I. Tamer, and D. Coksert, “Relative vitamin D insufficiency in Hashimoto’s thyroiditis”, *Thyroid*, vol. 2, n. 8, pp. 891-896, 2011.

[19] Q. Zhang, Z. Wang, M.Sun, M. Cao, Z.Zhu, Q. Fu, Y.Gao, J. Mao, Y.Li, Y.Shi, F.Yang, S.Zheng, W.Tang, Y.Duan, X. Huang, W.He, and T.Yang, “Association of high vitamin D status with low circulating thyroid-stimulating hormone independent of thyroid hormone levels in middle-aged and elderly males”, *International Journal of Endocrinology*, doi: 10.1155/2014/631819.

[20] R. Goswami, R. K. Marwaha, N. Gupta, N. Tandon, V. Sreenivas, N. Tomar, D. Ray, R. Kanwar and R. Agarwal, “Prevalence of vitamin D deficiency and its relationship with thyroid autoimmunity in Asian Indians: a community-based survey”, *British Journal of Nutrition*, vol.102, n. 3, pp. 382-386, 2009.

[21] L.O. Chailurkit, W. Aekplakorn, and B. Ongphiphadhanakul, “High vitamin D status in younger individuals is associated with low circulating thyrotropin”, *Thyroid*, vol. 23, n. 1, pp. 25-30, 2013.

**Table 1:** serum TSH and FT4 according to 25(OH)D levels

25(OH) D range	No. of patients	Age mean (years)	Serum F4 (ng/dL)	Serum TSH (uIU/mL)	r with FT4	r with TSH	p ft4	p tsh
<20	27 (28%)	30	0,98	1,53	0,005	-0,103	<0,01	<0,01
21-30	51 (52%)	32	0,93	1,69	-0,184	0,023	<0,01	<0,01
>30	20 (20%)	29	0,85	2,06	-0,071	-0,541	<0,01	<0,01
total	98	31	0,93	1,72	-0,210	0,077	<0,01	<0,01