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VITAMIN D DEFICIENCY AND VITAMIN D RECEPTOR GENE POLYMORPHISMS – RISK FACTORS IN DIFFERENTIATED THYROID CARCINOMA

THESIS SUMMARY

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Cuprins

Introduction

Thyroid carcinoma is the most common malignant endocrine pathology representing approximately 3.2% of all newly diagnosed cancers [1]. Its incidence has increased significantly in recent years, being estimated at 13.7/100,000 people/year in 2017 and 14.6/100,000 people/year in 2022, but the mortality rate has remained constant at 0.5/100,000 people/year [2.3]. This difference is supposed to be primarily due to the intensive ultrasound screening, but recent studies incriminate the involvement of new genetic mechanisms and aim to discover factors that can be used for diagnostic, prognostic and monitoring purposes [4].

Regarding the relationship of vitamin D level with differentiated thyroid carcinoma, Zhao et al. carried out an analysis that included 14 studies that confirmed that the level of 25-hydroxy-vitamin D was significantly lower in patients with thyroid cancer, vitamin D deficiency can be considered a risk factor for thyroid carcinoma [5, 6] . The most recent hypothesis regarding the association between vitamin D and differentiated thyroid carcinoma is represented by the study of vitamin D receptor (VDR) gene polymorphisms. This gene located on chromosome 12q12-q14 shows 4 main polymorphisms, namely: FokI (in exon 2), BsmI and ApaI (in intron 8) and TaqI (in exon 9) [7]. The results of these studies are controversial, but precisely the principle of genetic diversity can lead to conclusive results.

The obtained results show that ApaI and FokI polymorphisms (AA and FF genotypes) can confer protection against follicular carcinoma, while FokI TT genotype is correlated with T3/T4 stages, extrathyroidal invasion, multifocality and tumor sizes ≥ 1cm [8, 9].

All collected data confirm that vitamin D interferes and regulates the entire tumorigenesis process including cell proliferation and differentiation, apoptosis and autophagy processes as well as angiogenesis, inflammation and immunity [8, 9, 10].

The conclusions of these studies indicate the need for similar studies in thyroid carcinoma, especially that resistant to radioiodine therapy. I therefore consider that the chosen topic is one of great scientific interest, being the first paper aimed at the study of vitamin D and VDR gene polymorphisms in differentiated thyroid carcinoma in Romania.

General data

Chapter 1 – Thyroid cancer

1.1.Definition

Thyroid nodules are a common pathology, with a prevalence of 4-7% in the United States population [11].

1.2.Epidemiology

It represents the main endocrine cancer, with a recorded incidence of 3.4% of all diagnosed cancers [12, 13].

1.3. Risk factors

They are considered: radiation exposure followed by family history, iodine intake, pre-existing thyroid pathologies, vitamin D deficiency and endocrine disruptors [12, 13].

1.4.Differentiated thyroid carcinoma classification

CPT accounts for over 80% of CT cases, and CFT is the second most common type of CT, accounting for 10% of all cases [14].

1.5.Differentiated thyroid cancer risk stratification

The 8th edition of the TNM classification brings changes such as increasing the age limit for staging from 45 to 55 years at the time of diagnosis and the fact that minimal extrathyroidal extension detectable only on histopathological examination has been excluded from the T3 category and no longer has an impact on staging [15].

1.6.Diagnosis

Diagnosis of CT is clinical, imaging and by fine needle biopsy [14].

1.7.Differentiated thyroid cancer management

Surgical treatment is the first choice, usually followed by radioiodine therapy and levothyroxine suppression therapy and rarely by cytotoxic chemotherapy [16]. Molecular therapy targets multikinase inhibitors (MKIs) and selective ones [17].

1.8.Differentiated thyroid cancer genetics

The most incriminated mutations in thyroid carcinoma are: RET/PTC rearrangements, TRK and ALK rearrangements, RAS mutations and BRAF mutation [18].

Chapter 2-Vitamin D and thyroid cancer

2.1. Vitamin D- synthesis and metabolism

The synthesis and metabolism of vitamin D consists of three main enzymatic steps (25-hydroxylation, 1α-hydroxylation and 24-hydroxylation) carried out by means of the genes encoding the specific enzymes CYP27A1 (sterol 27-hydroxylase), CYP27B1 (1-α hydroxylase), and CYP24A1 (24 hydroxylase) [19].

2.2.Mechanism of action

The 1,25(OH)2 vitamin D molecule enters the target cell and binds to the VDR receptor that heterodimerizes with the retinoid X receptor, increasing the affinity for the VDRE [20].

2.3.Vitamin D receptor gene polymorphisms

The most investigated polymorphisms of the VDR gene are: FokI (rs2228570), BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236) and Cdx2 (rs11568820) [20].

2.4.Vitamin D functions

2.4.1. Vitamin D and bone metabolism

Serum vitamin D level correlates with skeletal mineralization, bone turnover rate, and fracture risk [21].

2.4.2. Extraskeletal actions of vitamin D

Vitamin D is considered to be involved in autoimmune pathologies including thyroid, it is correlated with neuropsychiatric and cardiovascular pathologies as well as metabolic and malignant diseases [23].

2.5.Vitamin D and thyroid cancer

2.5.1. Antineoplastic effects of vitamin D

The most important effects of calcitriol are:

- It inhibits the proliferation of malignant cells by blocking the cell cycle in the G0/G1 phase
- Induces cell differentiation by regulating signaling pathways
- Induces cell apoptosis by disrupting mitochondrial function, cytochrome release and production of reactive oxygen species
- It causes autophagy mode change from cell survival to cell death in malignant cells
- Inhibits DNA damage induced by oxidative stress through antioxidant activity

- Reduces the ability of malignant cells to invade and metastasize by inhibiting angiogenesis and controlling some molecules involved in this process

- It inhibits the pathway of prostaglandins involved in the pro-inflammatory process [24].

2.5.2. Differentiated thyroid cancer and vitamin D levels

Preclinical studies have shown arrest of malignant cell growth in differentiated thyroid carcinoma after administration of pharmacological doses of 1,25(OH)2D or its analogs.

VDR gene polymorphisms and differentiated thyroid carcinoma

In addition to a significantly lower serum level of 1,25(OH)2D in patients with CTD compared to the healthy group, Penna-Martinez et al., highlights the association between VDR polymorphisms and the increased incidence of thyroid carcinoma, an increased risk for CTD being conferred by CYP24A1 gene haplotypes with 25(OH)D deficiency and reduced conversion to the active form. This study also concluded that the AA and FF alleles of the ApaI and FokI polymorphisms and the tABF haplotype confer protection against susceptibility to follicular carcinoma, while the Tabf haplotype appears to be correlated with increased risk of CFT [25]. Beysel S, et al. demonstrate that the TT genotype of FokI is associated with T3/T4 thyroid carcinoma stages, extrathyroidal invasion, multifocality, and a tumor diameter ≥ 10 mm [26].

Personal contribution

Chapter 3- Hypothesis and general objectives

Study objectives:

1. **Primary objectives:**

The project aims to:

- Identification of the level of vitamin D expressed by 25OHvitamin D3 in thyroidectomized patients with differentiated thyroid carcinoma and benign thyroid pathology
- Identification of vitamin D receptor gene polymorphisms in thyroidectomized patients with differentiated thyroid carcinoma and benign thyroid pathology
- Estimation of the prevalence of vitamin D deficiency in both categories of patients
- Correlation of vitamin D level with different clinical, imaging and histopathological parameters in patients with thyroid carcinoma versus those with benign pathology
- Correlation of vitamin D receptor gene polymorphisms with vitamin D level
- Correlation of vitamin D receptor gene polymorphisms with different clinical, hormonal, imaging and histopathological parameters in patients with thyroid carcinoma compared to those with benign pathology
- Synthesizing all information with the aim of establishing vitamin D deficiency and vitamin D receptor gene polymorphisms as risk factors for differentiated thyroid carcinoma

2. **Secondary objectives:**

Evaluation by a comparative, controlled study of the differences between clinical, biochemical, hormonal and imaging parameters in patients with differentiated thyroid carcinoma versus benign thyroid pathology

Chapter 4 – General research metodology

1.1. Patients

We performed a retrospective, controlled, observational, non-interventional and non-randomized study with a cross-sectional analysis element on a sample of 506 thyroidectomized patients with CTD and benign thyroid pathology between January 2017 and December 2021 in within the National Institute of Endocrinology "C.I. Parhon". The patients were divided into two groups: group A included 206 patients with CTD, respectively group B (control) included 300 patients with benign thyroid pathology.

1.2. Inclusion criteria

The inclusion criteria were: women and men aged > 18 years, obtaining informed written consent, total thyroidectomy with histopathological result of CTD or benign thyroid pathology and obtaining a complete anamnestic, paraclinical investigation and the possibility of genetic testing of VDR gene polymorphisms.

1.3. Exclusion criteria

Exclusion criteria were: age <18 years, absence of written informed consent or its withdrawal by the patient, patients with other types of CT, current or previous recent treatment with vitamin D preparations, antiosteoporotic medication, systemic glucocorticoids and antiepileptics, patients with pathologies or clinical signs associated with vitamin D deficiency.

1.4. Materials and methods

1. Anamnestic and demographic data
2. Paraclinical data: hematological and biochemical analyses, immunological and hormonal analyses, genetic analyzes included VDR gene polymorphisms: ApaI, BsmI, FokI, TaqI.
3. Imaging tests: preoperative thyroid ultrasound ¬ The result of the postoperative histopathological examination

1.5. Statistical analysis

The R program was used with the following packages loaded: effects, ggplot2, ggpubr, gtsummary, HardyWeinberg, table. The investigation of the existence of the Hardy-Weinberg equilibrium for the 4 genotypes was carried out using the 4 tests available in the HardyWeinberg package. P values lower than 0.05 were considered statistically significant.

Chapter 5 – Results

5.1.Demographic data analysis

Regarding age, the patients in the group of benign pathology had a higher average age compared to the group of those with cancer of 55.57 ± 12.07 years vs. 50 ± 14.18 years. Statistically significant results were recorded between the groups in the case of the environment of origin ($p=0.0268$), gender ($p=0.0044$) and average age ($p<0.0001$).

5.2.Biochemical data analysis

The value of total calcium is statistically significantly lower in patients with thyroid cancer compared to those with benign pathology ($p=0.002$), as well as that of serum magnesium ($p=0.040$) and HDL-cholesterol ($p=0.006$) and the value of ALT was statistically significantly higher in those with cancer compared to the control group ($p=0.036$).

5.3.Hormonal and immunologic data analysis

The values of fT4, ATPO and TRab were significantly higher in group B, and those of calcitonin and PTH were significantly higher in group A patients.

5.4.Ultrasound features analysis

Statistically significant differences were observed regarding the echogenicity of the nodules so that in the cancer group the proportion of those with hypoechoic nodules was higher compared to the benign pathology group. In group A, the presence of calcifications was more frequent compared to group B ($p<0.0001$), as were laterocervical adenopathies ($p<0.0001$).

5.5.Histopathologic result analysis

The histopathological result confirmed the multifocality of thyroid carcinoma in 98 cases (47.57%). Thyroid carcinoma was invasive in fibroconjunctive and muscle tissue in 25 cases (12.13%), angioinvasive in 42 cases (20.38%), lymphatic invasive in 48 cases (23.30%) and capsular invasive in more than half of cases (55.82%). Distant metastases were identified in 5 cases (2.43%) The average size of the cancer focus was 1.8±1.45 cm.

5.6.Comorbidities analysis

The most frequent associated pathologies were arterial hypertension (HT) and dyslipidemia in both groups representing 25.72% vs. 26.67% for HTN and 26.70% vs. 29% for dyslipidemia.

5.7.Vitamin D status evaluation

The comparative analysis of the vitamin D level between the two studied groups showed a statistically significant difference (p<0.001), with a higher average level in the case of the subjects of the group with benign thyroid pathology (Table 5.11).

Table 5.11 – Statistical data for vitamin D level

Data	Lot A N=206	Lot B N=300	Difference (95% CI)[1,2]	p[1] *value*
Vitamin D Mean ± DS	16.85 ± 7.10	19.04 ± 7.41	-2.2 (-3.5 la -0.90)	<0.001

[1] *Welch Two Sample t-test*
[2] *CI = Confidence interval*

5.8.Vitamin D gene receptor polymorphisms analysis

5.8.1. Hardy-Weinberg equilibrum analysis

VDR ApaI polymorphism analysis

In group A, the null hypothesis (H0) of the existence of equilibrium cannot be rejected, therefore the alleles for this genotype are considered to be in Hardy-Weinberg equilibrium. Similarly, for batch B.

VDR BsmI polymorphism analysis

For batch A, the calculated $\chi 2$ value is 0.7782 (p=0.520) and cannot reject the null hypothesis of the existence of equilibrium, therefore the alleles for this genotype are considered to be in Hardy-Weinberg equilibrium. Similarly, also in group B the BsmI alleles are considered in equilibrium.

VDR TaqI polymorphism analysis

The calculated $\chi 2$ value is 0.8671 (p=0688) and cannot reject the null hypothesis of the existence of equilibrium, therefore the alleles for this genotype are considered to be in Hardy-Weinberg equilibrium. Similarly, in batch B.

VDR FokI polymorphism analysis

In this group, the test results allow the rejection of the H0 hypothesis, thus confirming that the sample of patients with thyroid cancer is not in Hardy-Weinberg equilibrium for the FokI polymorphism. In pool B, the FokI alleles are considered in equilibrium.

Patients in the group with benign thyroid pathologies are in Hardy-Weinberg equilibrium for all 4 genotypes, so it can be stated that they are equivalent to the general population regarding

VDR polymorphisms and also the 4 genotypes do not seem to be involved in the etiopathogenesis of thyroid conditions from this lot.

5.8.2. VDR gene polymorphisms and thyroid cancer risk

VDR ApaI polymorphism analysis

The statistical analysis identified that in subjects with aa alleles the risk of neoplasm is lower, the OR being half compared to subjects with AA alleles, the effect being statistically significant (p=0.033). The Aa variant does not influence the risk of thyroid neoplasm (Fig. 5.21). Therefore, the aa genotype appears to have a protective effect against thyroid carcinoma.

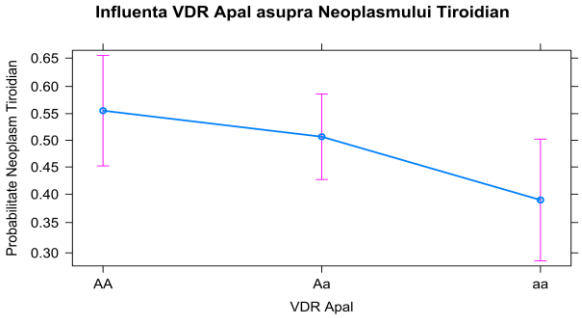

Fig. 5.21- Thyroid cancer risk for ApaI polymorphism

VDR BsmI polymorphism analysis

Regarding the BsmI polymorphism, 27 patients were homozygous BB, 80 were heterozygous Bb, and 48 were homozygous bb. In patients with the bb phenotype, the risk of differentiated thyroid carcinoma is lower, the OR being half compared to subjects carrying the BB phenotype, the effect being marginally insignificant (p=0.060). In addition, Bb does not influence the risk of thyroid cancer (Fig. 5.22).

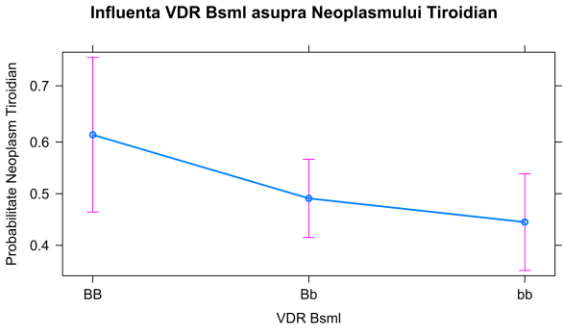

Fig. 5.22- Thyroid cancer risk for BsmI polymorphism

VDR TaqI polymorphism analysis

Regarding the involvement of the VDR TaqI polymorphism, no statistically significant influences were highlighted (Fig. 5.23). Of group A patients, 54 were TT homozygous, 77 were Tt heterozygous, and 24 were tt homozygous.

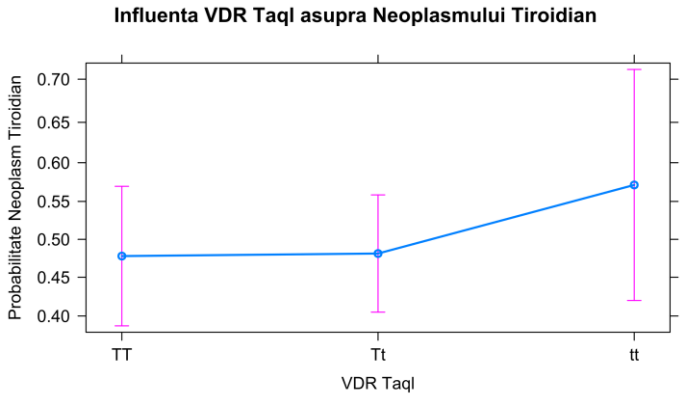

Fig. 5.23- Thyroid cancer risk for TaqI polymorphism

VDR FokI polymorphism analysis

The statistical analysis of the VDR FokI polymorphism showed that in patients with ff phenotype the risk of neoplasm is higher compared to those carrying FF, the OR being 3 times higher (p=0.004). Ff does not influence the risk of thyroid neoplasm (Fig. 5.24). Among group A subjects, 63 were homozygous FF, 62 were heterozygous Ff, and 30 were homozygous ff.

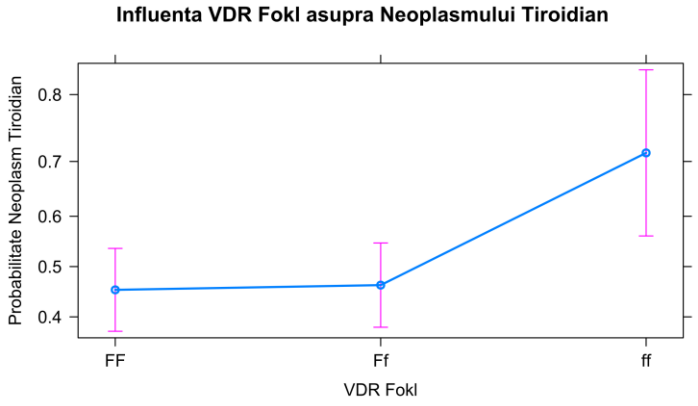

Fig. 5.24- Thyroid cancer risk for FokI polymorphism

5.8.3. VDR gene polymorphisms and vitamin D deficiency risk

VDR ApaI polymorphism analysis

By comparing the means we obtained a statistically significantly higher value in group B for the Aa genotype (p=0.0029). It can also be seen that all medians and means are higher in batch B compared to batch A.

VDR BsmI polymorphism analysis

According to the analysis, both factors have statistically significant p-values, thyroid cancer and the VDR BsmI genotype acting synergistically, and the lowest vitamin D values were recorded for the bb allelic variant.

VDR FokI polymorphism analysis

Thyroid cancer and the FokI genotype act synergistically, with the lowest vitamin D values found in cancer patients with the f allele present.

VDR TaqI polymorphism analysis

The analysis demonstrated that the source of the differences is secondary to the existence of the thyroid neoplasm, the influence of the VDR TaqI genotype being marginally insignificant. In conclusion, the bb genotype increases the risk of vitamin D deficiency by 3.22 times (p=0.0195) and the Ff genotype increases this risk approximately 5 times (p=0.0014). Simple univariate binomial logistic regression was used for this analysis.

5.9.Vitamin D and clinical-histopathologic features association analysis in thyroid cancer patients

1. **Study of the association between vitamin D level and TNM staging**

From this descriptive statistical analysis, the lower values of the mean level emerge of vitamin D in stage 3 and 4 patients compared to stages 1 and 2.

2. **Study of the association between the level of vitamin D and the presence of calcifications**

The evaluation shows a mean value of vitamin D of 18.5 ng/mL ± 6.92 SD in patients without microcalcifications, of 13.5 ng/mL ± 5.94 SD in those with macrocalcifications and of 15.8 ng/mL ± 7.18 SD in those with microcalcifications (Fig. 5.37) ($p<0.01$).

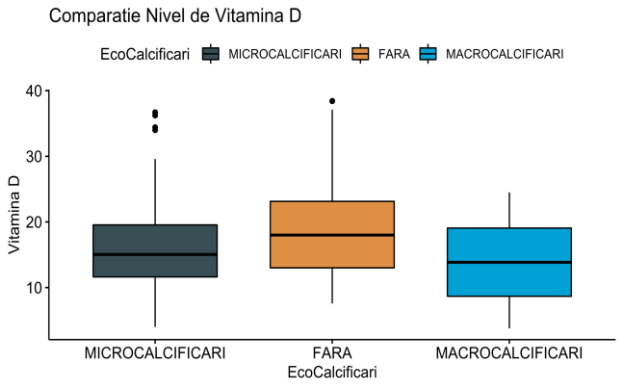

Fig. 5.37-Vitamin D and ultrasound calcifications

3. **Study of the association between the level of vitamin D and the ultrasound size of the nodule**

The result (r = -0.22, p= 0.001) confirms a weak, negative association with statistical significance, with patients with larger nodular sizes having a lower vitamin D level.

4. **Study of the association between vitamin D level and the presence of suspicious adenopathies**

The mean vitamin D level was significantly lower in patients with adenopathies suspicious at the preoperative ultrasound evaluation (p=0.007).

5. The association between the level of vitamin D and the maximum ultrasound size of adenopathies - without statistical significance (r=0.14, p=0.108).

6. The association between vitamin D level and the size of the focus of differentiated thyroid carcinoma – negative correlation (r=-0.07) but without statistical significance.

7. Association between vitamin D level and invasiveness of differentiated thyroid carcinoma

- Invasiveness in fibroconjunctive and muscle tissue

The mean value of vitamin D was statistically significantly (p=0.047) lower at patients with invasion in fibroconjunctival and/or muscle tissue (14.21 ng/mL vs. 17.21 ng/mL).

- Angioinvasiveness

The mean value of vitamin D was statistically significantly (p=0.037) lower at patients with angioinvasion (14.82 ng/mL vs. 17.37 ng/mL).

- Capsular invasiveness

The average value of vitamin D was statistically significantly (p=0.005) lower at patients with capsular invasion.

- Lymphatic invasiveness

The mean value of vitamin D was lower in patients with lymphatic invasion (15.66 ng/mL vs. 17.21 ng/mL), but the association had no statistical significance (p=0.185).

8. The association between the level of vitamin D and the presence of psammoma bodies

The average level of vitamin D was higher in patients who had bodies described psammoma,but the p value was statistically insignificant (p=0.64).

9. The association between the level of vitamin D and the number of outbreaks was investigated with using a Pearson r test, the result: r = -0.22, p = 0.001, demonstrating a weak, negative and statistically significant association between vitamin D and the number of outbreaks.

10. The association between the level of vitamin D and the presence of locoregional lymph node metastases was statistically significant (p=0.032) confirming a lower level of of vitamin D in patients who presented such determinations. The average number of nodal metastases was 2.85 ± 3.30 SD, and the median was 2 with a minimum value of 1 metastasis and a maximum of 20 metastases. According to Pearson's coefficient, r=-0.146, p=0.032, a weak, negative correlation between the 2 parameters is confirmed.

11. Association between vitamin D level and multifocality

The average level of vitamin D was marginally significantly (p=0.056) lower at patients with multiple foci of thyroid carcinoma compared to those who had a single foci.

12. Association between vitamin D level and histopathological type of thyroid carcinoma

The lowest mean value of vitamin D was found in patients with carcinoma follicular, but the small number of such patients do not allow issuing a firm conclusion

5.10. VDR gene polymorphisms and clinical-histopathologic features associations study in thyroid cancer patients

1. **ApaI polymorphism associations**
   a. **With ultrasound features**

There were no statistically significant differences in attendance calcifications (p=0.723). The smallest average size of ultrasound suspicious adenopathies was identified in the AA genotype (p=0.013 by ANOVA).

b. With histopathologic features

There were marginally insignificant associations identified for angioinvasiveness (p=0.088) where the lowest proportion was found in patients carrying the aa genotype and the highest in those with the Aa genotype.

2. **BsmI polymorphisms associations**

a. With ultrasound features

Associations with ultrasound parameters demonstrated the presence calcifications that the minimum proportion of microcalcifications was found in the Bb genotype and of macrocalcifications, in BB (p=0.286 by Fisher's exact test).

b. With histopatholgic features – no statistically significant correlations between genotypes were identified.

3. **TaqI polymorphisms associations**

a. With ultrasound features – no statistically significant correlations between genotypes were identified.

b. With histopatholgic features – no statistically significant correlations between genotypes were identified.

4. **FokI polymorphisms associations**

a. With ultrasound features

Macrocalcifications were most frequently present in those with genotype FF, and microcalcifications in those with Ff genotype (p=0.713 by Fisher's exact test). The average nodule size was higher for the ff genotype (p=0.866 by ANOVA test). The most frequent, ultrasound-suspected adenopathies were found in the case of the Ff genotype and their largest average size was found in ff (p=0.329 by ANOVA test).

b. With histopathologic features

Invasiveness in muscle and fibroconjunctive tissue has been identified the most frequent in Ff patients (p=0.132 at the $\chi 2$ test). Angioinvasiveness and capsular invasiveness were most frequently found in ff carriers (p=0.147, respectively p=0.301 at the $\chi 2$ test). The mean number of carcinoma foci was higher in the ff genotype (p=0.747), as was the mean foci size (p=0.414). All patients with the ff genotype had distant metastases (p=0.892).

Chapter 6 – Discussions

The comparative analysis of vitamin D level between the two groups showed a statistically significant difference ($p<0.001$) with a mean level of 16.85 ± 7.10 ng/mL in group A and 19.04 ± 7.41 ng/mL in group B, association found and in specialized literature. Zhao et al. showed in a meta-analysis of 14 studies that the preoperative level of 25-hydroxy vitamin D is lower in patients with thyroid carcinoma compared to controls (-0.22, 95% CI -0.36 to -0.09, p=0.001) and that the deficiency of vitamin D can increase the risk of thyroid cancer by almost 30% [5].

Analysis of genotypes in patients with thyroid cancer showed that VDR FokI is in Hardy-Weinberg disequilibrium, confirming the implication of this polymorphism in this pathology. The aa allele of the ApaI polymorphism is found more frequently in group B (p=0.033). Similarly, with a marginally insignificant p value (p=0.062) and the BB genotype was more frequent in the group with cancer as was the t allele. Statistical analysis confirmed that subjects with the aa allele have a significantly lower risk of thyroid carcinoma (p =0.033), this seems to have a protective effect against thyroid cancer. In patients with the bb phenotype the risk of cancer is lower, the OR being half comparable to subjects carrying the BB phenotype. VDR FokI polymorphism analysis showed that patients with ff phenotype have a 3 times higher risk of developing thyroid cancer (p=0.004). By analyzing the role of VDR gene polymorphisms on the risk of vitamin D deficiency, we obtained a significantly (p=0.0025) higher value in group B for the Aa genotype (Table 5.35), suggesting a possible protective role of this genotype against vitamin D deficiency. the VDR BsmI genotype, the allelic variant bb showed the lowest statistically significant values (p=0.034) of the vitamin D level, this allele increasing the risk of vitamin D deficiency by 3.22 times (p=0.0195). The VDR FokI genotype acts synergistically with thyroid cancer, the lowest values being found in patients carrying the f allele with p=0.001 values, the Ff genotype increasing the risk of vitamin D deficiency almost 5 times (p=0.0014). The mean value of vitamin D was significantly lower (p=0.047) in patients with invasiveness in the fibroconjunctive and muscle tissue described in the histopathological result, in those with angioinvasion (p=0.03, Table 5.56) and in those with capsular invasion (p= 0.005). A weakly negative association (r=-0.22, p=0.001) was also identified between the level of vitamin D and the number of tumor foci (Fig. 5.45). A lower level of vitamin D (p=0.032) was also identified in patients with locoregional lymph node metastases compared to those without.

Chapter 7 – Conclusions

1. Patients with differentiated thyroid carcinoma have a deficient level of vitamin D
2. Patients with follicular carcinoma had significantly lower vitamin D levels
3. Vitamin D status correlates negatively with female gender in the thyroid carcinoma group
4. The status of vitamin D does not correlate with the environment of residence.
5. Male patients have a 2-fold increased risk for differentiated thyroid carcinoma
6. Patients from the group of benign pathology can be considered equivalent to the general population regarding VDR polymorphisms, as they are not involved in the etiopathogenesis of diseases from this group
7. The aa genotype in the ApaI polymorphism has a protective effect against thyroid carcinoma
8. The bb genotype in the BsmI polymorphism has a protective effect against thyroid carcinoma
9. The ff genotype of the FokI polymorphism increases the risk of CTD by 3.02 times
10. The Aa genotype can be considered a protective factor against vitamin D deficiency
11. The bb genotype predisposes to vitamin D deficiency. Carrier patients have a 3-fold higher risk of vitamin D deficiency
12. The f allele present in the Ff and ff genotype increases the risk of vitamin D deficiency, with carrier patients having an almost 5-fold increased risk of vitamin D deficiency
13. Vitamin D status correlates negatively with the presence of markers of aggressiveness (presence of macrocalcifications, nodular sizes, presence of adenopathies, invasiveness, number of CT foci, presence and number of locoregional lymph node metastases)
14. Certain polymorphisms may be associated with aggressive characteristics in papillary carcinoma but without definite statistical significance

Personal contribution

The study conducted demonstrated the existence of a lower level of vitamin D in patients with differentiated thyroid carcinoma from Romania, as well as negative correlations between the level of vitamin D and the ultrasound and histopathological parameters of aggressiveness. The element of absolute novelty is the evaluation of vitamin D receptor gene polymorphisms and the identification of some correlations between the genetic pattern of patients and the predisposition to differentiated thyroid carcinoma and aggressiveness criteria, thus carrying out the first study on the heterogeneity of VDR polymorphisms in thyroid carcinoma in our country.

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1. **Cocoloș AM,** Vladoiu S, Caragheorgheopol A, Ghemigian AM, Ioachim D, Poiană C. Vitamin D Level and its Relationship aith Cancer Stage in Patients with Differentiated Thyroid Carcinoma. Acta Endo (Buc) 2022, 18 (2): 168-173. ISSN (print): 1841 – 0987, ISSN (online): 1843 - 066X; IF (2021): 1.104; indexat PMC; doi: 10.4183/aeb.2022.168; https://acta-endo.ro/2022/numarul2/fulltext/168-173%20A.M.%20Cocolos.pdf; (capitolul 4)
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BDI (B+) articles/studies in extenso

1. **Cocoloș AM,** Ghemigian A, Poianî C. " Vitamin D Receptor Gene and Vitamin D Binding Protein Gene Polymorphisms in Differentiated Thyroid Carcinoma – Still an Issue?" . Medicina Modernă, 2022, vol 29(3); 175-180; ISSN-online 2360-2473 ISSN-L 1223-0472, ISSN-print 1223-0472; indexată SCOPUS, EBSCO, DOAJ, CiteFactor, Scipio of UEFISCDI, INDEX COPERNICUS;

Vitamin D Receptor Gene and Vitamin D Binding Protein Gene Polymorphisms in Differentiated Thyroid Carcinoma – Still an Issue - Medicina Moderna; (capitolul 2)

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