Since 2002

Anxiety Disorders and Prediction of Their Development in Patients with Hypothyroidism and Autoimmune Thyroiditis

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Abstract

AIM: Since the association between thyroid dysfunction and neuropsychiatric disorders has been known for many years, it is important to analyze the associations of the BDNF gene polymorphism (rs6265), the VDR gene polymorphism (rs2228570), and the NMDA gene polymorphism (rs4880213) with the anxiety in patients with autoimmune thyroiditis and hypothyroidism in the Western Ukrainian population and predict the development of anxiety disorders in these patients.

METHODS: The study involved a total of 153 patients with various forms of thyroid pathology. BDNF levels in the sera of the patients and healthy individuals were quantified using enzyme-linked immunosorbent assay with highly sensitive Human BDNF ELISA Kit (Elabscience®, United States, Catalog No: E-EL-H0010) on E.I.A. Reader Sirio S (Seac, Italy). Genotyping of the VDR (rs2228570), BDNF (rs6265), and NMDA (rs4880213) gene polymorphism using TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA). Polymerase chain reaction (PCR) for TaqMan genotyping was carried out according to the kit instructions (Applied Biosystems, USA).

RESULTS: According to the data obtained when comparing of the presence of anxiety on Hamilton scale on SNP, statistically significant differences were revealed depending on BDNF gene polymorphism (rs6265) ($p < 0.001$). When comparing of the presence of anxiety on Hamilton scale on SNP depending on VDR gene polymorphism (rs2228570) and NMDA gene polymorphism (rs4880213), no statistically significant differences were revealed ($p = 0.782$ and $p = 0.677$, respectively). We identified an inverse strong correlation between the presence of anxiety on Hamilton scale and BDNF, 25-OH Vitamin D levels, and fT4 in the blood ($p < 0.001$) and a direct moderate correlation between the presence of anxiety on Hamilton scale and TSH, GRIN2B, and anti-TPO ($p < 0.001$).

CONCLUSIONS: Indicators such as BDNF, GRIN2B, fT4, anti-TG, and 25-OH levels of Vitamin D are prognostically significant risk criteria for anxiety.

Introduction

Hashimoto thyroiditis (HT) is a commonly spread endocrine disorder that causes hypothyroidism. This disease is the most common among autoimmune diseases [1].

The thyroid gland significantly influences human cognitive function and mood. Overt hypothyroidism is closely connected with multiple neuropsychological and psychiatric disorders, such as attention deficits, lack concentration and memory problems, psychomotor retardation, depressive mood, anxiety, and persecutory delusions [2].

Since the link between thyroid dysfunction and neuropsychiatric consequences has been known for many years [3], only in the past few years, a variety of studies revealed a link between AIT and cognitive and affective disorders in the euthyroid state.

Anxiety is defined as a negative, uncertain, and unpleasant emotional state that arises from the expectation of potential danger and generates increased vigilance and alertness even in the absence of immediate threat [4]. A study reported considerable comorbidity between anxiety disorders and thyroid disorders [5].

It is important to identify the role of genetic factors in the development of multifactorial diseases [6], [7] and the search for new targets for therapy [8]. Effective methods of detecting hereditary predisposition to certain conditions are the analysis of transcriptome [9] and single-nucleotide polymorphism [10].

We have previously noted that autoimmune thyroiditis (AIT) and hypothyroidism can disrupt the transcription of genes involved in neurogenesis [11], [12], the transmission of nerve impulses [13], and the regulation of the cell cycle [14] that may affect the development of neurological complications associated with pathology

of the thyroid gland [15], [16], [17], [18].

The work aims to analyze the associations of the BDNF gene polymorphism (rs6265), the VDR gene polymorphism (rs2228570), and the NMDA gene polymorphism (rs4880213) with the anxiety in patients with autoimmune thyroiditis and hypothyroidism in the Western Ukrainian population and predict the development of anxiety disorders in these patients.

Materials and Methods

Our research was conducted in Bukovinian State Medical University, Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University, Ukraine. The study included a total of 153 patients with different types of thyroid pathology. The subjects were distributed into three groups. Group 1 (n = 16) comprised patients with post-operative hypothyroidism (PO); Group 2 (n = 65) included patients with hypothyroidism (H) caused by autoimmune thyroiditis (AIT); and Group 3 (n = 72) included patients with both AIT and elevated serum antibodies anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO). Twenty-five healthy individuals were randomly selected as a control group without adjusting for age or sex.

Ethical approval

The study fully ensured standards described in the 1975 Helsinki Declaration of Human Rights (amended in 2008). The participants completed and signed a written informed consent before enrolling voluntarily in the research. The Ethics Committee of the HSEEU "Bukovinian State Medical University," I. Horbachevsky Ternopil National Medical University, and Chernivtsi Regional Endocrinology Center, Ukraine, have approved this study (approval ID: 11-07.11.2017).

To diagnose hypothyroidism, we were guided by recommendations required by the American Association of Clinical Endocrinologists 2012. The corresponding clinical features were considered when verifying AIT, namely, the results of a sonogram of the thyroid gland (reduced echogenicity) and circulating antibodies to thyroid antigens, anti-TPO, and anti-TG were detected [19].

Blood samples from patients and controls were taken in the morning (8–10 am) after a night fast. Using STAT FAX303/Plus analyzer (Awareness Technology Inc., USA), we determined levels of thyroxine (fT4, normal range 6.0–13.0 pmol/L for males and 7.0–13.5 pmol/L for females), thyroid-stimulating hormone (TSH, normal range 0.3–4.0 mIU/mL), anti-thyroid peroxidase (anti-TPO, normal range 0-30 IU/mL), and anti-thyroglobulin (anti-TG, normal range 0-65 IU/mL) in each individual who participated in the study.

Study exclusion criteria were the following: Less than 18 years of age, malignancy, inflammation resulting from rheumatic diseases or acute/chronic infection, diabetes mellitus, vascular, chronic diseases of liver and kidneys, and pregnancy. Individuals administering drugs that could influence thyroid function were also ruled out from the study.

We identified the severity of anxiety levels using the Hamilton rating scale for anxiety (HAMA), which is reliable for anxiety assessment. Due to the HAMA, each item is scored on a basic numeric scoring of 0 (not present) to 4 (severe) [20].

The optimal HAM-A score ranges were mild anxiety = 8–14; moderate = 15–23; and severe ≥ 24 (scores ≤ 7 were considered to represent no/minimal anxiety) [21].

To quantify BDNF levels in the sera of the patients and healthy individuals, we used enzyme-linked immunosorbent assay with highly sensitive Human BDNF (Brain-Derived Neurotrophic Factor) ELISA Kit (Elabscience®, United States, Catalog No: E-EL-H0010) on E.I.A. Reader Sirio S (Seac, Italy).

When determining 25-OH Vitamin D levels in the serum of the patients and healthy individuals, we applied the ELISA using the 25-OH Vitamin D Total (Vitamin D-Direct) Test System ELISA Kit (Monobind Inc.®, United States, Product Code: 9425-300) on E.I.A. Reader Sirio S (Seac, Italy).

GRIN2B levels in the sera of the patients and healthy subjects were assessed by enzyme-linked immunosorbent assay, which is highly sensitive to Human GRIN2B (Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate 2B) ELISA Kit (Elabscience®, United States, Catalog No E-EL-H1631).

Genotyping of the VDR (rs2228570), BDNF (rs6265), and NMDA (rs4880213) gene polymorphism

DNA isolation

When collecting venous blood, we used a sterile Vacutainer and stabilized it with K2EDTA. To isolate total DNA from peripheral blood, we applied PREP-RAPID-GENETICS DNA Extraction Kit (DNA-TECHNOLOGY, Russian Federation), adhering to the manufacturer's instructions.

DNA amplification and genotyping

The samples were genotyped by TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA). Polymerase chain reaction (PCR) for TaqMan genotyping was

conducted due to the kit instructions (Applied Biosystems, USA). TaqMan Genotyping Master Mix contains DNA polymerase AmpliTaq Gold®, dNTPs, reference dye ROX™, and buffer ingredients. TaqMan probes are target-specific oligonucleotides with reporter dyes attached to the 5' end of each probe: (VIC® dye on the 5' end of the Allele 1 probe and 6FAM™ dye on the 5' end of the Allele 2 probe), and a non-fluorescent quencher (NFQ) the 3' end of the probe. Genomic DNA was intensified in a 10 µL reaction mix comprising genomic DNA, forward and reverse primers, fluorescent probes, and TaqMan Genotyping Master Mix. Genotyping of the samples conducted on the CFX-Manager™ software using allelic discrimination assays based on the magnitude of relative fluorescence units.

Statistical analysis

We used the Student's t-test, ANOVA, Pearson's Chi-square test, ROC analysis, odds ratio test, relative odds ratio test, and equality 0 correlation test to determine the difference between groups. The odds ratio and 95% confidence interval (CI) were computed by binary logistic regression where $p < 0.05$ was regarded as a statistically significant difference between the two groups (Statsoft Statistica v.12.0).

Results

In our present study, we inspected the relationship between BDNF gene polymorphism (rs6265), VDR gene polymorphism (rs2228570), and NMDA Gene Polymorphism (rs4880213) with anxiety disorders in patients with autoimmune thyroiditis and hypothyroidism in the population of Western Ukraine.

The demographic, clinical, and biochemical characteristics of the participants are displayed in Table 1.

In our study, we assessed the presence of anxiety on the Hamilton scale in the examined patients (Table 2).

According to our study, in the assessment of anxiety on the Hamilton scale, 50% of patients with PO had mild anxiety and 50% had moderate anxiety. Mild anxiety was found in 50.8% of patients with AIT with hypothyroidism. About 49.2% of patients in this group had moderate anxiety on the Hamilton scale. There was no pronounced severe anxiety in the examined patients. In the group of patients with AIT, mild anxiety was detected in 87.5%, while 4.2% of patients had moderate anxiety.

We performed the analysis of the presence of anxiety on Hamilton scale on SNP (Table 3).

According to the data obtained when comparing of the presence of anxiety on Hamilton scale on SNP, statistically significant differences were revealed depending on BDNF gene polymorphism (rs6265) ($p < 0.001$).

When comparing of the presence of anxiety on Hamilton scale on SNP depending on VDR gene polymorphism (rs2228570) and NMDA gene polymorphism (rs4880213), no statistically significant differences were revealed ($p = 0.782$ and $p = 0.677$, respectively) (applied methods: Pearson's Chi-square test).

Correlation analysis of the association between hormones levels and anxiety was performed (Table 4).

Table 1: Demographic, clinical, and biochemical characteristics of study participants

Variable	Control group (n = 25)	Patients with post-operative hypothyroidism (PO, n = 16)	Patients with AIT-induced hypothyroidism (AIT with hypothyroidism, n = 65)	Patients with AIT and elevated anti-Tg and anti-TPO antibodies (AIT, n = 72)
Age (years)	46.08 ± 14.58	47.30 ± 12.27	46.72 ± 15.49	45.02 ± 13.65
fT4 (pmol/L)	8.91 ± 0.97	3.44 ± 0.31	4.13 ± 0.52	8.51 ± 0.82
TSH (mIU/mL)	2.67 ± 0.52	8.61 ± 0.84	7.09 ± 0.50	2.38 ± 0.62
Anti-TPO (IU/mL)	34.04 ± 3.70	36.13 ± 2.78	380.62 ± 73.42	330.36 ± 50.23
Anti-TG (IU/mL)	15.32 ± 1.97	15.50 ± 1.90	32.97 ± 4.27	36.38 ± 7.70
Current dose of L-thyroxine (µg/day)	None	110.95 ± 5.25	88.46 ± 1.55	None
25-OH Vitamin D ng/mL	39.2 ± 6.58	20.69 ± 3.09 (p < 0.001)	19.08 ± 3.144 (p < 0.001)	21.48 ± 2.83 (p < 0.001)
BDNF (pg/ml)	1037.8 ± 361.83	310.19 ± 112.84 (p < 0.001)	310.19 ± 112.84 (p < 0.001)	1031.34 ± 385 (p = 0.95)
GRIN2B ng/ml	6.234 ± 0.729	1.7915 ± 0.36 (p < 0.001)	9.866 ± 0.943 (p < 0.001)	6.062 ± 06 (0.4487)

Data are presented as a mean ± standard deviation.

Table 2: Analysis of the presence of anxiety on the Hamilton scale in groups of subjects

Variable	Categories	Groups of subjects				P
		Control	Post-operative H	AIT with H	AIT	
Presence of anxiety on Hamilton rating scale	No symptoms of anxiety	23 (92.0)	0 (0.0)	0 (0.0)	6 (8.3)	< 0.001*
	Mild anxiety	2 (8.0)	8 (50.0)	33 (50.8)	63 (87.5)	$p_{Control – Post-operative H} < 0.001$
	Moderate anxiety	0 (0.0)	8 (50.0)	32 (49.2)	3 (4.2)	$p_{Control – AIT with H} < 0.001$
						$p_{Control – AIT} < 0.001$
						$p_{Post-operative H – AIT} < 0.001$
						$p_{AIT with H – AIT} < 0.001$

* – Differences are statistically significant ($p < 0.05$)

Analysis of the correlation between the presence of anxiety on Hamilton scale and the levels of TSH, fT4, anti-TG, and anti-TPO antibodies, 25-OH Vitamin D, and BDNF levels revealed an inverse strong correlation between the presence of anxiety on Hamilton scale and BDNF levels ($p < 0.001$) and a direct moderate correlation between the presence of anxiety on Hamilton scale and TSH, GRIN2B, and anti-TPO ($p < 0.001$). We also revealed direct weak correlation between presence of anxiety on Hamilton scale and anti-TG ($p = 0.01$). In addition, we identified inverse moderate correlation between the presence of anxiety on Hamilton scale and 25-OH Vitamin D levels and fT4 in the blood ($p < 0.001$).

Table 3: Analysis of the presence of anxiety on Hamilton scale on SNP

Variables	Categories	Presence of anxiety on Hamilton scale			p
		No symptoms of anxiety	Mild anxiety	Moderate anxiety	
BDNF gene polymorphism (rs6265)	CC	22 (18.2)	83 (68.6)	16 (13.2)	< 0.001*
	CT	7 (15.6)	17 (37.8)	21 (46.7)	p_{CC-CT} < 0.001
	TT	0 (0.0)	6 (50.0)	6 (50.0)	p_{CC-TT} = 0.006
VDR gene polymorphism (rs2228570)	AA	12 (17.6)	40 (58.8)	16 (23.5)	0.782
	AG	9 (12.3)	45 (61.6)	19 (26.0)	
	GG	8 (21.6)	21 (56.8)	8 (21.6)	
NMDA gene polymorphism (rs4880213)	CC	6 (11.1)	32 (59.3)	16 (29.6)	0.677
	CT	16 (18.8)	50 (58.8)	19 (22.4)	
	TT	7 (17.9)	24 (61.5)	8 (20.5)	

*Differences are statistically significant ($p < 0.05$).

The observed dependence of depression from BDNF is described by a linear regression equation:

Table 4: Results of the correlation analysis of the association between hormones levels and anxiety

Variables	Correlation characteristics		
	P	Strength of the association assessed using Chaddock scale	p
BDNF – anxiety	−0.783	Strong	< 0.001*
GRIN2B – anxiety	0.472	Moderate	< 0.001*
TSH – anxiety	0.582	Close	< 0.001*
fT4 – anxiety	−0.620	Close	< 0.001*
anti-TPO – anxiety	0.352	Moderate	< 0.001*
anti-TG – anxiety	0.201	Weak	0.010*
25-OH Vitamin D – anxiety	−0.407	Moderate	< 0.001*

*Differences are statistically significant ($p < 0.05$).

Observed dependence of anxiety from BDNF is described by a linear regression equation:

$$Y_{anxiety} = -0.009 \times X_{BDNF} + 17.982$$

With a 1 increase of BDNF −0.009, change of anxiety should be expected. According to the coefficient of determination R^2 of the resulting model, 59.7% of the observed variance of anxiety were explained (Figure 1).
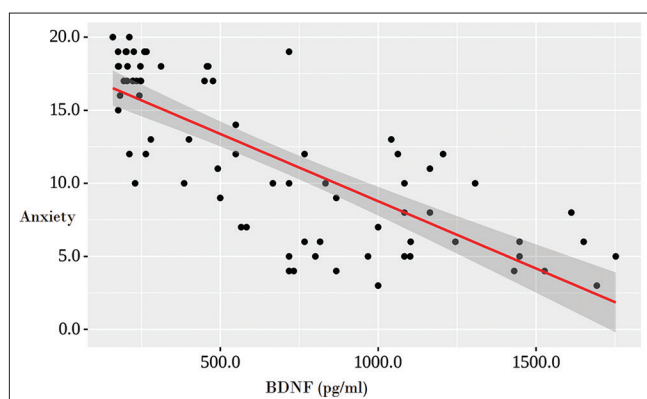

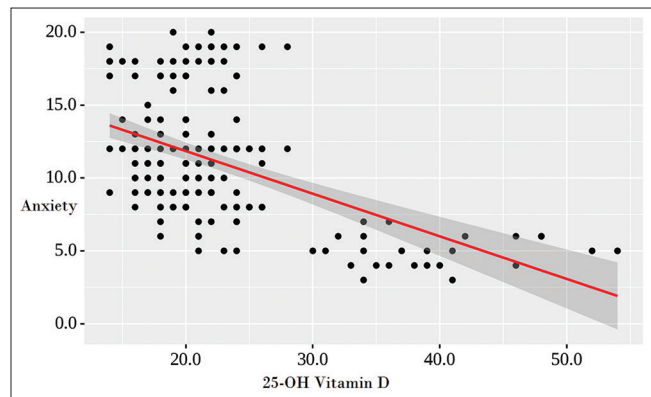*Figure 1: Regression line characterizing the dependence of anxiety from BDNF*

Figure 2: Regression line characterizing the dependence of anxiety from 25-OH Vitamin D

Observed dependence of anxiety from 25-OH Vitamin D is described by a linear regression equation:

$$Y_{anxiety} = -0.292 \times X_{25\text{-OH Vitamin D}} + 17.69$$

With a 1 increase of 25-OH Vitamin D −0.292, change of anxiety should be expected. According to the coefficient of determination R^2 of the resulting model, 26.6% of the observed variance of anxiety were explained (Figure 2).

In accordance with the presented Table 5, when comparing of the presence of anxiety on Hamilton scale, statistically significant differences were revealed depending on BDNF and 25-OH Vitamin D ($p < 0.001$) (applied methods: Pearson's Chi-square test).

We conducted a study of the prognostic value of plasma levels of a number of hormones as potential markers of anxiety.

The displayed curve was obtained during the estimation of the dependence of detecting the presence of depression conditioning on BDNF using ROC analysis (Figure 3).
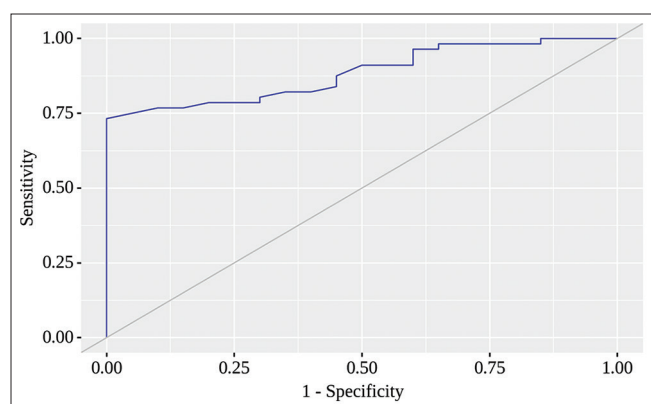

Figure 3: ROC curve characterizing the dependence of the probability presence of anxiety on BDNF

The area under the ROC curve comprised 0.884 ± 0.051 with 95% CI: 0.784-0.985. The resulting model was statistically significant ($p < 0.001$).

The cutoff value of BDNF which corresponds to the highest Youden's J statistic is 717.950. If BDNF

Table 5: Analysis of anxiety conditioning on BDNF and 25-OH Vitamin D Levels

Variables	Categories	Presence of anxiety on Hamilton scale			p
		No symptoms of anxiety	Mild anxiety	Moderate anxiety	
BDNF level	Normal level	17 (38.6)	5 (11.4)	22 (50.0)	< 0.001*
	Decrease level	3 (9.4)	21 (65.6)	8 (25.0)	
25-OH Vitamin D	Normal level	23 (92.0)	2 (8.0)	0 (0.0)	< 0.001*
	Vitamin D < 20 ng/mL (Vitamin D deficiency)	1 (1.3)	51 (66.2)	25 (32.5)	p1 < 0.001
	Vitamin D = 20–30 ng/mL (Suboptimal Vitamin D availability)	5 (6.6)	53 (69.7)	18 (23.7)	p2 < 0.001

*Differences are statistically significant (p < 0.05). P1 – P value between normal level and Vitamin D deficiency, P2 – P value between normal level and suboptimal Vitamin D provision.

was less than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 73.2% and 100.0%, respectively.

In addition to the BDNF level, indicators such as TSH, fT4, anti-TPO, and 25-OH Vitamin D levels were found to be prognostically significant criteria for the risk of developing anxiety.

Therefore, the estimation of the dependence of detecting the presence of anxiety conditioning on TSH using ROC analysis revealed that the area under the ROC curve comprised 0.764 ± 0.044 with 95% CI: 0.679-0.850. The resulting model was statistically significant (p < 0.001).

The cutoff value of TSH which corresponds to the highest Youden's J statistic is 6.000. If TSH was greater than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 59.6% and 100.0%, respectively.

The analysis of the dependence of detecting the presence of anxiety conditioning on fT4 using ROC analysis revealed that the area under the ROC curve comprised 0.843 ± 0.050 with 95% CI: 0.746-0.941. The resulting model was statistically significant (p < 0.001).

The cutoff value of fT4 which corresponds to the highest Youden's J statistic is 7.200. If fT4 was less than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 61.0% and 100.0%, respectively.

We found that the area under the ROC curve of the dependence of anxiety conditioning on anti-TPO using ROC analysis comprised 0.922 ± 0.022 with 95% CI: 0.879-0.964. The resulting model was statistically significant (p < 0.001).

The cutoff value of anti-TPO which corresponds to the highest Youden's J statistic is 42.000. If anti-TPO was greater than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 87.5% and 88.5%, respectively.

The analysis of the dependence of detecting the presence of anxiety conditioning on anti-TG using ROC analysis revealed that the area under the ROC curve comprised 0.894 ± 0.026 with 95% CI: 0.842-0.945. The resulting model was statistically significant (p < 0.001).

The cutoff value of anti-TG which corresponds to the highest Youden's J statistic is 26.000. If anti-TG

was greater than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 82.4% and 96.2%, respectively.

The following curve was obtained when estimating the dependence of detecting presence of anxiety conditioning on 25-OH Vitamin D using ROC analysis (Figure 4).
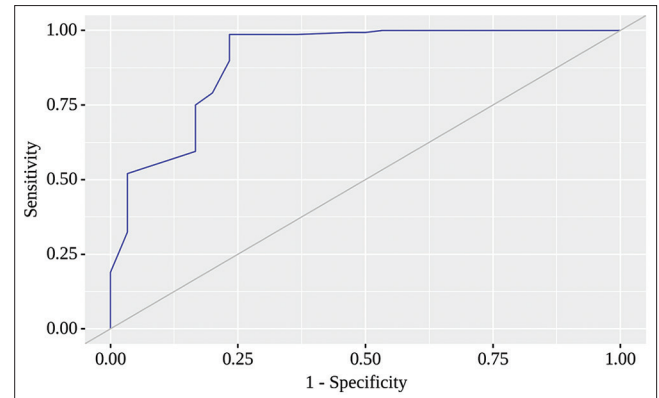

Figure 4: ROC curve characterizing the dependence of the probability presence of anxiety on 25-OH Vitamin D

The area under the ROC curve comprised 0.900 ± 0.039 with 95% CI: 0.825-0.976. The resulting model was statistically significant (p < 0.001).

The cutoff value of 25-OH Vitamin D which corresponds to the highest Youden's J statistic is 30.000. If 25-OH Vitamin D was less than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 98.6% and 76.7%, respectively.

The dependence of anxiety on quantitative variables was estimated using multiple linear regression (Table 6).

Table 6: Analysis of anxiety conditioning on BDNF, GRIN2B, fT4, anti-TG, and 25-OH Vitamin D

	B	Std. error	t	p
Intercept	18.132	1.538	11.793	< 0.001*
BDNF	−0.005	0.001	−6.322	< 0.001*
GRIN2B	0.269	0.118	2.276	0.026*
fT4	−0.764	0.150	−5.099	< 0.001*
anti-TG	0.104	0.042	2.501	0.015*
25-OH Vitamin D	−0.116	0.046	−2.516	0.014*

*Differences are statistically significant (p < 0.05).

The observed association of anxiety with BDNF, GRIN2B, fT4, anti-TG, and 25-OH Vitamin D is presented by a linear regression equation:

$$Y_{anxiety} = 18.132 - 0.005 \times X_{BDNF} + 0.269 \times X_{GRIN2B} - 0.764 \times X_{fT4} + 0.104 \times X_{anti\text{-}TG} - 0.116 \times X_{25\text{-}OH\ Vitamin\ D}$$

With an 1 increase of BDNF, an 0.005 of anxiety

should be expected, with an 1 increase of GRIN2B, an 0.269 of anxiety should be expected, with an 1 increase of fT4, an 0.764 of anxiety should be expected, with an 1 increase of anti-TG, an 0.104 of anxiety should be expected, and with an 1 increase of 25-OH Vitamin D, an 0.116 of anxiety should be expected.

The resulting regression model is characterized by a correlation coefficient r_{xy} = 0.9, which corresponds to the functional relationship on the Chaddock scale. The model was statistically significant ($p < 0.001$). Based on the value of the coefficient of determination R^2, the model accounts for 84.7% of anxiety variance.

Discussion

The association between thyroid hormone deficiency and anxiety has been widely discussed in various reliable studies [22], [23].

Fischer and Ehlert, 2017, reported considerable comorbidity between anxiety disorders and thyroid disorders [5]. This fact is proved routine screening indicated for thyroid disorders during the treatment of patients to anxiety disorders [24].

The meta-analysis identified a strong link between AIT, depression, and anxiety disorders, respectively [23]. More than that, a couple of studies depicted common anxiety signs and symptoms manifested in patients with AIT [22], [25].

According to our study, when assessing anxiety on the Hamilton scale, 50% of patients with software had mild anxiety and 50% – moderate. Mild anxiety was found in 50.8% of patients with AIT with hypothyroidism. About 49.2% of patients in this group had moderate anxiety on the Hamilton scale. The examined patients did not have severe anxiety.In the group with AIT, 87.5% of patients had mild anxiety and 4.2% had moderate anxiety.

According to research, higher TSH levels cause depression and anxiety [26].

Various genetic studies have focused on the effects of BDNF polymorphism on brain function and behavior in healthy individuals, as well as on pathological conditions, especially neuropsychiatric disorders [27].

Some outcomes related to animal studies prove that BDNF may facilitate the functional changes which accompany anxiety disorders. Compared with wild-type mice, BDNF mutants with conditional BDNF deletion in the brain after birth observed hyperactive response to stressors and elevated anxiety levels when assessed by the light/dark exploration test [28].

According to our study when comparing of the presence of anxiety on Hamilton scale on SNP,

statistically significant differences were revealed depending on BDNF gene polymorphism (rs6265) ($p < 0.001$).

Such researchers as Chen *et al.* [29] generated a variant BDNF mouse (BDNF(Met/Met)) that reproduces the phenotypic traits in subjects with allelic variations. In the case of putting in stressful medium, BDNF(Met/Met) mice demonstrated elevated anxiety-related behaviors, showing the influence of Met substitution of BDNF on anxiety behaviors.

In our study, when comparing anxiety on the Hamilton scale, patients with different thyroid pathology, depending on the rs6265 polymorphism genotype, showed the highest scores in TT and CT genotypes carriers in the experimental group compared to the CC genotype carriers.

To date, several studies focused on anxiety-related disorders have been carried out. In a family-based association study, a positive correlation was detected between the BDNF Val66Met polymorphism and predisposition to obsessive-compulsive disorder (OCD), and significant overtransmission was observed for the Val66 allele [30]. Ensuing studies failed to reproduce this finding [31], [32]. Versus, two assessorial studies detected that the Met66 allele could be a risk allele in OCD development, and this allele was related to an early onset of OCD in males [33], [34].

We can assume that against the background of pathology of the thyroid gland, genetic predisposition plays an important role in the occurrence of anxiety.

During the estimation of the dependence of detecting the presence of anxiety conditioning on BDNF using ROC analysis, the area under the ROC curve comprised 0.884 ± 0.051 with 95% CI: 0.784-0.985. The resulting model was statistically significant ($p < 0.001$).

The cutoff value of BDNF which corresponds to the highest Youden's J statistic is 717.950. If BDNF was less than or equal to this value, the presence of anxiety was predicted. The sensitivity and specificity of the method were 73.2% and 100.0%, respectively.

Another publication suggests data on a possible connection between Val66Met status and anxiety disorders, but without any marked association [35]. In an earlier study, post-traumatic stress disorder (PTSD) reported neither BDNF SNPs (C270T and Val66Met) observed to be associated with PTSD [36].

Due to ambivalent findings, the connection between the BDNF Val66Met polymorphism and panic disorder appears ambiguous and contradictory [37], [38]. A meta-analysis of six studies detected no relevant correlation between the polymorphism and panic disorder in the dominant model [39]. Although, in the recessive model, a notable association was detected between the BDNF Val66Met polymorphism and panic disorder.

During the past three decades, many publications have described a complicated interaction between peripheral immune activation, neuroinflammation, and changes in brain circuits which may lead to depression and anxiety [40]. Due to this, Vitamin D is known as a regulator of innate immunity, acting as transcription and a growth factor, and also interacting with surface receptors in different immune cells [4]. Consequently, Vitamin D may positively affect human behavior due to its possibility to regulate peripheral and central nervous system (CNS) immune responses.

Unlike the investigations that prove that depression depends on Vitamin D, only some publications have highlighted the association of anxiety disorders with Vitamin D levels [41]. A couple of them even decline the connection of Vitamin D deficiency with anxiety or stress [42].

Contrary to the findings suggested by Black *et al.* (2014), the connection of Vitamin D deficiency was reported for both depression and anxiety disorders with no significant differences within studied groups [43].

In our study, we identified an inverse strong correlation between the presence of anxiety on Hamilton scale and BDNF, 25-OH Vitamin D levels, and fT4 in the blood ($p < 0.001$) and a direct moderate correlation between the presence of anxiety on Hamilton scale and TSH, GRIN2B, and anti-TPO ($p < 0.001$).

Most studies that are focused on assessing anxiety-related symptoms in different populations point out a strong link between Vitamin D low levels and anxiety [44], [45], [46]; still, at the same time, there is some evidence reporting no association between anxiety symptoms and serum Vitamin D [47].

Despite the apparent essential function of Vitamin D in calcium metabolism and its role in proliferation, differentiation, and immunomodulation, there is reliable evidence on Vitamin D plays a critical role in the health and disease of the brain and nervous system, respectively [48], [49], [50].

VDR is widely expressed in the peripheral immune system in immune cells, including those categorized as B and T lymphocytes, monocytes/macrophages, dendritic cells, and natural killer cells [51], [52]. Vitamin D is implicated in the modulation of innate and adaptive immune responses [53]. In this setting, depression and anxiety are those conditions that are often associated with a state of low-grade inflammation and peripheral increase in acute-phase proteins and inflammatory cytokines [54].

Conclusions

Patients with AIT and hypothyroidism show an increased chance of developing symptoms of anxiety disorders. The outcomes of this study are essential for patients with AIT and could contribute not only to early treatment choices but also psychotherapeutic treatment for organic disease.

Since that mood and anxiety disorders are relatively frequently spread and are related to significant health impairment due to limited current treatments, new compounds possessing ample properties, namely, antidepressants and anxiolytics, are required. Within this framework, Vitamin D has been studied as a promising approach in treating these disorders.

Ethical Approval

The approval for this study was obtained from the Ethics Committee of the Bukovinian State Medical University, Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University, Ukraine (approval ID: 11-07.11.2017).

Our study was conducted according to the Declaration of Helsinki adopted in 1975 and revised in 2008, and the ethical principles were entirely respected.

Consent to Participate

Written informed consent was obtained from the participants.

Data Availability

The data of this study are available by request.

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