Hashimoto’s thyroiditis is a chronic autoimmune thyroiditis characterized by lymphocytic infiltration that may result in the gradual loss of thyroid tissue [1]. According to a study, even with normal thyroid function, autoimmune thyroiditis can cause neuroinflammation, leading to emotional changes and mental disorders [2].

Although the mechanism is unclear, some research indicates that vitamin D has a beneficial effect on autoimmune thyroid diseases [3]. A case-control study found that patients with HT had lower levels of vitamin D [4]. Another meta-analysis has linked vitamin D deficiency with autoimmune thyroid disease as well [5]. Clinical studies have indicated a strong correlation between serum vitamin D levels and cognitive impairment in patients with autoimmune thyroiditis [6]. Vitamin D is regarded as a neurosteroid that regulates immunomodulation, brain development, and function in adulthood [7, 8].

The vitamin D receptor (VDR) and the enzyme that transforms 25(OH)D to the active form of the vitamin, 1,25-dihydroxyvitamin D, are expressed in all organs, including the brain [9]. According to a recent study conducted by the Institute of Medicine, only individuals with 25(OH)D levels below 10 ng/mL are at a permanent risk of cognitive decline, regardless of other factors [10, 11].

**Objective** — to investigate the impact of cholecalciferol on cognitive function in patients with hypothyroidism and autoimmune thyroiditis in the Western Ukrainian population.

**MATERIALS AND METHODS**

Our research was conducted at Bukovinian State Medical University, Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University, Ukraine. The prospective cohort study design included 56 patients with hypothyroidism (H) caused by autoimmune thyroiditis (AIT). These patients were distributed into two groups. Patients in the Group 1 (n = 28) received cholecalciferol at a dose of 4,000 IU/day (28,000 IU/week) and L-thyroxine (88.39 ± 12.70 μg/day). Patients in the Group 2 (n = 28) were prescribed only L-thyroxine (87.50 ± 12.73 μg/day). During the treatment, all patients were visited and interviewed about possible side effects, and to determine the degree of compliance Examinations were performed at the beginning and end of the 12-week treatment. After 12 weeks of treatment, all laboratory tests and clinical evaluations were repeated as per the initial visit.

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 were detected [12].

Blood samples from patients and controls were taken in the morning (8 to 10 am) after a night fast. Using STAT FAX303/Plus analyzer (Awareness Technology Inc, USA),
we determined levels of free 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 detect a decline in cognitive function using the Mini-Mental State Examination (MMSE), which has been the most used screening instrument throughout decades [13, 14].

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 (VitD-Direct) Test System ELISA Kit (Monobind Inc., United States, Product Code: 9425-300) on E.I.A. Reader Sirio S (Seca, Italy).

Statistical analysis. Quantitative variables were assessed for normality using the Shapiro-Wilk test (when the number of subjects was less than 50) or the Kolmogorov-Smirnov test (when the number of subjects was more than 50). Quantitative variables following non-normal distribution were described using median (Me) and lower and upper quartiles (Q₁ — Q₃). Comparisons of three or more groups on a quantitative variable whose distribution differed from normal were made using the Kruskal-Wallis test and Dunn’s criterion with Holm correction as a post-hoc method. A comparison of frequencies in the analysis of multifield contingency tables was performed using Pearson’s chi-square test (for expected values greater than 10).

Ethical approval. The study fully ensured the 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.

**RESULTS**

We performed the analysis of TSH and fT₄ levels before and after vitamin D treatment (Table 1).

Table 1 presented in the previous context suggests that there was a statistically significant decrease in TSH levels in both groups of patients after therapy (p < 0.001). Additionally, there were statistically significant differences in fT₄ levels depending on their initial level (p < 0.001). The table also indicates that after treatment, fT₄ levels were normalized in both patient groups. We performed the analysis of anti-TPO and anti-TG levels before and after treatment (Figure 1, 2).

According to the data obtained when comparing anti-TPO statistically significant differences were revealed depending on the level of anti-TPO (p < 0.001).

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
<th>Level of TSH, mIU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 28) Before treatment</td>
<td>7.10 (6.80 — 7.60)</td>
<td></td>
</tr>
<tr>
<td>After treatment</td>
<td>4.00 (3.90 — 4.10)*</td>
<td></td>
</tr>
<tr>
<td>2 (n = 28) Before treatment</td>
<td>7.10 (6.80 — 7.40)</td>
<td></td>
</tr>
<tr>
<td>After treatment</td>
<td>3.90 (3.60 — 4.10)*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
<th>Level of fT₄, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 28) Before treatment</td>
<td>4.10 (3.40 — 4.80)</td>
<td></td>
</tr>
<tr>
<td>After treatment</td>
<td>7.30 (7.10 — 7.50)*</td>
<td></td>
</tr>
<tr>
<td>2 (n = 28) Before treatment</td>
<td>4.00 (3.70 — 4.60)</td>
<td></td>
</tr>
<tr>
<td>After treatment</td>
<td>7.35 (7.10 — 8.10)*</td>
<td></td>
</tr>
</tbody>
</table>

Note. Data are presented as median and lower and upper quartiles (Me (Q₁ — Q₃)).

* The difference to the value before treatment is statistically significant (p < 0.001).
In this study, the group of patients who received cholecalciferol and L-thyroxine showed a greater decrease in anti-TPO levels compared to the group of patients who received only L-thyroxine. Specifically, the group that received cholecalciferol and L-thyroxine showed a decrease of 31.25% in anti-TPO levels after treatment, while the group that received only L-thyroxine showed a decrease of only 14.1%.

Based on the data analysis, it was found that there was a potential reduction in anti-TG levels in patients who received both cholecalciferol and L-thyroxine by 18.84% after treatment. On the other hand, in Group 2 patients who only received L-thyroxine, anti-TG levels decreased by only 8.82% after treatment (see Figure 2).

Analysis of 25(OH)D levels was performed before and after treatment. Table 2 shows that after treatment in patients of Group 1 there was a normalization of the level of 25(OH)D, at the same time in patients of Group 2 level of 25(OH)D decreased compared to its level before treatment.

Analysis of MMSE was performed conditioning on MMSE test (Table 3).

After treatment, there is a statistically significant improvement in cognitive function according to the MMSE test in a group of patients who took cholecalciferol and L-thyroxine compared with patients who received only L-thyroxine (p = 0.011) (applied method: The Kruskal-Wallis test).

Statistically significant differences were revealed when comparing Cognitive impairment depending on the MMSE test (p < 0.001) (applied method: Pearson’s chi-square test).

After the course of treatment, patients showed improvement in cognitive function. Thus, in both groups, according to the MMSE test, patients with mild dementia improved cognitive performance to moderate (pre-dementia) cognitive impairment. In Group 1 patients who received cholecalciferol and L-thyroxine after treatment, the percentage of patients with moderate (pre-dementia) cognitive impairment decreased from 50.0% to 21.4% (Table 4). At the same time, no statistically significant changes were found in the group of patients taking L-thyroxine alone. Normalization of cognitive functions according to the MMSE test increased from 28.6% to 78.6% in the first group, and from 21.4% to 50.0% in patients of Group 2. Thus, treatment with cholecalciferol supplementation was statistically more effective than L-thyroxine alone.
DISCUSSION

Thyroid disease is a prevalent endocrine disorder, and autoimmune thyroid disease (AITD) is thought to be the most common autoimmune disease [15]. Vitamin D, as an immunomodulator, plays a role in the development and progression of AITD [16]. Due to the increasing evidence of the association between vitamin D deficiency and thyroid disorders, there is a growing interest in using vitamin D supplementation for the prevention and treatment of thyroid disorders.

These are some examples of studies that have investigated the relationship between vitamin D and thyroid function. The first study, conducted by F. Ucar et al. in Turkey, found lower levels of 25(OH)D in elderly patients with subclinical hypothyroidism compared to healthy individuals [17]. A case-control study by N. J. Aljohani et al. found an inverse relationship between vitamin D status and free triiodothyronine levels [18]. A Polish pilot study that monitored vitamin D status during summer months in patients on L-thyroxine treatment reported that vitamin D sufficiency did not improve even during the summer. These studies suggest that there may be a relationship between vitamin D deficiency and thyroid dysfunction, but further research is needed to fully understand this relationship [19].

The study by I. M. B. Botelho et al. suggested that a decreased level of FT₄ could be a predictor of vitamin D deficiency in patients with Hashimoto’s thyroiditis, which is in line with the findings of the current study that suggest that thyroid hormone may play a role in regulating autoimmune thyroid function in the presence of sufficient vitamin D levels [20]. Another study reported a positive interaction between FT₄ and 25(OH)D levels, suggesting that supplementation with either vitamin D or thyroid hormones could help regulate the balance between these two parameters [21].

Our study showed a statistically significant decrease in anti-TPO levels in patients who received both cholecalciferol and L-thyroxine, with a reduction of 31.25 %, compared to patients who only received L-thyroxine, where anti-TPO levels decreased by 14.1 %. Additionally, we observed a decrease in anti-TG levels in patients receiving cholecalciferol and L-thyroxine by 18.84 %, while patients in Group 2 who only received L-thyroxine experienced a decrease in anti-TG levels by only 8.82 %.

Vitamin D plays an important role in regulating various vital physiological processes in the central nervous system, including cell differentiation, neurotransmitter biosynthesis, and neurotrophic release [22, 23]. Studies have shown that vitamin D supplementation can prevent cognitive impairment and improve hippocampal plasticity in aged rats by increasing the expression of potential key genes involved in neuroplasticity or enhancing the function of major neurotransmitter receptors such as dopamine, serotonin, and glutamate [24].

According to our study, treatment with cholecalciferol and L-thyroxine resulted in a significant improvement in cognitive function, as assessed by the MMCE test, compared to patients who received only L-thyroxine. In the group that received cholecalciferol and L-thyroxine, there was a decrease in the percentage of patients with moderate cognitive impairment (pre-dementia) from 50.0 % to 21.4 % after treatment. In contrast, no significant changes were observed in the group that received L-thyroxine alone. The percentage of patients with normalized cognitive function, as assessed by the MMCE test, increased from 50.0 % to 21.4 % after treatment. In contrast, no significant changes were observed in the group that received L-thyroxine alone. The percentage of patients with normalized cognitive function, as assessed by the MMCE test, increased from 50.0 % to 21.4 % after treatment. In contrast, no significant changes were observed in the group that received L-thyroxine alone.

<table>
<thead>
<tr>
<th>Impairment</th>
<th>Group 1 Before treatment</th>
<th>Group 1 After treatment*</th>
<th>Group 2 Before treatment</th>
<th>Group 2 After treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild dementia</td>
<td>6 (21.4 %)</td>
<td>0</td>
<td>2 (7.1 %)</td>
<td>0</td>
</tr>
<tr>
<td>Moderate (pre-dementia) cognitive impairment</td>
<td>14 (50.0 %)</td>
<td>6 (21.4 %)</td>
<td>20 (71.4 %)</td>
<td>14 (50.0 %)</td>
</tr>
<tr>
<td>No cognitive impairment</td>
<td>8 (28.6 %)</td>
<td>22 (78.6 %)</td>
<td>6 (21.4 %)</td>
<td>14 (50.0 %)</td>
</tr>
</tbody>
</table>

Note:* The difference to the value before treatment is statistically significant (p < 0.01).

# The difference to the indicator value of Group 1 after treatment is statistically significant (p < 0.001).
The results mentioned earlier suggest that vitamin D may have a positive effect on the connectivity of certain neural circuits involved in reward-dependent and motor behavior, such as the ventral tegmental area-accumbens nucleus-prefrontal cortex circuit and the nigro-striatal circuit. Additionally, the presence of vitamin D receptors and enzymes involved in vitamin D metabolism in brain regions involved in cognitive processes, such as complex planning and memory formation, suggests a potential role for vitamin D in neuro-cognition [10].

**CONCLUSIONS**

Based on the results of our study, which showed a significant improvement in cognitive function and a reduction in thyroid autoantibodies in patients receiving vitamin D supplementation, it can be concluded that vitamin D may have a positive influence on improving cognitive function in patients withAIT and hypothyroidism. Additionally, our findings suggest that vitamin D therapy may be a safe and effective treatment option for these conditions, particularly in patients with vitamin D deficiency.

**Ethics approval.** 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 is available by request.

**Conflicts of interest:** none.

**ЛІТЕРАТУРА/REFERENCES**

ABSTRACT
Hashimoto’s thyroiditis is a chronic autoimmune thyroiditis characterized by lymphocytic infiltration that may result in the gradual loss of thyroid tissue. According to a study, even with normal thyroid function, autoimmune thyroiditis can cause neuroinflammation, leading to emotional changes and mental disorders. Although the mechanism is unclear, some research indicates that vitamin D has a beneficial effect on autoimmune thyroid diseases. The relationship between serum vitamin D levels and cognitive impairment in patients with autoimmune thyroiditis has been established in clinical studies.

Objective — to investigate the impact of cholecalciferol on cognitive function in patients with hypothyroidism and autoimmune thyroiditis in the Western Ukrainian population.

Materials and methods. The study involved 56 patients diagnosed with hypothyroidism resulting from autoimmune thyroiditis. These patients were divided into two groups. Group 1 (n = 28) received cholecalciferol at a dose of 4000 IU/day (28.000 IU/week) and L-thyroxine (88.39 ± 12.70 μg/day), while Group 2 (n = 28) received only L-thyroxine (87.50 ± 12.73 μg/day). Assessments were conducted at the start and end of a 12-week treatment period, and cognitive function was measured using the Mini-Mental State Examination. The results showed a decline in cognitive function.

Results. Following the treatment course, patients demonstrated an improvement in cognitive function. In Group 1, where patients were treated with cholecalciferol and L-thyroxine, the proportion of patients with moderate (pre-dementia) cognitive impairment decreased from 50 % to 21.4 %. However, there were no significant changes observed in the group that received only L-thyroxine. Normalization of cognitive function as determined by the MMCE test increased from 28.6 % to 78.6 % in Group 1, and from 21.4 % to 50 % in Group 2 patients.

Conclusions. Based on our findings, vitamin D supplements should be given to patients diagnosed with autoimmune thyroiditis and hypothyroidism in order to enhance cognitive function.

Keywords: autoimmune thyroiditis, hypothyroidism, cognitive function, cholecalciferol.
Матеріали та методи. У дослідженні взяли участь 56 пацієнтів з гіпотиреозом, який виник унаслідок автоімунного тиреоїдиту. Пацієнтів розподілили на дві групи. Хворі першої групи (n = 28) отримували холекальциферол у дозі 4000 МО/добу (28 000 МО/тиж) і левотироксин ((88,39 ± 12,70) мкг/добу), хворі другої групи (n = 28) — лише левотироксин ((87,50 ± 12,73) мкг/добу). Когнітивні функції оцінювали за допомогою Mini-Mental State Examination (MMSE) на початку і в кінці 12-тижневого курсу лікування.

Результати. За даними тесту MMSE, у пацієнтів з легким ступенем деменції когнітивні показники поліпшилися до помірних (переддементних) порушень. У групі пацієнтів, які отримували холекальциферол і левотироксин, після лікування частка хворих з помірними (переддементними) когнітивними порушеннями зменшилася з 50,0 до 21,4 %, тоді як у групі пацієнтів, які приймали лише левотироксин, статистично значущих змін не відзначено. Нормалізацію когнітивної функції за результатами тесту MMSE зареєстровано у 78,6 % пацієнтів першої групи (до лікування — у 28,6 %) та 50,0 % — другої групи (до лікування — у 21,4 %).

Висновки. Пацієнтам з гіпотиреозом на тлі автоімунного тиреоїдиту слід призначати додаткові дози вітаміну D для поліпшення когнітивної функції.

Ключові слова: автоімунний тиреоїдит, гіпотиреоз, когнітивна функція, холекальциферол.