THE INTERRELATION BETWEEN THYROID DISEASE PHYSIOPATHOLOGY, ITS TREATMENT AND THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS, THE AUTONOMIC NERVOUS SYSTEM AND SEX HORMONES

PhD Thesis Abstract

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The table of contents and the abbreviations list in this abstract are kept in the same form as in the PhD thesis. A limited number of tables and figures were selected for this abstract and numbered the same way as in the thesis.

**Key words:** stress, hypothalamic-pituitary-adrenal axis, sympathetic adreno-medullary system, salivary cortisol, salivary alpha-amylase, autoimmune thyroid diseases, psychometric questionnaire.

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ABBREVIATIONS

1,25(OH)₂D₃  Calcitriol (1,25-dihydroxivitamine D₃)
AAs            Salivary α-amylase
ACTH           Adrenocorticotropic Hormone
AITD           Autoimmune Thyroid Disease
ANS            Autonomic Nervous System
APC            Antigen Presenting Cells
APS            Autoimmune Polyglandular Syndrome
ATA            Anti-thyroid antibodies
ATA            American Thyroid Association
ATD            Anti-thyroid drugs
ATg            Anti-Tg antibodies
ATPO           Anti-TPO antibodies
AUC            Area Under the Curve
BMI            Body Mass Index
CAR            Cortisol Awakening Response
CBG            Corticosteroid Binding Globulin
CBZ            Carbimazole
CD             Cluster of Differentiation
CRH            Corticotropin-Releasing Hormone
CTLA4          Cytotoxic T-Lymphocyte-Associated Protein 4
CYP27B1        Cytochrome P450 family 27 subfamily B member 1
D₂             Type 2 deiodinase
DH             Daily hassles
DHEA-S         Dihydroepiandrosterone-sulphate
DXM            Dexamethasone
ECLIA          Electro-chemiluminescence immunoassay
ELISA          Enzyme-Linked Immunosorbent Assay
FNAB           Fine Needle Aspiration Biopsy
fT₃            Free T₃
fT₄            Free T₄
GD             Graves’ Disease
GPx3           Glutathione peroxidase 3
H₂O₂           Hydrogen peroxidase
HAART          Highly Active Antiretroviral Therapy
HAM-A          Hamilton Anxiety scale
HAM-D          Hamilton Depression scale
<table>
<thead>
<tr>
<th>Term</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Human Leukocyte Antigen - antigen D Related</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic–Pituitary–Adrenal axis</td>
</tr>
<tr>
<td>Hsp70</td>
<td>heat shock protein 70</td>
</tr>
<tr>
<td>HT</td>
<td>Hashimoto’s thyroiditis</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>L-T3</td>
<td>Liothyronine</td>
</tr>
<tr>
<td>L-T4</td>
<td>Levothyroxine</td>
</tr>
<tr>
<td>mARN</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>MMI</td>
<td>Methimazole</td>
</tr>
<tr>
<td>NK</td>
<td>Natural Killer</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PANAS</td>
<td>Positive and Negative Affect Schedule</td>
</tr>
<tr>
<td>PSS</td>
<td>Perceived Stress Scale</td>
</tr>
<tr>
<td>PTPN-22</td>
<td>Protein Tyrosine Phosphatase, Non-Receptor Type 22</td>
</tr>
<tr>
<td>PTU</td>
<td>Propylthiouracil</td>
</tr>
<tr>
<td>rT3</td>
<td>Reverse T3</td>
</tr>
<tr>
<td>SAM</td>
<td>Sympathetic Adreno-Medullary System</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>SST</td>
<td>Somatostatin</td>
</tr>
<tr>
<td>T1/2</td>
<td>Half-life</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>TBAb</td>
<td>TSH receptor blocking antibodies</td>
</tr>
<tr>
<td>TBG</td>
<td>Thyroxine-Binding Protein</td>
</tr>
<tr>
<td>Tg</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>Th</td>
<td>T helper (lymphocyte)</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TPA</td>
<td>Tripropylamine</td>
</tr>
<tr>
<td>TPO</td>
<td>Thyroperoxidase</td>
</tr>
<tr>
<td>TRAb</td>
<td>TSH-R Antibodies</td>
</tr>
<tr>
<td>TRH</td>
<td>Thyrotropin-Releasing Hormone</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid Stimulating Hormone</td>
</tr>
<tr>
<td>TSH-R</td>
<td>Thyroid Stimulating Hormone-Receptor</td>
</tr>
<tr>
<td>YE</td>
<td>Yersinia enterocolitica</td>
</tr>
</tbody>
</table>

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Chapter 5

THE MOTIVATION AND OBJECTIVES OF THE RESEARCH PROJECT

Thyroid dysfunctions are amongst the most common endocrine disorders, with an estimated prevalence of 20 to 76% in the general population. Furthermore, up to 60% of the individuals with thyroid dysfunctions are not aware of their condition. Thyroid diseases are often diagnosed fortuitously, when the patients seek medical advice for non-specific symptoms, such as heart rate dysfunctions, sleep disturbances, mood disorders or alterations of the hair or skin. Women are 5 to 8 times more prone to thyroid disorders compared to men and it is estimated that one woman in eight will develop a thyroid disorder during her lifetime (Gardner, Shoback, 2011, Vanderpump, 2011).

The pathogenesis of thyroid dysfunctions varies with the geographical areas and is mostly caused by the presence or absence of iodine in peoples’ diets. Nowadays, a third of the world’s population is considered to live in areas with insufficient iodine, and the prevalence of endemic goiter in these people can reach up to 80% (Vanderpump, 2011).

In developed countries, where programs for the correction of iodine deficit have been implemented, there is a higher prevalence of autoimmune thyroid pathologies. These conditions affect approximately 2-12% of the general population, particularly the female sex (Giagourta et al., 2004).

Autoimmune thyroid diseases (AITD) are the main cause of hypothyroidism and hyperthyroidism in the United States of America and in other developed countries, where programs for iodine deficit corrections have been implemented. Autoimmune based thyroid dysfunctions have an especially negative impact on the quality of life of the patients, mostly through the augmentation of the risks to cardiovascular, phycho-behavioral
or reproductive disorders. Moreover, AITDs are the most common cause of hypothyroidism in the pediatric population in non-endemic areas, with negative consequences on growth, somatic and neuronal development (Vanderpump, 2011, Brown, 2013).

Similar to other autoimmune disorders, the etiopathogenesis of AITD is still incompletely decoded, due to its intricate genetic, immunologic, existential and environmental factors. Numerous studies have indicated the involvement of stress, such as posttraumatic stress or sexual abuse, in the autoimmune dysfunctions of the thyroid axis (Sgarbi, Maciel, 2009, Tomer, Huber, 2009).

In stressful conditions, the production of glucocorticoids and catecholamines are enhanced in order to maintain homeostasis, through the activation of the hypothalamic-pituitary-adrenal (HPA) axis and, respectively, of the sympathetic adreno-medullary (SAM) system. The continuous activation of these axes leads to metabolic diseases such as high blood pressure, heart rate abnormalities or high blood sugar. Moreover, together with the dysfunction of the SAM system, autoimmune thyroid dysfunctions can cause cardiovascular diseases, which, in time, amplify the risk of cardiovascular mortality (Cozma et al., 2017).

Cortisol is the most important steroid secreted by the adrenal gland and a well-known marker of the HPA axis activity. Also, the salivary production of α-amylase (AAs) has been proposed as a marker of the changes in plasma catecholamines during the activation of the SAM system in certain stressful conditions, particularly during acute stress (McEwen, 2003, Cozma et al., 2017).

The dysfunctional activations of the HPA axis and SAM system have been involved in numerous chronic diseases, particularly inflammatory disorders (Kassi et al., 2012).

The literature contains several arguments, mostly contradictory, regarding the involvement of stress in the
development of autoimmune thyroid pathologies. Nevertheless, most of these arguments are based on experimental studies or on clinical research based on self-administered questionnaires evaluating the subjective perception of stress.

To this day, the interactions between autoimmune thyroid dysfunctions, their treatment and the psycho-neuro-endocrine system have not been fully observed. Also, data regarding the hormonal changes in the HPA and SAM systems in AITDs and the influence of the specific treatment for AITDs on the stress markers is insufficient at the moment.

Since pharmacological therapies available today for the treatment of autoimmune thyroid dysfunctions are not causal, but target the normalization of thyroid function, the main objective for this pilot study was to evaluate the feasibility of a prospective study meant to evaluate the effect of levothyroxine and anti-thyroid drugs, respectively, on the diurnal trajectories of salivary cortisol and α-amylase, as markers of the HPA and SAM systems activities, as well as on the perception of stress.

In order to realize this main objective, the following secondary objectives were drawn:

1. the evaluation of subjective stress perception through standardized questionnaires in female sex patients with AITDs, differentiated on thyroid function (hypo-/hyperthyroidism or euthyroidism) and on reproductive status (reproductively active and post-menopausal women), as well as the influence of specific treatments of thyroid dysfunctions on the subjective stress perception;

2. the evaluation of non-invasive biomarkers (salivary cortisol and AAs) as possible predictive parameters of the interrelationship between stress and AITDs in female sex patients;

3. the evaluation of the possible correlations between the two biomarkers of the activities of the HPA axis and SAM system, as well as between these biomarkers and the psychometric parameters;
4. the evaluation of the impact the treatment with levothyroxine (L-T₄) and anti-thyroid drugs (ATDs), respectively, have on the diurnal production of salivary cortisol and AAs, based on thyroid function.

Through realizing these objectives, the present study aims to contribute to the clarification of the physiological mechanisms behind the development of AITDs in the female sex, during the reproductive years and post-menopause.

Knowledge of this data will allow the discovery of new methods to follow-up and predict the efficacy of pharmacological treatments in patients with these types of disorders.

Chapter 6

MATERIALS AND METHODS

In order to achieve the intended objectives, we performed an observational, prospective study, that has been conducted over a period of 22 months. This study has received approval (No. 14750 from July 17th 2014) from the Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy, Iași.

The subjects included in this study have been selected from among patients of the Endocrinology Clinic of the “St. Spiridon” Emergency Clinical Hospital in Iași, that had been diagnosed de novo with autoimmune thyroiditis, with or without thyroid dysfunction, had met the inclusion criteria and had agreed to participate in the study by signing the Informed Consent form.

The patients have been selected to participate to the study based on the following inclusion criteria:

- female sex
- 18 to 65 years old
• body mass index (BMI) of <30 kg/m²
• *de novo* diagnosis of autoimmune thyroiditis, irrespective of thyroid function
  • cooperative patients
  • patients that were intellectually capable of understanding and signing the informed consent

Patients were excluded from the study if they met at least one of the **exclusion criteria**:

• male patients
• under 18 years or over 65 years old
• obesity (BMI of >30 kg/m²)
• treatment for any thyroid disease at enrollment date or in their medical history
  • major comorbidities
  • history of partial or total thyroidectomy
  • documented psychiatric disorders
  • psychiatric treatment, treatment with anti-depressives, anxiolytic drugs, immunomodulators, anabolic steroids, corticosteroids, oral contraceptives or hormonal drug therapy during post-menopause at the enrollment moment, in the past 90 days before the enrollment or during the participation in the study
  • pregnancy or breastfeeding
  • the lack of cooperation or the inability to read or understand the informed consent

We included consecutive subjects, who were enrolled as they came to the Endocrinology Clinic. The study group included 42 patients with AITD, who met the inclusion criteria:

• group I: 20 patients with HT and euthyroidism;
• group II: 18 patients with HT and hypothyroidism;
• group III: 4 patients with active GD.

We estimated, with statistical analysis, that for the first study (comparison between patients with autoimmune hypothyroidism and euthyroid patients) are necessary at least 15 patients per group, while for the second study (comparison between patients with hyperthyroidism and euthyroid patients) are necessary a minimum of 4 patients with hyperthyroidism
and 8 patients with euthyroidism. We arrived at these numbers considering that this is a pilot study, with an $\alpha$ coefficient of 0.05 and a statistical power of 80% and in order to obtain an absolute difference of minimum 50% for the concentrations of salivary cortisol.

For each participant to the study, we evaluated at baseline and, for groups II and III, also at the follow-up visit, (approximately 3 months after initiating specific treatment, after reaching euthyroidism), the following parameters:

- general evaluation (history, clinical examination);
- thyroid evaluation (clinical, biochemical and imaging);
- evaluation of the subjective stress perception through four self-administered standardized questionnaires;
- evaluation of the HPA axis and SAM system activities through the determination of specific salivary biomarkers: salivary cortisol and salivary $\alpha$-amylase, respectively.

The subjective stress perception was evaluated through four standardized, self-administered questionnaires: the Perceived Stress Scale (PSS) (Cohen et al., 1983), the Daily hassles (DH) questionnaire (Kanner et al., 1981), Hamilton depression (HAM-D) (Hamilton, 1960) and anxiety (HAM-A) (Hamilton, 1959) scales. The questionnaires were administered before the clinical examination, in order to minimize the influence on the psychological status of the patients, since the psychometric scales tested the depressive/anxiety symptoms, the stress perceiving manner, as well as the modality to face the daily hassles, loneliness or major (stressful) life events.

For the collection of saliva, the Salivette® (Sarsted, Italia) device was used, which permitted the rapid, hygienic and non-invasive passive collection of saliva, through a synthetic swab, without salivation stimulatory factors. The saliva was subsequently recovered after the centrifugation of the devices for 5 minutes at 3000 rpm.

The collection of saliva was made by the patients at their own homes, during one day, in 5 pre-established moments and
the Salivette® devices were returned to the investigators the next day. Following centrifugation, the saliva was frozen until the laboratory quantitative determinations. The moments of saliva collection were as follows:

- in the morning, at awakening;
- 30 minutes after awakening;
- 60 minutes after awakening;
- before lunch (12:00 – 14:00 hours);
- In the evening, before dinner (19:00 – 21:00 hours).

The database was created with Microsoft Office Excel - 2010-2016 versions and the statistical analysis and figures were performed with SPSS v.20 for Windows (SPSS Inc., Chicago, Illinois) and Sigma Plot 11 (Systat Software Inc., San Jose, California).

Chapter 7

GENERAL RESULTS

The study group included 42 women newly diagnosed with AITD at the Endocrinology Clinic of the “Sf. Spiridon” Emergency Clinical County Hospital of Iași.

The ages of patients enrolled in our study varied between 20 and 63 years old and the peak of frequency was recorded in the 3rd and 4th decade (approximatively 60% of patients).

Following the clinical, ultrasonographical and biochemical evaluations of the thyroid gland, the study group was divided in 3 groups, based on the value of TSH (Thyroid Stimulating Hormone) at baseline:

- patients with normal thyroid function = euthyroidism: normal TSH level (0.4 - 4 µUI/ml);
- patients with hyperthyroidism: low TSH levels (<0.4 µUI/ml);
• patients with hypothyroidism: high TSH levels (>4 µIU/ml).

Thus, based on TSH levels and the need to initiate specific treatment (ATDs or L-T₄) and based on the reproductive status, the study group was divided in 6 subgroups:
1. reproductively active patients with hyperthyroidism (n=3);
2. post-menopausal patients with hyperthyroidism (n=1);
3. reproductively active patients with euthyroidism (n=14);
4. post-menopausal patients with euthyroidism (n=6);
5. reproductively active patients with hypothyroidism (n=11);
6. post-menopausal patients with hypothyroidism (n=7)

**Chapter 8**

**RESEARCH CONCERNING THE INFLUENCE OF LEVOTHYROXINE TREATMENT ON THE SUBJECTIVE STRESS PERCEPTION AND ON THE HPA AND SAM SYSTEMS IN FEMALE PATIENTS WITH AUTOIMMUNE HYPOTHYROIDISM**

**8.1. Evaluation of the levothyroxine treatment effect on the subjective stress perception in female patients with autoimmune hypothyroidism**

At the beginning of the study enrollment, the euthyroid patient group scored lower means compared to hypothyroid patients at all four stress perception questionnaires. However, differences were not statistically significant. The mean scores on PSS and DH questionnaires, at baseline, corresponded to a medium stress level, both in the
reproductively active and post-menopausal subgroups, while the HAM-A scores pointed to no anxiety disorder. However, regarding the HAM-D scale, the reproductively active women had normal mean scores, while the results of post-menopausal women tests can be associated with mild depression.

During the follow-up visit, there was a reduction in the mean scores for all four stress perception questionnaires for the hypothyroid group (n=18), after the restauration of euthyroidism with L-T4 treatment.

![Graph showing mean scores for psychometric scales](image)

**Fig. 8.7.** The progress of psychometric scores in hypothyroid patients after the restauration of euthyroidism with L-T4 treatment (*: p<0.05)

In the reproductively active group, after restoring euthyroidism, hypothyroid patients scored lower at all four psychometric scales compared to euthyroid patients at baseline. However, the differences were not statistically significant.

In the post-menopausal group, hypothyroid women showed a non-significant reduction of subjective stress
perception after restoring euthyroidism, as quantified by the PSS questionnaire, compared to baseline (42.28 ± 6.1 vs. 41.86 ± 5.0, p=0.817). Conversely, for all the other questionnaires, the post-menopausal women scored higher after the normalization of the thyroid function with L-T₄ treatment, compared to baseline, when they were in a hypothyroid state.

8.2. Evaluation of the levothyroxine treatment effect on the hypothalamic-pituitary-adrenal axis in female patients with autoimmune hypothyroidism

The daily fluctuations of salivary cortisol were observed at baseline both in euthyroid and hypothyroid groups, with higher levels in the morning than in the evening. Furthermore, both groups had a typical cortisol awakening response (CAR), characterized by an augmentation in the first 30 minutes after waking up, followed by a reduction in the next 30 minutes.

In the hypothyroid group, we observed lower mean cortisol levels, at baseline, compared to the euthyroid group. More precisely, hypothyroid patients exhibited a blunted cortisol response during the first hour after awakening (AUC_CAR: F₁, 36=4.575, p=0.039) and a significantly flatter daily curve (AUC_Circadian: F₁, 36=7.696, p=0.009) compared to euthyroid women (figure 8.13.).

Unlike reproductively active hypothyroid patients, who recorded a typical CAR curve, with a 10.05% raise (p=0.636) in the first 30 minutes after waking and a 7% reduction (p=0.760) in the following 30 minutes, the post-menopausal group presented a demeaning CAR: an 8.6% (p=0.747) decrease 30 minutes after awakening and another 39.3% (p=0.51) decrease 60 minutes after awakening (figure 8.16.).
Fig. 8.13. The circadian profile of salivary cortisol in hypothyroid and euthyroid patients at baseline. The data is presented as mean ± SE. (*:p<0.05)

After approximately 3 months of L-T4 therapy and after restoring the euthyroid status, the hypothyroid group recorded a partial restauration of CAR, compared to baseline, when the mean salivary cortisol levels increased with only 1.7% in the first 30 minutes after awakening (p=0.915), followed by a 23.8% decrease 60 minutes after awakening (p=0.205).
8.3. Evaluation of the levothyroxine treatment effect on the sympathetic adreno-medullary system in female patients with autoimmune hypothyroidism

There were no significant differences at study enrollment regarding the daily activity of AAs between hypothyroid and euthyroid patients (p=0.990) (figure 8.27.). Once euthyroidism was achieved, after approximately 3 months of L-T4 treatment, we observed a significant increase of AAs activity during lunchtime for the hypothyroid groups, compared to the same moment at study enrollment (p=0.023) and the highest level during the day (40.39 ± 17.1 U/ml) (figure 8.30.). Moreover, the lowest AAs mean level during the day after the restauration of euthyroidism was one hour after waking (22.1 ± 11 U/ml), compared to baseline and the euthyroid group, when the lowest daily AAs concentrations were recorded 30 minutes after waking up (23.87 ± 16.1 U/ml and 28.61 ± 18.9 U/ml, respectively).
Fig. 8.27. The circadian profile of AAs in hypothyroid and euthyroid patients at baseline. The data is presented as mean ± SE

Fig. 8.30. The AAs daily trajectories in the euthyroid group at baseline and in the hypothyroid group at baseline and after the restoration of euthyroidism with L-T₄ treatment
Chapter 9

RESEARCH CONCERNING THE INFLUENCE OF ANTI-THYROID DRUG TREATMENT ON THE SUBJECTIVE STRESS PERCEPTION AND ON THE HPA AND SAM SYSTEMS IN FEMALE PATIENTS WITH AUTOIMMUNE HYPERTHYROIDISM

9.1. Evaluation of the anti-thyroid drug treatment effect on the subjective stress perception in female patients with autoimmune hyperthyroidism

At enrollment, both hyperthyroid and euthyroid patients had mean PSS and DH scales scores that corresponded to a medium level of stress, without significant differences between groups. The euthyroid patient group had normal mean levels in the Hamilton depression and anxiety scales. However, in the hyperthyroid group, we recorded higher levels, that corresponded to mild depressive disorder and anxiety disorder, respectively.

After the restoration of euthyroidism with ATDs, we recorded a descending trend for PSS, HAM-D and HAM-A scores in the hyperthyroid patient group, as compared to the baseline scores. During the follow-up visit, these mean scores were closer to the euthyroid group, but still slightly higher. It was only in the DH questionnaire, that evaluated the well-being status of the patients, that an increased stress level was recorded, even higher than that of the euthyroid group (figure 9.3.).
9.2. Evaluation of the anti-thyroid drug treatment effect on the hypothalamic-pituitary-adrenal axis in female patients with autoimmune hyperthyroidism

We recorded higher mean salivary cortisol levels for the hyperthyroid patients group, compared to euthyroid patients, during all five moments of the day when saliva was collected.

After the normalization of the thyroid function with ATDs, as compared to baseline, patients had a significant reduction of CAR (4.65 ± 3.1 ng/ml vs. 10.34 ± 3.7 ng/ml, p=0.034) (figure 9.5.). Comparing the two moments (the enrollment and the follow-up visits) for the hyperthyroid group and the euthyroid group at enrollment, the One-Way ANOVA test indicated a statistically significant difference, both for the secretion of cortisol during the first hour after waking (F2, 13=4.671, p=0.03), and for the daily cortisol production (F2, 13=3.908, p=0.047).
9.3. Evaluation of the anti-thyroid drug treatment effect on the sympathetic adrenergic-medullary system in women with autoimmune hyperthyroidism

There were no significant differences between hyperthyroid and euthyroid female patients regarding the daily production of AAs (p=0.827) at the enrollment visit.

After the normalization of the thyroid function with ATDs, there was an increase in AAs activity during 4 of the 5 moments of the day, that translated in an increase of the diurnal AAs production, as compared to baseline (506.57 ± 102.2 U/ml vs. 445.05 ± 93.1 U/ml, p=0.284) (figure 9.7.).
Fig. 9.7. The diurnal trajectories of AAs in the euthyroid group at baseline and in the hyperthyroid group at baseline and after the restoration of euthyroidism with ATD. The data are presented as mean ± SE. (*: p<0.05 - hyperthyroidism before and after the ATD treatment)

Chapter 10

DISCUSSIONS

The first section of this pilot study showcased the distribution of the patients included in the study, according to thyroid parameters. This research included only patients with autoimmune thyroid disease, regardless of the presence or absence of hormonal dysfunction. Considering the design of the pilot study, as well as the narrow inclusion and exclusion criteria and the lack of a healthy control group, we cannot
extrapolate epidemiological details on the incidence and/or prevalence of AITDs in the region of Moldova.

The evaluation of stress perception and depressive-anxiety disorders in the general population is usually done based on validated and standardized questionnaires. In order to shorten the duration of interviews, most of these scales were self-administrated. In this study, we used 4 standardized psychometric questionnaires, in order to evaluate both the subjective stress perception and the presence or absence of depressive-anxiety disorders, in a population of 42 women newly diagnosed with AITD.

Stress is one of the factors commonly incriminated for the initiation and propagation of several chronical disorders, as a part of the modern lifestyle (McEwen, 2007, Kassi et al., 2012). Furthermore, according to Becker et al. (2007), it seems that the female sex holds a higher susceptibility to stress and emotion-based imbalances. Therefore, it was in no way surprising that in the female population included in our study, the subjective stress perception scores corresponded to a medium level.

Moreover, even though the stress perception scores, as quantified by the PSS and DH scales, were slightly higher for hypothyroid than euthyroid patients at baseline. Depressive disorder euthyroid patients recorded normal mean scores, while the hypothyroid patients had mean scores corresponding to mild depression. On the other hand, Carta et al. (2005) found in a group of 18 women with euthyroid Hashimoto’s Thyroiditis (HT), in comparison with 16 patients with euthyroid nodular goiter, that psychiatric disorders, among which, generalized depressive and anxiety symptoms, were more common in patients with autoimmune thyroiditis (OR=6.6 and OR=4.9, respectively, confidence interval of 95%), irrespective of the thyroid function.

Another important aspect highlighted in the first section of this study was the different modality of perceiving stress.
between the reproductively active and the post-menopausal female population included in this study. Therefore, the results we obtained in this study showed higher, but not significant scores, in the PSS, HAM-D and HAM-A questionnaires for post-menopausal patients. These results agree with the hypothesis that age is an important factor that influences both quantitative and qualitative stress perception. Therefore, our findings are in agreement with the results of Strieder et al. (2005), who found, on the Amsterdam cohort, that age differences were strongly related to the amount and type of self-reported stress, as ATPO positive older patients tended to report fewer stressful events and daily hassles compared to younger patients.

After the correction of hypothyroidism with L-T4 treatment, all hypothyroid patients showed a reduction of all four psychometric questionnaires scores. Moreover, regarding the subjective stress perception quantified by the PSS scale, we observed a significant reduction (p=0.030) of the mean perceived stress levels, even though values remained in the medium stress level range. Also, although the reduction of the mean score of the HAM-D scale after the restoration of euthyroidism was not statistically significant (p=0.185), it seems that it was clinically significant, as it decreased from a level corresponding to mild depressive disorder, to a normal level.

In the hyperthyroid patient group, we did not find any significant differences regarding the scores of the PSS and DH questionnaires, compared to the euthyroid group. Similar data was reported by Effraimidis et al. (2012a), who showed the lack of a difference regarding the subjective perception of stress as quantified by the Dutch and PANAS (Watson et al., 1988) scales, between the GD patients and the control group. However, regarding the depression and anxiety Hamilton scales, hyperthyroid patients had scores corresponding to mild depression and anxiety disorder, respectively, while the
euthyroid group recorded normal mean scores at both questionnaires.

After the restoration of euthyroidism with ATDs, there was a tendency towards the normalization of psychometric scores, particularly for the HAM-D and HAM-A scales. Therefore, the mean scores for all 4 psychometric questionnaires were closer, during the follow-up visit, with those of the euthyroid group at baseline. These results are partly concordant to the data found in the present literature, as Fukao et al. (2003) showed, in a group of 69 patients with autoimmune hyperthyroidism that were being treated with ATDs, that after the cessation of the treatment, the scores of psychometric tests increased significantly for the relapsed patients, compared to patients who maintained the euthyroid status.

The HPA axis, with cortisol as the main secretion product, is considered one of the most important regulator of adaptive processes in the human body and maintenance of homeostasis during psychological and/or psychopathological challenges (McEwen, 2003). It seems that during illnesses, there is an over-stimulation of the HPA axis, that translates to chronically high levels of cortisol in the periphery, as well as an under-stimulation (inadequate response), characterized by a flat cortisol curve (Adam, Kumari, 2009).

We showed in this study that the typical profile of cortisol diurnal secretion was present in all patient groups, both in patients with thyroid dysfunction (hypothyroidism or hyperthyroidism) and in euthyroid patients. Higher levels were recorded in the morning, during the first hour after awakening, and the lowest values were observed in the evening. Moreover, the typical cortisol awakening response (CAR) was obvious, in all patient groups, with an increase of mean cortisol values during the first 30-60 minutes after waking up. The maximum daily concentrations of salivary cortisol were observed in the euthyroid and hypothyroid groups, 30 minutes after waking up, while in the hyperthyroid group, the maximum concentration of
salivary cortisol was recorded, at the time of enrollment, at 60 minutes after waking up.

In the first section of the study, hypothyroid women recorded lower levels at baseline than the euthyroid group, regarding both CAR and the daily production of cortisol. Therefore, considering that there were no significant differences between the two groups regarding ATPO levels and the only distinct factor was the TSH concentration, we can argue that autoimmune hypothyroidism, irrespective of ATPO, is associated with an attenuated CAR and a flat diurnal cortisol curve.

On the other hand, in the second section of the study we found that hyperthyroid female patients had significantly higher salivary cortisol levels than euthyroid patients, both during the first hour after waking up and during the day.

Three months of L-T4 treatment led to the normalization of the thyroid function in the hypothyroid group. The salivary cortisol production maintained its physiological curve, with significantly higher concentrations in the morning than in the evening and a CAR was partially restored. Furthermore, during the follow-up visit, the hypothyroid group had a significantly higher diurnal cortisol production compared to baseline, however lower compared to the euthyroid group at baseline (p=0.022).

Interestingly, in the hypothyroid reproductively active group, after the restauration of euthyroidism, we noticed a continuous attenuation of CAR, as well as a lower diurnal salivary cortisol secretion compared to baseline.

There are few and contradictory data, in literature, regarding the integrity of the HPA axis in GD. In theory, hyperthyroidism in active GD leads to an acceleration of all metabolic processes, resulting in an increase in both the production and clearance of cortisol, and therefore serum cortisol remains in the normal range (Gardner, Shoback, 2011). However, in order for this hypothesis to be true, it needs all the
systems involved in the production of cortisol, such as hepatic cortisol binding proteins, as well as the clearance systems to function in normal parameters (Price et al., 2012).

Our study suggests there is a hyper-activation of HPA axis associated to a hyperactive thyroid, irrespective of anti-thyroid antibody concentrations. Therefore, corroborating this data with high psychometric scores, particularly regarding depressive, anxiety symptoms and the reduction of all these parameters along with the normalization of the thyroid function, we could consider hyperthyroidism itself as a stressor for the body.

In this pilot research, we have, for the first time, evaluated the SAM system activity through the quantitative determination of AAs in a newly diagnosed AITD female population.

The results shown in this pilot study revealed, at study enrollment, the presence of a physiological circadian cycle of AAs (with lower levels in the morning and maximum levels at the end of the day), without any significant differences between the hypothyroid and euthyroid patients or between the hyperthyroid and euthyroid patients, respectively.

The findings of this research have shown opposite circadian trajectories for salivary cortisol and AAs. In the morning there is a high secretion of salivary cortisol, that decreases during the day, while the AAs activity has an ascending line during the day. These results are concordant to the data found in the literature (Chatterton et al., 1996, Yamaguchi et al., 2006, Nater et al., 2007) and suggests the distinct origin of the two systems: cortisol is the representative biomarker of the HPA axis, while the AAs is the projection of the SAM system (Wolf et al., 2008).
Chapter 11

CONCLUSIONS

The following conclusions can be drawn from this research:
1. Patients with autoimmune thyroiditis, irrespective of age, reproductive status or thyroid function, fell into a medium level of stress, according to the scores of the self-administered psychometric questionnaire.
2. In the autoimmune hypothyroid group, at study enrollment, patients had higher mean scores in all 4 psychometric tests than the euthyroid patients, with a reduction of all these scores after the correction of hypothyroidism L-T4 therapy. Also, hyperthyroid patients had higher scores in 3 out of the 4 psychometric tests compared to euthyroid patients, that decreased after the restauration of euthyroidism with ATDs.
3. There are different modalities of subjective stress perception between reproductively active and post-menopausal women with autoimmune hypothyroidism:
   - Younger women had a tendency to score higher regarding daily hassles and the well-being status questions;
   - Post-menopausal women had higher scores at the scales that evaluated depressive and anxiety symptoms and the perception of unforeseen life events.
4. All hypothyroid patients had psychometric scores corresponding to a mild depressive disorder at baseline, while in the post-menopausal group, these depressive symptoms were apparent also in the euthyroid group. Moreover, the L-T4 treatment effect was different according to the thyroid function, therefore suggesting the involvement of the sex hormones in the emotion-based disorders, the subjective perception of stress and, accordingly, a possible benefit of starting hormonal replacement therapy during peri-menopause.
The normal circadian rhythm of salivary cortisol secretion was present in all study groups, irrespective of the thyroid function, both in reproductively active and post-menopausal women, with higher levels in the morning and lower in the evening. Also, the typical cortisol awakening response (CAR), with a significant increase of the salivary cortisol concentrations during the first 30 and 60 minutes after waking, was evident in all patients of the study group.

Autoimmune hypothyroid patients recorded significantly lower curves of salivary cortisol, for both CAR and diurnal cortisol production, compared to euthyroid patients, while, conversely, hyperthyroid patients had significantly lower salivary cortisol levels during the first hour after waking, as well as during the day.

After the correction of the thyroid function with L-T4 and ATD treatment, respectively, both hypothyroid and hyperthyroid patients had a partial restoration of CAR, as well as a tendency of the circadian cortisol production to approach the concentrations recorded at baseline in the euthyroid group.

The L-T4 treatment had a different effect on the production of salivary cortisol, based on the reproductive status: while in the post-menopausal group there is a restoration of the CAR, in the reproductively active women there was a continuous blunting of this response, as well as a lower diurnal secretion compared to enrollment. After the normalization of the thyroid function, in both reproductively active and post-menopausal patients there was an increase of the salivary cortisol concentration at the end of the day, indicating the inability of the body to relax during less-challenging periods.

All the patients included in our study, irrespective of the reproductive status and thyroid function, had a physiologic diurnal AAs trajectory, with lower levels during the morning and maximum levels at the end of the day, with no significant differences among groups.
10. Similar to salivary cortisol, there was an awakening response in the case of AAs, the mean levels of AAs decreasing during the first 30-60 minutes after waking up. This response was more pronounced at baseline in the hypothyroid and the hyperthyroid patients than in the euthyroid group, irrespective of the reproductive status.

11. In the hyperthyroid group, we saw a paradoxical increase in the AAs activity after the restauration of euthyroidism with ATD treatment.

12. The salivary cortisol and AAs had opposite trajectories in all patient groups, confirming the current literature, that suggested a distinct origin of the two systems involved in the stress response.

Chapter 12

ORIGINALITY AND FUTURE RESEARCH PERSPECTIVES

The research that was done during the development of the doctoral thesis brings important contributions to the clarification of the complex interactions between the hypothalamic-pituitary-adrenal axis and the sympathetic adreno-medullary system, as key systems involved in the organism’s ability to adapt to variate acute or chronic stress, in patients with autoimmune thyroid disorders. To our knowledge, from the existent data in the medical literature, this is the first study to evaluate the (dys)functionality of the HPA axis and the SAM system through their characteristic salivary biomarkers in patients with AITDs, therefore justifying the pilot design of the study.

This pilot research indicates for the first time the existence of specific salivary cortisol and AA secretion curves
in the autoimmune thyroid pathology and their association with the subjective perception of stress in female subjects.

Also, this is the first study of this type that shows differences regarding the stress perception and the functionality of the systems involved in stress adaptation between reproductively active and post-menopausal women.

The results obtained during the doctoral research are encouraging and justify the intention to continue and deepen the studies on the physiopathology and pharmacology of autoimmune thyroid disorders, with important perspectives in the diagnosis and monitoring of the specific medical treatment for these diseases.

First, it is important to establish a real incidence of AITDs. The majority of the epidemiological data regarding autoimmune thyroid diseases comes from studies on large cohorts in countries with very high economical and education levels such as the United States of America (NHANES III), Australia (The Busselton Thyroid Study), Denmark or Great Britain (The Wickham Study) (Hollowel et al., 2002, O’Leary et al., 2006, Pedersen et al., 2003, Vanderpump et al., 1995). In Romania, there is a clear lack of epidemiological research data regarding the incidence and prevalence of AITD. Therefore, the development of a national (multicentric), prospective study, is of great importance, and results could contribute to a wider knowledge of the real frequency of these pathologies in this country.

In order to better evaluate the involvement of stress in the pathogenesis of AITDs through the self-report of the subjective perception of stress, there is a need for prospective studies on ATPO-naïve patients, with evaluation of the development of ATPO in time and associations with the periodic self-administration of psychometric questionnaires. Also, in order to test the hypothesis according to which thyroid autoimmunity, irrespective of thyroid functionality, is involved in the development of depressive-anxiety disorders, further
studies should include a healthy, age and body-type matched control group.

Considering the clear association of autoimmune hyperthyroidism with an exaggerated cortisol response at awakening, as well as during the day, and the link between autoimmune hypothyroidism and a blunted salivary cortisol awakening response in both reproductively active and post-menopausal women, which have been shown in this study, with the purpose of proving the relationship between this altered HPA axis response and thyroid autoimmunity, larger studies are needed, that include both a control group and a group of patients with thyroid dysfunctions other than autoimmune.
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