"OVIDIUS" UNIVERSITY IN CONSTANŢA
MEDICAL DOCTORAL SCHOOL
DOCTORAL FIELD MEDICINE

SUMMARY OF POSTGRADUATE THESIS

SCIENTIFIC COORDINATOR:
Prof. Univ. Dr. CIRCO EDUARD

***PhD CANDIDATE*:**
AXENTE (STANCIU) LILIANA-ELENA

CONSTANŢA
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THE EFFECT OF PELOIDOTHERAPY IN FIGHTING AGAINST THE BIOLOGICAL AGEING PROCESS - CLINICAL, BIO-CHEMICAL AND HORMONAL CONSIDERATIONS

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This PhD thesis contains:

- PART I: ***Current knowledge stage***, containing 4 chapters

- PART II: ***Personal contributions***, containing 5 chapters

- 363 references

- 247 tables

- 105 pictures

- Two articles from the thesis subject, published as first author, in extenso: an article published in an ISI magazine and an article in a B+ magazine, both magazines indexed in international data bases.

KEY WORDS: ageing, endocrinology, antioxidant, mud, peloidotherapy, Techirghiol.

NOTE: Tables, figures and references in this summary maintain the original numbers in the postgraduate thesis. The table of contents in the summary is the original one in the thesis.

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INTRODUCTION

"Healthy ageing by a healthy life style"

Healthy ageing means the optimisation of opportunities for a good general health condition, so that elders can play an active role in the society and enjoy a high and independent quality of life. Th ageing of population is present in all geographic areas of the world and this results from the reduction of mortality and reduced fertility. The process entails the relative reduction of young population and an increase in the ratio of adult and old population.

The originality of this scientific research results from the investigation of some determining factors [insulin 1 growth factor (IGF-1), Serum cortisol, dehydroepiandrosterone-sulfate (DHEA-S), glutathione-peroxidasis (GPx)], that seem to play a major role in the beginning and evolution of the biological ageing process and their behaviour under the effect of balnear therapy, a method recognised by international bodies, standardised along time or certain pathological conditions, in order to achieve the maximum benefit for the old patient therapeutic approach.

The main purpose of this paper was to investigate the effect of the therapy with Techirghiol sapropelic mud, a natural factor used within Techirghiol Balnear and Recovery Sanatorium for the old patient.

We have carried out this study in order to find a a new perspective on the know-how of pathogenic mechanisms of the human ageing process subject to the therapy with Techirghiol sapropelic mud given that we have found **no study** in the specialised literature studied, both national and international, investigating the evolution of serum levels of IGF-1, serum cortisol, DHEA-S, GPx during the therapy with Techirghiol sapropelic mud in old patients and the follow-up of effects achieved after the balnear therapy.

Over the last two decades, the medical research has known a remarkable progress in terms of demonstrating the role of IGF-1 in the evolution of biological ageing process, 3013 clinical studies being published in the international literature approaching the subject from various perspectives. However, the mud therapy and its effect on the ageing process has only been modestly represented in 6 works published, while IGF-1 variations during mud therapy, mentioned in 4 studies published up to date, suggest that IGF-1 exercises a protecting influence on the cartilage structure, only being studied in osteoarticular pathologies at elders. So far, no scientific research has been published in the specialised literature assessing the effects of mud therapy on human ageing from the IGF-1 perspective.

Over the last years, medical research has known a remarkable development in terms of glucocorticoids implications in the physiological evolution of biological ageing process, 888 clinical studies being published in the international literature approaching the subject from various perspectives. The mud therapy and its effect on the biological ageing process, as mentioned above, has only been modestly represented in 6 works published up to date. Merely 4 works published in the specialised literature analyse the homoeostasis of hypothalamic-hypophyseal-adrenal axis during mud therapy, suggesting the beneficial effect of glucocorticoids in diseases with a major inflammatory component, rheumatological diseases (osteoarthritis, fibromyalgia) being studied in 3 studies, while the pelvic inflammatory disease has only been included in a single study. So far, no scientific research has been published in the specialised literature assessing the effects of mud therapy on human ageing with the inclusion of cortisol variation.

The medical research includes many clinical studies aiming to define the role of DHEA-S in the biological ageing process, thus 1247 scientific have been published in the international literature, so far, approaching the subject from various perspectives; of these, 68 articles have studied the biological ageing process with one of the monitoring variables being the cortisol / DHEA-S ratio. So far, no scientific research has been published in the specialised literature assessing the effects of mud therapy on human ageing assessed under the serum variations of DHEA-S. Given all such data, the mud therapy and the consequences on the ageing process have been modestly represented by as little as 6 works published.

As regards the implication of the enzyme family of glutathione peroxidasis (GPx) in the development of the human ageing process, 1489 clinical studies have been published in the specialised literature reporting such issue. Thus, the mud therapy and the effect on the biological ageing have been represented in 6 works published up to date. As little as 3 works published in the specialised literature analyse GPx variation during mud therapy, suggesting the beneficial effect of this enzyme family in diseases with a major inflammatory component, mainly patients with osteoarthritis being monitored. So far, no clinical study has been published in the specialised literature reporting the effects of mud therapy on human ageing with the direct inclusion of GPx.

CHAPTER 1

Ageing theories

Table no. 1– Classification and brief description of main ageing theories

Biological level / Theory	Description
Evolutionist Theory [1]	
Accumulation of mutations *	Mutations affecting health at old ages are not selected.
Antagonistic (Pleiotropy) *	Somatic cells are only maintained to ensure the ongoing success of reproduction; after reproduction, the soma becomes disposable. Beneficial genes at young ages become harmful at old ages.
Molecular Theory [1]	
Gene adjustment *	Ageing is caused by changes in the gene expression influencing both development and ageing.
Restriction of codons	The fidelity / accuracy of RNAm transcription is altered by the incapacity to decode codons in RNAm.
"Catastrophic" errors	The decline in the old age gene expression fidelity entails the increase in the fraction of abnormal proteins.
Somatic mutations	Accumulations of molecular damage, mainly in the genetic material / DNA.
Dysdifferentiation	Gradual accumulation of random molecular damage preventing the gene expression adjustment.
Cellular Theory [1]	
Cellular ageing - Telomere theory *	Ageing phenotypes are caused by the increase of old cells frequency. Ageing can result from the loss of telomeres (replication ageing) or the cellular stress (cellular ageing).
Free radicals *	Oxidation metabolism produces highly reactive free radicals subsequently affecting the structure of lipids, proteins and DNA.
Body ageing	Accumulation of physiological damage.
Apoptosis	Cellular programmed death from genetic events to genomic crises.
Systemic Theory [1]	
Neuroendocrine *	Disorders in the neuroendocrine control of homoeostasis entail physiological changes related to ageing.
Immunological *	The decline of immune function during ageing entails the reduction of infectious diseases incidence, however to an increase of the self-immunity incidence.
Life expectancy	It refers to a fix dimension of metabolic potential for each live body.

* Approached in the text

CHAPTER 2

Ageing neuroendocrine theory

Endocrine changes - Considering the changes on the endocrine function at elders, it is important to distinguish between the effects of ageing per se (biological ageing) on the endocrine physiology and the effects caused by age related diseases, given the high incidence of disorders, both symptomatic and non-symptomatic in elders [2]. The distinction between age and diseases related changes does not necessarily entail therapeutic intervention [2]. So far, it is not clear whether the treatment of several age related endocrine changes is beneficial [10].

Several general comments regarding ageing related endocrine changes are relevant: the single endocrine system for which there is a well-defined, sudden and universal change according to age is the hypothalamic-hypophyseal-gonadal axis at women [2]. The function of hormonal growth - insulin like growth factor 1 (IGF-1) system, the male hypothalamic-hypophyseal-gonadal and the portion (reticular area) in the adrenal cortex providing the synthesis of dehydroepiandrosterone, all these are subject to a progressive decline, along ageing at most people [2]. Normal values, adjusted according to age for serum IGF-1, total and free or bio-available testosterone and the dehydroepiandrosterone concentrations may be defined, but it is not known whether such values are physiologically optimal [2]. The secretion of other hormones is also subject to changes with age, however changes are less predictable and there are not well-defined normal values adjusted with age [2].

CHAPTER 3

Molecular mechanisms of oxidation stress in ageing

"Ageing is the result of progressive accumulation of cellular changes reducing the capacity of the body to withstand the stress, thus causing a reduction of the surviving possibilities"[1,2].

It has been demonstrated that a rapid functional decline occurs during the ageing process [11]. The actual mechanisms causing such functional decline are not clear. The theory of free radicals of the ageing

process shows that an increase in the production of free radicals of oxygen, with age, by the mitochondria causes the growth of cellular damage[12-15].

In humans, the first line of antioxidant defence are antioxidant enzymes, especially SOD, glutathione peroxidasis (GPX) and, to a lesser extent, catalase, as well as tripeptide glutathione (GSH) [60]. Such enzymes help destroying SOR, H2O2 and lipid peroxides, while GSH protects against oxidised proteins.

CHAPTER 4

Techirghiol sapropelic mud

The legend says the lake became famous after a Turk miraculously healed. Techir, an old and crippled man, stopped together with his donkey at Techirghiol lake, being stuck in the smelly mud together with his animal. After a while in the water, he recovered sight and he was no longer a cripple. The news of such miracle was heard of by many and people from all over came and used the water in the "lake of Techir"- today, Lake Techirghiol[6].

The endocrine mechanism entails peloidotherapy specific anti-inflammatory effects by activating the hypothalamic-hypophyseal-adrenal axis and by the general improvement of the endocrine balance with post-therapy effects also and the defence mechanisms are activated by the thermal factor. Only oestrogen effects have been recognised for a long time, then Ştefan Milcu has signalled progesterone properties, too[26], and later the tendency to restore the functional thyroid balance is outlined (also by the elective intervention and fixing in the thyroid of iodine in the mud composition). Starting from these results, the action of mud and mud extracts on other endocrine glands has been studied. The study is both interesting and valuable because it studied "by hysto-chemical methods, the possible changes in enzymes, metabolism, secretions of morphology of endocrine glands (hypophysis, thyroid, adrenal, gonad)" in a time (early 60's) when the literature concerning mud action on endocrine glands was poor and the existing one almost exclusively referred to the genital endocrine area[14]. Enzyme and metabolic changes in endocrine glands entailed by the mud treatment differ according to the specific secretion of the gland, the functional stage and the therapeutic method used: integral

mud baths or extract injections. It results in a secretion optimisation within the endocrine system by increasing the enzyme and synthesis activity. Similar gland changes have been possible within experiments carried out with turf mud from Poiana Stampei and the resulting effects are metabolic, in general, and cellular, non-specific. It results in the enrichment of teguments in sulphur-sulfhydryl groups, alkaline phosphatase, hyaluronic acid, the reduction of glycogen in the liver and muscles, of glutathione in the blood, the increase of serum transaminases, the increase of vitamin C in the brain, liver, testicle, lung and adrenal, the increase of A.T.P. in the muscles.

CHAPTER 5

Subject selection motive and paper objectives

There were three motives to select this subject: as a young specialist doctor in Recovery, Physical Medicine and Balneology, I started my medical activity at Techirghiol Balnear and Recovery Sanatorium, a medical unit where the main form of treatment is represented by the use of natural therapeutic factors: the stimulating environment, the sapropelic mud and the chlorine-sodium-iodine water in the lake[1]. The studies of therapeutic factors in Techirghiol balnear area are only a few some of them carried out many years ago, given that the environment is subject to a perpetual change. As a doctor, I carry out my activity in the current medical conditions of scientific evidence based medicine and I felt the urge to deepen the scientific research regarding Techirghiol sapropelic mud started by my piers, doctors at Techirghiol Balnear and Recovery Sanatorium. The therapeutic mud in Lake Techirghiol has unique features, given by its physical-chemical structure, properties that can only be resembled, however not matched, to other therapeutic muds, such properties being also influenced by the climate in the area, each balnear resort being thus unique. The aforementioned aspect entails the urgent need to study the natural therapeutic factors as independent entities.

A second matter urging this scientific paper is the importance of studying the ageing process under the current demographic conditions, with national application of the "ageing prophylaxis" concept [2], a process started

and achieved, in our country, through the product Gerovital H3, by Professor Doctor Ana Aslan, a word wide known medical and scientific personality. This scientific research started from the idea of a connection between the importance of the therapy with Techirghiol sapropelic mud in the ageing process under the logo "giving life to years", started since early 1982 in Vienna during the "World Meeting for Elders", by Professor Doctor Ana Aslan[2].

A third reason to prepare this postgraduate thesis is the interest I have in holistic, natural treatments supported in time in terms of their efficacy on longevity. Acupuncture is seen by the Chinese people as national patrimony[3], as I believe the treatment with Techirghiol mud used as therapy for more than 117 years, together with the treatment with Gerovital H3, the most long-lasting geriatric treatment with therapeutic virtues endorsed by the passage of time[2], should take its well deserved place in the Romanian national patrimony.

The study main objective:

Assessment of endocrine changes in the hypothalamic-hypophyseal-corticoadrenal axis as biological ageing index, under peloidotherapy To achieve this objective, we dosed IGF-1, serum cortisol, DHEA-S for all patients included in the study and determined the ratio cortisol/ DHEA-S at two different moments in time: at the time of admission and at the time of discharge and we studied the variation of these parameters.

Secondary objectives of the paper:

1. Assessment of persistence of endocrine changes in the hypothalamic-hypophyseal-corticoadrenal axis after the balnear treatment To achieve this secondary objective, we carried out hormonal determinations (IGF-1, serum cortisol, DHEA-S) and determined the ratio cortisol/DHEA-S after 1 month and 4 months, respectively, from the time of discharge.

2. Assessment of antioxidant condition under the treatment with Techirghiol sapropelic mud We dynamically monitored the value of glutathione peroxidasis (GPx) at the time of admission, discharge, one month after the balnear treatment and four months after discharge.

3. Assessment of bio-psycho-social status of patients proposed for research within Techirghiol Balnear and Recovery Sanatorium. To achieve this objective, we carried out some haematologic and bio-chemical analyses for the patients included in the study (complete blood count, VSH, PCR, alkaline phosphatase, glycaemia, uric acid, total cholesterol, triglycerides, TGO, TGP, urea and serum creatinine) both at the time of admission and at the time of discharge. We recorded data regarding the frequency of the treatment within Techirghiol Balnear and Recovery Sanatorium, the duration of the degenerative disease, the physical activity during life, identification of muscle atrophy or reduced tone clinically outlined by the physician. We determined the value of VAS scale, the BMI and abdominal circumference for each patient at the time of admission, at the time of discharge and demographic data. The assessment of the psychological condition included the analysis of the stress level by means of a psychological examination, the subjective assessment of the stress level felt by the patients at admission and discharge and by means of the subjective assessment of the sleep quality of patients at admission to and discharge from Techirghiol Balnear and Recovery Sanatorium.

CHAPTER 6

Material and method

Therapeutic intervention

The treatment applied to patients on a daily basis during the admission to Techirghiol Balnear and Recovery Sanatorium consists of peloidotherapy, hydrokinetotherapy in the therapy basin with water from lake Techirghiol or in the lake itself during summer and additional procedures (electrotherapy, kinetotherapy, massage therapy).

The unction with cold mud is a therapeutic complex consisting of the successive contrast warm-cold. The unction with cold mud is practised during the warm season, on the specially arranged beach, where mud extracted from the deposit is brought on a daily basis or every 2 days. After the sun exposure for 15-20 minutes, the patient applies a 1-1.5 cm thick layer of mud on the entire body surface. Under the environmental

temperature, the mud dries in 15-30 minutes. Then, the complete immersion in the lake water occurs to remove the mud, associated with active movements of all body segments. At the end of the application, the patient takes a shower with fresh water. The duration of the sun exposure is increased daily (from 5-10 minutes on the first day, to 30-40 minutes at the end of the treatment), as well as the number of immersion in the lake. The number of mud application per day is constant. A single mud application per day is carried out during the treatment period[5].

The general warm mud bath is carried out using 10 kg of mud diluted in 120-150 l of chlorine-sodium water from the lake. The application temperature is of 37^0C-39^0C, the mud thermal neutrality being at 38^0C. The bath takes 20-25 minutes and the temperature is maintained relatively constant by adding hot water at the middle of the bath. After the bathing time, the patient takes a warm shower to remove the mud, then a short cold shower to prevent systemic vessel dilatation. The general mud bath is carried out every two days, alternatively with a saline bath (in the hydrokinetotherapy pool or in a bathtub) or with plant extract[6].

Th study group included a number of 52 patients, observing the inclusion and exclusion criteria and the norms regarding the preparation of a scientific paper.

The subjects were divided into two batches, as follows:

- **Batch 1**: carried out 5 peloid thermally neuter applications / general mud baths (BN), alternatively with 5 general saline baths (BS), 3 additional electric procedures, one regional massage therapy session per day and one kinetotherapy session per day.

- **Batch 2**: carried out 10 mud applications as mud unction - therapy with contrasting factors (general progressive sun therapy, mud unction, immersion in the lake) during summer, 3 additional electric procedures per day, one regional massage therapy session per day and one kinetotherapy session per day.

Research project performance method

This scientific paper is a group prospective clinical study, carried out during 15.07.2013-25.02.2016 and has included a total number of 1377

patients admitted to Techirghiol Balnear and Recovery Sanatorium, physician Doctor Stanciu Liliana-Elena. Of these, only 52 patients met the the inclusion and exclusion criteria for the study, as set for admission, discharge, one month and 4 months, respectively, intervals from discharge.

Of the 1377 patients studied during the entire scientific research, 1000 patients were older than 50, inclusion criterion. Of the 1000 potential patients meeting the acceptance criteria to participate to the study, only 64 were not under medication at the time of the case history being carried out. The remaining 377 patients admitted were younger than 50.

The total number of patients meeting the criteria set at admission was of 64, 12 of them being subsequently excluded from the study for the following reasons: 7 patients did not follow the hormonal determinations set to monitor the hormonal variations one month after discharge, for personal reasons; 3 patients showed positive results for the PCR quality determination at discharge; 2 patients showed values of VSH > 10 mm/h at discharge.

A. Study inclusion criteria:

- patients older than 50, with the correct indication of balnear treatment, both women and men, admitted to SBRT;

- patient with algic symptoms of mechanical nature, degenerative, at axial and peripheral joint level.

B. Study exclusion criteria:

- any of the situations stated as counter-indications for balnear treatment; presence or occurrence of inflammatory phenomena (the blood level of DHEA-S not only reduces with age, but also in almost all systemic disorders); cardiac and / or blood pressure disorders during treatment; skin injuries, known respiratory, endocrine, neurological disorders.

- cortisol treatment or any other treatment that can interfere with the determined parameters (diuretics, food supplements with mineral content or sugar content);

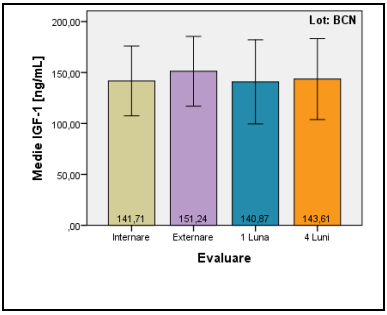
- balnear treatment, physiotherapy at least 4 weeks prior to entering the study.

The subject assessment included:

- Oral explanations and reading the information form regarding the performance of this scientific research.
- Verification of the patient's personal signature on the Patient Informed Consent.
- The Patient Informed Consent is also signed by the nurse of the medical cabinet, employee of SBRT, in the presence of whom the patient is orally informed on the performance of this medical study.
- The patient's general clinical examination.
- Completion of an admission questionnaire containing the patient's identification data: initials, personal number, age, sex, for female patients - the presence or absence of menopause and the age menopause occurred at, if present, the level of education, age of degenerative disease, VAS scale, balnear treatment frequency at SBRT, BMI, abdominal circumference, stress level psychologically assessed, sleep quality at the time of admission subjectively assessed, physical activity during life, identification of muscle atrophy or reduced tone clinically outlined by the physician.
- Completion of a discharge questionnaire containing: VAS scale, BMI, abdominal circumference, subjective assessment of the stress level felt at the time of discharge, sleep quality at the time of discharge subjectively assessed.
- Hormonal determinations: IGF-1, serum cortisol, DHEA-S, GPx analysis, carried out at admission, discharge, one month and 4 months, respectively, after discharge.
- Haematological and bio-chemical determinations carried out at admission and discharge: Complete blood count, VSH, PCR, alkaline phosphatase, glycaemia, uric acid, total cholesterol, triglycerides, TGO, TGP, serum urea and serum creatinine - to correctly select patients according to the inclusion-exclusion criteria.
- The psychological assessment was carried out when admitting the patient, by the psychology cabinet of Techirghiol Balnear and Recovery Sanatorium, by psychologist Mariana State, employed by the medical unit, using: Manual for depression, anxiety and stress scales[7].

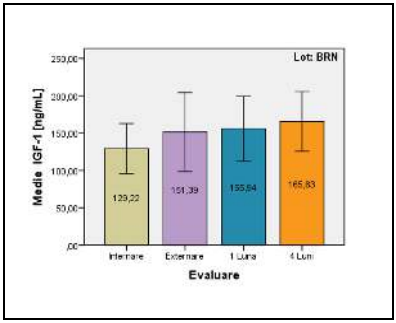
CHAPTER 7

Statistical results



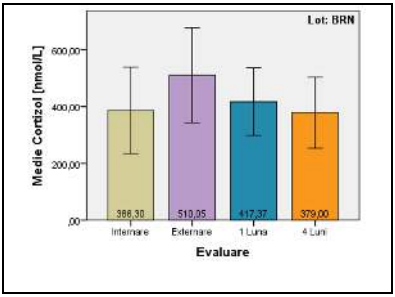
Picture 46

Picture no. 46 - Graphic representation of IGF-1 variable at the four moments of time, at patients in the batch of cold mud baths (BRN)



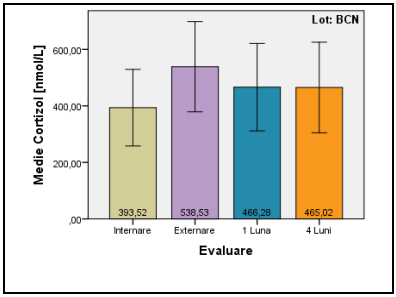
Picture 47

Picture no. 47 - Graphic representation of IGF-1 variable at the four moments of time, at patients in the batch of warm mud baths (BCN)



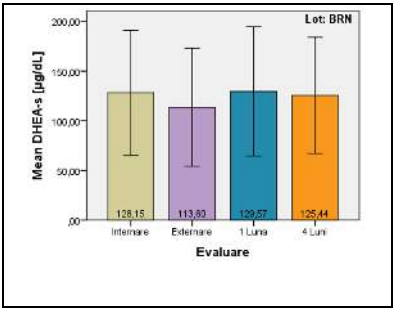
Picture 48

Picture no. 48 - Graphic representation of serum cortisol variable at the four moments of time, at patients in the batch of cold mud baths (BRN)



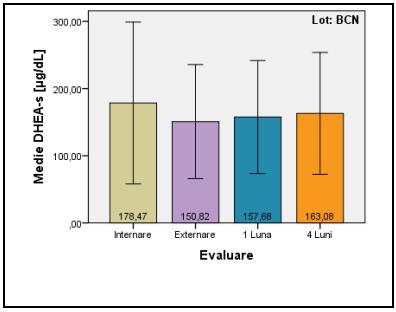
Picture 49

Picture no. 49 - Graphic representation of serum cortisol variable at the four moments of time, at patients in the batch of warm mud baths (BCN)



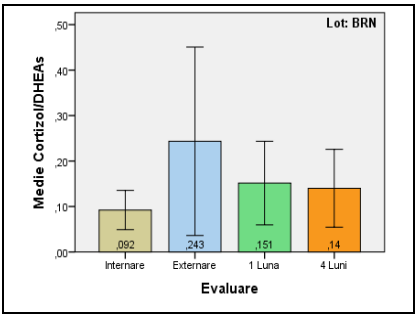
Picture 50

Picture no. 50 - Graphic representation of DHEA-S variable at the four moments of time, at patients in the batch of cold mud baths (BRN)



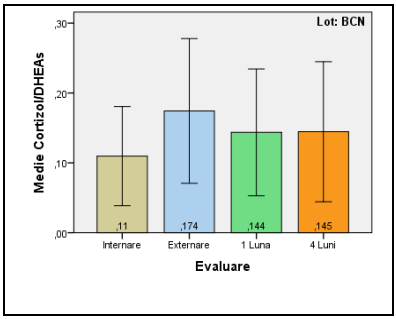
Picture 51

Picture no. 51 - Graphic representation of DHEA-S variable at the four moments of time, at patients in the batch of warm mud baths (BCN)



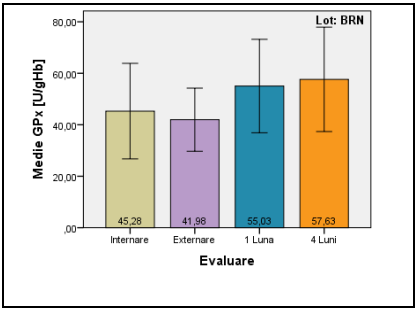
Picture 52

Picture no. 52 - Graphic representation of cortisol/DHEA-S variable at the four moments of time, at patients in the batch of cold mud baths (BRN)



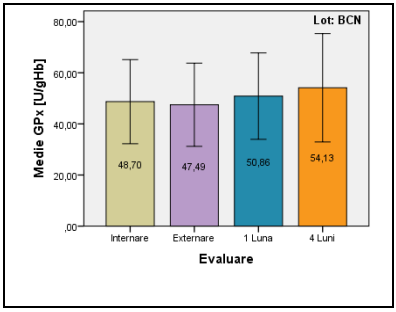
Picture 53

Picture no. 53 - Graphic representation of cortisol/DHEA-S variable at the four moments of time, at patients in the batch of warm mud baths (BCN)



Picture 54

Picture no. 54 - Graphic representation of GPx variable at the four moments of time, at patients in the batch of cold mud baths (BRN)



Picture 55

Picture no. 55 - Graphic representation of GPx variable at the four moments of time, at patients in the batch of warm mud baths (BCN)

CHAPTER 8

Discussions and result interpretation

As a result of outlining the complexity of the population ageing phenomenon, there has been a higher and higher concern over the last decades in the study of gerontology, in examining the issues related to the ageing process diversity within the various population groups. Such concerns are also reflected by the scientific development of this study, outlining the clinical, bio-chemical and hormonal aspects of ageing.

The originality of this thesis results from the research of the biological ageing process evolution under the effect of balnear therapy, a treatment method of recognised age, improved along time, to obtain the maximum benefit for the therapeutic approach of the old patient, as well as from the fact that it is **the only study** dynamically monitoring the evolution of some hormonal parameters and antioxidants involved in the ageing process for a period of 4 months after the the balnear treatment with Techirghiol sapropelic mud.

We have organised the analysis of the data collected starting from the objectives set at the beginning of this paper, comparing the same with data from the specialised literature.

8.1. Assessment of endocrine changes in the hypothalamic-hypophyseal-corticoadrenal axis under peloidotherapy

To achieve this objective, we dosed IGF-1, serum cortisol, DHEA-S for all patients included in the study and determined the ratio cortisol/ DHEA-S at two different moments in time: at the time of admission and at the time of discharge and we studied the variation of these parameters.

8.1.1. Results showed, for the batch carrying out **COLD MUD BATHS,** a statistically significant growth (**p=0.044**) of **IGF-1**, the variation of this hormone demonstrating the positive effect of the balnear treatment with contrasting factors in the biological ageing process. The IGF-1 low activity is associated with a significant morbidity in adults, with a high risk of cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases, with certain implication in ageing modulation. There is the hypothesis that maximum human life expectancy depends on the strict regulation of the GH-IGF axis and on maintaining the optimal action of IGF-1[1]. The optimal activity of this hormonal axis is involved both in the extension of life expectancy and in the increased resistance to the oxidative stress[2].

Also, the variation of **serum CORTISOL** recorded a statistically significant growth (**p=0.044**), the variation corresponding to the specialised literature. Stress entails the increase of the cortisol secretion, the process being directly dependant on the type and duration of the stress factor[3].

The variation of **DHEA-S** showed a statistically significant reduction (**p=0.004**), corresponding to the studied literature. DHEA-S is a form of DHEA "inactivation" and, at the same time, a form of storage for it. The reduction of DHEA-S within the BRN batch can also be seen as a modality of redistribution, reactivation, consumption under metabolic stimulation assuming the response hypercortisolism. Such evolving behaviour creates the theoretical premises of a future study assuming the harvesting and comparison of the variation for the two hormones: DHEA and DHEA-S.

The variation of the ratio **Cortisol/DHEA-S** recorded a statistically significant growth (**p=0.012**).

The ageing process, according to the evolutionist theory, is characterised by the chronic production of inflammatory cytokines. Such inflammatory mechanism is fought by the hypothalamic-hypophyseal-adrenal axis[5]. Cortisol and DHEA-S show opposite effects as regards the action on the immune system. Cortisol entails immunity suppression and its concentration increases with age, while DHEA-S antagonises the cortisol effects and is immunity modulating, its concentration reducing with age. Therefore, when analysing the effects of the hypothalamic-hypophyseal-adrenal axis on the inflammation, the ratio between cortisol and DHEA-S[6] can reflect the endocrine activity more accurately as compared to the variation of cortisol and DHEA-S analysed individually, this being the reason for which we have analysed the variation of this ratio in this study.

8.1.2. Results showed, for the batch carrying out **WARM MUD BATHS**, an growth close to the statistical significance (**p=0.067**) of **IGF-1**. The growth tendency at the time of discharge, however not at statistically significant values, observed during the peloidotherapy with thermal contrast shows, as a whole, the general positive effect of the balnear treatment in the ageing process. I believe that additional studies including a higher number of participants are necessary for statistical validation. It must be mentioned that, within both study batches, the hormonal variation is within physiological limits, in the optimal hormonal function, being known that high levels of IGF-1 were connected by some studies to the risk of cancer, given the role of this hormone in the facilitation of normal and malign tissue growth[7].

The variation of **serum CORTISOL** recorded a statistically significant growth (**p<0.001**) corresponding to the specialised studies[3].

DHEA-S showed a statistically insignificant reduction (**p=0.121**), possibly due to the high dispersion of the values obtained, however corresponding to the variation of serum cortisol in terms of evolution pattern, the opposite physiological actions of the two hormones being known[3].

The variation of the ratio **Cortisol/DHEA-S** recorded a statistically significant growth (**p=0.018**), the evolution corresponding to the patients subject to contrasting therapy.

8.2. Assessment of persistence of endocrine changes in the hypothalamic-hypophyseal-corticoadrenal axis after the balnear treatment

To achieve this secondary objective, we carried out hormonal determinations (IGF-1, serum cortisol, DHEA-S) and determined the ratio cortisol/DHEA-S after 1 month and 4 months, respectively, from the time of discharge.

Results showed, for the batch carrying out **COLD MUD BATHS,** that **IGF-1** recorded a statistically insignificant growth from the time of discharge until the final moment of this study, thus raising the premises regarding the persistence of the balnear treatment positive effect for at least 4 months after discharge.

The variation of **serum cortisol** recorded a statistically insignificant reduction from the time of discharge until the final moment of this study.

DHEA-S showed a statistically insignificant growth between discharge and 1 month after, respectively a statistically insignificant reduction between 1 month and 4 months after discharge. This evolution pattern supports the major function of DHEA-S of antagonising the cortisol action imposed by the balnear treatment, suggesting the hormonal balance achieved under the therapeutic procedure described.

The ratio **Cortisol/DHEA-S** shows, as evolution pattern, a statistically insignificant reduction between discharge and 4 months after the balnear treatment.

For the **batch subject to WARM MUD BATHS, IGF-1** showed a statistically insignificant reduction between discharge and 1 month, respectively a statistically insignificant growth between 1 month and 4 months after discharge. Considering that 4 months after discharge the evolution tendency is of hormonal growth, we can conclude on the positive effects of the balnear treatment also under thermally neuter temperature in the biological ageing process, raising the premises for new studies including a higher number of volunteers and a longer period of the balnear treatment for statistical validation.

The variation of **serum cortisol** recorded a statistically insignificant reduction from the time of discharge until the final moment of

this study, confirming the persistent stimulating effect on the hypothalamic-hypophyseal-adrenal axis.

DHEA-S showed a statistically insignificant growth between discharge and 1 month after, respectively a statistically insignificant reduction between 1 month and 4 months after discharge. This evolution pattern supports the persistent nature of corticoadrenal functional adaptive changes under peloidotherapy.

The ratio **Cortisol/DHEA-S** shows, as evolution pattern, a statistically insignificant reduction between discharge and 4 months after the balnear treatment. The hormonal evolution behaviour analysed after the balnear treatment suggests that, after the important hormonal effect of acute stage, hormonal changes are smaller, however an important issue we must outline is their persistence within physiological limits, with extended action in time. The data in the specialised literature studied confirms that the adaptive support of the body response to balnear factors is hormonal in nature, the modulation of endocrine secretion fixing the adaptive response in time[9].

8.3. Assessment of antioxidant condition under the treatment with Techirghiol sapropelic mud

We dynamically monitored the value of glutathione peroxidasis (GPx) at the time of admission, discharge, one month after the balnear treatment and four months after discharge. The evolution pattern of this antioxidant enzyme is the same, regardless of the method of application of the balnear therapeutic factor. It shows a statistically insignificant reduction during the balnear treatment, with a subsequent growth, statistically insignificant at discharge and until the final moment of evolution monitoring within the study. We must mention that, within the batch subject to cold mud baths, the variation of average values of **GPx** at the 4 moments of time showed a very close value to the statistical significance (**p= 0.057)**. The reduction of GPx level, as antioxidant status during the balnear treatment, corresponds to the existing data in the literature studied[10]. Glucocorticoids released from the adrenal gland as a response to the stress induced activation of the hypothalamic-hypophyseal-adrenal axis induce a high activity in the reduction-oxidation cellular system, entailing a high oxidative stress and the consumption of enzymes involved in the antioxidant process. Thus, I believe the low value of GPx during the study are the result of the

antioxidant process stimulated by the adaptive mechanisms under peloidotherapy, maintaining some physiological parameters.

8.4. Assessment of bio-psycho-social condition of patients proposed for research within Techirghiol Balnear and Recovery Sanatorium

To achieve this objective, we carried out some haematologic and bio-chemical analyses for the patients included in the study (complete blood count, VSH, PCR, alkaline phosphatase, glycaemia, uric acid, total cholesterol, triglycerides, TGO, TGP, urea and serum creatinine) both at the time of admission and at the time of discharge. We recorded data regarding the frequency of the treatment within Techirghiol Balnear and Recovery Sanatorium, the duration of the degenerative disease, the physical activity during life, identification of muscle atrophy or reduced tone clinically outlined by the physician. We determined the value of VAS scale, the BMI and abdominal circumference for each patient at the time of admission, at the time of discharge and also demographic data. Patients were assessed by the psychology cabinet at admission, the stress level and the quality of sleep being also assessed subjectively by the patients at the time of admission to and discharge from Techirghiol Balnear and Recovery Sanatorium.

A. The statistical analysis performed for the 52 patients validated for the study according to the inclusion-exclusion criteria allows the following comments:

8.4.1. Demographic results, professional status analysis and pain nature:
- relative predominance of female patients (51.9%)
- the case percentage analysis per age groups showed that the highest ratio is held by patients in the age group 50- 65 years old (82.7%), as compared to patients between 65-80 years old (17.3%)
- 84.6% of the patients are from urban environments
- 55.8% of the patients have occupations entailing physical activity, while 44.2% of the patients have intellectual activities. The specialised literature outlines that persons from urban environments with occupations requiring higher education have easier access to medical information and are more concerned in adopting healthy life styles[11]
- 80.8% of the patients recorded prior admissions to the study unit, while as little as 19.2% of the patients were admitted for the first time, the patients'

regular admission for balnear treatment being notable; most patients (86.5%) were admitted with pains older than one year.

8.4.2. The psychological assessment outlined that 44.2% of the patients showed some stress level at admission, 36.5% were patients with some level of depression and anxiety was noted in 46.2% of the patients; here was some correspondence between the subjectively assessed stress level and the psychologically assessed stress level, outlining that patients in this study are aware of the stress level felt, **p = 0.005** for the BRN batch, **p = 0.001** for the patients subject to warm mud baths. Specialised studies show the importance of stress resistance mechanisms at old ages, of the general health condition and of the life quality[12].

8.4.3. The level of stress, depression, anxiety and sleep quality did not show statistically significant correlations with the analysed hormonal parameters, nor with the variation of GPx, the result being interpreted under the variation within physiological limits of the parameters studied during the balnear treatment.

8.4.4. 34.6% of the patients showed muscle trophic changes at the time of admission. One of the clinical distinctive features of the population ageing is the loss of muscle mass and force, with implications on the life quality and independence[13].

8.4.5. The assessment of the **VAS scale** showed a statistically high significant reduction for both study batches: cold mud baths, **p=0.001**; warm mud baths, **p<0.001**, interpreted as positive effect of the balnear treatment on the patient's life quality, a positive effect also within the biological ageing process. A number of hormones are involved in pain management: cortisol, pregnenolone, dehydroepiandrosterone (DHEA), progesterone, testosterone, oestrogens and thyroid hormones[14].

- 80.8% of the patients recorded prior admissions to the study unit, while as little as 19.2% of the patients were admitted for the first time, the patients' regular admission for balnear treatment being notable; most patients (86.5%) were admitted with pains older than one year.

8.4.6. Glycaemia concentration showed a reduction at both study batches, statistically significant for the warm mud baths (**p=0.020**) corresponding to the data in the specialised medical literature: the stress induced growth of the cortisol secretion expedites sugar metabolism and the production of oxygen reactive types[15]. Thus, the balnear treatment is useful to control glycaemia by activating the adaptive, antioxidant process with energy consumption.

8.4.7. The analysis of **alkaline phosphatase** showed a discordant evolution at the two study batches, with a statistically significant growth at the batch subject to cold mud baths (**p=0.032**), in relation with metabolic and enzymatic tissue changes at skin level [16].

8.4.8. Urea showed a discordant evolution at the at the two study batches, with a statistically significant reduction at the batch subject to warm mud baths (**p<0.001**), maintaining the values obtained within the normal age limits. Over 99% of the urea synthesis occurs in the liver. The amount of urea varies according to the substrate provided to the liver, the liver function and renal perfusion. It varies inversely proportional to cellular anabolism[17].

8.4.9. Within the batch subject to warm mud batch, there was a statistically significant correlation between the values of serum cortisol and BMI at the time of admission. The specialised literature outlines the role of serum cortisol metabolic stimulation acting on the metabolism of lipids, proteins and sugars[18]. Specialised studies have shown that high levels of adiposity can increase the cortisol stress response[19].

8.4.10. Within the batch subject to warm mud baths, there was a statistically significant correlation between the values of DHEA-S and Triglycerides at discharge (**p=0.011**). In specialised studies, some authors reported significant correlations between the two parameters[20,21], while other authors did not show any statistical correlation between these variables[22].

8.4.11. Within the batch subject to cold mud baths, there are statistically significant differences (**p = 0.020**) between the average values at discharge for the abdominal circumference variable (CA) measured in male patients as

compared to female patients. CA remains, thus, a simple and valid marker of the adipose tissue at abdominal and visceral level[23]. Abdominal circumference provides a highly feasible and cheap method to monitor the body fat distribution and to identify persons with a high risk of illness[23]. Although trends in men and women were similar in terms of age, women showed a lower CA than men in the studies carried out[23].

B. We must mention a number of social-medical findings: of the 1377 patients studied during the entire scientific research, 1000 patients were older than 50, inclusion criterion. Of the 1000 potential patients meeting the acceptance criteria to participate to the study, only 64 were not under medication at the time of the case history being carried out. This raises a major issue for the population in our country, the one of diverse pathologies present in persons of approximately 50 years old and the use of drugs for such diseases, the population ageing level being high, with organic substrate, of some initially functional disorders. The remaining 377 patients were younger than 50, an issue outlining the high level of admission at SRBT for balnear therapies of young patients, as well.

CHAPTER 9

Conclusions

- The beneficial effects of the balnear treatment with Techirghiol sapropelic mud include the changes in the hormonal secretions and antioxidant process, both with potentially positive effect on the control of some biological ageing processes.

- The results of the study confirm different bio-hormonal changes for several of the variables analysed comparatively between cold baths (contrasting factors) and warm baths (thermally neuter application), respectively, with Techirghiol sapropelic mud, however within the physiological secretion limits.

- During the cold baths with sapropelic mud, we noted a statistically significant growth of the values of **IGF-1** during the balnear treatment, with the persistence of the stimulated level also 4 months after the balnear treatment. Comparatively, during the warm baths with Techirghiol sapropelic mud, the growth of serum IGF-1 recorded values close to the statistical significance at the end of the treatment. Post treatment follow-up, 1 month and 4 months, respectively, after discharge recorded quantity variations, statistically insignificant, within physiological limits with a growth trend towards the study final moment.

- **Serum cortisol** recorded a statistically significant growth in patients analysed in both study batches during the treatment and a statistically insignificant reduction between the time of discharge and the final assessment after the balnear treatment. Both methods of application of the therapeutic factor significantly and persistently stimulated the cortisol secretion as endocrine-metabolic adaptive positive mechanism.

- The **DHEA-S** serum level variation recorded a statistically significant reduction for both methods of application of the balnear therapeutic factor, with a statistically
  significant growth of the ratio **CORTISOL/ DHEA-S** during treatment.
  During the follow-up period after the balnear treatment, no statistically significant values were recorded for the serum level of DHEA-S and for the ratio CORTISOL/DHEA-S.
  This evolution pattern suggests the role of balnear treatment in maintaining the hormonal balance.

- As regards the antioxidant status, during the treatment and after the balnear treatment, **GPx** quantity fluctuations were noted for both therapy methods with a significance close to the statistical values for the cold mud baths and without statistical significance for the warm mud baths. The low values of GPx during treatment were seen as an adaptive mechanism to peloidotherapy, activating the antioxidant mechanism after the balnear treatment. This finding outlines the

importance of extended assessments in patients' subject to balnear treatment.

- The assessment of the VAS scale outlined a statistically highly significant reduction at both study batches during treatment, reducing the pain symptoms, thus outlining an increase in the patient's life quality, including within the ageing process.

- The statistically insignificant variation, however within physiological limits of other parameters analysed, shows the metabolic safety of the application of natural factors in the area of Techirghiol at old patients.

- Discordant results obtained comparatively between the two study batches can be generated by several factors, the thermal conditions of the balnear factor application having a major importance.

- The hormonal evolution behaviour outlines that, after the important hormonal effect of acute stage, hormonal changes are smaller, but with extended action in time.

- Patients assessed during the study showed multiple affections, with drug administration, an observation raising the issue of a higher ageing level in the population, even at middle ages. The high level of admission of patients under 50 years old to Techirghiol Balnear and Recovery Sanatorium suggests the idea that the use of the natural factor as balnear treatment factor should not depend on the age.

- The study carried out raises the premises for future research carried out involving a higher number of patients, a longer treatment period and a longer post treatment follow-up, with simultaneous dosing both of DHEA-S and DHEA.

- Given the data obtained during the study, I support the hypothesis that peloidotherapy with Techirghiol sapropelic mud has a **beneficial effect on the biological ageing process**, that it is a **therapy carried out under metabolic safety parameters**, and that the performance of future research shall add to the **development of new concepts and approaches of the ageing process**.