

URINARY STEROID PROFILES IN OSTEOPOROSIS AND GYNECOLOGICAL DISEASES

PhD Thesis

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Introduction

Diseases especially threatening the female population include osteoporosis, currently regarded as an important endemic disease, tumors of the female genital organs which even nowadays belong to the leading causes of death, and endometriosis significant due to its rising incidence. Several risk factors have been identified in their development, however, their pathophysiology has not been completely cleared and can be associated with modified steroid metabolism.

The qualitative and quantitative determination of steroid hormones -due to their complex role in the physiological processes of the organism - is important in medical diagnostics and investigation into the pathophysiology of various diseases.

From among the various methods that may be applied to measure steroid hormones and their metabolites, currently chromatographic methods and the use of immunoassays are the most widespread.

Individual steroid measurements (generally immunoassays) from serum, plasma and in the case of certain components from saliva and urine can be performed rapidly and easily and can mainly be automatized, but these provide information about the steroids present in the circulation only at the moment of sampling.

However, the results of urinary measurements provide information relating to steroid metabolism in the glands and the periphery as well. Although the gas-chromatographic (GC) method known as the urinary steroid profile requires longer time and a more up-to-date technical background, it makes possible to measure simultaneously several steroid groups. By eliminating the daily fluctuations that are characteristic for certain steroids, measurements from urine collected during a period of 24 hours provide an overall picture concerning one-day steroid metabolism, the metabolic routes of steroids and also the functioning of the enzymes taking part in the synthesis. Another advantage of the gaschromatographic-mass-spectrometric method is that the structure of certain compounds can be clearly determined, while immunoassays in some cases are not completely compound-specific.

The urinary steroid profile method has outstanding importance in the differential diagnostics and research of several endocrine diseases. In Hungary, with medical diagnostic aims, urinary steroid profile tests are allowed to perform only at the Institute of Bioanalysis, Faculty of Medicine, University of Pécs, being the member of an international organisation that coordinates laboratories using this same method.

Aims

In our investigations urinary steroid profiles were determined in various diseases affecting women. Our aim was a better understanding of the clinical features of these gynecological diseases and also improving our knowledge relating to their hormonal background. We wanted to examine whether a specific urinary steroid profile of these pathological pictures existed or whether the detected changes could be interpreted in the framework of diagnostics and therapy.

1. We aimed to investigate the role of androgens and corticosteroids in osteoporosis in postmenopausal women.
2. Our aim was to recognize the changes of steroid metabolism in myomas in premenopausal women in the pre and post operative period.
3. We intended to examine the involvement of androgens and overweight/obesity as risk factors in endometrial cancer in postmenopausal women.
4. Our aim was to investigate the level of androgen and progesterone metabolites, and establish individual urinary steroid profiles in epithelial ovarian tumors in postmenopausal women.
- 5., 6. We wanted to investigate the probable role of androgens in granulosa cell ovarian tumor in premenopausal women, and in cervical cancer in postmenopausal women.
7. We analysed the urinary steroid profiles of young women with endometriosis during GnRH analog treatment. We aimed to examine the changes in steroid metabolism before and after the treatment.

Materials and methods

1. Subjects

24-hour collection of urine samples was applied. Patients from Department of Obstetrics and Gynecology of the Faculty of Medicine, University of Pécs were chosen, with the help of clinicians.

The patients did not receive medication influencing steroid metabolism, did not have endocrine and other serious diseases before the collection of urine samples, collected correctly the urine samples and agreed to participate in the research.

The controls were age and sex-matched healthy people from Department of Obstetrics and Gynecology and from Institute of Bioanalysis of the Faculty of Medicine, University of Pécs.

The concentrations of serum progesterone (P), dehydroepiandrosterone-sulphate, (DHEAS), estradiol (E_2), testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by electrochemiluminescence immunoassay.

2. Determination of urinary steroid-profile

Before the gas chromatographic measurements we performed sample pre-treatment consisting of the following steps: extraction of steroids from urine, releasing of metabolites secreted as conjugates by enzymatic hydrolysis, derivatization of the polar groups (hydroxyl and keto groups) influencing heat degradation and volatility. We removed the silylating reagent excess by column chromatography.

We used Agilent Technologies system consisted of a gas chromatograph (6890N) and mass spectrometer (5975) (Agilent Technologies, Santa Clara, USA). The steroid derivatives were injected into the GC in solvent phase. The separations were carried out in a capillary column (HP-1-MS) with nonpolar stationary phase (25 m* 0.2 mm* 0.33 μ m), (Hewlett-Packard Company, USA). The quantitation of the samples was carried out in the selected ion-monitoring mode. The Target Ion was used for the quantitative analysis of the steroid metabolites and the steroid identification in each sample was based on the ratio of Qualifier Ions to Target Ion and the difference between the retention times of each steroid. The

quantification was obtained by relating the peak areas of the individual components to the area of the internal standard.

Results and discussion

24-hour urinary steroid profiles and serum-hormone values of patients were analysed in seven female pathological pictures affecting their steroid metabolisms. The 24-hour urinary steroid volumes and serum-hormone values measured in patient samples were compared to those of healthy control-subjects of similar ages by way of statistical tests in compliance with the distribution of samples.

1. In the majority of medical literature data concerning osteoporosis a positive correlation was found between serum, and plasma androgens and bone density, thus the expected significantly decreased androgen metabolite levels experienced by us confirm the possibility that besides oestrogens androgens may also play a role in the process of bone loss after menopause. Androgens exert a positive effect on bones, have an antireabsorptive effect, and they may exert their effect by transforming themselves into oestrogens, or directly through androgen receptors.

The decrease in DHEA level experienced confirms the research results that with appropriate caution this component may be therapeutically applicable in postmenopausal osteoporosis.

The decrease in cortisol metabolites seems to be controversial to the known effects of glucocorticoids inducing osteoporosis, for the explanation of which further investigations will be necessary. Is it possible that this decrease means no other than the internal compensation of the organism?

2. Based on our results, different changes were found in patients with myoma before and after surgery. The significance of potential oestrogen hormones in the development and progression of the disease is supported by several factors, however, in some cases the tumor-inducing effects of oestrogen hormones have not been proved. Serum E₂ level was not significantly different in patients with those of premenopausal controls. Similarly, in serum P, DHEAS, T, FSH and LH levels were not significantly different in patients before the surgical treatment compared to premenopausal controls. As expected, after surgical treatment significantly lower E₂ levels and significantly higher FSH and LH levels were found.

Analysing urinary steroid profiles in patients before surgery, the androgen and progesterone metabolite levels were not significantly different, however, significantly higher values were found in the metabolite level of cortisol, one cortisone and also one corticosterone metabolite level. The significantly higher levels of corticosteroid metabolites support the fact that glucocorticoids may play a role in the process of myoma development. In the patients more cortisone transforms into active cortisol, than in the case of premenopausal controls, which signals altered function of 11β -hydroxysteroid-dehydrogenase enzyme system.

Investigating the postoperative steroid profiles of patients we found that the level of several androgen metabolites and PD progesterone metabolit were significantly lower compared to that of the premenopausal controls. From among the corticosteroids the metabolite level of only one corticosterone was significantly lower, which fact may indicate that as a result of surgery the functioning of 11β -hydroxysteroid-dehydrogenase enzyme system created a balance in the cortisone-cortisol transformation.

Comparing the postoperative urinary steroide profiles of patients with myoma to those of the postmenopausal controls we found that the metabolite levels of several androgens were significantly lower.

Our results suggest that in the case of myomas developing during premenopause, as a result of the surgical removal of the ovaries changes will occur in the metabolism of steroids.

3. A complex correlation among oestrogens, androgens and overweight/obesity is assumed during the development of endometrial cancer after menopause. In the postmenopausal stage of females, due to the ceased hormone production of the ovaries the production of oestrogene hormones decreases to minimum. To a lesser extent, androgens with a low plasma concentration originate from the ovaries that have transformed into androgen-producing glands, however, predominantly they originate from the adrenal cortex. At this point, oestrogens are produced by aromatisation of the androgens of the adrenal cortex in the fatty tissue. In the case of an overweight there is a higher probability of the development of endometrial cancer that may be associated with elevated oestrogen levels. The probable role of oestrogen hormones in the development of tumors may be regarded as evident, however, in certain cases raised levels of oestrogens cannot be detected. In our examinations the serum E_2 level was not significantly different compared to that of the controls. It was found that the serum androgens are also controversial. The major part of data supports elevated levels of serum androgens, contrary to this the results of serum T and DHEAS levels investigated by us are consistent with those research results that do not justify the higher level of androgens.

Investigating the urinary steroid profiles, significantly lower values were found in the metabolite levels of several androgens and cortisol metabolites with weak androgen effects in women with endometrial cancer compared to the control group. Is it possible that this statement may justify the aromatisation of androgens to oestrogens? In addition, is it possible that the decrease means no other than the internal compensation of the organism?

We can assume that progesterone may have a protective effect in tumor development. The metabolite level of progesterone was not significantly different in patients than in the controls. This finding was supported by serum P levels examined by us.

According to our results the metabolite levels of the majority of corticosteroids were significantly lower in the patient group than in the control group, which fact directs attention to the possible role of glucocorticoids and raises the possibility of medicinal therapy to be applied.

4. Based on scientific data it could be assumed that steroid hormones may also play a part in the development of epithelial ovarian tumors. Regarding the role of oestrogens, we mainly find medical data, according to which an elevated oestrogen effect may play a part in tumor development, however, there are controversial assumptions as well. During our examinations we found that the patient serum E₂ level was not significantly different.

Our results justify the assumptions according to which elevated levels of androgens may promote the development of a tumor. During urinary steroid profile examinations we found that from among the androgens the level of DHEA and the level of its metabolites were significantly higher in postmenopausal patients than in the controls. However, the serum T and DHEAS levels were not significantly different. Our finding supports the view that androgens exert their effect by aromatisation to oestrogens in tumor development.

It is assumed that progesterone may have a protective effect on epithelial ovarian tumor. We found that the metabolite level of progesterone was significantly higher in the patient group than in the control group, on the other hand, the serum P level did not justify this. Is it possible that with a higher progesterone level the organism tries to compensate the effects of oestrogens developed by aromatisation of androgens?

During our examinations from among corticosteroids significantly higher values were found in the levels of one cortisol and corticosterone metabolites in the patient group compared to the control group, and the serum LH level was also significantly higher in the patient group. Our results justify the finding that there is a positive correlation between the cortisol production of the adrenal cortex and raised serum LH level in postmenopausal

women. Our findings raises the potential role of corticosteroids in the epithelial ovarian tumor.

5. Our results concerning the level of serum E₂ are conform to literature data which justify those granulosa cell ovarian tumors are hormonally active and produce mainly oestrogens. According to others results in some cases the tumor produces androgens and not oestrogens. This finding could not be confirmed during the examination of the serum T and DHEAS levels.

Our results show that from urinary androgen metabolites the volume of only one metabolite exceeded the maximum value measured in the same metabolite controls. However, the cortisol metabolites with weak androgen effect also exceeded the maximum values measured in the controls.

A higher level of the progesterone metabolite amount was found in one patient with granulosa cell ovarian tumor, but this could not be confirmed by the serum P level test. This finding can direct attention to the fact that granulosa cell ovarian tumors can produce progesterone as well.

Our results show that in both patients with granulosa cell ovarian tumor the amount of almost all corticosteroid metabolites exceeded the maximum values for corticosteroid metabolites measured in the controls. This finding emphasizes the possible role of glucocorticoids as well in the development of tumors. Important and expected result - based on recent information - is the elevation of serum E₂ levels of patients. The fact that there was no difference found in the serum FSH and LH level of the patients compared to the control-subjects proposes that the urinary steroid profile test can be a more suitable method for the follow up of patients with granulosa cell ovarian tumor.

6. According to literature data the modified metabolism of the oestrogens can be a risk factor for cervical cancer, and the elevated oestrogen level can increase the spread of the papilloma virus infection that is crucial in the development of the tumor. We found that the level of serum E₂ was not significantly different in the patients compared to the control group.

Results were available from former literature concerning the urinary steroid profile in premenopausal women with cervical cancer. Differences were found when analyzing the steroid profile of postmenopausal women with cervical cancer who were examined by us. In compliance with previous results concerning the metabolite level of androgens and cortisol meatabolites with weak androgen effect, significantly lower values were detected. However, ths serum T and DHEAS levels were not significantly different.

Contrary to former results, significantly lower values were found in the levels of several cortisone and cortisol metabolites. This finding can indicate that corticosteroids can have a different role in the cervical cancer developed in pre and postmenopause.

7. Endometrial nodules react to oestrogen and progesterone hormones during the menstrual cycle like the endometrium. In the treatment of the disease, the role of various medications inhibiting the function of the ovaries (oestrogen production) is important. GnRH analogs are most frequently used, since it has been proved that endometriosis regresses and symptoms improve in an oestrogen-poor setting.

The decrease in serum E₂, FSH and LH levels is known from the literature in the case of patient groups with GnRH analog treatment, which was confirmed by our results as well.

Analyzing the urinary steroid metabolite levels the metabolite level of one androgen was significantly lower in the patient group compared to the control group, which can be explained by the expected effect of the treatment. Among corticosteroids the metabolite level of one cortisol was significantly lower. The metabolite level of one corticosterone was significantly lower and that of another one was significantly higher. The result shows that the GnRH analog treatment has an effect on the metabolism of the glucocorticoids. An important and expected result, based on information gained so far, is the decrease in the E₂, FSH and LH levels of the serum. Our results demonstrate that due to the GnRH analog treatment of endometriosis changes occur in the metabolism of steroids.

Conclusions

To sum up our results, among investigated diseases, affecting women changes occurred in the urinary steroid profile compared to the control group consisting of patients of the same sex and similar age.

The urinary steroid profile method successfully improved by us can be used for examining several steroid groups at the same time. It is applicable for both clinical and research purposes. Its advantage is to give a clear picture about the daily synthesis of steroids including metabolism in the glands and the periphery as well. Changes in the level of some metabolites and their proportion to each other direct attention to the role of given enzymes and steroid groups, thus giving help in the recognition and handling of abnormal health conditions.

Major Results:

1. In postmenopausal osteoporosis we observed a significant decrease in the level of several androgen metabolites which can indicate that after menopause the effect of sexual steroids on the bone loss is not only connected to estrogens. The unexpected decrease of cortisol and its metabolites seems to be contradictory with the fact that the increased level of glucocorticoids can induce osteoporosis.
2. We pointed out that due to the surgical treatment of myomas developing in pre-menopause, the metabolism of steroids was altered. Attention was drawn to the fact that more cortisone is transformed into active cortisol in untreated patients, which signals the change in the function of the 11β -hydroxysteroid dehydrogenase enzyme system. After the operation a decrease in the level of androgen and progesteron metabolites was confirmed.
3. In the case of postmenopausal endometrial cancer the risk of overweight was confirmed. A decrease was confirmed in the level of androgen metabolites which shows the supposed aromatization to oestrogens. Attention was called to the role of glucocorticoids as well.
4. In the case of postmenopausal epithelial ovarian tumors a significant elevation was confirmed in the metabolite level of several androgens which can also mark the role of

androgens in the development of tumors. The significant elevation confirmed in the metabolite level of progesterone justified the protective effect of the hormone. It was confirmed that the elevated serum LH level had an effect on the cortisol production by the adrenal cortex in the case of postmenopausal women.

5. In the case of premenopausal granulosa cell ovarian tumors examined as a case study oestrogen production by the tumor has been confirmed. Besides the possibility of progesterone production was also assumed. Furthermore, elevation in the metabolite levels of several androgens, progesterones and glucocorticoids was confirmed. This raises the possibility that the method developed for the examination of urinary steroid metabolites to follow up the disease can be more suitable than the methods used for determining serum hormones.

6. Attention was drawn to the fact that different hormonal changes occurred in women with cervical cancer who contracted the disease in different phases of menopause. In the case of cervical cancers developing during postmenopause, a significant decrease in the metabolite level of several cortisones and cortisols was confirmed which shows the diverse role of glucocorticoids.

7. It has been demonstrated that due to the effect of GnRH analog treatment of endometriosis changes occur in the metabolism of steroids. The expected, significant decrease in serum E, FSH and LH levels due to the treatment was also confirmed.

8. Further developing the urinary steroid profile, a new method has been established by which the evaluation of samples has become quicker, and the quantitative and qualitative analysis of steroid metabolites has become more accurate.

Similar changes experienced in the level of certain metabolites are accompanied by various symptoms in different diseases. Therefore, the urinary steroid profile method is suitable for detecting the changes, however, to clarify the exact role of individual components further tissue-specific examinations are required.

Significant contributions to Thesis

Articles:

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