Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 62; Numer/Number 3/2011
ISSN 0423–104X

Clinical observations and hormone screenings of patients with non-standard hypertrophy of the adrenal cortex

Obserwacje kliniczne i badania hormonalne chorych z zespołem nieklasycznego przerostu kory nadnerczy

Grzegorz Franik, Piotr Skałba

Gynaecological Endocrinology Clinic of the Silesian Medical University in Katowice, Poland

Abstract

Background: Non-standard hypertrophy of the adrenal cortex is a rare endocrinopathy causing the incidence of hyperandrogenism among women of procreative age.
The primary objective of this paper is the specification of the clinical picture and modifications of the concentration of pituitary, ovarian and adrenal hormones in the blood of female patients with the syndrome of non-standard hypertrophy of the adrenal cortex (NPKN).
Material and methods: In the Gynaecological Endocrinology Clinic of the Silesian Medical University in Katowice, Poland, 2,353 female patients were hospitalised between 1 January 2003 and 30 June 2009 with symptoms of hyperandrogenism. Of these, 55 were selected for the study. Eventually, 25 female patients with diagnosed NPKN, and 30 randomly selected patients with the polycystic ovarian disease polycystic ovary syndrome (PCOS) were enrolled in the study.
Results: Of the 2,353 female patients hospitalised in the Gynaecological Endocrinology Clinic with symptoms of hyperandrogenism between 1 January 2003 and 30 June 2009, NPKN was found in 1.2% of them. Patients with NPKN displayed a strong hirsutism, which was significantly more intense than in the comparative group. Insulin resistance was found more frequently in the group of female patients with PCOS (67%) compared to the group with NPKN (40%). Polycystic ovarian disease was more frequently observed in the group of patients with PCOS (93%), compared to the group with NPKN (72%). The average concentration of androstendione in the blood serum in the group of patients with NPKN amounted to 7.60 ng/ml (SD = 3.57) and was significantly higher than in the group of patients with PCOS where it was 3.46 ng/ml (SD = 1.53). The average concentration of free testosterone in the blood serum in the group of patients with NPKN amounted to 7.30 pg/ml (SD = 4.13) and was significantly higher than in the group of patients with PCOS, where it was 2.90 pg/ml (SD =1.43). The average concentration of DHEAS in the blood serum in the group of patients with NPKN accounted for 403.23 μg/dl (SD = 192.59), and in the group with PCOS it was 257.39 μg/dl (SD = 63.67). This concentration was statistically significantly higher in the group with NPKN than in the group with PCOS. The average concentration of estradiole in the blood serum in the group with NPKN amounted to 111.98 pg/ml (SD = 113.68), while in the group with PCOS it was 62.39 pg/ml (SD = 31.18). The difference of concentrations between the groups NPKN and PCOS was statistically significant. We found a positive correlation between the 17-OHP concentration after 60 minutes of the ACTH test and the severity of hirsutism in the group of patients with NPKN (r = 0.77896). In addition, we found a correlation between the free testosterone and the 17-OHP concentration after 60 minutes of the ACTH test in the group of patients with NPKN (r = 0.48149). A positive correlation was also reported between the symptom of hypertrophy of the clitoris and the 17-OHP concentration after 60 minutes of the ACTH stimulation test in the group of patients with NPKN (r = 0.77221).
In the comparative group of patients with PCOS, there was no correlation between the free testosterone and 17-OHP concentration after 60 minutes of the ACTH test (r = 0.3059). There was also no correlation between the severity of hirsutism and the concentration of 17-OHP concentration analysed after 60 minutes of the ACTH test. In all female patients from the PCOS group, there was a correct size of clitoris.
Conclusions: Analysing the clinical picture of the examined population of patients with NPKN enabled us to specify symptoms of disease which were significant for diagnosis, and which helped differentiate NPKN from other endocrinopathies involving hyperandrogenism, including in particular PCOS. Taking everything into consideration, non-standard hypertrophy of the adrenal cortex is a rare cause of hyperandrogenism in women of procreative age. Intense hirsutism and features of virilisation presenting as hypertrophy of the clitoris predominate in the clinical picture of non-standard hypertrophy of the adrenal cortex.
The laboratory confirmation of diagnosis of NPKN constitutes the analysis of the 17-OHP level in blood in the ACTH stimulation test. The analyses of free testosterone and its unbound fraction, androstendione and estradiole, help differentiate NPKN from polycystic ovarian disease. **(Pol J Endocrinol 2011; 62 (3): 230–237)**

Key words: *non-standard hypertrophy of the adrenal cortex, ACTH test, 21-hydroxylase, 17-hydroxyprogesteron, polycystic ovarian disease, hypertrophy of the clitoris*

Streszczenie

Wstęp: Nieklasyczny przerost kory nadnerczy jest rzadką endokrynopatią powodującą wystąpienie hiperandrogenizmu u kobiet w wieku rozrodczym. Celem pracy jest ustalenie obrazu klinicznego i zmian stężeń hormonów przysadkowych, jajnikowych i nadnerczowych we krwi pacjentek z zespołem nieklasycznego przerostu kory nadnerczy (NPKN).
Materiał i metody: W Klinice Endokrynologii Ginekologicznej Śląskiego Uniwersytetu Medycznego w Katowicach hospitalizowano 2353 pacjentki z objawami hiperandrogenizmu w okresie od 01.01.2003–01.07.2009 r. Spośród nich do badań zakwalifikowano 55 kobiet. Ostatecznie do badań włączono 25 pacjentek z rozpoznanym NPKN i 30 wybranych losowo pacjentek z zespołem policystycznych jajników (PCOS).

Grzegorz Franik MD, Gynaecological Endocrinology Clinic of the Silesian Medical University in Katowice, Poland, ul. Medyków 14, 40–752 Katowice, e-mail: gfranik@poczta.onet.pl

Wyniki: W liczbie 2353 pacjentek hospitalizowanych w Klinice Endokrynologii Ginekologicznej z objawami hiperandrogenizmu w okresie od 01.01.2003–01.07.2009 r. NPKN wystąpił u 1,2% z nich. U pacjentek z NPKN odnotowano silny hirsutyzm, istotnie bardziej nasilony niż w grupie porównawczej. Insulinooporność występowała częściej w grupie pacjentek z PCOS (67%) w porównaniu z grupą z NPKN (40%). Obraz policystystyczności jajników częściej obserwowano w grupie pacjentek z PCOS (93%) w porównaniu z grupą z NPKN (72%). Średnie stężenie androstendionu w surowicy krwi w grupie z NPKN wynosiło 7,60 ng/ml (SD = 3,57) i było istotnie wyższe niż w grupie z PCOS, w której wynosiło 3,46 ng/ml (SD = 1,53). Średnie stężenie testosteronu wolnego w surowicy krwi w grupie z NPKN wynosiło 7,30 pg/ml (SD = 4,13) i było istotnie wyższe niż w grupie z PCOS, w której to wynosiło 2,90 pg/ml (SD = 1,43). Średnie stężenie DHEAS w surowicy krwi w grupie z NPKN wynosiło 403,23 μg/dl (SD = 192,59), a w grupie z PCOS 257,39 μg/dl (SD = 63,67). Stężenie to było istotnie statystycznie wyższe w grupie z NPKN niż PCOS. Średnie stężenie estradiolu w surowicy krwi w grupie z NPKN wynosiło 111,98 pg/ml (SD = 113,68), a w grupie z PCOS 62,39 pg/ml (SD = 31,18). Różnica stężeń pomiędzy grupami z NPKN i PCOS była istotna statystycznie. Stwierdzono dodatnią korelację pomiędzy stężeniem 17OHP w 60. minucie trwania testu z ACTH a nasileniem hirsutyzmu w grupie pacjentek z NPKN (r = 0,77896). Ponadto stwierdzono korelację pomiędzy stężeniami wolnego testosteronu i 17OHP badanego w 60. minucie trwania testu z ACTH w grupie pacjentek z NPKN (r = 0,48149). Dodatnią korelację wykazano również pomiędzy objawem przerostu łechtaczki a stężeniem 17OHP w 60. minucie trwania testu stymulacyjnego z ACTH w grupie pacjentek z NPKN (r = 0,77221). W grupie porównawczej pacjentek z PCOS nie wykazano korelacji pomiędzy stężeniami wolnego testosteronu i 17OHP badanego w 60. minucie trwania testu z ACTH (r=0,3059). Brak było także korelacji pomiędzy nasileniem hirsutyzmu a stężeniem 17OHP oznaczonego w 60. minucie trwania testu z ACTH. U wszystkich pacjentek z grupy PCOS odnotowano prawidłowe wymiary łechtaczki.
Wnioski: Analiza obrazu klinicznego badanej populacji pacjentek z NPKN pozwoliła wytypować istotne dla rozpoznania objawy chorobowe, pomocne w różnicowaniu choroby z innymi endokrynopatiami przebiegającymi z hiperandrogenizmem, w tym głównie z PCOS. Podsumowując, nieklasyczny przerost kory nadnerczy jest rzadką przyczyną hiperandrogenizacji kobiet w okresie rozrodczym. W obrazie klinicznym nieklasycznego przerostu kory nadnerczy dominuje silnie nasilony hirsutyzm oraz cechy wirylizacji pod postacią przerostu łechtaczki. Potwierdzeniem laboratoryjnym rozpoznania nieklasycznego przerostu kory nadnerczy jest oznaczenie stężenia 17-OHP we krwi w teście stymulacyjnym z ACTH. Pomocnymi w różnicowaniu zespołu nieklasycznego przerostu kory nadnerczy z zespołem policystycznych jajników są oznaczenia wolnego testosteronu i jego niezwiązanej frakcji, androstendionu i estradiolu.
(Endokrynol Pol 2011; 62 (3): 230–237)

Słowa kluczowe: *nieklasyczny przerost kory nadnerczy, test z ACTH, 21-hydroksylaza, 17-hydroksyprogesteron, zespół policystycznych jajników, przerost łechtaczki*

Introduction

Hyperandrogenism affects between 7% and 12% of women of procreative age [1]. In most young women, hyperandrogenism is linked to polycystic ovarian disease (PCOS). Other causes of incidences of disorders occur much more rarely. The mutation of the gene CYP21, transmitted by around 0.1% of the overall white population, is responsible for the prevalence of NPKN, also known as 'late revealing syndrome of hypertrophy of the adrenal cortex' or 'partial syndrome' or 'weakened syndrome'. Mutations lead to an enzymatic defect which results in a deficiency of 21-hydroxylase. Clinical manifestations of the syndrome remain in close correlation with the grade of deficiency of 21-hydroxylase. On the basis of clinical manifestations, determining of the genotype is not possible, and the diversity of phenotypes indicates the influences of factors modifying the gene functions. NPKN is an autosomal recessive disease and the clinical presentation of this disease varies.

The primary objective of this paper was to specify the clinical presentation and modifications profile of concentrations of pituitary, ovarian and adrenal hormones in the blood of female patients with the syndrome of non-standard hypertrophy of the adrenal cortex (NPKN). It was assumed that the clinical presentation of NPKN is similar to that of PCOS and, accordingly, the comparative group comprising women with this syndrome was included in this study.

Material and methods

In the Gynaecological Endocrinology Clinic of the Silesian Medical University in Katowice, Poland, 2,353 female patients were hospitalised between 1 January 2003 and 30 June 2009 with symptoms of hyperandrogenism. Of these, 55 women were selected for inclusion in the study. Inclusion criteria were: female patients aged between 18 and 33, a NPKN diagnosis and a PCOS syndrome diagnosis. According to expert opinion, NPKN is diagnosed when:

— clinical symptoms of hyperandrogenisation;
— family history includes incidents of classic hypertrophy of the adrenal cortex;
— membership of ethnic groups carrying a high degree of risk;
— increased level of 17-OHP (> 10 ng/ml) in the morning hours;
— increased concentration of 17-OHP in the blood after corticotrophin stimulation (ACTH) (≥ 10 ng/ml after 60 test minutes) [2].

Finally, NPKN was diagnosed in 28 female patients, of whom 25 were selected for study. The other three patients were excluded from analysis because they met exclusion criteria. PCOS was diagnosed in 2,046 female

patients based on the ESHRE/ASRM criteria devised in Rotterdam in 2003 [3].

For our study, 30 PCOS patients were selected by means of a digital number generator based on a computer program: StatSoft STATISTICA (data analysis software system), version 7.1 (www.statsoft.com).

Finally, two groups were formed:

— the examined group, comprising 25 women with diagnosed NPKN;
— the comparative group, comprising 30 women with diagnosed PCOS.

All patients were subject to a medical and gynaecological examination, and to a USG examination of the pelvis minor. The profiles of glycaemia, insulin resistance and lipidogram were analysed among all of them, and anthropometric examinations (height, body mass and BMI evaluation, measurement of clitoris) were carried out. Rates of glucose and insulin concentration carried on an empty stomach were used to evaluate insulin resistance: Homeostasis Model Assessment (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI) and the glucose/insulin rate (G/I). The examined patients from both groups did not differ in terms of age: the average age of NPKN patients was 24.4 years, and of patients with PCOS it was 23.97 years.

Hormonal examinations were conducted on an empty stomach within the first five days of the oestrous cycle. In blood serum, FSH, LH, PRL, TSH and cortisole were determined. The determination of androstendione and 17α-hydroxyprogesterone (17-OHP) in the blood serum was carried out by means of the ELISA method using ready sets (IBL). DHEAS was determined in the blood serum by means of the immunofluorescence method on a DPC Immuno-lite 2000 analyser. The determination of free testosterone in the blood serum was carried out by means of the ELISA method using ready sets (DRG). The radioimmunologic studies were conducted in the Radiodiagnostic and Nuclear Medicine Institute of the Silesian Medical University in Katowice, Poland.

For the analysis of the present work, the following determinations were used: androstendione, DHEAS, free testosterone and 17-OHP. The results of the remaining analyses served for the determination of diagnosis. In female patients, among whom in the morning hours in the first five days of the oestrous cycle, 17-OHP concentration amounted to ≥ 1.7 ng/ml, an ACTH stimulation test was conducted. This was carried out by intravenous application of 0.25 mg/ml of Synasthen (Novartis Pharma) with blood samples taken shortly before, and 30 and 60 minutes after, the hormone was injected. 17-OHP was determined from all samples., and a 17-OHP concentration after 60 minutes of 10 ng/ml or more was the basis for a diagnosis of NPKN.

All results were subject to statistical analysis, which was carried out using StatSoft STATISTICA program. Because the parametric test comparing mean values, the t-Student test, requires a conformity with normal distribution and variance homogeneity, the Mann-Whitney U-test was used.

Findings

Of the 2,353 female patients hospitalised in the Gynaecological Endocrinology Clinic with symptoms of hyperandrogenism between 1 January 2003 and 30 June 2009, NPKN was diagnosed in 28 patients, meaning 1.2% of all hospitalised patients showed symptoms of hyperandrogenism. Among the female patients with NPKN, a severe hirsutism was observed, significantly more intense than in the comparative group (Table I).

In the group of NPKN patients, there was a greater percentage of corpulence and adiposis than in the group of PCOS patients. The BMI indicators were within normal limits in both groups, and differences were not statistically significant. In the group of PCOS patients, insulin resistance was found in 67% of patients, whereas in the NPKN group it was 40%. In the NPKN group, symptoms of polycystic ovaries were found in 72% of patients, and in the PCOS group the figure was 93%. The average concentrations of examined steroid hormones in the blood, and differences between the groups of patients with NPKN and PCOS, are presented in Table II.

The average concentration of androstendione in the blood serum in the NPKN group amounted to 7.60 ng/ml (SD = 3.57) and was significantly higher compared to the PCOS group, where it accounted for 3.46 ng/ml (SD = 1.53). The average concentration of free testosterone in blood serum in the group with

Table I. *Concentration of hirsutism among patients of the examined and comparative groups*

Tabela I. *Nasilenie hirsutyzmu u pacjentek z grupy badanej i porównawczej*

	Number	Average concentration of hirsutism (acc. to Ferriman-Gallwey score)
Examined group with NPKN	25	23 (SD = 7.06)
Comparative group with PCOS	30	11.27 (SD = 3.25)
Difference of mean values		–11.73
U-M-W Test		p = 0.001

Table II. *Average concentrations of examined steroid hormones and differences of mean values between the groups*
Tabela II. *Średnie stężenia badanych hormonów steroidowych i różnice średnich pomiędzy grupami*

	Examined group with NPKN	Control group with PCOS	Difference of mean values	Statistical significance
Average concentration of androstendione	7.60 ng/ml (SD = 3.57)	3.46 ng/ml (SD = 1.53)	–4.15 ng/ml	p = 0.001
Average concentration of free testosterone	7.30 ng/ml (SD = 4.13)	2.90 pglm/l (SD = 1.43)	–4.40 ng/ml	p = 0.001
Average concentration of DHEAS	403.23 μg/dl (SD = 192.59)	257.39 μg/dl (SD = 63.67)	–145.83 μg/dl	p = 0.001
Average concentration of estradiole	111.98 ng/ml (SD = 113.68)	62.39 ng/ml (SD = 31.18)	–49.59 ng/ml	p = 0.001

Table III. *Average 17-OHP concentrations in the ACTH stimulation test [ng/ml]*
Tabela III. *Średnie stężenia 17-OHP w teście z ACTH [ng/ml]*

	p = 0.001 0 minutes ng/ml		p = 0.001 30 minutes		p = 0.001 60 minutes	
	X	SD	X	SD	X	SD
Examined group with NPKN	10.48	6.52	23.96	17.73	30.40	18.77
Control group with PCOS	1.22	0.79	2.07	0.65	2.70	0.85

NPKN was 7.30 pg/ml (SD = 4.13) and was significantly higher compared to the PCOS group, where it was 2.90 pg/ml (SD = 1.43). The average concentration of DHEAS in the blood serum of the group with NPKN amounted to 403.23 μg/dl (SD = 192.59), and in the group with PCOS it was 257.39 μg/dl (SD = 63.67). This concentration was significantly higher in the group with NPKN then in the PCOS group. The average concentration of estradiole in the blood serum in the NPKN group accounted for 111.98 pg/ml (SD = 113.68) and in the group with PCOS it was 62.39 pg/ml (SD = 31.18). This difference was statistically significant. The average 17-OHP concentrations in the ACTH stimulation test are presented in Table III.

We found a positive correlation between the 17-OHP concentration after 60 minutes of the ACTH test and the severity of hirsutism in the group of patients with NPKN (r = 0.77896) (Figure 1).

In addition, we found a correlation between the free testosterone and 17-OHP concentrations examined after 60 minutes of the ACTH test in the group of patients with NPKN (r = 0.48149) (Figure 2).

A positive correlation was also found between the symptom of hypertrophy of the clitoris and the 17-OHP concentration after 60 minutes of the ACTH stimulation test in the group of patients with NPKN (r = 0.77221) (Figure 3).

We did not find a correlation between the free testosterone and 17-OHP concentrations examined after 60 minutes of the ACTH test in the group of patients with PCOS (r = 0.3059).

There was also no correlation between severity of hirsutism and the 17-OHP concentration determined after 60 minutes of the ACTH test in the group of patients with PCOS. Among all patients from the PCOS group, we found correct clitoris dimensions.

Discussion

Non-standard hypertrophy of the adrenal cortex (NPKN) belongs to a group of rare diseases which are characterised by an overproduction of androgens [4–8].

According to several authors, less than 5% of women with excessive androgens suffer from non-standard hypertrophy of the adrenal cortex in the general population [9]. Moran et al. [10] stated that NPKN dependent on a deficiency of 21-hydroxylase is the most frequent autosomal recessive disease, occurring in 1/1,000–2,000 of the whole population excluding Ashkenazi Jews. Azziz et al. [4] pointed out the ethnic groups at a high degree of risk, among which 17-OHP

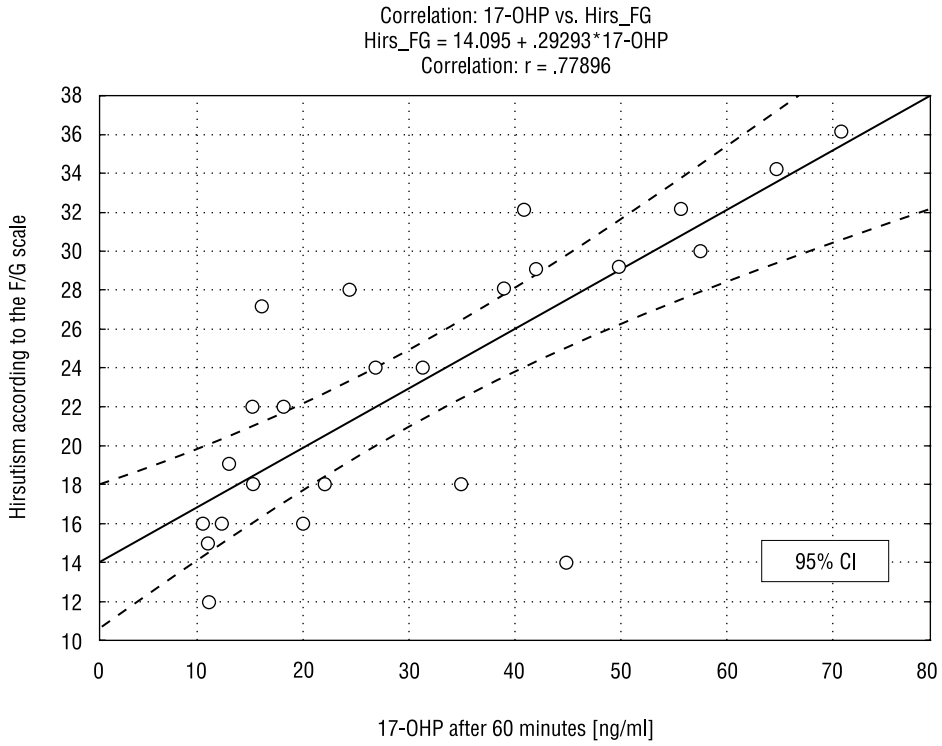

Figure 1. *Positive correlation between the 17-OHP concentration after 60 minutes of the ACTH test and the severity of hirsutism in the group of patients with NPKN*

Rycina 1. *Dodatnia korelacja pomiędzy stężeniem 17-OHP w 60. minucie trwania testu z ACTH a nasileniem hirsutyzmu w grupie pacjentek z NPKN*

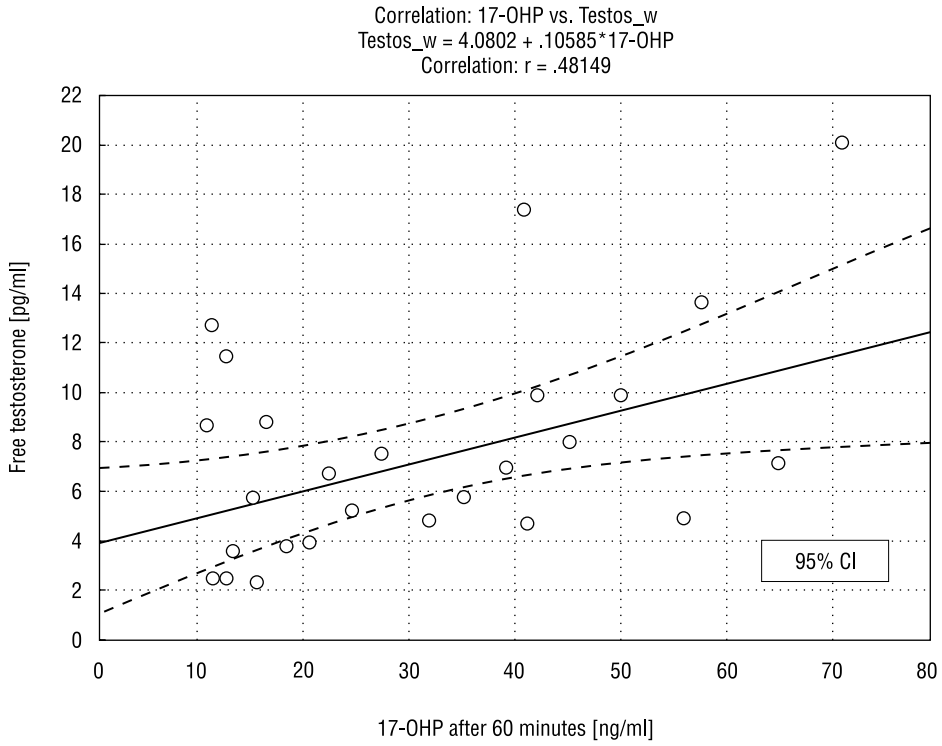

Figure 2. *Correlation between the free testosterone and 17-OHP concentrations examined after 60 minutes of the ACTH test in the group of patients with NPKN*

Rycina 2. *Korelacja pomiędzy stężeniami wolnego testosteronu i 17-OHP w 60. minucie trwania testu z ACTH w grupie pacjentek z NPKN*

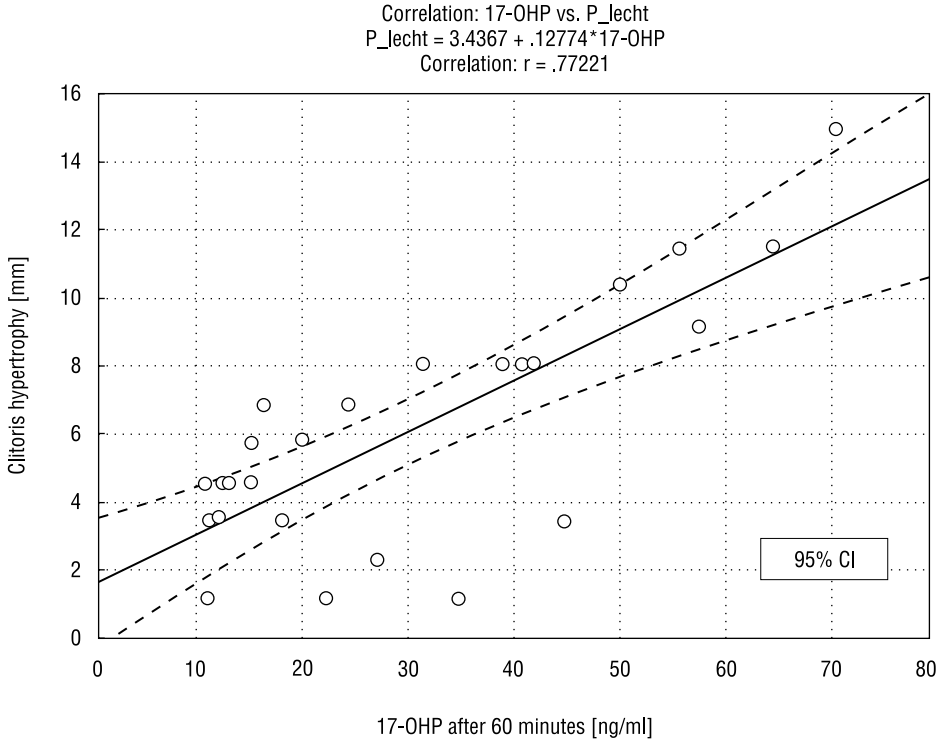

Figure 3. *Positive correlation between hypertrophy of the clitoris and the 17-OHP concentration after 60 minutes of the ACTH test in the group of patients with NPKN*

Rycina 3. *Dodatnia korelacja pomiędzy przerostem łechtaczki a stężeniem 17-OHP w 60. minucie trwania testu z ACTH w grupie pacjentek z NPKN*

needs to be determined in order to exclude NPKN: Ashkenazi Jews (frequency of incidence 1/27), people of Spanish descent (1/140) and Slavs (1/50). This is in contrast to Italians (1/300) and the Caucasian population in the USA (1/1,000). This disease occurs rarely in African-Americans [4].

In the examined group of women visiting the Endocrinology Clinic due to hyperandrogenism, mainly living in Upper Silesia, the incidence of NPKN was 1/120. This seems lower than expected on the basis of information in the literature [4, 6, 11]. This discrepancy may be explained by the fact that the non-classic deficiency of 21-hydroxylase is based on partial insufficiency of 21-hydroxylation, which in fact means that in a certain proportion of sufferers, this disease shows no symptoms.

Wild et al. [12, 13] reported that an increased concentration of testosterone can slightly disturb the lipid profile, and they also observed a tendency towards hyperinsulinaemia with a positive correlation between free testosterone and triglyceride concentration and a negative correlation with HDL cholesterol concentration [12, 13]. The authors found a high percentage of corpulent women in diseases accompanied by hyperandrogenism, including NPKN. In our own study, we did not find statistically relevant differences in BMI between the group of patients with NPKN and the comparative group with PCOS, although the frequency of incidence of corpulence in the NPKN patients is higher than in the comparative group. Consequently, it seems that the incidence of corpulence among female patients with accompanying hyperandrogenism symptoms is not a factor facilitating the identification of patients with NPKN.

Insulin resistance is defined as a decreased ability to stimulate glucose conversions in target tissues, which leads to a compensatory increase of insulin concentrations in the serum. Hyperinsulinaemia can be found among women with hyperandrogenism and among corpulent women, but also among slim ones. Hyperinsulinaemia occurs more often, and its course is more difficult, among corpulent women. In our study, insulin resistance occurred more frequently in the comparative group with PCOS than among patients with NPKN. This shows that although hyperandrogenism is more strongly expressed in NPKN, insulin resistance is rarer. This implies that androgens are not the only factor disturbing the tissue metabolism of insulin.

We found in the comparative group of patients with PCOS that disturbances of ovary structure observed in the ultrasonographical study occurred more

frequently than in patients with NPKN. This confirms that polycystic symptoms in the ovary/ovaries structure is not characteristic for patients with the examined syndrome.

There is a deficiency of 21-hydroxylase enzyme among patients with NPKN. This deficiency causes an impairment of conversion of 17 -hydroxyprogesterone to 11-deoxycortisole and progesterone to deoxycorticosterone. As a consequence of these processes, androgen precursors are accumulated (including 17 -hydroxyprogesterone and deoxycorticosterone). The diagnosis of deficiency of 21-hydroxylase is based on the measurement of concentration in the blood of basic 17-OHP and – again after ACTH stimulation [14–18]. Three types of answers are possible in the ACTH stimulation test: the standard answer in congenital hypertrophy of the adrenal cortex, where basic concentrations, and again after ACTH stimulation, reach the highest levels (> 100 ng/ml basic and > 10,000 ng/ml after stimulation); the intermediate answer typical of patients with non-standard hypertrophy of the adrenal cortex (≥ 10 ng/ml basic and > 10– 100 ng/ml after ACTH stimulation); and a characteristic answer for heterozygote, where basic 17-OHP levels, and again after ACTH stimulation, are within normal limits.

The symptoms of hyperandrogenism in NPKN have a different level and there are three phenotype variants: the first one with disturbances of ovulation and PCOS characteristics; the second one with hirsutism symptoms, with slight and infrequent periods; and the third one with increased androgen levels in the blood but without clinical symptoms [19].

In the examined group of patients with NPKN, we found statistically significant higher concentrations of 17-OHP already in basic determinations, before carrying out the ACTH test. A characteristic of NPKN is also a significant concentration increase of 17-OHP in blood after ACTH stimulation [21]. The increased concentration of 17-OHP among some women with symptoms of hyperandrogenism caused by other reasons, is usually small, which is confirmed by findings of our results among women with PCOS [18, 21].

Biologically active androgens are 19-carbon steroids, of which testosterone is the strongest one. In the examined group of patients with NPKN, the average concentrations of free testosterone were significantly higher than in the comparative group of patients with PCOS. Similarly, the average concentrations of androstendione in the examined group were significantly higher than in the comparative group of patients with PCOS. It is to be assumed that among women with NPKN, the adrenal source of producing androgens predominates, although the participation of ovarian source in the overall pool of serous androgens cannot be ruled out. This is confirmed by the positive correlation between the concentration of free testosterone and 17-OHP after 60 minutes of the ACTH test.

Hirsutism is a significant symptom of androgen surplus. Among the female patients with NPKN, a severe hirsutism was observed, which was significantly more intense than that observed in the comparative group. This observation aligns with the information in the literature, where authors have emphasised that severe hirsutism is caused by an adrenal defect [21–23].

High concentrations of androgens in the blood can be an isolated reason for particularly severe hirsutism and can lead to virilism. We observed this in our study, where in the examined group of patients with NPKN we found a positive correlation between the concentration of 17-OHP after 60 minutes of the ACTH test and the severity of hirsutism. A significant severity of hirsutism should persuade clinicians to carry out 17-OHP determinations in the blood. Moreover, in the examined group of women, we found the characteristics of clitoris hypertrophy in18 of 25 (72%). We found also a positive correlation between hypertrophy of the clitoris and 17-OHP concentration after 60 minutes of the ACTH test. These results demonstrate that adrenal hyperandrogenism leads to virilisation.

The next androgen participating in the pathogenesis of hirsutism is sulphate of dehydroepiandrosterone (DHEAS). It occurs in significantly higher concentrations in the blood compared to other androgens, and comes almost exclusively from the adrenals. The determination of DHEAS concentration in the blood was not especially helpful as a screening assay in the NPKN diagnosis, because its level is increased among 16% of healthy women [4]. In the examined group of patients with NPKN, we found a higher DHEAS concentration in the blood serum compared to the group with PCOS. It is to be assumed that increased ACTH production among women with non-standard hypertrophy of the adrenal cortex, caused by an enzymatic synthesis deficiency of 21-hydroxylase, also affects the increase of DHEAS concentration in the blood serum.

We also found that the average concentration of estradiole in the blood serum in the group with NPKN was higher than in the group with PCOS. The difference of concentrations between the groups was significant. It seems that lower production of estradiole among women is attributed to a handicapped activity of aromatase in the granule cells. The adrenals do not produce significant numbers of oestrogenes in the blood; however, they increase indirectly the amount of the overall concentration of oestrogens by ex-gonadal conversion of precursors of C-19 androgens into oestrogens. This mechanism certainly occurs among patients with NPKN, among whom adrenals

produce increased amounts of pre-androgens, especially 17-OHP.

In summary, we found that the clinical manifestation of the examined population of patients with NPKN enabled us to specify significant symptoms of disease for diagnosis, which were of assistance in differentiating from other endocrinopathies proceeding with hyperandrogenism, including in particular PCOS.

These symptoms include severe hirsutism and the characteristics of virilism in the form of hypertrophy of the clitoris. The most important factor for diagnosis is the increase of concentrations of 17-OHP after ACTH stimulation, as well as high concentrations of testosterone and androstendione.

The specification of the appropriate diagnosis is significant for medical treatment for at least two reasons:

1. Treatment must be long-running.
2. Couples expecting offspring require genetic consultation in order to establish a pre-natal diagnosis and potential early implementation of appropriate treatment of congenital hypertrophy of the adrenal cortex (WPN).

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