Marija Miletić[1], Miloš Žarković[1],Jasmina Ćirić[1],
Biljana Nedeljković-Beleslin[1], Milina Tančić-Gajić[1], Miloš Stojanović[1],
Miomira Ivović[1], Mirjana Stojković[1], Ranko Rajović[2],Tanja Nišić[1],
Slavica Savić[1], Svetlana Vujović[1]

Mister XX

Abstract: Female pseudohermaphroditism represents discrepancy between karyotype and gonadal features on one side and a psychogenic phenotype on the other. Congenital adrenal hyperplasia is the part of the spectrum of female pseudohaermaofroditism and is due to an enzyme deficiency in steroidogenesis. More than 95% of patients have 21 hydroxylase deficiency which leads to a lack of cortisol and ACTH hypersecretion of pituitary, overproduction of 17 hydroxy progesterone (17OHP) and androgens and adrenal cortex hyperplasia . The clinical phenotype is classified as classical and nonclassical (aka. 'Late onset' form). The classic form is represented as salt-waisting and simply virilizing , depending on the degree of lack of aldosterone. Pathophysiology of CAH due to the lack of 21α hydroxylase is closely associated with the degree of enzyme deficiency. Overproduction of androgens is leading to accelerated virilisation.Classical form is manifested in childhood and is characterized by the overproduction of cortisol precursors and adrenal androgens. In the most severe form, co-aldosterone deficiency leads to loss of salt with all the complications. Girls with the classical form of CAH typically have am ambigous genitals at birth due to high concentrations of androgens in utero. CAH due to 21OH deficiency is the most common cause of ambigous genitals in 46XX newborns. Characteristically, the clitoris is enlarged, partially fused labia maiora and a common urogenital sinus at the site of the urethra and vagina. The uterus, Fallopian tubes and ovaries are present and normal, a structures of Wolfian duct are absent. When diagnosed in childhood 46XX CAH patients has been assigned femele gender so far, even in fully expressed in virilised external genitalia.This dogmatic approach is besed on preserving fertility, and if there was at least uterus, opting for female sex was considered justified. Only about 5% of 46 patients with XX KAH has a

[1] Marija Miletić, Clinic of Endocrinology, Diabetes and metabolic diseases, Clinical Center of Serbia, Belgrade, Medical Faculty, marija.barac@gmail.com.

[2] Department of Physiology, University of Koper, Slovenia.

psychogenic male gender, as described patient. In addition to mutations of CYP21A2 and effects of high concentration of androgen in the CAH, in the phenotypic expression of CAH different length of sequence of CAG androgen receptor could have an impact, which modulates the effect of androgens on the periphery. Also, while the cases of adaptation of sexual identity and the external genitalia from female to male were documented, not a single case of questionable male sexual identity is noted. In the presented patient testosterone levels are at the upper limit for men, which may be due to enzymatic block and synthesis of cortisol precursor, which is further metabolized to the active androgen: testosterone and dihydrotestosterone. High concentrations of T prenatally and in infancy has led to premature fusion epiphysis and low growth, and high concentrations of DHT to clitoromegaly. The LHRH test showed no stimulatory response of LH, which is probably suppressed by high concentrations of testosterone , but there still needs to be done endocraniaol MNR to rule out other possible etiologies of gonadotroph suppression. Dexamethasone supreesion test with measurement of testosterone could give an answer. The treatment of adult patients is based on hydrocortisone in dosages that provide permanent substitution of cortisol and supress ACTH hypersecretion. In 46 XX patients who are phenotypically male, and whose full identity is male, reconstructive surgical procedures are needed to enable adequate quality of life.

Key words: congenital adrenal hyperplasia, gender, adrenal incidentaloma

Case report

Patient DS , 39 years old, a laborer from Belgrade. The main complaints : the inability to achieve sexual relations and legal problems related to the identification birth list , as he was given a female name.He is the first child of five , mother had a proper pregnancy with vaginal delivery at term. Mother smoked during pregnancy , did not use drugs . At the birth he was asigned as a female. He is the only of the brothers and sisters of low growth . As a child playing with the boys , particularly wrestling and football. At the age of 17, hair on the face and body appeared and in the 18th he began to shave . Menstruation is never obtained. He feels like a man, sexually oriented toward the female sex . He has not served in the army . He had never been examined in terms of differentiation disorders sex. He did not use any kind of medical treatment .

Physical examination: Aware and oriented in space, time and the persons, actively driven, well-developed skeletal structure of the male type, low growth, TT 61.5 kg, TV 143cm, BMI 30 kg / m2, arm span 154cm. Eupnoic, acyanotic. The skin is constitutionally darker stained, subcutaneous tissue turgor is normal. Breasts are male

type. Examination of the heart, lungs and abdomen was normal. TA: 120/80 mmHg. Status genitalis localis: micropenis 3.5 x 2 cm, labia maiora present with absent labia minora. Unpalpable testes. Pubis: male type hair growth. Laboratory analysis were all in reference range, except elevated triglycerides. Hormonal analysis 0.2 FSH IU / L (2.5-15), LH 2.5 IU / L (4-20), PRL 811, ACTH 774ng / l (10-90), E2 139 (105 to 217) pmol / l, T 32 nmol / l (8.2-34.4 for men), DHEAS 9.2 nmol / l, 170HP 64 nmol / l, AMH 143. In an effort aldosterone response was found to rise from 199 to 689ng / l, PRA from 3.2 to 19.4 ng / ml / h, Pregnyl test: E2 from 118 to 157 pmol / l, T from 26 to 29.2 nmol / L FSH LHRH test- 0:18, 0.37, 0.91, 0.25 0.25 LH 0.25.AFP 3.22 IU / ml, βHCG 0.1 IU / L, CEA 1:42 ng / ml. Karyotype 46, XX. ECG: sinus rhythm 71 / min, with normal ST interval and T wave.Chest radiogram: mutual hilobazalno amplified pulmonary drawing, the shadow of the heart increased. Craniogram, profile recording: normal size. Rtg of right shoulder, right hip, right forearm, left lower leg and both hands: normal . Osteodensitometry: hip Z score - 0.4, spine: - 1.4. Abdominal US: At the projection of the right adrenal gland, there is a zone of lower echogenicity ,diameter 23mm. Other findings are physiological. 'Multislice scan' of the pelvis: Both adrenal gland are changed. Right is dominated by 2 changes that could be considered a bi-lobate tumor formation overall diameter of 78 mm. The left gland is multulocular, with fatty tissue that shows irregular zone of reduced opacification, with overall dimensions of 119x71mm. The liver is fatty, pancreas, spleen, bladder and kidneys physiologically adequate. Posterovesical left is a solid formation of 33x31x43 mm, which by its CT features resembles the uterus in involution. Strucrures that would fit the testes, ovaries, prostate and seminal vesicles are not differentiated.

Disscusion

Female pseudohaermaphroditism represents discrepancy between karyotype and gonadal geneder on one side and a psychogenic phenotype and, on the other. Congenital adrenal hyperplasia is the part of the spectrum of female pseudohaernaphroditism and is due to an enzyme deficiency in steroidogenesis. More than 95% of patients have 21 hydroxylase deficiency which leads to a lack of cortisol and ACTH hypersecretion of pituitary overproduction 17 hydroxy progesterone (17OHP) and androgens and adrenal cortex hyperplasia (1). The clinical phenotype CAH-a is classified as classical and nonclassical (aka. 'Late onset' form). The classic type presents as salt waisting and without loss of salt (virilizing form), depending on the degree of lack of aldosterone. For the classic form of the disease incidence in the world is 1: 12000 (2). Salt loosing form occurs in 67% and virilizing form in 33% of patients (3). The incidence of the classical form of the disease depends on the ethnic and geographic origin. The largest in Alaska (4), among Yupic Eskimos, Brazil and the Philippines (3). Neonatal 'screening' is not applyed to diagnose a nonclassical form of CAH-a, and is considered even

more prevalent than the classical form, with a prevalence of 1: 1000 among whites (5). Non-conventional form of the disease, often unrecognized, the most represented among Ashkenazi Jews, and in the former Yugoslavia, even 3.7% (5).

The enzyme 21-hydroxylase is α microsomal enzyme which belongs to the family of P450 enzymes and convert 17 OH progesterone into 11 deoxycortisol and deoxycorticosterone progesterone. The gene encoding the 21 alpha hydroxylase, CYP21A2, is located about 30 kb away from the inactive, non-functional pseudogene, CYP21A2P on the short arm of chromosome 6 (6p21.3) within the 'Human leukocytes antigen complexes' (HLA) histocompatibility. CYP21A2 genes and CYP21A2P, of which the second non-functional, they have 10 exons and showed a high degree of nucleotide sequence identity (between 98% and 96% of exons between introns) (6). The high degree of identity of active genes and pseudogenes enables conversion that occurs when the normal sequence present in the pseudogene is being moved to a functional gene, so that he loses the ability to physiological gene expression and the possibility of translation of the enzyme (7). The majority of patients (65-75%) were heterozygotes (having 2 different mutations at the two alleles), and the clinical phenotype correlates with the allele is mutated to a lesser extent, and, consequently, with partial function 21 hydroxylase (8). Most studies showed genotype-phenotype correlation, but it is less pronounced in milder forms of the disease (9). In addition, factors other than CYP21A2 mutations play a critical role in the phenotypic variability of the clinical effects of high concentration of androgen in the in-CAH. This variability could be in large part due to the different length of CAG sequence androgen receptor, which modulates the effect of androgens on the periphery. Also, the genotype-phenotype relationship loses its significance in adults, because at that age the clinical status result of the quality and adequacy of applied therapy.

Pathophysiology KAH-for lack of a 21α hydroxylase is closely associated with the degree of enzyme deficiency. Hypersecretion occurs and accumulation of precursors proximal to the level at which operates 21 hydroxylase. 17 hydroxy progesterone precursor is metabolized by the action of dehydroepiandrosterone in the 17,20 lyase (DHEAS) and 4Δ androstenedione, and testosterone, and. Overproduction of androgens leading accelerated virilization.

The classic form is manifested in childhood and is characterized by the overproduction of cortisol precursors and adrenal androgens. In the most severe form, aldosterone deficiency leads to loss of salt with all the complications. Girls with the classical form of CAH typically have a genital ambuiguty at birth due to high concentrations of androgens in utero. CAH due to 21OH deficiency is the most common cause of ambivalent genitalia in 46XX newborns. Characteristically, the clitoris is enlarged, partially fused labia maiora and a common urogenital sinus at the site of the urethra and vagina. The uterus, fallopian tubes and ovaries are present and normal, and Wolffian duct structures are absent. Boys with the classic form of CAH do not show signs at birth, except for mild hyperpigmentation and possible slight enlargement

of the penis. Disease in patients with non-classical form is manifested by premature pubic hairness, hirsutism (60%), oligomenorrhea or amenorrhea (54%), polycystic ovary syndrome (33%), and infertility (10).

The diagnostic criteria for the classic form due to the lack of 21 hydroxylase is a high concentration of 17 hydroxyprogesterone (which are normally less than 3 nmol / l in a newborn 3 days of life).Treatment of patients is based on hydrocortisone in dosages that provide substitution of permanent lack of cortisol and suppress hypersecretion of ACTH, cortisol and precursors of adrenal androgens. Since glucocorticoids in supraphysiological doses have suppressive effect on growth and androgens, it is necessary to ajust the dosages of hydrocortisone to children to provide normal speed of growth for the appropriate chronological age. The aim is to prevent long-term complications, such as adrenal tumors, metabolic syndrome, osteoporosis and infertility. Heterozygous carriers of the mutation OH 21 show subtle HPA axis function disorders. Also, increased risk of developing clinically unobservable adrenal tumors is present (15) and they show greater vulnerability in response to physiological stress (14). Glucocorticoids are essential for the further development and regulation function of the adrenal medulla, because they stimulate the expression of phenylethanolamine-N-metltransferase which converts norepinephrine to epinephrine (16). Adrenal hyperplasia in a loose-controlled disease leads to the formation of adrenal tumors, which are in most cases myelolypomas, which could be the case in our patient. R. Ravichandran et al have also demonstrated several cases of massive adrenal Incidentalomas in patiets with CAH (15).

Conclusion

Congenital adrenal hyperplasia belongs to the spectrum of female pseudohermafroditism. At the presented patient there is a clear dissonance between cariotype , which is female and the genital sex , gender identity and gender roles that are male . When diagnosed in childhood , 46 XX patients with CAH , has been assigned as females, even in fully expressed virilization in newborns . This dogma was oriented towards preserving fertility if there is at least uterus ,opting for female sex to be considered justified . Only about 5% of 46 patients with XX CAH has a psychogenic male gender, the feeling of belonging to male sex, as described patient . In addition to mutations of CYP21A2 and effects of high concentration of androgen in the CAH , the role in the phenotypic expression could possibly depend on different lengths of sequence of CAG androgen receptor which modulates the effect of androgens on the periphery. Also, while there are documented cases of sexual identity changes and external genitalia of a female in a male, not a single case of changes in male sexual identity is not detected(11). In the present patient testosterone levels are at the upper limit for men, which may be due to enzymatic block in synthesis of precursor of cortisol, which is

further metabolized to the active androgen: testosterone and dihydrotestosterone. High concentrations of T prenatally and in infancy has led to premature epiphysis fusion and low growth, and high concentrations of DHT to clitoromegaly. In the LHRH test there was no response to LH , which is probably suppressed by such a high concentrations of testosterone, but still NMR of endocranium is needed to rule out other etiologies of gonadortoph suppression. Dexamethasone test with measurement of testosterone could give an answer. The treatment of adult patients with CAH is based on hydrocortisone in dosages that provide permanent substitution of cortisol and supresses ACTH hypersecretion .In 46 XX patients, who are phenotypically male, and whose full identity is male, necessary reconstructive surgical procedures are needed, as phaloplasty and testical prothesis , that would enable adequate quality of life.

Reference:

1. Grumbach MM, Shaw EB. Further studies on the treatment of congenital adrenal hyperplasia with cortisone: IV, eefect of cortisone and compoubd B in infants with disturbed electrolyte metabolism. *Pediatrics 1*998; 102: 215-21.
2. Pang S, Clark A. Congenital adrenal hyperplasia due to 21-hydroxilase deficiency: newborn screening and its relationship to diagnosis and treatment of the disorder. *Screening* 1993; 2:105-39.
3. Therrell BL. Newborn screening for congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am* 2001; 30: 15-30.
4. Pang S, Murphey W, Levine LS et al. A pilot newborn screening in Alaska for congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 1982; 55: 413-20.
5. Speiser PW,Ndupont B, Rubinstein P, Piazza A, Kastelan A, New MI. High frequencyof nonclassical steroid 21-hydroxilase deficiency. *Am J Hum Genet* 1985; 37: 650-67.
6. Kawaguchi H, O'Huigin C, Klein J. Evolutionary origin of mutations in the primate cytochrome P450c21gene. *Am J Hum Gen* 1992; 50: 766-80.
7. Tajuima T, Fujeda K, Fujii-Kuryama Y. De novo mutation causes steroid 21-hydroxilase deficiency in one family of HLA identical affected and unaffected siblings. *J Clin Endorinol Metab* 1993; 77: 86-89.
8. Speiser PW, Dupont J, Zhu D et al. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxilase deficiency. *J Clin Invest* 1992; 90: 584-95.
9. Lee CT, Tung YC, Hsiao HP. Clinical characteristics of Taiwanese children with congenital adrenal hyperplasia caused by 21 hydroxilase deficiency in pre-screening era. J Formos Med Assoc 2010; 109: 148-55.
10. Moran C, Azziz R, Carmina E et al. 21-Hydroxilase deficient nonclassic adrenal hyperplasia is a progressive disorder: a multicentric study. *Am J Obstet Gynecol* 2000; 183: 1468-74.

11. Bidet M, Bellanne-Chantelot C, Galand-Portier MB, Tardy V, Morel Y, Kutten F et al. Fertility in women with nonclassical congenital adrenal hyperplasia due to 21 –hydroxilase defficiency. J Clin Endocrinol Metab. 2010; 85: 1182-90.
12. Forest M. Recent advances in the diagnosis and management of congenital adrenal hyperplasia due to 21- hydroxilase deficiency. *Hum Reprod Update* 2004; 10: 469-485.
13. Cutler CB, Laue L. Congenital adrenal hyperplasia due to 21-hydroxilase deficiency. *N Engl J Med* 1990; 323: 1806-13.
14. Charmandari E, Merke DP, Negro PJ. Endocrinologic and psychologic evaluation of 21-hydroxilase defficiency carriers and matched normal subjects; evidence for physical and psychological vulnerability to strss. *J Clin Endocrinol Metab* 2004; 89: 2228-36.
15. Baumgartner-Parzer SM, Pauschenwein S, Polzer K, Vierhapper H. Increased prevalence of heterozygous 21-hydroxilase deficiency germline mutations in patients with adrenal incidentalomas. *Clin Endocrinol (Oxf*) 2002; 56: 811-16.