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ORIGINAL ARTICLE

Trends in diagnostics and treatment of congenital adrenal hyperplasia

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Summary

Introduction: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases caused by a deficiency of enzymes responsible for the steroidogenesis. There are three forms of CAH due to 21-hydroxylase deficiency: the classic form with salt loss, the classic virilizing and the non-classic form. The aim of the paper was to analyze the changes in the diagnosis and treatment of children with CAH during previous 15 years.

Material and methods: This retrospective cohort study includes patients who were diagnosed with CAH due to 21-hydroxylase deficiency in the period from 2007 to 2021 in endocrinology department of the Institute for Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic". Respondents were divided into two groups - a group whose diagnosis was made in the period between 2007 and 2014 and another group of those whose diagnosis was made in the period between 2015 and 2021. Statistical analysis using Hi-square and Mann Whitney U test was conducted using the software IBM SPSS ver. 22, and p values <0.05 were considered significant.

Results: Out of the total of 55 patients included in the study, 46 patients (83.6%) had 46, XX karyotype. The diagnosis was made in all patients on the basis of biochemical analyzes. In the second group the diagnosis was confirmed by genetic analysis in statistically significantly higher number of children (p <0.05). 49 patients (89.1%) received hydrocortisone and 16 patients received fludrocortisone. In patients from the second group a statistically significantly higher frequency of fludrocortisone therapy was noticed in patients with classic CAH.

Conclusion: Having in mind the constant advancement in the field, frequent improvements in clinical care of children with CAH are needed.

Keywords: congenital adrenal hyperplasia, recommendations, diagnosis, treatment

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases caused by the disruption of enzymes responsible for cortisol synthesis (1). The most common cause is 21-hydroxylase deficiency (95-99% of patients), which is responsible for the conversion of 17-OH progesterone (17-OHP) into 11-deoxycortisol and progesterone into 11-deoxycorticosterone, which are precursors of cortisol and aldosterone (2). As a result of the block in steroidogenesis, there is an accumulation of 17-OHP, which is translated into androgens by three different enzymes, the most significant of which in patients with CAH is the alternative pathway, which is under control of 5α-reductase and 3α-reductase (3). There are three forms of the disease, the classic form with salt loss, the classic virilizing form and the non-classic form of CAH. In 75% of cases of the classic form of CAH, the enzyme 21-hydroxylase is completely inactive and there is a complete deficit of cortisol and aldosterone, which can lead to a salt-wasting crisis, and this is why this form was named salt wasting form -SW. In female newborns ambivalent genitalia are commonly noticed at birth, and in male newborns the disease is most often manifested by a salt-wasting crisis. This metabolic crisis most commonly occurs during the second or third week of life, presenting with vomiting, hyponatremia, hyperkalemia, dehydration, and shock. In the remaining 25% of patients the activity of the enzyme is reduced to 1-5%, enabling the synthesis of aldosterone and preventing salt wasting, which is why this is virilizing form of CAH (simple virilizing form -SV). The main manifestations of this form of CAH in female children are the ambivalent genitalia (in varying degrees), clitoromegaly, posterior labial fusion or hirsutism, and precocious puberty occurs in male children (4). The third form of the disease is non-classic KAH in which enzyme activity is reduced to 20-60%, which is why symptoms appear later, most often in adolescence. The disease is manifested by precocious pubarche, oligomenorrhea, hirsutism or reduced fertility (2).

What is common to all three forms of CAH is an elevated value of 17-OHP. In most patients with the classic form of CAH, the level of 17-OHP in the blood is over 30nmol/L, while the gold standard for diagnosis is The Short Synacthen Test. It is based on the application of synthetic ACTH and the measurement of the increase in the concentration of cortisol and 17-OHP after 30 and 60 minutes after the application of ACTH (3). The value of 17-OHP below 2.5 nmol/L excludes the diagnosis of CAH (5). It should be kept in mind that the level of 17OHP is high even in healthy newborns during the first 48 hours after birth, while after that the value drops in children with normal cortisol synthesis (6). Preterm children, children with infection or under stress have higher levels of 17OHP than healthy term children, therefore there are more false positive results (7).

In case of the classic form of CAH in female newborns, the diagnosis is usually made at birth due to the existence of ambivalent genitalia. The finding of ambivalent genitalia is described using the "External genitalia score" (EGS) or the Prader scale. EGS represents a modification "External masculinization score", which was introduced into literature in order to universally describe the finding of ambivalent genitalia in children with gender differentiation disorders. Within this score, 5 characteristics are described (labioscrotal fusion, length of the phallus, position of the opening of the urethra, positions of the right and left gonads) and the total score ranges from 0-12. The EGS value for the usual appearance of male genitalia is 12, while for female genitalia the score is 0. The Prader scale is used to describe the genitalia in female children with congenital adrenal hyperplasia and the values on this scale range from 1 (phenotypically female genitalia with clitoromegaly) to 5 (phenotypically male genitalia with nonpalpable gonads) (8).

In male newborns, the first symptom of the disease is often an adrenal crisis with salt loss. This metabolic disorder is most often seen in male infants with the most severe form of CAH, and at a later age it can be caused by infections or surgical interventions. It is manifested by hypotension, vomiting and electrolyte disturbances (hyponatremia and hyperkalemia). Unless this condition is promptly treated with parenteral administration of hydrocortisone and correction of hyponatremia and hypovolemia, it can lead to a fatal outcome. Due to the fact that infections are the most common causes of adrenal crisis, increasing hydrocortisone dose during intercurrent infections (''stress-dosing'') is recommended for all children with CAH.

The non-classic form of CAH has its own specificities in terms of clinical picture, diagnosis and therapy. The most common symptom in children under 10 years of age is premature pubarche and growth acceleration while in older girls irregular periods appear as the most common symptom (56% of patients), acne, hirsutism, clitoromegaly, irregular periods or primary amenorrhea, and later fertility problems. An important difference compared to the classical type of the disease is that there is no genital virilization in female newborns. The Short Synacthen Test is also used in the diagnosis of non-classic CAH, where a basal level of 17OHP above 7 nmol/L together with a level of 17-OHP above 30 nmol/L after ACTH administration is considered sufficient to make the diagnosis of non-classic CAH. The therapy of choice in pediatric age is hydrocortisone, most often in a dose of 10-15mg/m2 (5).

The therapeutic goal in patients with CAH is to compensate for cortisol, normalize androgen levels, preserve growth and fertility potential, and avoid complications (2). Hydrocortisone is the drug of choice for glucocorticoid replacement. The latest guidelines recommend hydrocortisone tablets at a dose of 10-15mg/m2 in growing children and 15-25mg in adults, divided into several doses during the day (10). In recent guidelines, fludrocorti-

sone therapy is recommended for all patients with classic CAH, including the simple virilizing (SV) form. Recommended doses are 0.05-0. 2mg per day. In addition to this, the use of sodium chloride is also recommended in infants for salt replacement, and the recommended dose is 1 mmol/kg per day. The guidelines do not recommend increasing the dose of therapy in case of daily emotional stress as well as minor infections (11).

Regarding the timing of genital surgery in CAH, there are still no sufficient data regarding the long term outcomes or randomized controlled studies of either the best age or the best methods for restoring functional female anatomy in virilized girls with CAH. However, during recent years there has been a shift towards later age at surgery in many centers, balancing the benefits and potential harms of early surgery.

Congenital adrenal hyperplasia is a complex disease with evolving diagnostic and management protocols. The aim of the present paper is to analyze the changes in the diagnostic and therapeutic approach to children with CAH in a tertiary center during the previous 15 years.

THE MATERIAL AND METHODS

In this retrospective cohort study, a total of 55 children diagnosed with congenital adrenal hyperplasia due to 21-hydroxylase deficiency were examined. The data were collected from the database of the endocrinology department of the Institute for Mother and Child Health Care of Serbia, "Dr. Vukan Čupić", and the study included patients who were diagnosed in the period from 2007 to 2021. The first group of respondents includes patients whose diagnosis was made in the period between 2007 and 2014, and the second group of respondents includes patients who were diagnosed with CAH in the period between 2015 and 2021.

The data that were analyzed included the diagnosis, the first symptoms, biochemical analyses at the time of diagnosis, the data regarding genetic findings and the timing of the first genital surgery in patients who had undergone the intervention, the medical treatment that was applied and the occurrence of adverse disease outcomes, including salt-wasting crisis and death. Statistical analysis of variables between the two groups of subjects was performed using Chi-square and the Mann Whitney U test was performed using the software *IBM SPSS ver. 22*, and p values <0.05 were considered significant.

RESULTS

In total, 55 patients were included in this study, of which 38 (69%) were patients from the first group, and 17 (31%) belonged to the second group. The most common type of disease was the non-classic form (NC) of CAH in 32 patients (58.2%), while the classic form with salt loss (SW) was diagnosed in 15 patients (27.3%), whereas the classic virilizing form (SV) was found in 8 patients (14.5%). A total of 46 (83.6%) of all CAH patients had the female karyotype (46,XX), and 9 (16.4%) had the male karyotype (46,XY), with a decreasing proportion of 46,XY karyotype in SV and NC forms compared to subjects with SW form **(Figure 1)**. The karyotype was determined by the G-bending technique, at 32 metaphases. The average age at which the diagnosis was made was 7.1 years, and

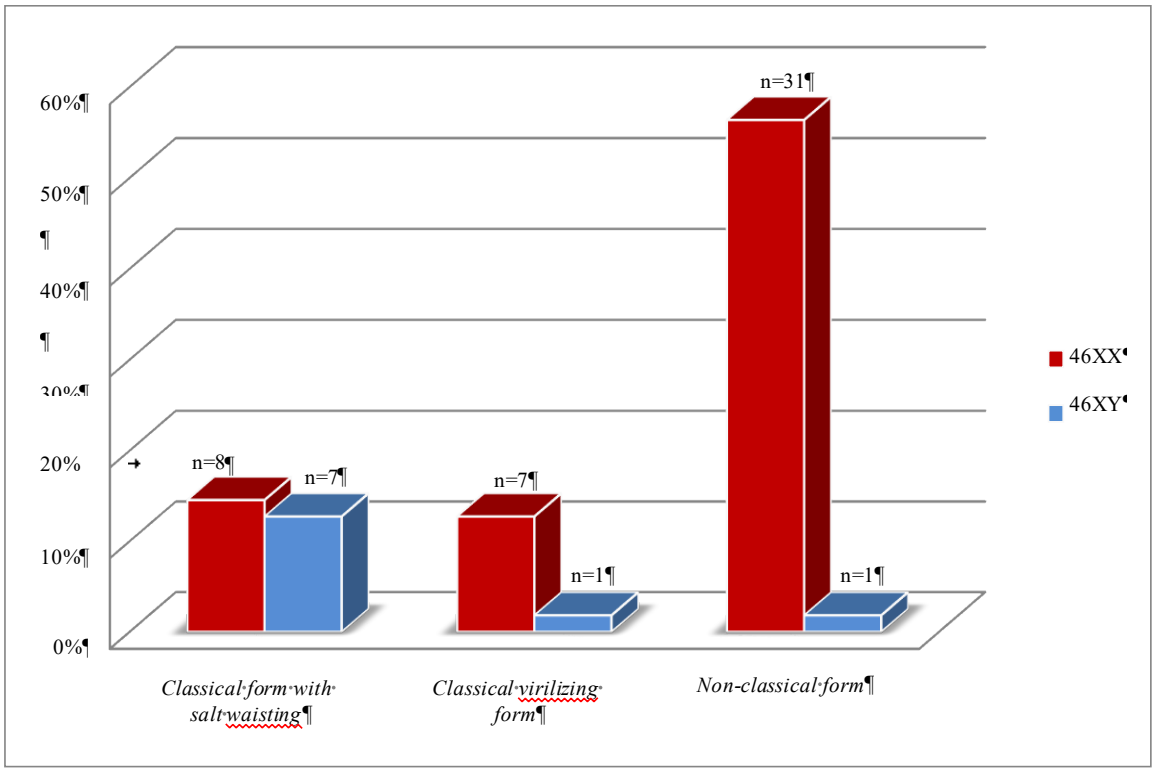

Figure 1. Frequency of CAH forms in children with female and male karyotype

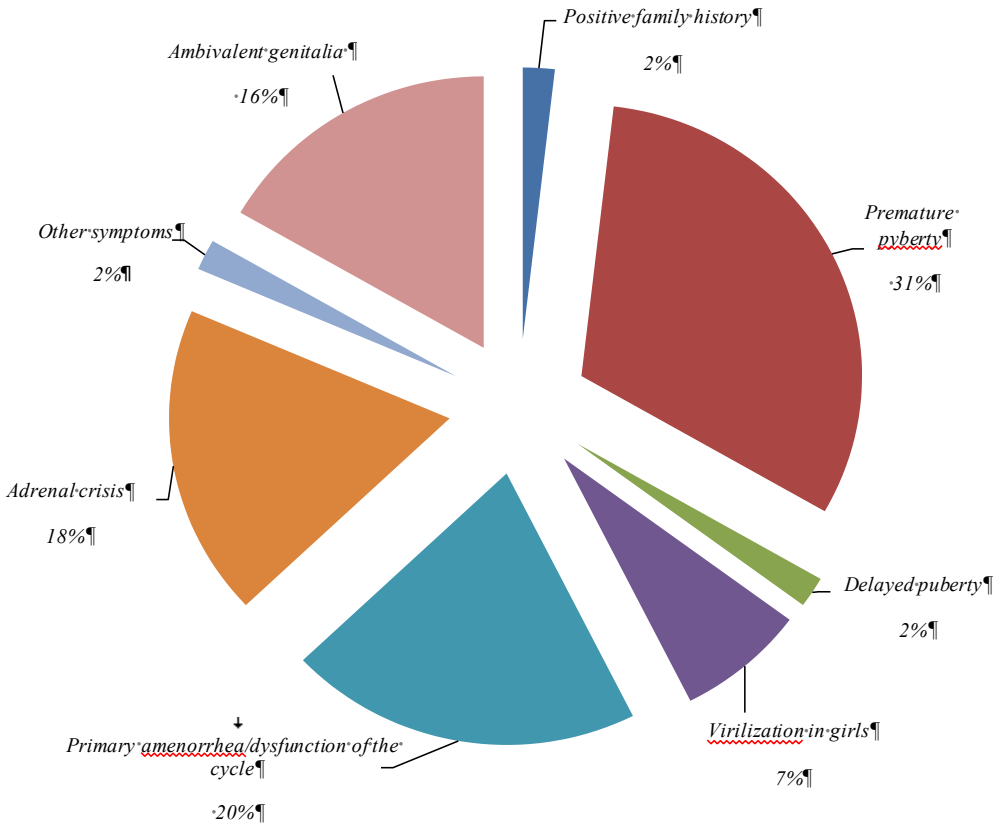

Figure 2. The first symptoms of the disease in children with congenital adrenal hyperplasia

in 22 patients (40%) the diagnosis was made during the first year of life. All patients were diagnosed on the basis of biochemical findings of elevated 17-OHP, while in 4 patients (7.3%) the diagnosis was confirmed by genetic analyses. A statistically significant difference ($p<0.05$) was observed regarding the percentage of patients from the first group whose diagnosis was confirmed by genetic analyzes (2.6%, n=1) compared to the number of patients from the second group whose diagnosis was confirmed by the existence of genetic mutations (17.6%, n=3).

In 10 patients, a salt loss crisis was the first recognized manifestation of the disease, and in the total of 15 patients (65.2% of all children with classic CAH) the salt loss syndrome developed before the definitive diagnosis of CAH. In addition, the most common first symptom of the disease was the appearance of signs of premature puberty in 17 patients, primary amenorrhea (or other disorders of the menstrual cycle) in 11 patients and ambivalent appearance of the external genitalia in 9 patients (**Figure 2**). The Prader scale and the External Genitalia Score (EGS) were used to describe genital masculinization in girls with classic CAH. The EGS values ranged from 0.5 to 11, and the values on the Prader scale from 0 to 4. The mean EGS value was 5.43 ± 4.48 and the Prader virilization score was 2.47 ± 1.73.

Of all patients, 6 patients with non-classic form of CAH did not receive any corticosteroid therapy. 49 patients (89.1%) received hydrocortisone, while 16 patients (29.1%) also received fludrocortisone. A statistically significant difference was noticed in the number of patients diagnosed with classic CAH (including both SW and SV forms) who received hydrocortisone and fludrocortisone when comparing the two groups of subjects ($p<0.05$). While 56.3% of children from the first group with classic CAH received fludrocortisone, all children from the second group with classic CAH received fludrocortisone.

Eight patients (14.5%) underwent genital surgery, with five patients belonging to the first group of subjects. Although the difference was not statistically significant, the average age at which genital surgery was performed was 9.6 months, while in the second group, the average age at the time of the first genital surgery was 2.2 years.

DISCUSSION

In the present study, a significantly higher proportion of subjects with CAH had 46,XX karyotype compared to the number of subjects with 46,XY karyotype. Considering that it is an autosomal recessive disease, the same risk for CAH is expected in children of both sexes. One of the possible explanations for this predominance of girls in children diagnosed with CAH is that in girls the early diagnosis is facilitated by the clinical finding of ambiguous genitalia at birth, while in boys the disease often manifests itself as an adrenal crisis which can have a fatal outcome before the diagnosis has even been made. This is exactly what reflects the importance of newborn screening. Currently neonatal screening for CAH is performed in more than 35 countries worldwide. The latest guidelines suggest double-check screening with an upper limit of 17 OHP adjusted for ges-

tational age along with birth weight. The first method used in screening for CAH is measuring the level of 17OHP by immunoassay method from blood on filter paper, which is used for screening other diseases. This method has a lot of false positive results, so liquid chromatography with mass spectrometry is used for the second screening method (7).

Genetic analyses represent an important factor when the next pregnancy is being planned as well as a possible prenatal therapy in the next pregnancy. Ten patients (18.2%) in our study had a positive family history. In patients belonging to the second group, a statistically significantly higher number of children whose analysis was confirmed by genetic analyses was observed compared to the number of patients from the first group who underwent genetic analyses, which is in accordance with the latest global recommendations. 21 hydroxylase deficiency is caused by mutations in the CYP21A2 gene, most often by intergenic recombination. 65-75% of patients with CAH are compound heterozygotes. Mutations are divided into 4 groups and each group is typical for a certain form of CAH. Group 0 is associated with the classic salt-wasting form, group A occurs in both classic types of the disease, group B in the classic virilizing form and group C in the non-classic form of CAH. The phenotype depends on the milder mutation (10).

Genital surgery is commonly used for restoring functional anatomy in female children with pronounced virilization of genitalia. Feminizing genitoplasty includes clitoroplasty, opening of the vaginal meatus if there is only an opening of the urogenital sinus, and labiaplasty. In our study, genital reconstructive surgery was performed in eight virilized female patients with CAH. There are still no clear recommendations about the ideal age for surgical intervention. In a study that processed data on patients who had been treated at the Institute for Mother and Child Health Care of Serbia with disorders of sexual differentiation of various causes, an older age at which the operation was performed was noticed, as well as a lower frequency of genital surgery as a treatment method in the last period (12). In patients who do not have a malformation in the form of the urogenital sinus but the vagina and bladder are normally developed, there are two approaches, earlier and later surgery. Earlier guidelines recommend surgery shortly after birth for tissue elasticity, reduction of parental stress due to ambivalent genitalia, and prevention of hydrometrocolpos (10). On the other side, the benefits of later surgery are the formation of the child's own opinion about the decision related to the surgery and the formation of gender identity. The most common complication that occurs after surgery is vaginal stenosis, while fistulas, urinary incontinence and infections occur less often (11). In our research, although statistical significance was not confirmed, a clear trend towards postponing genital surgery to a later age can be observed, in accordance with most world guidelines and trends in clinical practice.

Newer guidelines recommend the use of fludrocortisone in addition to hydrocortisone in all patients with the classic form, including SV without salt loss, because aldosterone replacement enables therapy with lower doses of hydrocortisone and thus reducing the side effects of corticosteroid use. Also, although the aldosterone deficit is clinically manifest only in the classic form of CAH with salt loss, even in the classic virilizing form of CAH there is a subclinical aldosterone deficit. Maintenance of electrolyte concentration is important due to euvolemia and reduction of vasopressin and ACTH secretion, which allows for lower doses of hydrocortisone. Therefore, fludrocortisone along with hydrocortisone is introduced as therapy in all patients with the classic form of CAH (11). In our study, a statistically significantly higher frequency of fludrocortisone administration was observed in patients from the second group with the classic form of CAH, in accordance with the latest international guidelines for the treatment of children with CAH.

CONCLUSION

Congenital adrenal hyperplasia is a condition that can be life-threating, and the outcome of which significantly depends on the moment of diagnosis. In present study, a significant difference was observed in the number of female and male children with CAH, which indicates a probable failure to establish a diagnosis in a significant number of boys with CAH. Timely diagnosis of CAH by means of newborn screening and early replacement therapy could prevent the occurrence of adrenal crisis, which can have a fatal outcome. Also, replacement therapy would allow for reduced testosterone production, which would suppress further masculinization of the genitals in female children. The importance of confirming the diagnosis with genetic analyses is observed when planning further offspring.

Performing genital surgery interventions at an older age allows parents, together with doctors, to monitor the child's psychological and gender development.

The observed changes in medical and surgical practice in our center during previous 15 years highlight the constant advancements in the field as well as the need for regular update of clinical practice protocols in the management of CAH.

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PROMENE U DIJAGNOSTICI I LEČENJU KONGENITALNE ADRENALNE HIPERPLAZIJE - ISKUSTVO TERCIJERNOG CENTRA

Jana Milenković[1], Tatjana Milenković[2], Katarina Sedlecki[2], Predrag Ilić[1,2], Vladimir Kojović[1,2], Jelena Martić[1,2], Katarina Mitrović[1,2], Slađana Todorović[2], Marko Marjanović[2], Jovana Tončev[2], Sanja Panić-Zarić[2], Danilo Pešić[1,3], Rade Vuković [1,2]

Sažetak

Uvod: Kongenitalna adrenalna hiperplazija (KAH) predstavlja skup autozomno recesivnih bolesti koje su prouzrokovane deficitom enzima odgovornih za sintezu hormona nadbubrežne žlezde. Postoje tri oblika KAH usled deficita 21-hidroksilaze: klasičan oblik sa gubitkom soli, klasičan virilizujući i neklasični oblik. Cilj ovog rada je analiza izmena u dijagnostici i terapiji dece sa KAH tokom prethodnih 15 godina.

Materijal i metode: U ovoj retrospektivnoj kohortnoj studiji izdvojeni su pacijenti kojima je u okviru rada službe za endokrinologiju Instituta za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić" utvrđena dijagnoza KAH usled deficita 21-hidroksilaze u periodu od 2007. do 2021. godine. Ispitanici su podeljeni u grupu čija je dijagnoza postavljena u periodu od 2007. do 2014. godine i drugu grupu onih kojima je dijagnoza postavljena od 2015. do 2021. godine. Statistička analiza upotrebom Hi-kvadrat i Mann Whitney U testa je sprovedena upotrebom softvera IBM SPSS ver. 22, a značajnim su smatrane p vrednosti <0,05.

Rezultati: Od ukupno 55 pacijenata uključenih u istraživanje, 46 pacijenata (83,6%) imalo je 46, XX kariotip. Dijagnoza je kod svih pacijenata postavljena na osnovu biohemijskih analiza, a u drugoj grupi pacijenata je dijagnoza potvrđena genetičkim analizama kod statistički značajno većeg broja dece (p<0,05). 49 pacijenata (89,1%) je primalo terapiju hidrokortizonom, a 16 pacijenata je dobijalo fludrokortizon. Kod pacijenata iz druge grupe uočena je statistički značajno češća učestalost primene fludrokortizona kod pacijenata sa klasičnim oblikom KAH.

Zaključak: Imajući u vidu konstantan napredak na ovom polju, potrebna su stalna unapređenja u dijagnostici i lečenju dece sa KAH.

Ključne reči: kongenitalna adrenalna hiperplazija, preporuke, dijagnostika, lečenje

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