BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Endocrinology Research
https://ojs.bilpublishing.com/index.php/jer

ARTICLE

Adrenomedullary Function in Cohort of Brazilian Pediatric Patients with Classic Congenital Adrenal Hyperplasia

Talita Soriano Cruz Hovland Izabel Calland Ricarte Beserra*

Universidade Federal do Rio de Janeiro, Brazil

ARTICLE INFO

Article history
Received: 16 November 2020
Accepted: 27 November 2020
Published Online: 30 December 2020

Keywords:
Classical congenital adrenal hyperplasia
Adrenal medulla
Normetanephrine
Metanephrine
Hospitalization

ABSTRACT

Congenital Adrenal Hyperplasia is a group of autosomal recessive disorders resulting from deficiency of enzymes essential for the synthesis of cortisol. Disease of the adrenal cortex, but there may be involvement adrenomedullary. Cortisol and epinephrine are directly related to the individual's stress response. Lower values of epinephrine in children with congenital adrenal hyperplasia could be related to increased clinical complications and hospitalizations rate. We evaluated the serum values of metanephrines and normetanephrines in children and adolescents with classic congenital adrenal hyperplasia and primary hypothyroidism and possible correlations with disease and hospitalizations. Cross-sectional study involved 29 patients (10 simple virilizing and 19 salt-wasting), and control group of 28 patients with primary hypothyroidism (10 overt and 18 subclinical). There were no differences in age ($p = 0.24$) and metanephrine ($p = 0.34$) or normetanephrine values ($p = 0.85$) between groups. Hospitalization rate was higher in the cases than in the controls (51 x 12). We conclude the serum values of metanephrine and normetanephrine in patients with congenital adrenal hyperplasia were within the normal values of reference, with no significant difference of group with primary hypothiroidism. The number of hospitalizations in the case was high in relation to the control, mainly in salt-wasting.

1. Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from genetic defects which cause a deficiency of enzymes essential for the synthesis of cortisol and, sometimes, aldosterone. The most frequent form of the disease, accounting for 90-95% of all cases of CAH, involves a mutation causing deficiency of the enzyme 21-hydroxylase (21-OHlase). The clinical manifestations of CAH depend on the degree of enzyme deficiency, ranging from milder to more severe forms[1].

Three major clinical phenotypes have been described: salt-wasting (SWCAH), which affects 75% of patients with the classic form; simple-virilizing (SVCAH), which affects the remaining 25%; and nonclassical CAH. Treatment aims at adequate glucocorticoid replacement, with or without mineralocorticoids as needed, in order to prevent adrenal crisis and minimize the virilizing effects of the disease [1-3].

The most common form of CAH (classic 21-hydroxylase deficiency) affects approximately 1:15,000 live births

**Corresponding Author:*
Izabel Calland Ricarte Beserra,
Universidade Federal do Rio de Janeiro, Brazil;
Email: izabelcalland@medicina.ufrj.br

DOI: https://doi.org/10.30564/jer.v2i1.2574

[4,5]. Since the introduction of cortisone therapy by Wilkins et al. in the early 1950s, children with CAH have been able to survive to adulthood [2]. In Brazil, the incidence of SWCAH ranges from 1: 7,500 to 1: 10,000 live births [6].

Although CAH is a disease of the adrenal cortex, there might be an impairment of the adrenomedullary function in these patients. The medulla represents about 10% of the adrenal gland and is responsible for the production of epinephrine (80%) and norepinephrine (20%), in addition to minimal amounts of dopamine. These hormones are secreted into the bloodstream upon direct stimulation of the adrenal glands by sympathetic nerves [7].

Epinephrine synthesis is related to the presence of cortisol, produced in the adrenal cortex. Cortisol induces the enzyme phenylethanolamine-N-methyl transferase enzyme, which catalyzes the conversion of norepinephrine to epinephrine in marrow chromaffin cells [7].

Cortisol and epinephrine are directly related to the individual stress response, and act to prevent hypoglycemia as counterregulatory hormones. Thus, lower values of epinephrine in children with CAH may be related to increased complication and hospitalization rates [8].

The objective of the study was to compare the serum values of metanephrine and normetanephrine in pediatrics patients with classic CAH to those of patients with primary hypothyroidism, as well as to assess potential correlations with disease control, clinical complications, and the hospitalization rate.

2. Patients and Methods

A cross-sectional study of 29 patients with classic CAH (*cases*) and 28 patients with primary hypothyroidism (*controls*) was conducted at the endocrinology outpatient clinic of a pediatric university hospital, from 2017 to 2019. Patients with other associated diseases and/or using medications that could alter the dosage of catecholamines, such as tricyclic antidepressants, levodopa, drugs containing adrenergic receptor agonists (eg decongestants), amphetamines, buspirone, psychoactive agents, prochlorperazine, reserpine were excluded. In addition, patients were instructed not to drink soft drinks, coffee, tea, or use tobacco before the exams were collected. All patients underwent a complete physical examination, and had blood collected for serum dosage. The levels of of androstenedione, testosterone, metanephrine, and normetanephrine were measured for *cases*, while free thyroxine (fT4), thyrotrophin (TSH), metanephrine, and normetanephrine were measured for controls.

Normetanephrine and metanephrine were measured in plasma by high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA), respectively. The reference ranges were < 90 pg/mL for metanephrine and < 196 pg/mL for normetanephrine.

Depending on whether their androstenedione levels were within or outside normal range, patients with CAH were classified as having good control or poor control, respectively.

The study was approved by the institution's research ethics committee.

3. Statistical Analysis

The hypothesis of normality was rejected by the Shapiro-Wilk test; thus, nonparametric measures were used. The Mann-Whitney *U* test was used for comparison of age, metanephrine, and normetanephrine between the two groups (case vs. control) and subgroups thereof, while the chi-square test was used to compare sex distribution.

The statistical significance threshold was set at 5%. All analyses were processed in the SAS® System software environment, version 6.11 (SAS Institute, Inc., Cary, North Carolina).

4. Results

The clinical characteristics of patients in the case group and control group are shown in table 1.

All patients were receiving glucocorticoid replacement therapy (22 were using prednisolone and 7 dexamethasone). The median hydrocortisone equivalent dose was 9.76 mg/m^2/day, and the mean was 10.57 ± 7.37 mg/m^2/day. Of the 10 patients with SVCAH, 5 also received fludrocortisone regularly for mineralocorticoid replacement, with the mean and median dose of 0.05 mg/day; of the 19 patients with SWCAH, 17 were also on fludrocortisone, at the mean dose of 0.22 mg/day (median, 0.20 mg/day).

Table 1. Clinical characteristics of cases and controls

Clinical features	Cases (CAH)	Controls (hypothyroidism)
N	29	28
Female	15	16
Prepubescent	16	8
Pubescent	13	20
Age (years)		
Range	0.41-20	4.9-15.25
Median	7.41	11.63
Mean ± SD	9.32 ± 6.31	11.25 ± 2.48
SWCAH / SVCAH	19 / 10	-
Median / mean hydrocortisone equivalent dose, mg/m^2/day	9.76 / 10.57 ± 7.37	-
Overt / subclinical hypothyroidism	-	10/18

Notes: SD, standard deviation; SWCAH, salt-wasting congenital adrenal hyperplasia; SVCAH, simple virilizing congenital adrenal hyperplasia.

DOI: https://doi.org/10.30564/jer.v2i1.2574

Of the 28 patients with hypothyroidism, 10 of them had overt hypothyroidism, diagnosed after 2 years of age, and had serum fT4 values within the normal range at the time of the study, with replacement doses of levothyroxine ranging from 1 to 2.17 mg/kg/day. The remaining 18 were diagnosed with subclinical hypothyroidism and maintained T4L values within the normal range throughout their follow-up, without the need for replacement with levothyroxine.

Table 2 reports the age and metanephrine and normetanephrine levels of cases and controls, and the corresponding descriptive statistic (p-value) of the Mann-Whitney test. The median age in the control group is higher than in the case group, because of the later age of onset of acquired hypothyroidism. Thus, as noted in Table 1, there are no infants or preschool children in the control group. In the case group, there were 2 infants and 2 adults (aged 20), which explains the greater deviation of age in this group. Nevertheless, the age difference was not significant ($p = 0.24$).

The median (interquartile range) plasma metanephrine level was 33 (25-42) in the case group, and 38 (31-42) in the control group ($p = 0.34$). The median normetanephrine level was 77 in both groups, with an interquartile range of 58-99 in the case group and 64-92 in the control group ($p = 0.85$).

According to the chi-square test, the proportion of females in the case group (51.7%) was not significantly different from that of the control group (57.1%), with $p = 0.68$.

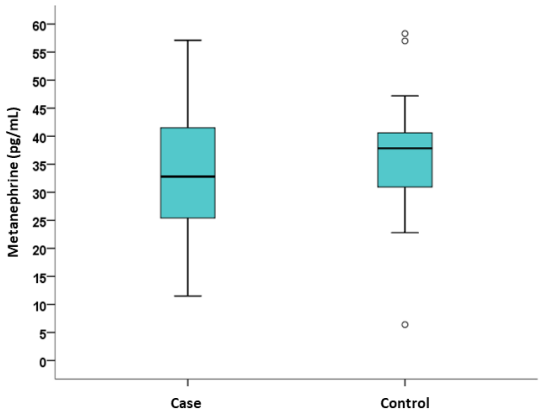
Table 2. Age, and metanephrine and normetanephrine values by group (cases versus controls)

Variable	Cases (n = 29)				Controls (n = 28)				p-value
	Median	IQR			Median	IQR			
Age (months)	89	49	-	174	140	121	-	160	0.24
Metanephrine (pg/mL)	33	25	-	42	38	31	-	41	0.34
Normetanephrine (pg/mL)	77	58	-	99	77	64	-	92	0.85

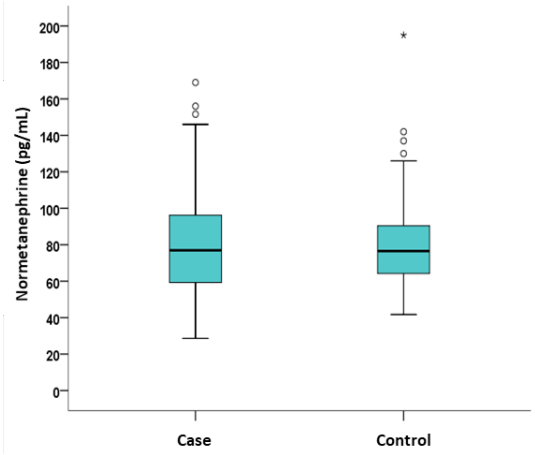
Note: Mann-Whitney test. IQR, interquartile range (Q1-Q3).

Figure 1(a) shows the trend of metanephrine values, which varied from approximately 25 to 40 pg/mL in the case group and from 30 to 40 pg/mL in the control group. Figure 1(b) shows the trend in normetanephrine values, which ranged from approximately 60 to 100 pg/mL in the case group and from 70 to 100 pg/mL in the control group. Outliers were disregarded in the box plot, since they would have skewed the analysis.

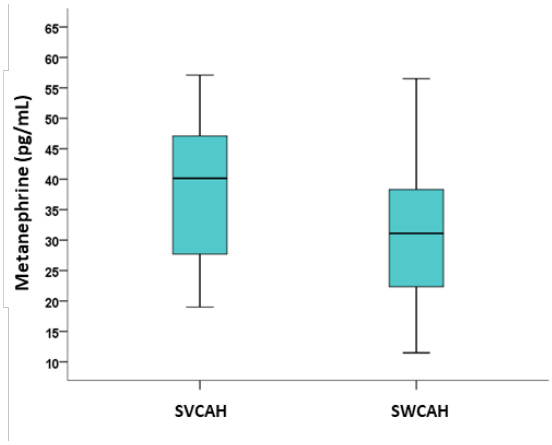
Figure 1(c) we observe the mean, maximum and minimum values of metanephrine (pg/mL) of the case group studied according to the disease form (SVCAH and SWCAH). The values varied in SVCAH and SWCAH approximately of 22 to 47 and from 23 to 40, respectively. In figure 1. (d) we observe the mean and maximum and minimum normetanephrine values (pg/mL) of the SVCAH and SWCAH subgroups studied. The trend of values in SVCAH varies from approximately 60 to 100 and from 60 to 90 in SWCAH. Again, outliers were disregarded in the box plot, since they would cause deviation in the analysis.



(a)

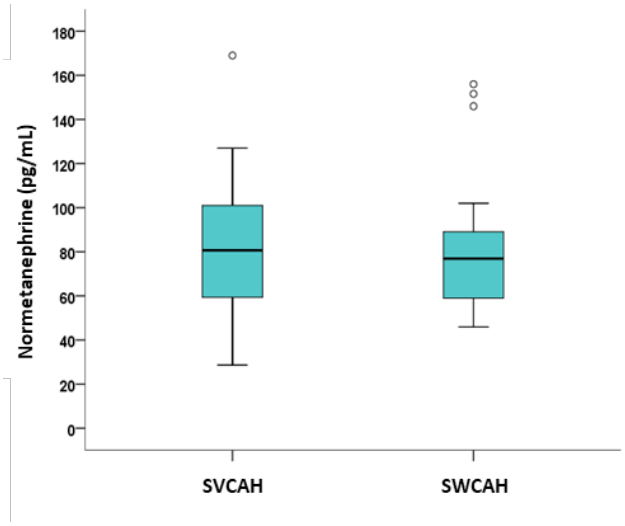


(b)



(c)

DOI: https://doi.org/10.30564/jer.v2i1.2574



(d)

Figure 1. Box plots of serum levels metanephrine (pg/mL) and normetanephrine s (pg/mL): (a) metanephrine, cases vs. controls; (b) normetanephrine, case vs. controls; (c) metanephrine, cases SVCAH vs. SWCAH; (d) normetanephrine, cases, SVCAH vs. SWCAH

The number of hospitalizations was significantly higher in the case group (n=51) than in the control group (n=12). Of the hospitalizations in the case group, 43 occurred in patients with SWCAH and 8 in those with SVCAH. (In this assessment, we excluded hospitalizations for elective procedures). Figure 2 shows this relationship.

As for reasons for hospitalization in the case group (SVCAH and SWCAH), 55% of them were due to infectious processes (n = 28), abdominal infections (n = 12) being the most frequent, followed by respiratory infections (n = 10), sepsis (n = 3), renal infections (n = 2), neurological infections (n = 1) and other infectious causes (n = 1). In 37% of cases, hospitalization occurred due to dehydration (n = 19) and in the remaining 8% (n = 4) other causes (low weight, acute hemorrhagic edema of infancy, trauma). In the control group, the reasons for hospitalization for infections were reported in 83% of patients, with respiratory infections being the most frequent (n = 6), followed by gastroenteritis (n = 1), neurological infection (n = 1) and other infectious causes (face cellulitis and bacterial superinfection in chickenpox) (n = 2). In the remaining hospitalizations, 17% (n = 2) were referred to as other causes and the reason for hospitalization was arthralgia.

The median number of hospitalizations was 2.0 in the case group and 1.5 the control group. The mean was 4.4 in the case group, versus 3.3 in the control group.

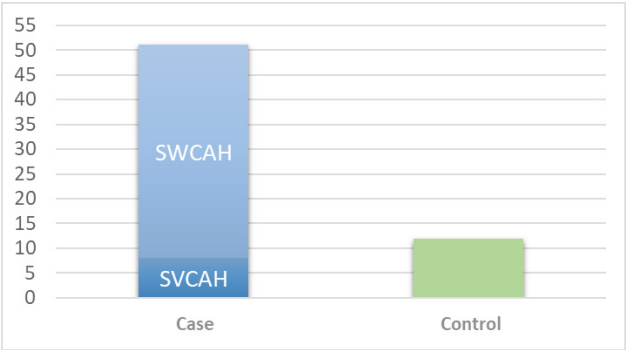


Figure 2. Number of hospitalizations in the case (SVCAH + SWCAH) and control groups

5. Discussion

Few studies have assessed the adrenomedullary function in patients with CAH. Ours is the first to assess a broader pediatric age group and compare it to a control group. In the group of patients with CAH, there are approximately twice as many patients with the salt-wasting form (65%) as compared to simple virilizing (34%). This proportion shows the higher prevalence of the most severe form of presentation of the disease, similar to the literature [1,4].

The control group of the study was composed of patients with primary hypothyroidism, whether overt or subclinical (only elevated TSH values), who presented serum fT4 values within the normal range at the time of assessment. The group was selected to be the control group, because, while clinically and laboratory compensated, they do not generally present problems that can interfere with the results. And, since collections are collected from exams regularly, their inclusion in the study would not cause extra procedures. None of the 18 patients with subclinical hypothyroidism used levothyroxine at the time of the study as they all maintained serum fT4 values within the normal range.

In our control group, we found no differences in metanephrine and normetanephrine serum values between patients with untreated subclinical hypothyroidism and those with overt hypothyroidism treated with levothyroxine. In a previous study by Kim et al, there was no difference in catecholamine levels between patients with congenital hypothyroidism and euthyroid controls [7].

In the case group, both patients with SWCAH and those with SVCAH showed plasma metanephrine and normetanephrine levels within the normal reference range, with no statistically significant difference. This differs from three published studies that studied adrenal medullary function in patients with equivalent epidemiological

 DOI: https://doi.org/10.30564/jer.v2i1.2574

profiles.

Kim and colleagues [7] evaluated adrenal medullary activity in 21 newborns, 9 with CAH (case group) and 12 with congenital hypothyroidism (control group). Newborns with CAH showed significantly lower plasma levels of adrenaline than controls, indicating that adrenomedullary function may be impaired during fetal development and at birth.

Merke *et al* [8] evaluated 97 individuals, 38 of whom had CAH, 39 healthy controls, and 20 who had undergone bilateral adrenalectomy. Significantly lower plasma levels of epinephrine, metanephrine, and normetanephrine were observed in patients with CAH and in adrenalectomized controls in relation to healthy individuals.

Lisá *et al* [9] studied 37 patients with moderate or severe SWCAH and found low plasma levels of metanephrine and normetanephrine[9]. It should be noted in our study that even patients with poor disease control had metanephrine and normetanephrine levels within the normal range.

In both the SVCAH and SWCAH subgroups, there was a greater variation in metanephrine levels than in normetanephrine, although they remained within the reference range, as shown in Figure 1(c).

When assessing the number of hospitalizations, we observed a 4.2-fold higher rate in the case group compared to the control group, being the main cause as infectious pathologies, among them gastroenteritis, followed by dehydration. In addition to a trend towards a higher number of hospitalizations in patients with SWCAH, when compared to SVCAH. This shows a greater number of clinical complications by patients with the most severe form of the disease. Similarly, Merke et al. described that the number of hospitalizations and hospitalizations in patients with CAH was significantly higher in the group with SWCAH than in those with SVCAH in the first 2 years after diagnosis, and that adrenal crisis was the main cause of hospitalization [8,10].

Probably, patients with CAH, when submitted to stressful situations, such as fever, infections and other clinical complications, present a deficit in the metabolic and hormonal response, reflecting the greater number of hospitalizations in this group [4,10,11].

In addition to another factor that may have influenced the number of hospitalizations between the case and control groups, it is the age group difference between them. The population of the case group in our study had a median age below that of the control group, 89 months and 140 months, respectively, the age group most prone to infectious processes. Moreira and Novaes [12] showed the highest number of hospitalizations in Brazil, in the age group of children under one year old, from 1 to 4 years old, and from 5 to 9 years old in relation to the age group of 10 to 19 years old12.

Finally, our study assessed plasma metanephrine and normetanephrine levels in a very broad age group of patients (up to 20 years old). Perhaps a study with a larger sample is needed to evaluate specific pediatric age groups, such as the neonatal period, childhood, and adolescence. This would provide a broader view of adrenal medullary function in the pediatric population and, consequently, broaden our understanding of the pathophysiology of adrenal medullary impairment in individuals with CAH.

We conclude that the plasma levels of metanephrine and normetanephrine in patients with CAH in this study were within normal reference range. There was no significant difference in metanephrine or normetanephrine between the case group (SVCAH and SWCAH) and the control group. However, the number of hospitalizations in the patients with CAH was much higher than in the group with hypothyroidism, and patients with SWCAH had a higher number of hospitalizations than those with SVCAH, reflecting the broad spectrum of disease severity.

References

[1] Merke D. P., Bornstein S. R. Congenital adrenal hyperplasia. The Lancet, 2005, 36: 2135-2136.

[2] Ambroziak U., Bednarczuk T., Malinowska M. G., Malunowicz E. M, Grzechocinska B., Kaminski P., Przedlacki J., Andziak E. B. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency - management in adults. Endokrynologia Polska/Polish Journal of Endocrinology, 2010, 61(1): 142-155. .

[3] Auchus R. J. Management of the adult with congenital adrenal hyperplasia. International Journal of Pediatric Endocrinology, 2010, 2010: 9. Article ID 614107

[4] Pang S., Clark A. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: newborn screening and its relationship to the diagnosis and treatment of the disorder. Molecular Genetics and Metabolism Reports. Screening, 1993, 2: 105-139.

[5] Bradfort, L.T. Newborn screening for congenital adrenal hyperplasia. Endocrinology Metabolism Clinics North America, 2001, 30: 15-30.

[6] Brasil. Ministério da Saúde. Portaria SAS/ MS no 16, Brasília, 15 Janeiro 2010. Protocolo Clínico e Diretrizes - Hiperplasia Adrenal Congênita. Disponível em: www.saude.gov.br. Access in: 20 mar 2018.

[7] Kim M. S., Lienhard-Ryabets A., Bali R., Lane C. J., Park A. H., Hall S., Geffner M. E. Decreased

DOI: https://doi.org/10.30564/jer.v2i1.2574

Adrenomedullary Function in Infants With Classical Congenital Adrenal Hyperplasia. Journal of Clinical Endocrinology and Metabolism, 2014, 99(8): 1597-1601.

[8] Merke D.P., Chrousos G P, Eisenhofer G., Weise M., Keil M. F.; Rogol A. D.; Van WYK J. J., Bornstein S. R. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hidroxilase deficiency. New England Journal of Medicine, 2000, 343: 1362-1368.

[9] Lisa, L., Bilelkl, R., Vrzalova, Z. Congenital adrenal hyperplasia and the function of adrenal medulla. Hormone Molecular Biology and Clinical Investigation, 2010, 2(2): 245-248.

[10] Bornstein, S.R., Tajima, T., Eisenhofer, G., Haindan A., Aguelera, G. Adrenomedullary function is severely impaired in 21/hydroxylase/deficient mice. The FASEB Journal, 1999, 13: 1185-1194.

[11] Kronenberg, H. M., Melmed, S., Polonsky, Larsen, P. R. Williams Tratado de Endocrinologia. Tradução da 11a edição. Editora Elsevier, 2010.

[12] Moreira, M. L., Novaes, H. M. D. Internações no Sistema de Serviços Hospitalares, SUS e não SUS: Brasil, 2006. Revista Brasileira de Epidemiologia, 2011, 14(3): 411-422.

 DOI: https://doi.org/10.30564/jer.v2i1.2574