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ORIGINAL ARTICLE

Vitamin D Deficiency in Children with Congenital Adrenal Hyperplasia (CAH): a Cross-sectional Study from Tertiary Health Care Center of Pakistan

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ABSTRACT

Objective: To find out vitamin D deficiency (VDD) in children with CAH who were receiving steroids. Recent studies show that steroids enhance the inactivation of 25(OH) D by up-regulating 24-hydroxylase activity.

Study Design: Prospective descriptive cross-sectional study using non-probability consecutive sampling technique.

Place and Duration of Study: The outpatient department of pediatric endocrinology at National Institute of Child Health (NICH) Karachi, during 2017-2018.

Material and Methods: Children of either gender, aged 5 to 20 years of age, who were the known case of CAH and on the treatment of hydrocortisone and fludrocortisone for more than 6 months were included. The study was approved by the Institutional Ethical Review Board. Written informed consent was obtained from the guardian or the patient. The data was analyzed using SPSS version 22.0. After stratification, a chi-square test was used to find the association between vitamin D deficiency and the relevant variables, with statistical significance defined at a P-value of ≤0.05.

Results: Fifty-four per cent (n=81) of patients with CAH had VDD. The mean age of patients was 11.85 ± 3.32 years. The mean duration of CAH and vitamin D levels were 11.85 ± 3.32 years and 14.6 ± 5.65 ng/mL, respectively. VDD was found more in female patients (55.56%, n=45), patients from low socioeconomic status (52.78%, n=38), those residing in urban area (53.40%, n=55) and children with inadequate sun-exposure (65.62%, n=63).

Conclusion: The frequency of VDD in children with CAH is comparable to our general population. Adolescent girls, patients from lower socioeconomic status, patients from the urban areas and patients with inadequate sun exposure are prone to VDD.

Key Words: *Congenital adrenal hyperplasia, Vitamin D deficiency, pediatric endocrine disorder*

Abbreviations: CAH: Congenital adrenal hyperplasia**;** VDD: vitamin D deficiency**;** BMI: body mass index**;** 25(OH)D: 25-hydroxyvitamin D

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an autosomal recessive endocrine disorder caused by single-gene mutations on chromosome 6, resulting in impaired adrenal steroidogenesis and

deficiencies in cortisol and/or aldosterone.[1, 2] This deficiency disrupts negative feedback inhibition, leading to adrenal gland hyperplasia and excessive androgen secretion. The imbalance in hormone levels can cause a range of symptoms, including abnormal genitalia, puberty irregularities, severe salt-wasting, and potentially fatal electrolyte disturbances.[3,4] The most common form of CAH (95% of cases) involves a defect in the 21-hydroxylase gene on 6p21.3, part of the HLA complex. Diagnosis is often confirmed through genetic analysis, but this is unnecessary with classic clinical and laboratory findings, which include hypoglycemia, low serum sodium and potassium, and elevated 17α-hydroxyprogesterone.[5-8] The prevalence of congenital adrenal hyperplasia (CAH) varies geographically, with a high incidence among Native Americans and Yupik Eskimos in the United States, while data from countries like Pakistan are lacking, and no screening programs exist there.[5,6,9]

Children with congenital adrenal hyperplasia (CAH) start glucocorticoid treatment at diagnosis, which may be at birth or even prenatally. While improved monitoring has reduced glucocorticoid doses and lessened cushingoid features, the treatment still affects growth, leading to shorter stature and delayed bone maturation. Many adults with CAH have heights 1.0–1.5 standard deviations below the average.[10-12]

Vitamin D deficiency (VDD) is a widely under recognized epidemic affecting numerous pediatric populations and has emerged as a global health concern, impacting an estimated one billion individuals globally.[13,14] VDD is on the rise in the Pakistani pediatric population as well.[15,16] Prolonged and severe form of VDD in infants and children leads to bone deformities resulting from inadequate mineralization, such as rickets. Milder form that is vitamin D insufficiency hinders the achievement of optimal peak bone mass in youth, potentially contributing to an elevated risk of fractures in later life. New research indicates that steroids boost the inactivation of 25(OH) D by increasing the activity of 24-hydroxylase.[17,18] The reported prevalence of VDD among patients with congenital adrenal hyperplasia (CAH) is 51.9 %.[19]

In Pakistan, a low-middle-income country grappling with nutritional deficiencies, VDD in children with CAH exacerbates health challenges. CAH patients, already at risk of growth issues and bone metabolism disorders due to steroid use, face heightened risks of short stature, osteoporosis, and fractures. This study addresses the gap in localized data by assessing vitamin D levels in CAH patients, examining socioeconomic status, habitat and sun exposure. By offering insights into these factors, the research aims to enhance understanding, inform clinical guidelines, and advocate for routine monitoring and targeted supplementation.

MATERIAL AND METHODS

Study Design and Participant: This descriptive cross-sectional study took place at the pediatric endocrinology outpatient department of the National Institute of Child Health (NICH) Karachi, spanning the period from 2017 to 2018. Inclusion criteria were the children of either gender who were the known case of CAH and on the treatment of hydrocortisone and fludrocortisone for more than 6 months. As most of the cases in our setup are diagnosed late except for a few cases diagnosed in early infant age, so to avoid any confounding effect, children aged 5 to 20 years were included. Children with any other endocrinological disorder like hyperpara-thyroidism, patients with deranged renal function with CAH and patients having a history of taking steroids for any disease through any route other than CAH were excluded from the study as were the patients whose parents refused to participate in the study.

Ethical consideration: The study was approved by the Institutional Ethical Review Board of the National Institute of Child Health Karachi. Written informed consent was obtained from the guardian (either the mother or father) or the patient, depending on the patient's age. For patients under 12 years old, only the guardian or parent signed the consent form. For children aged 12 to 18 years, both a parent or guardian consent form and a patient assent form were signed. For patients above 18 years, consent was obtained directly from the patient. All participants were provided with a comprehensive explanation of the study's purpose and procedures, and confidentiality was ensured. Patients and parents/guardians had complete autonomy to withdraw their participation

in the study at any juncture, with the assurance that such withdrawal would not impact their treatment or follow-ups.

Sampling and sample size: The sampling technique for our study was non-probability consecutive sampling. The sample size was n= 150 CAH patients. The sample size was calculated by taking the expected prevalence of vitamin D deficiency in CAH patients' as 51.9%[19], Confidence level: 95% and margin of error as 8%. We used formula: $n= Z^2 \times P \times (1-P)/ d^2$

Where:

n is the required sample size.

Z is the Z-score corresponding to the desired confidence level.

P is the hypothesized frequency (expressed as a decimal).

d is the margin of error (expressed as a decimal).

For a 95% confidence level, the Z-score is approximately 1.96.

Given:

Margin of error (d) = 8% = 0.08

Confidence level (Z) = 95% = 1.96

Hypothesized frequency (p) = 51.9% = 0.519

So, by using above formula:

$n= (1.96)^2 \times 0.519 \times (1-0.519) / (0.08)^2$

n= 3.842 x 0.519 x 0.481/0.0064

n=0.959/0.0064

n= 149.844

Therefore, the estimated sample size for our cross-sectional study was taken as 150 patients with CAH.

Diagnosis of vitamin D deficiency and operational definitions: Serum 25-hydroxyvitamin D (25-OHD) concentration was performed by the method of ELISA with ELISA READER STATFAX. Vitamin D deficiency was defined as a serum 25-OHD level of < 20 ng/mL (25 OHD <20 ng/ml). Exposure to sunlight was considered adequate if children had exposure of > 30 min a day for 5 days a week and for equal to or greater than 6 months otherwise, it was labeled as inadequate. Socioeconomic status was categorized into three classes: lower socioeconomic class for those with an income of less than PKR 30,000 per month, middle socioeconomic class with an income between PKR 30,000 and PKR 100,000 per month, and upper socioeconomic class with an income above PKR 100,000 per month.

Statistical analysis: Pre-designed proforma was used to record the findings of enrolled patients by a trained team member. Demographic characteristics including age, gender, weight, height, BMI, duration of CAH were recorded. The data was analyzed on SPSS version 22.0. The age, weight, height, BMI, vitamin D level, duration of CAH was expressed in mean±SD values. Gender, residence, sunlight exposure, socio-economic status, vitamin D deficiency were presented as frequencies along with percentages. Stratification was carried out by categorizing participants into different groups based on effect modifiers such as age, gender, BMI, residence, socioeconomic status, and sunlight exposure. We created strata by dividing participants into appropriate subgroups, including age groups, gender categories, BMI categories (normal or not normal), urban or rural residence, socioeconomic status categories, and levels of sunlight exposure. We then performed the chi-square test post-stratification within each of these subgroups to assess the impact of each effect modifier on the outcome variables. This method allowed us to control for confounding effects and examine the association between vitamin D deficiency and congenital adrenal hyperplasia more accurately. Statistical significance was determined with a P-value of ≤0.05.

RESULTS

The mean age of the patients in our study was 11.85 ± 3.32 years. The mean height of our patients was 0.8 ± 0.1 meters, mean weight was 35.97 ± 10.89 kilograms and mean BMI was 19.52 ± 5.89 kg/m^2. The mean duration of CAH was 11.85 ± 3.32 years and vitamin D level was 14.6 ± 5.65 ng/mL. Descriptive statistics of all quantitative variables are calculated in terms of mean and standard deviation in table 1.

Most patients in our study were from low socio-economic status i.e. 72 (48%) out of 150 CAH patients. The proportion of patients from the urban areas was higher in our study as compared to rural areas, i.e. 103/150 (68.67%) patients. The percentage of patients with inadequate sun

exposure was higher than the patients with adequate sun exposure (64 % vs. 36 %), table 2.

TABLE 1: Descriptive statistics of quantitative variables (n=150)

Variables	Mean	Std. Deviation
Age (years)	11.85	3.32
Height(m)	0.80	0.10
Weight(kg)	35.97	10.89
BMI (kg/m^2)	19.52	5.89
Duration of CAH (years)	11.85	3.32
Vitamin D level (ng/mL)	14.60	5.65

TABLE 2: Frequency distribution of socioeconomic status, residence and inadequate sun exposure (n=150)

Variables		Fre-quency	Percen-tage
Socio-economic status	Lower class	72	48.0
	Middle class	51	34.0
	Upper class	27	18.0
	Total	**150**	**100.0**
Residence	Urban	103	68.67
	Rural	47	31.33
	Total	**150**	**100.0**
Inadequate sun Exposure	Yes	96	64.0
	No	54	36.0
	Total	**150**	**100.0**

In our study, females were found to be affected with CAH in a frequency slightly higher than the males with a male to female ratio of 1:1.2, **Graph I.** We found that 81(54%) patients with CAH had vitamin D deficiency and 69(46%) had sufficient levels of vitamin D, fig 2.

Stratification for vitamin D deficiency in CAH patients for age, BMI, gender, residence, sunlight exposure, socioeconomic status, duration of CAH is done by applying the chi-square test. p-value of <0.05 is taken as significant, table 3.

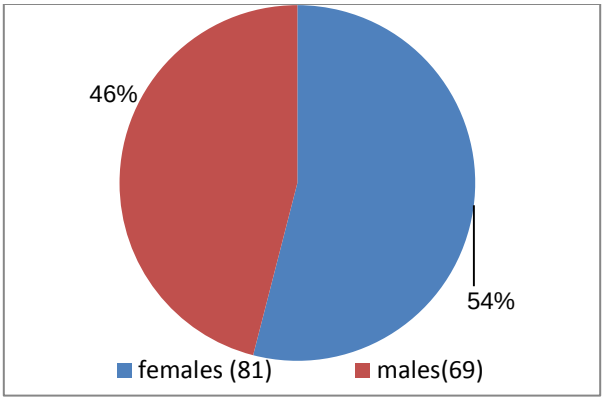

Fig 1: Frequency distribution of gender (n=150)

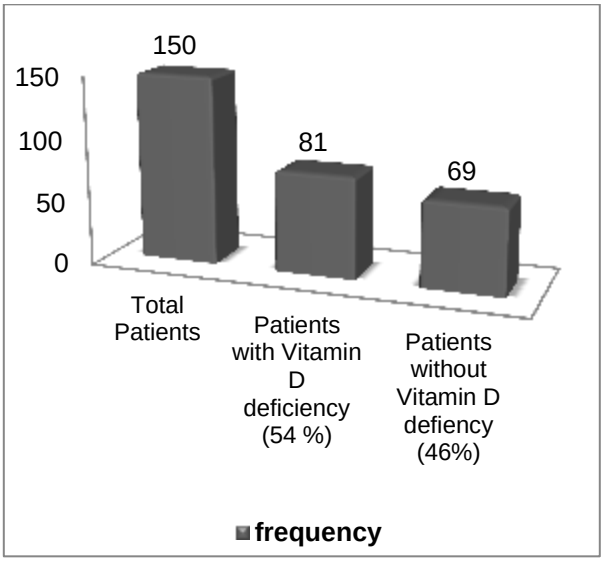

Fig 2: Frequency Distribution of Vitamin D Deficiency among CAH Patients (n=150)

TABLE 3: Stratification for vitamin D deficiency with respect to age groups, gender, BMI, residence, socioeconomic status and inadequate sun exposure (n=150)

Variable		Vitamin D Deficiency		Total
		No Frequency (%)	Yes Frequency (%)	Frequency (%)
Age group	<12 years	42 (45.16)	51 (54.84)	93 (100)
	12 or more years	27 (47.37)	30 (52.63)	57 (100)
	Total	69 (46)	81 (54)	150 (100)
	p- value		0.79	
Gender	Female	36 (44.44)	45 (55.56)	81 (100)

	Male	33 (47.83)	36 (52.17)	69 (100)
	Total	69 (46)	81 (54)	150 (100)
	p-value		0.67	
Normal (<25BMI)	No	39 (39.39)	60 (60.61)	99 (100)
	Yes	30 (58.82)	21 (41.18)	51 (100)
	Total	69 (46)	81 (54)	150 (100)
	p- Value		0.024*	
Residence	Rural	21 (44.68)	26 (55.32)	47 (100)
	Urban	48 (46.60)	55 (53.40)	103 (100)
	Total	69 (46)	81 (54)	150 (100)
	p-value		0.82	
Socioeconomic Status	Lower	34 (47.22)	38 (52.78)	72 (100)
	Middle	21 (41.18)	30 (58.82)	51 (100)
	Upper	14 (51.85)	13 (48.15)	27 (100)
	Total	69 (46%)	81 (54)	150 (100)
	p-value		0.64	
Inadequate Sun exposure	Yes	33 (34.38)	63 (65.62)	96 (100)
	No	36 (67.67)	18 (33.33)	54 (100)
	Total	69 (46)	81 (54.0)	150 (100)
	p- value		0.0001*	

*p-value significant <0.05

DISCUSSION

The relationship between steroid use and VDD is well-documented. Skversky *et al* through the National Health and Nutrition Examination Survey (2001-2006), found that individuals who chronically use steroids are at a higher risk of VDD compared to the general population.[17] Further research has confirmed that patients receiving steroids, especially higher doses of glucocorticoids, tend to have lower levels of vitamin D.[20,21] In addition to the deactivation of vitamin D by steroids by enhancing the activity of 24-hydroxylase, CAH patients are more susceptible to VDD due to decreased sun exposure—often a result of the psychological effects of chronic illness, which is exacerbated if genital surgery and sex determination are delayed. Furthermore, intercurrent illnesses can lead to reduced nutritional intake of provitamin D substrates, coupled with a need for higher doses of steroids.

Our study highlights a significant prevalence of VDD among children and adolescents with congenital adrenal hyperplasia (CAH). We found that approximately 54% of the patients with CAH aged between 5 and 20 years who were followed up at our pediatric endocrinology clinic had VDD. This prevalence is similar to the 51.9% reported by Demirel *et al* in a study conducted in Turkey on children with CAH, indicating a higher rate of VDD in these patients compared to the general pediatric population of the same age.[19] Notably, in our study, even among those classified as "not deficient," around half were actually insufficient in their vitamin D levels due to our study's binary outcome measure of "deficient" or "not deficient." Interestingly, our findings are comparable to those of Moorani *et al* who studied VDD in the general pediatric population where routine vitamin D prophylaxis is not practiced. This suggests that in our setting, children with CAH do not have a significantly higher risk of VDD compared to their peers.[16]

We observed that girls were more frequently vitamin D deficient compared to boys, and the pre-pubertal age group had higher rates of VDD than the pubertal age group. This aligns with global concerns about vitamin D deficiency among children and adolescents, which has led many countries to implement vitamin D prophylaxis as part of their national health policies.[22] For example, Turkey begins vitamin D supplementation in the neonatal stage due to the high prevalence of deficiency.[23,24] However, many developing countries lack such systematic policies, resulting in notably higher VDD rates compared to developed nations.

In our study, we found that over 60% of patients had inadequate sun exposure, which significantly contributed to vitamin D deficiency (VDD). As patients age, their reluctance to adhere to medication regimens increases, further elevating their risk of developing illnesses and VDD. The prevalence of VDD was notably higher among urban-dwelling patients compared to those from rural areas, likely due to a more sedentary lifestyle and limited sun exposure in urban environments. Interestingly, VDD was less common in CAH patients with a normal BMI, but it remained prevalent among children from lower socioeconomic backgrounds, even if their BMI was normal or near normal. This suggests that inadequate intake of vitamin D-rich foods, reflecting broader nutritional challenges, is a significant factor contributing to VDD in developing countries like Pakistan.

Strengths and limitations of the study: This study included a larger cohort of patients with congenital adrenal hyperplasia (CAH) compared to previous studies, allowing for more comprehensive data analysis and increased statistical power, which enhances the reliability of our findings. Although our study is single-centered, the substantial number of participants provides valuable insights into the prevalence of vitamin D deficiency in children with CAH. This large sample size, drawn from a high-volume pediatric endocrinology clinic, also captures a diverse range of clinical presentations, contributing to the generalizability of our findings. However, to draw more definitive conclusions, multicenter, large-scale studies with consistent standards are warranted.

A significant limitation of our study was its single-center design and the absence of a control group, which limits the generalizability of our results to other settings and makes direct comparisons with healthy children challenging. Nonetheless, leveraging recent vitamin D research on healthy children within our province enabled us to contextualize and interpret our findings. Furthermore, patients were predominantly recruited from the outpatient department, where most were under regular follow-up care. This facilitated early detection and management of vitamin D deficiencies. Additionally, the presence of a nutrition specialist in the outpatient department provided counseling on dietary modifications, contributing to the maintenance of normal vitamin D levels among the patients.

CONCLUSION

The frequency of VDD in children with CAH is comparable to our general population. Adolescent girls, patients from lower socioeconomic status, patients from the urban areas and patients with inadequate sun exposure are more prone to VDD. Hence, special emphasis should be placed on monitoring these patients closely, and it is imperative to conduct early vitamin D testing and administer supplementation as necessary.

Recommendation: We recommend that all children with CAH should be followed on a regular basis at the pediatric endocrinology clinics and checked by a qualified pediatric endocrinologist. Early vitamin D testing and dietary counselling by a qualified dietician should be done in high-risk patients like adolescent girls, patients from lower socioeconomic status, patients from the urban areas and patients with inadequate sun exposure due to any reason.

Conflict of interest: The authors declare that they have no conflict of interest

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