ORIGINAL ARTICLE

Bone Mineral Density in Children with Congenital Adrenal Hyperplasia

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Abstract

Background: Treatment in all forms of Congenital Adrenal Hyperplasia (CAH) includes lifelong replacement of steroids. Steroids have an impact on bone health in multiple ways and are known to cause osteoporosis when given in high doses or for a longer duration.

Objective: To evaluate bone mineral density (BMD), using dual-energy X-ray absorptiometry (DEXA) scan in children with CAH taking long-term steroids presenting in the pediatric endocrinology ward of National Institute of Child Health, (NICH) Karachi, Pakistan.

Materials and Methods: This cross-sectional study was performed at the Department of Pediatric Endocrinology, National Institute of Child Health, Karachi from October 2021 to July 2022. A total of 47 diagnosed cases of CAH taking steroids for more than 5 years were enrolled. Assessment of BMD was done using a DEXA scan. Lumbar spine BMD was done and Z-score was modified for height for age z-score. The dose of steroids and duration was calculated.

Results: Out of 47 patients, low BMD was observed in 8 (17.02%) patients. Individuals with low BMD had significantly higher median duration, (p=0.017), dose (p=0.003), and median alkaline phosphate level (p=0.036),) but low median BMD value (p=0.009) and z score (p<0.001) than normal BMD individuals. Although median bone age (p=0.009) was appropriate for chronologic age in low BMD patients. A moderate negative significant correlation was observed between z score and age (rho=-0.319, p=0.029), z-score and duration of steroid treatment (rho=-0.364, p=0.012), z score and alkaline phosphate (rho=-0.466, p=0.001), z score and bone age (rho=-0.378, p=0.009).

Conclusion: Low BMD was observed in 17% of children on the DEXA scan. Moreover, these individuals had significantly higher median average duration and dose of hydrocortisone.

Keywords: *Bone Mineral Density (BMD), Congenital Adrenal Hyperplasia (CAH), Dual Energy X-Ray Absorptiometry (DEXA), steroids.*

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of rare genetic disorders characterized by a deficiency of enzymes, responsible for the formation of cortisol, aldosterone, or both [1]. Its incidence is 1:10000 to 1:20000 births [1]. 21-hydroxylase deficiency is the most common genetic mutation and is found in more than 90% of cases. Disease presentation is variable depending on the severity of the enzymatic defect. Phenotypically it is divided into classic and non-classic forms [2]. The error in the synthetic pathway causes a deficiency of cortisol and sometimes aldosterone with the excess synthesis of androgen, resulting in salt wasting and ambiguous genitalia in female born. Treatment in all forms of CAH includes life-long replacement of steroids along with mineralocorticoids when needed [3].

Steroids have an impact on bone health in multiple ways and are known to cause osteoporosis when given in high doses or for a longer duration. Steroids tend to reduce the gut's ability to absorb calcium [4], altering the balance between osteoblasts and osteoclasts cells [5] while androgens have a good effect on bone health as they cause stimulation of bone-forming cells [6]. Patients with CAH assume a lifelong glucocorticoid therapy which may increase the risk of osteoporosis and non-traumatic bone fractures with significant morbidity and reduction in quality of life. So, the bone health of these patients needs monitoring.

Multiple studies have been conducted on children with CAH in different parts of the world which have shown variable results of steroid effect on BMD but no such study has been conducted in Pakistan so far. Therefore, the present study aimed to know the effect of steroids on bone health in children with CAH. The objective of this study was to evaluate bone mineral density (BMD), using a dual-energy X-ray absorptiometry (DEXA) scan in children with congenital adrenal hyperplasia (CAH) taking long-term steroids.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Endocrinology Department of "The National Institute of Child Health (NICH)", Karachi, Pakistan from October 2021 to July 2022. The sample size was calculated using WHO software of sample size calculation, using the mean BMD of patients with CAH at the spine as 0.96 g/cm2 [4]. Ethical approval was obtained from the NICH before conducting the study Ref No IERB-31/2021.

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Received: March 05, 2023; Revised: May 08, 2023; Accepted: June 05, 2023
DOI: https://doi.org/10.37184/lnjpc.2707-3521.5.43

Signed informed consent was taken from the parents/ guardian before enrolment of the children.

Inclusion criteria: All diagnosed cases of CAH (diagnosed clinically and on biochemical analysis), of either gender taking steroids for more than 5 years were consecutively enrolled. Exclusion criteria: Children whose hepatic or renal disease affects bone mineral density, as well as those with other endocrine issues, chronic illness, or other conditions, were excluded. As patients with CAH have a deficiency in steroids so synthetic steroids are given in physiologic doses and this study aimed to see the risk of osteoporosis in children taking steroids in physiological doses for longer duration that do not require any supplements with vitamin D so we excluded patients already taking calcium or vitamin D supplementations.

Detailed medical history of each patient including age, and duration of steroid treatment was obtained. All the children enrolled in the study were taking hydrocortisone as glucocorticoid replacement therapy and their dose was calculated. Anthropometric measures including height, weight, surface area, and BMI were measured. The blood sample of each child was collected for the evaluation of calcium, phosphorus, vitamin D levels, and alkaline phosphatase as markers of bone turnover. Bone age was calculated using the "Greulich and Pyle method" by performing X-rays of the left hand and wrist. A "Dual-energy X-ray absorptiometry (DEXA)" scan for BMD was done using a Hologic QDR machine. Using paediatric software, "total body bone mineral density (TBMD)" Z-scores were computed, and TBMD Z-scores were calibrated for height for age Z-scores. All the cases underwent the same laboratory and machine. Z-score between -1 to -2.5 SD was defined as osteopenia and below -2.5 SD as osteoporosis while Z-scores above -1 SD were considered normal. DEXA was used to evaluate the BMD of the lumbar spine (L1-L4). Patient data were compared with standard data from the program and absolute values were transformed to Z-scores. DEXA scan assessed BMD utilizing spectral imaging. A special proforma was formed to record study information.

For statistical analysis, "Statistical Package for Social Sciences (SPSS)", version 24.0 was used. Quantitative variables like age, weight, height, BMI, surface area, age at the time of diagnosis, age of starting of the treatment, average dose of hydrocortisone, duration of steroids treatment, and serum calcium, serum phosphorus, alkaline phosphate, vitamin D3, bone age, lumbar spine, and z-score were expressed as median and interquartile range. Frequencies and percentages were reported for qualitative variables like gender, residence, clinical manifestation, and low BMD. The median difference of quantitative variables with low BMD was explored using the Mann-Whitney-U test whereas the chi-square/Fisher-exact test was applied to see the association of low BMD with categorical variables. In addition, Spearman's correlation test was applied to see the relationship of the z-score with predicting variables. The p-value ≤0.05 was considered significant.

RESULTS

Of 47 patients, the median age was 9.50 (7.0-14.0) years while 27 (57.4%) patients were aged ≤10 years and 20 (42.6%) with >10 years of age. Females were predominantly higher as compared to males, *i.e.* 34 (72.3%) vs. 13 (27.7%). The median weight, height, and BMI were 35.0 (19.0-40.0) kg, 134.0 (113.0-140.0) cm, and 18.4 (15.7-21.6) kg/m2 respectively. There were 27 (57.4%) patients with <18.5 kg/m2 BMI. The residents of 36 (76.6%) patients were from Sindh followed by Baluchistan 9 (19.1%) and Khyber Pakhtunkhwa 2 (4.3%). Clinical manifestation showed that bone pain was observed in 5 (10.6%) while bone fracture was reported in none of the patients.

The frequency of low BMD was observed in 8 (17.0%) children. The median age was significantly higher in low BMD children than those with normal BMD, 16.5 (7.8-19.3) years vs. 8 (7-12) years (p=0.026). However, gender (p=0.085), BMI (p=0.147), residence (p=0.229), and clinical manifestation (p=0.196) were insignificantly associated with low BMD as shown in Table **1**.

Table 1: Comparison of characteristics of the children with Low Bone Mineral Density (N=47).

Variables		Total (n=47)	Low BMD		P-value
			Yes (n=8)	No (n=39)	
Age (years)	Median (IQR)	9.50 (7.0-14.0)	16.5 (7.8-19.3)	8 (7-12)	0.026
	≤10 years, n(%)	27 (57.4%)	2 (25.0%)	25 (86.2%)	0.057
	>10 years, n(%)	20 (42.6%)	6 (75.0%)	14 (13.8%)	
Gender	Male, n(%)	13 (27.7%)	0 (0)	13 (33.3%)	0.085
	Female, n(%)	34 (72.3%)	8 (100%)	26 (66.7%)	
BMI (kg/m^2)	Median (IQR)	18.4 (15.7-21.6)	22.8 (15.3-29.8)	17.9 (15.7-21.3)	0.147
	<18.5, n(%)	27 (57.4%)	2 (25.0%)	25 (64.1%)	0.062
	18.5-22.5, n(%)	10 (21.3%)	2 (25.0%)	8 (20.5%)	
	>22.5, n(%)	10 (21.3%)	4 (50.0%)	6 (15.4%)	
Clinical Manifestations	No symptoms, n(%)	42 (89.4)	6 (75.0%)	36 (92.3%)	0.196
	Bone Pain, n(%)	5 (10.6)	2 (25.0%)	3 (7.7%)	
	Bone Fracture, n(%)	0 (0)	0 (0)	0 (0)	

Mann-Whitney U test applied for the comparisons of the median (IQR)
Chi-square/Fisher-Exact test applied for categorical data

Table 2: Median difference of quantitative therapeutic variables with low BMD.

Variables	Total (n=47)	Low BMD		p-value
		Yes (n=8)	No (n=39)	
Surface Area, m^2	1.12 (0.77-1.23)	1.38 (0.77-1.48)	1.03 (0.77-1.20)	0.072
Age at the time of diagnosis, years	1 (0.20-4.00)	0.55 (0.10-6.25)	2.01 (0.20-4.01)	0.365
Age of starting of the treatment, years	1 (0.20-4.00)	0.55 (0.10-6.25)	2.01 (0.30-4.01)	0.308
The average dose of glucocorticosteroids, mg/m^2/day	12 (9-15)	13.01 (10.01-16.01)	9.01 (4.25-10.01)	0.003
Duration of steroids treatment, years	6 (5-11)	13.50 (6.75-16.50)	6.01 (5.01-10.01)	0.017

Data is expressed as median (inter-quartile range). Mann-Whitney U test applied

Table 3: Comparison of median (IQR) radiological and biochemical parameters of Children (N=47).

	Total (n=47)	Low BMD		p-value
		Yes (n=8)	No (n=39)	
Serum Calcium (mg/dl)	9.6 (9.2-10.20)	9.6 (8.9-10.3)	9.6 (9.5-10.05)	0.749
Serum Phosphorous (mg/dl)	4.30 (4.10-4.90)	4.6 (4.1-5.0)	4.3 (4.1-4.8)	0.395
Alkaline Phosphate (U/L)	194.0 (100-230)	225 (167-282)	170 (93-230)	0.036
Vitamin D3 (ng/ml)	19.46 (15.6-25.80)	19.81 (17.39-20.79)	18.70 (14.9-26.5)	0.923
Bone Age (years)	12.0 (8.0-15.5)	16.5 (10.8-19.3)	12.01 (8.0-14.0)	0.009
Lumbar Spine BMD (g/cm2)	0.69 (0.56-0.84)	0.51(0.37-0.64)	0.70 (0.58-0.85)	0.009
Z-score	0.30 (-0.90-0.90)	-1.6 (-1.8--1.6)	0.50 (-0.30-1.0)	<0.001

Data is expressed as median (inter-quartile range). Mann-Whitney U test applied; BMD: Bone mineral density

The median age at the time of diagnosis was 1 (0.20-4.00) years, the age of starting of treatment was 1 (0.20-4.00) years, the average dose of hydrocortisone was 12 (9-15) years and the duration of steroids treatment was 6 (5-11) years. The median average dose of hydrocortisone was significantly high among patients with low BMD than those with normal BMD, 13.01 (10.01-16.01) mg vs. 9.01 (4.25-10.01) mg, p=0.003. The median duration of steroid treatment was significantly higher among patients with low BMD than those with normal BMD, 13.50 (6.75-16.50) years *vs* 6.01 (5.01-10.01) years, p=0.017 as shown in Table **2**.

The median serum calcium level was 9.6 (9.2-10.20) mg/dl, serum phosphate level was 4.30 (4.10-4.90) mg// dl, alkaline phosphate level was 194.0 (100-230) U/L, vitamin D3 level was 19.46 (15.6-25.80) ng/ml, bone age was 12.0 (8.0-15.5) years, lumbar spine was 0.69 (0.56-0.84) g/cm2, and z score was 0.30 (-0.90-0.90).

Eight children had low BMD, of which, 6 (75%) had aged more than fifteen years. All of them were females. Two (25%) were seventeen years, 2 (25%) were twenty years, and 2 (25%) had sixteen years of age. Moreover, the majority of them had a duration of glucocorticoid treatment of more than ten years, *i.e.* 6 (75%). Further data revealed that calcium and phosphorus level was normal, however, vitamin D level was low in most of the children. The median alkaline phosphate level was significantly higher among patients with low BMD than those with normal BMD, *i.e.* 225 (167-282) U/L vs. 170 (93-230) U/L (p-value 0.036). Table **3** is shows comparisons of radiological and biochemical profiles concerning BMD levels.

A moderately negative significant correlation was observed between Z-score and age (rho=-0.319, p=0.029), Z-score and duration of steroid treatment (rho=-0.364, p=0.012), Z-score and alkaline phosphate (rho=-0.466, p=0.001) and Z-score and bone age (rho=-0.378, p=0.009) as shown in Table **4**.

Table 4: Correlation of Z-score with baseline clinical and clinical parameters (n=47).

Variables	rho	p-value
Age, years	-0.319	0.029
BMI, kg/m^2	-0.075	0.618
Surface Area, m^2	-0.153	0.305
Age at the time of diagnosis, years	-0.03	0.840
Age of starting of the treatment, years	-0.038	0.798
The average dose of glucocorticosteroids, mg/m^2/day	0.105	0.482
Duration of steroids treatment, years	-0.364	0.012
Serum Calcium, mg/dl	-0.018	0.902
Serum Phosphorous, mg/dl	-0.273	0.064
Alkaline Phosphate, U/L	-0.466	0.001
Vitamin D3, ng/ml	-0.127	0.394
Bone Age, years	-0.378	0.009
Lumbar Spine, g/cm^2	-0.378	0.009

Spearman's correlation test applied

DISCUSSION

According to the current study findings, the frequency of low BMD was observed in 17.0% of patients with CAH. The findings of the current study showed that the median average dose and period of taking steroids were convincingly higher in patients with low BMD than in normal BMD. Furthermore, in the current study, individuals with low BMD had significantly lower BMD values and Z-scores for BMD when adjusted with height-for-age and bone age consistent with chronologic age. However, there was no difference in serum calcium

phosphorus, and vitamin D levels. A study analyzing BMD in patients with CAH was conducted in Egypt and revealed low BMD and high bone metabolism in CAH patients when compared to healthy individuals [7]. The same study showed that the variability was more eminent in patients having poor control and taking prednisolone as a replacement therapy. Another study showed that there is no difference in BMD in children having CAH when compared to controls [8]. The differences among different studies may be due to different subtypes of CAH, differences in severity and age of children, and variability of total dose and type of steroids used [7]. Researchers in the past have reported lower BMD in patients with CAH [4]. Some others have also reported decreased BMD in CAH children compared to controls [7] while Zimmermann *et al.* [9] found that higher hydrocortisone dose was associated with lower BMD values. The variations in age, the type and degree of 21-hydroxylase deficiency, the cumulative dosage, and the duration of the short- and long-acting glucocorticoids may be the cause of these inconsistencies between studies. Frey *et al.* in their study reported that taking prednisolone as steroid therapy results in markedly lower BMD in contrast to hydrocortisone [10]. The age at the time of diagnosis was 0.55 years for those with decreased BMD. Those with normal BMD aged 2.01 years. This could be explained as children of the first group having the severer form of CAH and they present earlier and need higher steroid doses.

In our study, a moderately negative significant correlation was noticed between the Z-score and age, Z-score and duration of steroid treatment, Z-score and alkaline phosphate, and Z-score and bone age of the patients. Throughout childhood, hormonal monitoring must be done carefully for prompt development and to avert the effects of excess androgens [11, 12]. Fleischman *et al.* [13] described that there was no association between current levels of androgen and glucocorticoids and BMD. BMD correlates more with total steroid dosages than with individual doses. These and other findings [13] therefore imply that a variety of parameters, including age, duration, and dose of medication, as well as the number of examined cases, affect the bone state in children with CAH [14].

A study done by de Almeida Freire *et al.* showed that individuals with CAH had higher BMD Z-scores when their bone ages were advanced [15]. This result is not unexpected given that higher bone age Z-scores suggest more androgen exposure, resulting in enhanced BMD by stimulating osteoblastic activity [16]. Advanced bone age is linked to excess androgen exposure which is not expected in children taking proper treatment for CAH however this may be attributable to various factors that may cause poor control including drugs compliance, parent's knowledge about the disease, availability of drugs, and affordability issues.

The results of the current study could be highlighted in light of a major limitation that this study did not include controls. The majority of the previously conducted studies on the topic have included non-CAH patients as controlled [4, 17, 18]. Moreover, certain important predictor variables were not studied in this study. As monitoring of 17-hydroxyprogesterone is required annually for children with CAH and kept 2-3 times of normal range, due to affordability issues we could not perform this test. However, it was done in very few numbers of patients that could not be generalized for overall study results. The sample size was also small. Despite these drawbacks, this study represents a substantial effort in documenting the results of low BMD in CAH patients who were undergoing treatment at tertiary care public hospital in Pakistan. It is suggested that more large-scale, multicenter analytical investigations be conducted to further verify the findings of this study.

CONCLUSION

Low BMD was observed in seventeen percent of children with CAH on a DEXA scan. Moreover, these individuals had significantly higher median average dose and duration of glucocorticoids.

ETHICAL APPROVAL

Ethical approval was obtained from the NICH before conducting the study Ref. No. IERB-31/2021. Signed informed consent was taken from the parents/guardian before enrolment of the children. All procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration.

CONSENT OF PUBLICATION

Written informed consent was taken from the parents/guardians of all the participants.

AVAILABILITY OF DATA

The authors confirm that data supporting the results of this study are available upon request to the corresponding author.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

AUTHORS' CONTRIBUTION

All authors contributed significantly to the study.

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