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# The Effect of Glucocorticoid Therapy on Bone Mineral Density in Children and Adolescents with Congenital Adrenal Hyperplasia

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***Abstract***

***Background:*** This study aimed to investigate the effect of the treatment with corticosteroids, and to identify different factor associating to the bone density of the spine and femoral head increases. To this end, the bone mineral densities of the spine and femoral head in children suffering from different types of congenital adrenal hyperplasia were assessed; and their association with some other factors, along with the effects of the treatment was investigated.

***Method:*** This retrospective and descriptive cohort study was done in Iran from 2020 until 2021, on 44 patients with mean age of 10 years old. Forty-four patients, including 18 males and 26 females with a mean age of 10 years were enrolled in this study. They were treated with corticosteroids with a physiological dose of 15-20 mg/m2 since infancy. Patients (parents) filled a form providing demographic information and the disease history and responded to questions about taking supplements and any other type of medications, doing exercise, having a history of fractures in patients or other family members.

***Result:*** The bone density level was not correlated to the duration and dose of corticosteroid consumption, age, sex, time of diagnosis, 17-hydroxyprogesterone level, calcium, and phosphorus. Other factors such as height, weight, puberty stage, and vitamin D levels had significant roles in determining the bone density level. There was a positive correlation between height and bone density, which means that the taller the patient, the higher the bone density of the spine and femoral head. As patients gain weight, the bone density of the spine and femoral head increases. With increasing vitamin D levels in patients, the femoral head bone density increases and vice versa.

***Conclusion:*** Bone densitometry should regularly be done, osteoporosis prophylaxis should be considered using weight-bearing exercises and calcium and vitamin D supplements, and the level of vitamin D should be monitored. The dose of corticosteroids should be adjusted.

***Key Words:*** Bone mineral density, Corticosteroid, Congenital adrenal hyperplasia.

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## 1- INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive diseases that are caused by a deficiency in adrenal enzymes, and leads to changes in cortisol and aldosterone levels. The classic form of CAH which is due to 21-hydroxylase deficiency (21-OHD) accounts for almost 90% of cases. The 21-hydroxylase enzyme is needed for converting progesterone to deoxycorticosterone and 17-hydroxyprogesterone to 11-deoxycortisol. The deficiency of this enzyme leads to increased feedback of the pituitary-hypothalamic-adrenal axis and eventually adrenal hyperplasia. These precursors of cortisol and aldosterone are converted to androgens, leading to virilization and a hyperandrogenic state. If left untreated, this condition can lead to electrolyte disturbances, shock, and even death (1).

The incidence of the classic form of CAH is around 1 among 14,000 to 18,000 births, while 75% of them are patients with salt loss (salt-wasting) (2). Other common forms of CAH are due to deficiency of 17 alpha-hydroxylase (17α), 11 beta-hydroxylase (11β), 3-beta-hydroxy (3β) steroid dehydrogenase, and P450 oxidoreductase deficiency (2).

An accurate hormonal balance must be maintained in these patients during childhood so that these children can have normal growth and that eventually, the hyperandrogenic state disappears. Treatment thus aims to prevent adrenal deficiency and optimize growth. Complete suppression of androgens and precursors would only be possible with excessive treatment that may lead to cognitive iatrogenic syndrome and growth and development disorders (3). These potential disorders increase the risk of changes in bone mineral density (BMD) and body composition (4).

The classic form of CAH requires long-term treatment with glucocorticoids, and they are usually administered since infancy (1, 3, 4). Glucocorticoids have a catabolic effect on muscles and lead to fat accumulation and decreased muscle mass (3). However, the increased level of androgens has opposite effects compared to corticosteroids and has protective effects on body composition (4). Therefore, in this study, we investigated the management of CAH and the impact of corticosteroids on BMD.

Since the classic form of CAH needs long-term treatment, an early evaluation and identification of side effects of corticosteroids, including its impact on bone density, is vital to prevent or mitigate the occurrence of these complications and their consequences. Considering the reduced bone density, prevention of osteoporosis and bone fractures should be prioritized.

Nevertheless, the results in the literature regarding the association of reduced BMD with CAH have not been conclusive. Ganesh et al., in a study on children aged 0 to 132 months with CAH treated with hydrocortisone, reported a lower BMD for patients who were treated with corticosteroid for more than five years (5). However, in the study by Girgis et al., BMD was reported normal (6). In another study by Elnecave et al., the BMD in 60 girls, with CAH of type 21-hydroxylase deficiency, was evaluated, and BMD of the lumbar spine and whole body was normal compared to the control group (7). Similarly, in a study reviewed by Cetinkaya et al., corticosteroid treatment had no effect on BMD in patients with CAH (8). In a study by Sciannamblo et al. on patients aged 16-29 years, they measured BMD using a lunar dual-energy X-ray absorptiometry (DXA) device in Italy. They reported that the lumbar BMD was normal in CAH patients, but the total body BMD was below normal (9). Note that among the methods used to assess BMD, the DXA method is preferred

considering its availability, speed, and minimum exposure and radiation (10).

In a study on 28 children and adults with CAH in Romania, BMD was assessed only in the spinal region, but no correlation was found between BMD and adrenal metabolites, including dehydroepiandrosterone (11). However, another study by Paganini et al. on 50 CAH patients (23 boys and 27 girls) aged 1-28 years reported a decrease in BMD and bone growth in adolescents and young adults with the classic form of CAH, but not in children. But, the markers of bone formation and bone resorption were normal (12). In a study by Christiansen et al., 28 patients aged 2.5 to 27 years were studied. They examined the profile of urinary steroids and BMD of the lumbar vertebrae, and BMD was found to be lower in relation to bone age (13).

Ventra et al. evaluated the markers of bone activity, and it was found that the activity and serum level of osteocalcin were positively correlated with growth velocity and negatively correlated with BMD (14). In a study conducted by El-Maouche et al. on 80 CAH patients over 20 years of age, 47 patients with a classic type and 33 patients with a non-classic type, the BMD of the forearm was examined, and it was found to be 52% below normal (15). There were patients with a more severe decline in the classic type group. Moreover, the classic type group had a lower level of dehydroepiandrosterone than the non-classic type group and a higher rate of non-traumatic fractures. This study also showed a positive effect of dehydroepiandrosterone (DHEA) on BMD (15). A study by Demirel et al. showed positive effects of vitamin D levels on BMD in children with CAH (16).

Considering these heterogeneous findings, and as very few studies have been conducted on the link between BMD and glucocorticoid therapy in CAH disease among Iranian children, we aimed to investigate this association. In this study, patients' BMD was assessed during the corticosteroid therapy. We evaluated the effect of several variables such as age, gender, and the duration of treatment on BMD. Since the level of vitamin D can affect BMD (17), in this study, we also examined the level of vitamin D in patients and its relation to BMD. Accordingly, it could be decided if vitamin D supplements should be prescribed for these patients to prevent osteoporosis and bone fractures. The ultimate goal is to help CAH patients by properly controlling the underlying disease and the complications of the treatment, to improve their quality of life. To this end, this work attempts to answer the research question that whether long-term consumption of corticosteroids leads to a decrease in bone density and whether other factors such as vitamin D level and sex affect it. In general, we aimed at evaluating the effect of glucocorticoid therapy on bone mineral density in children and adolescents with congenital adrenal hyperplasia.

## 2- METHODS

### 2-1. Study design and population

In this cross-sectional study, we enrolled 44 children and adolescents, including 26 girls and 18 boys, aged 5-20 years, who had been treated with corticosteroids for more than 36 months usually since their infancy. The sample size was based on available patients who met the inclusion criteria, and could match with the timeline of the study.

Patients (parents) filled a form providing demographic information and the disease history and responded to questions about taking supplements and any other type of medications leading to impaired bone mineralization, doing exercise, having a history of fractures in patients or other family members.

The quality of the questionnaire was confirmed by experts, and its design was approved by the Research Committee.

### 2-2. Methods

To go under investigation and follow-up, the participants were referred to the Clinic of Pediatric Endocrinology of Imam Reza Hospital, associated with the Mashhad University of Medical Sciences, Mashhad, Iran. These patients suffered from congenital adrenal hyperplasia of various types of enzyme deficiency (21α-hydroxylase, 17α-hydroxylase, 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, etc.), and included patients with salt loss and non-classic form. Note that some of these patients, in addition to hydrocortisone and other corticosteroids (dexamethasone and prednisolone), received fludrocortisone as a mineralocorticoid, in the range of 5-20 mg/m2 daily, on different treatment days.

### 2-3. Measuring tools: Laboratory measurements

Patients underwent a medical examination at admission, and their height, weight, and the stage of puberty were determined using Tanner stages. The onset of puberty was considered based on the Tanner stage of II for breast and testes equal to 20x30 mm (18). The level of 17-hydroxyprogesterone (17-OHP) was measured to evaluate the course of disease and the response to treatment. The measurements were conducted on a morning sample before taking the drug, using the ELISA method. The level of 25-hydroxyvitamin D was also measured as a marker of bone mineralization, using the Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (16). The level of calcium, phosphorus, and alkaline phosphatase were also measured (7ml blood sample was drawn from elbow).

After obtaining the informed consent from the parents, the BMD levels in the femoral neck and lumbar vertebrae were measured by dual-energy X-ray absorptiometry (DXA, DEXA) method.

### 2-4. Intervention

Some of these patients, in addition to hydrocortisone and other corticosteroids (dexamethasone and prednisolone), received fludrocortisone as a mineralocorticoid, in the range of 5-20 mg/m2 daily**.**

### 2.5-Ethical consideration

This study was approved by the Ethics Committee of the Mashhad University of Medical Sciences with the code 951245, and the research protocol was approved by the Research Committee under the code 5062T. And informed consents were obtained from the parents.

### 2-6. Inclusion and exclusion criteria

In the data collection form, the dose of corticosteroids, duration of use, and the time of the onset of the disease were recorded. To be included in the study, the patients have had to be treated with corticosteroids for at least three years; and vitamin D and other supplements have had to be already eliminated from their medication regimen.

People who did not consent to participate in the study, children who were receiving corticosteroids orally or by inhalation for other reasons, such as asthma, rheumatic diseases, and other autoimmune and inflammatory diseases, were excluded from the study.

### 2-7. Data Analyses

The statistical analyses of the data were performed with the SPSS software version 22.0. Normality of the distributions was assessed with Kolmogorov-Smirnov and Shapiro–Wilk tests; for the variables that were normally distributed, Pearson correlation coefficient (age, height, Calcium (Ca), Phosphorus (P), alkaline phosphatase) was used, and the other

correlations were calculated through Spearman's correlation coefficient (weight, also the level of 17-OHP, vitamin D). In all statistical tests, P-values under 0.05 were considered as statistically significant. Independent t-tests and one-way ANOVA were used for the comparison of the categories of the quantitative variables in two or more groups.

## 3- RESULTS

As previously mentioned, 44 patients, including 18 males (44.9%) and 26 females (59.9%) were enrolled in this study. According to the results of Kolmogorov-Smirnov and Shapiro–Wilk tests, the data regarding the following variables were found to be normally distributed: Age (year) (p=0.118), Height (cm) (p=0.365), Calcium (Ca) (ng/ml) (p=0.056), Phosphorus (P) (ng/ml) (p=0.885), Alkaline phosphatase (ng/ml) (p=0.229), BMD of the spine (p=0.446), BMD of the femoral head. (p=0.612). The data of the other variables including weight, the level of 17-OHP, and Vitamin D were not normally distributed. Non-parametric tests were thus used for these three variables. Descriptive statistics along with the correlation coefficients of the quantitative variables with the bone density in the spine and in the femoral head are presented in **Table 1**.

**Table-1:** Descriptive analysis and correlation coefficients of the quantitative variables

| | Descriptive statistics | | | | Correlation with Spine BD | | Correlation with FH BD | |
|---|---|---|---|---|---|---|---|---|
| Variables | MEAN | SD | MIN | MAX | Correlation (r) | P-value[a] | Correlation | P-value[a] |
| Age (year) | 10.8 | 4.02 | 5 | 20 | 0.217 | 0.24[b] | 0.376 | 0.037[b] |
| Height (cm) | 141.65 | 16.96 | 100 | 166 | 0.435 | 0.014[b] | 0.677 | 0.001[b] |
| Weight (kg) | 40.85 | 18.19 | 15 | 80 | 0.46 | 0.017[c] | 0.681 | 0.001[c] |
| 17-hydroxyprogesterone (ng/ml) | 15.04 | 12.04 | 0.07 | 60 | -0.036 | 0.885[c] | -0.13 | 0.5[c] |
| Vitamin D (ng/ml) | 25.01 | 17.36 | 0.6 | 73 | 0.252 | 0.188[c] | 0.377 | 0.044[c] |
| Calcium (Ca) (ng/ml) | 9.78 | 0.32 | 8.8 | 10.5 | 0.241 | 0.236[b] | 0.37 | 0.063[h] |
| Phosphorus (P) (ng/ml) | 4.87 | 0.98 | 3.5 | 8.5 | 0.044 | 0.83[b] | 0.029 | 0.887[b] |
| Alkaline phosphatase (ng/ml) | 380.62 | 219.84 | 47 | 979 | 0.133 | 0.527[b] | -0.14 | 0.504[b] |
| BMD of the spine | -2.41 | 1.18 | -5.58 | 0.17 | - | | - | |
| BMD of the femoral head | -1.52 | 1.36 | 4- | 0.46 | - | | - | |

a: The level of statistical significance was less than 0.05
b: Pearson correlation coefficient
c: Spearman's correlation coefficient
BMD: Bone mineral density

As can be seen in the correlation columns of **Table 1**, the age of the patient has a significant correlation with the femoral head BMD but not with the spine BMD. The femoral head BMD is, therefore, higher in older patients. Height and weight of the patient had also a statistically significant correlation with both of the spine and the femoral head BMD. The positive correlation indicates that the taller or the heavier the patient, the higher is the bone density of the spine and femoral head. Note that the negative correlation coefficient of 17-hydroxyprogesterone with both spine and femoral head BMD indicates an inverse relationship, i.e., as the level of 17-hydroxyprogesterone increases, the bone mineral density

decreases. However, this relationship was not significant.

The level of vitamin D was significantly correlated only with the femoral head BMD, indicating that with a higher vitamin D level, a higher femoral head BMD is expected. Its correlation with the spine BMD was also positive, but not significant. Even though it was not statistically significant, it is worth to mention the positive correlation of Alkaline phosphatase with the spine BMD, in contrast to its negative correlation with the femoral head BMD.

For the categorical variables of sex, diagnosis time, CAH type, the stage of puberty, the comparison of both the spine and femoral head BMD among different categories, using an independent t-test or one-way ANOVA, showed no significant difference between categories. Based on the results of the independent t-test, we might suppose that the mean spinal bone density is higher in men, but femoral head bone density is higher in women; however, this difference was not significant in either case. The mean BMD of the spine and femoral head in patients with classic CAH type 21-OHD was higher than those in patients with the CAH type of 11β. This difference was not also significant.

The results of the independent t-tests demonstrated that the Spine bone density is not significantly correlated to Sex (p=0.682) and Diagnosis time (p=0.816). Likewise, the Femoral head bone density isn't significantly correlated to Sex (p=0.37) and Diagnosis time (p=0.316). According to the results of the one-way ANOVA tests, the CAH type is not significantly correlated to Spine bone density (p=0.851) and Femoral head bone density (p= 0.68)**.** Puberty stage, similarly, was not significantly correlated to Spine bone density (p=0.691), and Femoral head bone density (p= 0.964).The one-way ANOVA test, did not also confirm any significant correlation between the Glucocorticoid and Spine bone density (p=0.172) or Femoral head bone density (p= 0.021)**.** All data are summarized in **Table 2**.

As can be seen in **Table 3**, the highest femoral head bone density is seen in patients with prednisolone glucocorticoids and the lowest in patients with hydrocortisone. A Duncan's multiple range test (DMRT) test was performed to compare groups in pairs. The results revealed that the hydrocortisone and dexamethasone categories should both be included in subgroup (a) because their difference was not significant (P-value = 0.063), but their difference with the other group was significant. The prednisolone group with the highest femoral head bone density, then, falls into subgroup (b). It can be concluded that the femoral head bone density in patients with prednisolone glucocorticoids is significantly higher than that in other patients. But patients with glucocorticoids of hydrocortisone and dexamethasone are not, in this respect, statistically different from each other.

## 4- DISCUSSIONS

In this study, bone density in the femoral head and spine were investigated in regard to their possible associations with several factors, among 44 children and adolescents aged 5-20 years with different types of CAH. All the participants had been treated with corticosteroids for more than three years. Accordingly, the mean bone density in the spine area was found to be -2.41, and in the femoral head area was -1.52, which indicates that the effect of corticosteroid treatment on BMD was somewhat higher in the spine area than in the femoral head. We also found that the height and weight were significantly associated with the BMD in both spine and femoral head, while age, the level of vitamin D, and the type of corticosteroid had a significant relationship only with the femoral head BMD.

**Table-2:** Descriptive analysis for different categories of qualitative variables

| | | | Categories | | p-value | |
|---|---|---|---|---|---|---|
| | | | Spine bone density | Femoral head bone density | | |
| Variables | | N (%) | Mean | Mean | Spine bone density | Femoral head bone density |
| Sex | Male | 18 (40.91) | -2.85 | -1.98 | 0.68[a] | 0.37[a] |
| | Female | 26(59.09 ) | -3.03 | -1.53 | | |
| Diagnosis time | Newborn | 39(88.63) | -3 | -1.67 | 0.82[a] | 0.32[a] |
| | Infancy & Childhood | 5(11.37) | -3.2 | -2.7 | | |
| CAH type | 11□ | 2(4.54) | -3.11 | -2.04 | 0.85[b] | 0.68[b] |
| | 21-OHD | 38(86.38) | -2.94 | -1.61 | | |
| | 3□ | 2(4.54) | -2.83 | -1.765 | | |
| | 17**α** | 1(2.27) | -3.15 | -1.44 | | |
| | Star | 1(2.27) | -4.02 | -3.37 | | |
| Puberty stage | II | 19(43.18) | -2.47 | -1.42 | 0.69[b] | 0.96[b] |
| | III | 14(31.82) | -2.68 | -1.74 | | |
| | IV | 11(25) | -3.05 | -2.15 | | |
| Glucocorticoid | Prednisolone | 1(2.27) | -2.13 | 1.56 | 0.17[b] | 0.02[b] |
| | Dexamethasone | 10(22.72) | -2.07 | -1.25 | | |
| | Hydrocortisone | 33(75.01) | -2.48 | -1.9 | | |

A: Using an independent t-test
B: Using a one-way ANOVA test
CAH type: congenital adrenal hyperplasia

**Table-3:** Femoral head BMD (*bone mineral density*) of three Glucocorticoid groups and the results of DMRT

| | Descriptive statistics | | | | DMRT results | | |
|---|---|---|---|---|---|---|---|
| Group | MEAN | SD | MIN | MAX | N | A | b |
| Hydrocortisone | -1.9 | 1.17 | -3.58 | 0.78 | 33 | -1.9 | - |
| Dexamethasone | -1.25 | 1.52 | -4 | 0.61 | 10 | -1.27 | - |
| Prednisolone | 1.56 | 0 | 1.56 | 1.56 | 1 | - | 1.56 |
| | | | | | P-value | 0.063 | 1 |

DMRT: Duncan's multiple range test

In the dual-energy X-ray, absorptiometry (DXA) method is usually used to measure BMD, the BMD of the lumbar spine or whole body. If possible, the skull should be eliminated from the analysis of the whole body; because, with growth and disease progress, the skull changes very slightly. Measuring the BMD of the hip or neck of the femur is not a reliable method for young patients due to the difficulty of

finding the right place and identifying a suitable bone marker. Therefore, in these cases, the BMD is measured in the lateral of the distal femur. Moreover, in children, using T-score is not valuable and Z-score is mostly used. Accordingly, Z-score < -2 is considered severe osteoporosis, -2 < Z-score < -1 as osteopenia, and Z-score > -1 as normal. Using Z-score is actually equivalent to comparing a patient's BMD with a normal person of the same age and gender.

In a study by Cetinkaya et al., the BMD values were reported to be normal among CAH patients (8), while in two other studies on patients with CAH, the BMD was below normal, similar to our results (19, 20). In a study by Cameron et al. on 13 men and 8 women aged 8-32 years, BMD was normal in women but below normal in men (21).

In our study, 43.1% had BMDs less than -2 in the spine or the femoral head (27% had spine BMDs below -2). Also, 25.5% had BMDs between -2 and -1 in one of the areas (25% had BMDs between -2 and -1 in the femoral head). Finally, five patients had BMDs above -1, in both the femoral head and the spine. Regarding the type of CAH, one of the cases having 17α-hydroxylase deficiency showed BMDs below -2 in the spine and femoral head. In another case with an unknown diagnosis (considered as star deficiency), the BMD of the femoral head and spine was less than -2. Considering the 3β-hydroxylase deficiency, in 2 cases, the BMDs in the spine were less than -2, and those in the femoral head were between 1- and -2. Regarding the cases without salt loss (wasting) with 21-hydroxylase deficiency, one case had BMDs between -1 and -2 in the spine area and above -1in the femoral head area. Another case had a spine BMD of -5.29 and femoral head BMD of above -1. Moreover, one case in both areas had a BMD of less than -2.

In a study by El-Maouche et al., BMDs below normal in the spinal cord and hip were reported more for the classic types of CAH, compared to the non-classic types; BMDs below -1 were seen only in classic types (15). Similar findings have been also reported in other studies (21, 22). According to the study of El-Maouche et al., the patients with a classic type of CAH had higher levels of 17-hydroxyprogesterone than the non-classic patients, as well as lower levels of dehydroepiandrosterone hormone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) (15). Almost half of the classic and non-classic patients had a normal level of androstenedione and testosterone. DHEAS is thought to play an important role in determining the bone mass, and its effect has especially been in the radius area (15).

In another study on 26 adult women with CAH, including 12 postmenopausal women, it was reported that excessive adrenal androgen suppression was associated with an increased risk of bone loss. Further, those with osteopenia had lower levels of DHEAS and had taken higher levels of corticosteroids, compared to ones who had normal BMD (20).

Regarding the type and dose of corticosteroids consumption (33 cases of hydrocortisone, 10 cases of dexamethasone, and 1 case of prednisolone), the highest amount of prescribed corticosteroids was 20 mg/m2 in two patients with 21-hydroxylase deficiency. The maximum duration of treatment with corticosteroids among our patients was 18 years, since infancy. The lowest dose of corticosteroids used was 6 mg⁄m2, and the minimum duration of use was five years. The average dose of corticosteroids used by our patients was 13 mg⁄m2 with different treatment durations. The BMD difference between patients who took hydrocortisone and dexamethasone was not significant.

In a study by Chakhtoura et al., there was a negative correlation between the mean dose of corticosteroids used and the BMD (22). It was reported in another study that the corticosteroids dose of 10-15 mg/m2 resulted in adverse effects on bone and subnormal androgen levels (23). In that study, the patients with a non-classic form had a lower dose of corticosteroids and higher BMD; However, using a higher dose of fludrocortisone for the patients with the classic form, eventually, reduced the need for hydrocortisone, thus less impact on BMD (23).

In our study, there was no significant correlation between the dose of corticosteroids and BMD. Our findings are similar to the results of the study by Fleischman et al., where no association between corticosteroids and BMD was reported (24). However, Bachelot et al. reported a negative relationship between corticosteroids and BMD. Two other studies, likewise, had the same conclusion (18, 25). Due to this diversity in results and the difference in the prescribed dose of corticosteroids and the duration of treatment, no firm conclusion can be drawn.

In our patients, the average height was 150 cm (range: 100 - 166 cm), which could not be assessed considering the age difference of patients. For the growth, 7 patients were below the 3rd growth percentile, 5 girls and 2 boys, and no relationship between the growth and disease progress and corticosteroid dose was found. However, in measuring the bone density, there was a strong relationship between bone density and height, the taller the patient, the higher the bone density. In the study of Chakhtoura et al., the height was shorter than expected, and the patients had a reduced volumetric height during puberty, while the age of the onset of puberty was normal (22). Similar results have been reported in the studies by Stikkelbroeck et al. and Van der Kamp et al., where a small difference between the bone age and chronological age of the patients was reported (26, 27). Unfortunately in our study, due to the lack of cooperation of patients, their bone age was not examined.

Regarding the weight, we had 5 patients with around 80 kg of weight, 3 girls and 2 boys, above the 95th percentile. Four of them were 10 years old, and one was 8, all in the puberty stages of II and III. And our results showed that weight was positively correlated to both of the femoral head and spine BMDs. In the study by Chakhtoura et al., a higher body mass index (BMI) had protective effects on bone loss (22). Other studies, such as by Cetinkaya et al., have shown that lean tissue mass is more important than fat mass in increasing bone density, and that lean body mass (LBM) is normal in children and young adults with adrenal insufficiency (8).

In our patients, 25 patients were in the puberty stage (in stages III and IV), and the rest were pre-pubertal. We had 8 boys with a normal average puberty at the age of 9 and 18 female patients with an average puberty age of 8 years. Only one case of puberty at 6 years of age was reported. Based on our findings, different stages of puberty were not significantly correlated to the femoral head and spine BMDs, but in other studies, such as by Chakhtoura et al., they were found to be correlated (22). Note that maximum bone density is supposed to be reached during puberty, so we expect the BMD to be higher during this period. This discrepancy in our results is probably due to the diversity in CAH types and different doses of corticosteroids.

Regarding the relationship between sex and BMD, there was no significant difference between girls and boys. In the study by Chakhtoura et al., on 16 to 39-year-old patients, a large difference in the T-scores of both the femoral head and the spine BMDs was reported (22). According to this study and similar studies, Estrogen

was found to have a protective effect on BMD (28, 29), so women in premenopausal ages have higher BMDs.

We found a significant positive correlation between age and the femoral head BMD. In the study by Bachelot et al. (19), no significant difference in BMD by age was reported, and given the small number of studies on children and adolescents with CAH (only 4 studies to the best of our knowledge) (11, 12, 20, 30), no rigid conclusion can be reached.

For the 17-hydroxyprogesterone level, the lowest level of 17-OHP was 0.07 ng and the maximum level was 5.52 ng/ml. According to guidelines for 17-OHP, a value below 10 ng/ml was considered normal and above that abnormal. No significant correlation between 17-OHP and the spinal or femoral head BMDs was found. However, in some studies such as Cetinkaya et al. (8), individuals with increased 17-OHP had decreased femoral head BMD. In fact, an increase in the 17-OHP level indicates resistance to treatment; therefore, these patients had been treated with corticosteroids for a longer period of time. It may also indicate non-compliance of patients and irregularity in taking corticosteroids. Considering these conflicting results, no conclusion can be made about the association of the spinal or femoral head BMDs with 17-hydroxyprogesterone.

We analyzed the relationship between BMD and several bone activity markers [Ca, P, alkaline phosphatase, and vitamin D (25-OHD3)]. The level of Ca, P, and alkaline phosphatase were normal in these patients. However, vitamin D had an average level of 23.8 ng/ml. Only vitamin D had a significant positive correlation with the femoral head BMD. Cetinkaya et al. found that children with CAH had reduced bone turnover (8). In these patients, the level of osteocalcin decreased and the level of N-telopeptide increased, resulting in decreased bone density. However, changes in the level of these markers are more frequent in adults with CAH than in children. In the study of Girgis et al. (6) and Guo et al. (31), an increase in bone activity markers was reported in CAH patients. According to these studies, there is a positive correlation between osteocalcin level and growth velocity and a negative correlation with BMD.

## 5- LIMITATIONS

One of the limitations of our study was the diversity of age, type of CAH, and form and dose of corticosteroids in, somehow, a small population. Lack of cooperation and compliance of patients was another issue. In other studies, bone age is also usually assessed, but in our study, it was not done due to the lack of cooperation of patients. Note that in a study by Paulo Alonso et al., the Z-score of BMD in patients with classic CAH was lower when considering the bone age versus the chronological age; therefore, bone age can be an important factor in the evaluation of BMD (32). Lack of control group is the biggest limitation of this study.

## 6- CONCLUSION

Evaluating bone mineral density (BMD) in children and adolescents with congenital adrenal hyperplasia (CAH), no significant correlation was found between the BMD and corticosteroid use, duration of treatment, type of CAH, sex, age of the patients, and the level of 17-OHP. However, it was significantly associated with weight, height, and the level of vitamin D. When treatment with corticosteroids is started, future side effects such as osteoporosis should be taken into account. Bone densitometry should regularly be performed in these patients in their adulthood, osteoporosis prophylaxis should be considered using weight-bearing exercises along with calcium and vitamin D supplements; and

the level of vitamin D should be monitored. Finally, determining an appropriate dose of corticosteroids and controlling the disease in patients with CAH is the first step to prevent complications. Considering their need for lifelong treatment with corticosteroids, new solutions to prevent these complications should also be offered.

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