The Prevalence of Δ508 in Cystic Fibrosis Patients with Low Bone Mineral Density in Republic of Macedonia

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Abstract: Introduction: Reduced bone mass density (BMD) is frequent in patients with cystic fibrosis (CF). Pathogenesis of CF bone disease is multifactorial. Many studies suggest that there is genetic component, independent of the disease severity and nutritional deficits. Aim: To determine the prevalence and identify determinants of reduced BMD in CF patients. Material and methods: The study included 80 CF patients (range 5-36 y). BMD was measured via dual-energy x-ray absorptiometry (DXA) scan. Vitamin D level was assessed by plasma 25OHD levels (<15 ng/ml) was defined as deficiency. Results: 42 CF patients were homozygote for ΔF508 mutation and 26.2% have lower BMD (-0.43 ±0.99SD), five with osteoporosis. 25OHD was <15 ng/ml in 21.4%, homozygote CF patients. In group with heterozygote for ΔF508 (28 CF patients) low BMD have 32.1% (-0.53±1.13SD), two with osteoporosis. Severe deficiency of 25OHD < 15 ng/ml in heterozygous group have 39.2%. Ten CF patients have other mutations, 50% have low BMD (-0.45±1.2SD), one have osteoporosis. There was one patient with severe deficiency of 25OHD (10%). Conclusion: ΔF508 genotype was associated with impaired bone turnover (decreased osteoblast activity). Reduced bone mineral density in cystic fibrosis is associated with a number of factors including ΔF508 genotype, deficiency of vitamin D, lung disease severity and malnutrition.

Keywords: bone mineral density, cystic fibrosis, ΔF508 genotype, vitamin D deficiency

1. Introduction

Cystic fibrosis (CF) is potentially lethal autosomal recessive disease in white population determined by genetic mutation of CFTR gene. Above 1500 mutation are reported, but the most frequent is Δ508del mutation. Advances in medical care for patients with cystic fibrosis prolonged their life. The median age of survival has increased from 10 years to the fourth decade. These leads to additional complications like osteoporosis, diabetes mellitus, cirrhosis, and infertility. Bone disease in CF patients (CFBD) was first described by Hann in 1979 year and is the result of disturbed bone turnover, decreased osteoblast activity and increased osteoclast bone resorption. Incidence of CF bone disease in the world is estimated to be 30% (2, 3). The causes of CFBD are multifactorial: malabsorption of calcium and liposoluble vitamins D and K as a result of pancreatic insufficiency, malnutrition, physical inactivity as a result of impaired lung function, use of corticosteroids and inflammation. Many studies suggest that there is genetic component, independent from disease severity and nutritional deficits (6, 7). They suggest that there is direct link between Δ508 mutation and CFTR protein in molecular process involved in bone formation and resorption. In studies on mice and humans is found that CFTR is expressed on the surface of osteoblasts. Dysfunction of CFTR chloride canal in bone cells may have influence over disturbed regulation of expression of the genes involved in the process of bone formation. CF patients who have at least one allele for Δ508 have significant lower Z score (10). Clinical manifestations are kyphosis, vertebral and rib fractures who cause pain and disability to clear airways, inhibit effective cough and reduce lung function (11). Aim of the study was to assess prevalence of reduced bone mineral density and vitamin D deficiency in pediatric and adult CF patients who regular visit the CF center at the University Pediatric Clinic in Skopje, Macedonia despite the daily supplementation of 800 IU vitamin D and to identify the influence of Δ508 over reduced BMD in these patients.

2. Materials and Methods

2.1 Patients

The study included 80 clinically stable CF patients (range 5-36 y) who regularly attended the CF center at the Pediatric Clinic in Skopje. The diagnosis of CF was made by the presence of typical clinical characteristics of CF (chronic respiratory disease and/or pancreatic insufficiency) together with abnormal sweat chloride test (>60 moll/l) and/or the presence of two CFTR gene mutations. They were divided in 3 groups depending on CF genotype who was determined in the laboratory of MANU (Macedonian Academy of Science and Arts) by characterization of the molecular defect in two CFTR genes. The aim was to determine whether Δ508 del mutation contributes to reduced bone mineral density as independent factor. The three groups were:

- Homozygote for Δ508 del mutation
- Heterozygote for Δ508 del mutation
- Without alleles for Δ508 del mutation

2.2 Clinical Assessment

The nutritional status of CF patients was expressed as body mass index (BMI) index for weight and height (kg/m²). Values are compared with standard percentiles for age and sex. Pulmonary functional tests were measured by Flow Screen-Jaeger Spiro meter. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were analyzed. The values were expressed as percent of predicted values for sex, age, weight and height. Cystic fibrosis disease severity was assessed using the Shwachman-Kulczycki (S-K) system, which rates general activity level,
pulmonary physical findings, growth and nutrition, and chest radiographic findings. Total S-K scores may range from 20 to 100; low scores representing greater illness severity.

2.3 Laboratory Measurements

Calcium, phosphorus, and alkaline phospatase were measured in serum at the University Pediatric Clinic in Skopje. Serum osteocalcin (OC), β cross laps, 25OHD and PTH were determined electrohemiluminiscent method on the automatic immune analyzer elecsys 2010 roche at the University Clinic for Biochemistry in Skopje. Referral values for 25OHD are 15-44 ng/ml. According Cystic Fibrosis Foundation levels for 25 OHD in CF patients below 30 ng/ml are consider insufficient and levels beyond 15 mg/ml for severe deficiency.

2.4 Bone Density Measurements

BMD was measured via dual energy-ray absorptiometry (DXA) scans with spinal scores recorded. They were expressed by Z or T scores depending of the age of patients. Densitometry definition of osteoporosis is accepted by the European Foundation for Osteoporosis and World Health Organization (WHO) and is the golden standard for definition for osteoporosis. Osteoporosis is defined as a bone density <2 SD of the mean BMD of a gender-matched, young healthy population. Osteopenia is an intermediate category of reduced bone density defined as a Z or T score between -1 SD and -2 SD.

2.5 Statistical Analysis

Results are reported as mean value (M) and standard deviations (SD) for each group. Student's t-test was used for calculating significant differences between CF and control group. Pearson scores were used to determine correlation analysis between BMD and various clinical variables. Statistical significance was defined as p<0.01.

3. Results

The study included total of 80 patients with CF, who were divided in three groups depending on CF genotype (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Distribution of ΔF508 mutation in CF patients</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>FEV1</td>
</tr>
<tr>
<td>FVC</td>
</tr>
<tr>
<td>S-K score</td>
</tr>
<tr>
<td>BMI</td>
</tr>
</tbody>
</table>

We didn’t find statistically significant difference in pulmonary and nutritional status in CF patients despite having two, one or none alleles for ΔF508 mutation (Table 2).

Table 2: Average values for clinical parameters in CF patients depending on CF genotype

<table>
<thead>
<tr>
<th>CF genotype</th>
<th>N</th>
<th>Average age</th>
<th>Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous for ΔF508 mutation</td>
<td>42</td>
<td>13.5±6.8</td>
<td>52.5</td>
</tr>
<tr>
<td>Heterozygous for ΔF508 mutation</td>
<td>28</td>
<td>15.6±7.6</td>
<td>35</td>
</tr>
<tr>
<td>No allele for ΔF508 mutation</td>
<td>10</td>
<td>13.4±7.1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

50% of the CF patients with PI had serum vitamin D >20 ng (range 10-44ng/ml) with no difference of age. In CF group we found 26% < 15 ng/ml.We didn’t find statistically significant difference for vitamin D between the groups (Figure 1).

![Figure 1: Distribution for levels of 25OHD in CF patients depending on CF genotype](image)

We found statistically significant difference for serum osteocalcin between the groups with two or one alleles for ΔF508 mutation and the group without ΔF508 (p=0.002) (table 3). Osteocalcin levels in homozygous and heterozygous CF patients are lower which suggest that they have abnormal bone turnover and ΔF508 may have influence over decreased osteoblasts activity in these patients. There was no statistically significance for PTH in CF groups besides abnormal bone turnover in CF patients (Table 3).

Table 3: Average values for 25OHD, osteocalcin, βcrosslaps, PTH, BMD, calcium, phosphorus and alkaline phosphatase in serum for CF patients depending on CF genotype

<table>
<thead>
<tr>
<th></th>
<th>Homozygous for ΔF508 mutation</th>
<th>Heterozygous for ΔF508 mutation</th>
<th>No allele for ΔF508 mutation</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>25OHD</td>
<td>23.5±10.65</td>
<td>19.39±8.1</td>
<td>27.2±10.06</td>
<td>p=0.12</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>62.9±38.4</td>
<td>58.7±31.88</td>
<td>105.8±65.04</td>
<td>p=0.002*</td>
</tr>
<tr>
<td>β cross laps</td>
<td>1.3±0.8</td>
<td>1.0±0.67</td>
<td>1.3±0.72</td>
<td>p=0.7</td>
</tr>
<tr>
<td>PTH</td>
<td>40.2±29.44</td>
<td>47.58±23.51</td>
<td>44.3±29.6</td>
<td>p=0.92</td>
</tr>
<tr>
<td>BMD Z-score</td>
<td>-0.44±0.98</td>
<td>-0.54±1.11</td>
<td>-0.45±1.15</td>
<td>p=0.94</td>
</tr>
<tr>
<td>Ca</td>
<td>2.35±0.15</td>
<td>2.34±0.16</td>
<td>2.35±0.081</td>
<td>p=0.87</td>
</tr>
<tr>
<td>PAlk,phosphatase</td>
<td>249.4±79.86</td>
<td>199.4±74.56</td>
<td>226.7±69.37</td>
<td>p=0.92</td>
</tr>
</tbody>
</table>

* Statistically significant

Osteopenia (Z or T score < -1SD) was determined in 32% of patients. We found 8 patients are with osteoporosis (10%) (Figure 2).
Most studies suggest a higher incidence of vertebral fractures and rib fractures (11). Elkin, 1998, found 51% of adult CF patients with vertebral fractures (10).

More than 20 reports suggest that vitamin D insufficiency is common among individuals with CF (23%-75%), irrespective of season and despite supplementation with 800-1000 IE/day (15, 16).

Low serum 25 OHD concentrations were associated with lower BMD, suggesting that vitamin D deficiency may play a significant role in the pathogenesis of demineralization in cystic fibrosis. (19, 21, 23, 24, 27). In our study we found that 26% of CF patients are vitamin D deficient, despite supplementation with 800 IE/day.

5. Conclusion

- Prevalence of osteopenia and vitamin D deficiency in our study is about 30% which are similar to other studies.
- Levels of markers for bone formation in serum were decreased in CF patients with two or one allele for ΔF508 and this may contribute to impaired bone turnover. There is a possibility that genetic factor may have influence over reduced bone density.
- The mechanism of action of ΔF508 in reducing bone density in cystic fibrosis remains uncertain.
- Further studies are needed to determine optimal treatment strategies.

References


