



## Δ508 MUTATION IN PATIENTS WITH CYSTIC FIBROSIS WITH LOW BONE MINERAL DENSITY AND VITAMIN D DEFICIENCY

### Δ508 МУТАЦИЈА КАЈ ПАЦИЕНТИ СО ЦИСТИЧНА ФИБРОЗА СО НИЗОК КОСКЕН МИНЕРАЛЕН ДЕНЗИТЕТ И ДЕФИЦИТ НА ВИТАМИН Д

Jakovska T.<sup>1</sup>, Mecevska-Jovcevska J.<sup>2</sup>, Fustik S.<sup>1</sup>, Zorcec T.<sup>1</sup>

<sup>1</sup>University Children's Hospital, Medical Faculty, Ss. Cyril and Methodius University, Skopje, R. of Macedonia

<sup>2</sup>University Clinic for clinical biochemistry, Skopje, R. of Macedonia

Corresponding author: e-mail: maretti98@yahoo.com

Medicus 2014, Vol. 19 (2): 251-256

#### 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 5-36y). 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< 15ng/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.

**Key words:** bone mineral density, cystic fibrosis, ΔF508 genotype, vitamin D deficiency

#### 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 Δ508 del mutation (1). 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 (2). 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 (3) and is the result of disturbed bone turnover,

decreased osteoblast activity and increased osteoclast bone resorption (4). 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 (5). 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 (8). 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 (9). CF patients who have at least one allele for  $\Delta 508$  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  $\Delta 508$  over reduced BMD in these patients.

## MATERIALS AND METHODS

### 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 ( $>60$  mol/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  $\Delta 508$  del mutation contributes to reduced bone mineral density as independent factor. The three groups were:

- Homozygote for  $\Delta 508$  del mutation
- Heterozygote for  $\Delta 508$  del mutation
- Without alleles for  $\Delta 508$  del mutation

### Clinical assessment

The nutritional status of CF patients was expressed as body mass index (BMI) index for weight and height ( $\text{kg}/\text{m}^2$ ). 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 (FEV<sub>1</sub>) 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.

### Laboratory measurements

Calcium, phosphorus, and alkaline phosphatase were measured in serum at the University Pediatric Clinic in Skopje. Serum osteocalcin (OC),  $\beta$  cross laps, 25OHD and PTH were determined electrochemiluminescent 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.

### 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 within  $-1$  SD and  $-2$  SD.

### 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  $p < 0, 01$ .

## RESULTS

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

Table 1. Distribution of  $\Delta F508$  mutation in CF patients

CF genotype	N	Average age	Frequency %
Homozygous for $\Delta F508$ mutation	42	13.57±6.8	52.5
Heterozygous for $\Delta F508$ mutation	28	15.67±7.6	35
No allele for $\Delta F508$ mutation	10	13±4.7	12.5

We didn't find statistically significant difference in pulmonary and nutritional status in CF patients despite having two, one or none alleles for  $\Delta F508$  mutation (Table 2).

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

	Homozygous for $\Delta F508$ mutation	Heterozygous for $\Delta F508$ mutation	No allele for $\Delta F508$ mutation	t-test
FEV1	84.5±24.7	84.21±27.3	92.41±15.2	p=0.34
FVC	93.5±16.5	90.01±23.8	97.32±18.07	p=0.44
S-K score	80.34±22.76	82.57±18.8	88.4±17.4	p=0.44
BMI	19.76±2.78	19.46±2.85	20.41±2.11	p=0.33

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 (Figure1).

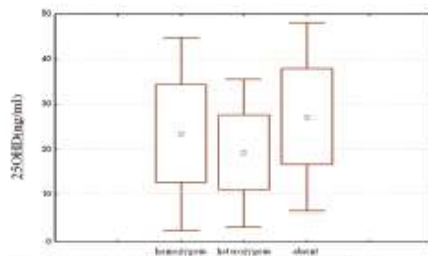


Figure 1. Distribution for levels of 25OHD in CF patients depending on CF genotype

We found statistically significant difference for serum osteocalcin between the groups with two or one alleles for  $\Delta F508$  mutation and the group without  $\Delta F508$  ( $p=0.002$ ) (table 3). Osteocalcin levels in homozygous and heterozygous CF patients are lower which suggest that they have abnormal bone turnover and  $\Delta F508$  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,  $\beta$ crosslaps, PTH, BMD, calcium, phosphorus and alkaline phosphatase in serum for CF patients depending on CF genotype

	Homozygous for $\Delta F508$ mutation	Heterozygous for $\Delta F508$ mutation	No allele for $\Delta F508$ mutation	t-test
25OHD	23.51±10.65	19.39±8.1	27.2±10.06	p=0.12
Osteocalcin	62.91±38.4	58.79±31.88	105.8±65.04	p=0.002*
$\beta$ cross laps	1.3±0.8	1±0.67	1.3±0.72	p=0.7
PTH	40.2±37.26	47.58±23.51	44.2±29.44	p=0.92
BMD Z score	-0.44±0.98	-0.54±1.11	-0.45±1.15	p=0.94
Ca	2.35±0.15	2.34±0.16	2.35±0.081	p=0.87
P	1.5±0.28	1.44±0.25	1.55±0.16	p=0.42
Alk. phosphatase	249.4±79.86	199.4±74.56	226.7±69.37	p=0.92

\* statistically significant

Osteopenia (Z or T score < -1SD) was determined in 32% of patients. We found 8 patients are with osteoporosis (10%) (Figure 2).

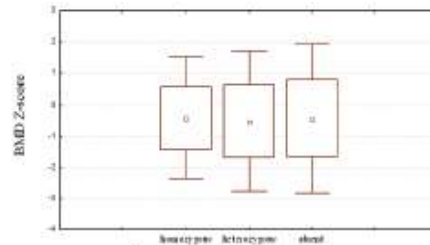


Figure 2. Distribution for Z or T scores from DXA scans in CF patients depending on CF genotype

We didn't find significantly difference between Z scores for spinal BMD depending on CF genotype.

## DISCUSSION

The possibility of managing CF with new medicines extended the life of patients to adulthood. The present average age of survival is 36.8 years (1). Because CF patients are now older, age related complications appear like fractures of the spine, hip and forearm (2). The origin of the low bone mass in patients with CF is not completely understood. Pancreatic insufficiency,

malabsorption of liposoluble vitamins D and K, malnutrition, hypogonadism, glucocorticoids, chronic inflammation, physical inactivity and genetic factors may be responsible for reduced bone mass in CF patients (3, 4). King et al. for the first time reported direct link between reduced bone density and  $\Delta F508$ , suggesting that CFTR mutations may be responsible for low bone density in CF patients (5). Bone formation is significantly reduced in adults with CF (6). Animal models of CFBD show that CFTR expression on osteoblasts leads to reduced osteoblast differentiation (7,8,9). In our study we found significant difference between patients who were homozygous and heterozygous for  $\Delta F508$  and those who have other mutations for osteocalcin, a product derived from osteoblasts. But we didn't find any difference for BMD Z scores. Osteopenia and osteoporosis are common in the adult CF population. They are result of abnormal turnover, decreased osteosynthesis and increased bone resorption (10). Vertebral fractures in patients with CF may contribute to an accelerated decline in lung function and can be a contraindication to lung transplantation (11). That is why is particularly important to promote the screening of osteoporosis in these patients. CF patients in our study presented high prevalence of reduced bone density (32%), and 10% have osteoporosis. Osteoporosis is systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk. Meta analysis from Paccou and al. reports that the mean prevalence of osteoporosis in adults with CF was 23.5% (in different studies from 9-59.1%) (5). The prevalence of CF bone disease increase with severity of lung disease and malnutrition (10). Most studies suggest on higher incidence on vertebral fractures and ribs fractures (11, 12, and 13). Elkin in 2001 found 17% of CF patients with vertebral fractures, and 8% with rib fractures (11). Aris, 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 deficit, despite supplementation with 800 IE/day.

#### 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  $\Delta F508$  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  $\Delta F508$  in reducing bone density in cystic fibrosis remains uncertain.
- Further studies are needed to determine optimal treatment strategies.

#### REFERENCES

1. Buzzetti R, Salvatore D, Baldo E, et al. An overview of international literature from cystic fibrosis registries. 1. Mortality and survival studies in cystic fibrosis. *J Cyst Fibros* 2009;8:229-37
2. Cystic Fibrosis Foundation, Patient Registry 2001 Annual Report. Bethesda, MD: Cystic Fibrosis Foundation, 2002
3. Paccou J, et al. The prevalence of osteoporosis, osteopenia, and fractures among adults with cystic fibrosis: a systematic literature review with meta-analysis *Calcif Tissue Int* 2010;86:1-7
4. Aris RM, Merkel PA, Bachrach LK, et al. Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab* 2005;90:1888-96
5. King SJ et al. Reduced bone density in cystic fibrosis:  $\Delta F508$  mutation is an independent risk factor. *Eur Respir J* 2005;25:54-61
6. Aris RM, Guise TA. Cystic fibrosis and bone disease: are we missing a genetic link? *Eur Respir J* 2005;25:9-11
7. Sermet-Gaudelus I, Castanet M, Retsch-Bogart G, Aris RM. Update on cystic fibrosis-related bone disease: a special focus on children. *Paediatric Respiratory Reviews* 10(2009) 134-142
8. Treszl A, et al. The prevalence of  $\Delta F508$  in primary osteoporotic patients. *Eur Respir J* 2004;23:32-34
9. Stalvey MS, et al. Osteoblast CFTR inactivation reduces differentiation and osteoprotegerin expression in a mouse model of cystic fibrosis-related bone disease. *PLoS one*, November 2013; volume 8, issue 11; e80098

10. Aris RM, Renner JB, Winders AD, et al. Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. *Ann Intern Med* 1998;128:186-93
11. Elkin SL, Fairney A, Burnett S, et al. Vertebral deformities and low bone mineral density in adults with cystic fibrosis: a cross-sectional study. *Osteoporos Int* 2001;12:366-72
12. Stephenson A, Jamal S, Dowdell T, et al. Prevalence of vertebral fractures in adults with cystic fibrosis and their relationship to bone mineral density. *Chest* 2006;130:539-44
13. Lewiecki EM, Gordon CM, Bain S, et al. International society for clinical densitometry 2007 adult and pediatric official positions. *Bone* 2008;43:1115-21 Hahn TJ, et al. Reduced serum 25-hydroxyvitamin D concentration and disordered mineral metabolism in patients with cystic fibrosis. *J Pediatr*, 1979; 94(1): p.38-42
14. Hall WB, Sparks AA, Aris RM. Vitamin D deficiency in cystic fibrosis. *Int J Endocrinol* 2010;2010:218691 [Epub 2010 Jan 28]
15. Rovner AJ, Stallings VA, Schall JI, et al. Vitamin D insufficiency in children, adolescents, and young adults with cystic fibrosis despite routine oral supplementation. *Am J Clin Nutr* 2007;86:1694-9.
16. Khazal NB, Judd SE, Jeng L, et al. Treatment and prevention of vitamin D insufficiency in cystic fibrosis patients: comparative efficacy of ergo calciferol, cholecalciferol and UV light. *J Clin Endocrinol Metab* 2009;94:2037-43
17. Grey AB, Ames RW, Matthews RJ, et al. Bone mineral density and body composition in adult patients with cystic fibrosis. *Thorax* 1993;48:589-93
18. Buntain HM, Greer RM, Schluter PJ, et al. Bone mineral density in Australian children, adolescents and adults with cystic fibrosis: a controlled cross sectional study. *Thorax* 2004;59:149-55
19. Sermet-Gaudelus I, Souberbielle JC, Ruiz JC, et al. Low bone mineral density in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2007;175:951-7
20. Grey V, Atkinson S, Drury D, et al. Prevalence of low bone mass and deficiencies of vitamins D and K in pediatric patients with cystic fibrosis from 3 Canadian centers. *Pediatrics* 2008;122:1014-20
21. Bianchi ML, Romano G, Sarrafogor S, et al. BMD and body composition in children and young patients affected by cystic fibrosis. *J Bone Miner Res* 2006;21:388-96
22. Javier R-M, Jacquot J. Bone disease in cystic fibrosis: What's new? *Joint Bone Spine* (2011), doi:10.1016/j.jbspin.2010.11.015
23. Henderson RC, Madsen CD. Bone mineral content and body composition in children and young adults with cystic fibrosis. *Pediatr Pulmonol* 1999;27:80-4
24. Conway SP, Oldroyd B, Brownlee KG, et al. A cross-sectional study of bone mineral density in children and adolescents attending a Cystic Fibrosis Centre. *J Cyst Fibros* 2008;7:469-76
25. Robertson J, and MacDonald IC. Prevalence of bone loss in a population with cystic fibrosis, *British Journal of Nursing*, vol. 19, no. 10, pp. 636-639, 2010
26. Bhudhikanok G, et al. Bone acquisition and loss in children and adults with cystic fibrosis: a longitudinal study, *Journal of Pediatrics*, vol. 133, no. 1, pp. 18-27, 1998
27. Henderson R, and Madsen C. Bone density in children and adolescents with cystic fibrosis, *Journal of Pediatrics*, vol. 128, no. 1, pp. 28-34, 1996