http://dx.doi.org/10.5272/jimab.2015214.901

Journal of IMAB
ISSN: 1312-773X
http://www.journal-imab-bg.org

Journal of IMAB - *Annual Proceeding (Scientific Papers)* 2015, vol. 21, issue 4

MARKERS OF BONE METABOLISM IN PATIENTS WITH CYSTIC FIBROSIS

Bojana Naydenova- Stoeva
Pediatric Clinic UMHAT "Alexandrovska", Sofia, Bulgaria",

ABSTRACT:

Aim: Some scientific studies show decreased bone mineral density and increased fracture frequency in patients with cystic fibrosis. The mechanism for early bone loss in CF patients are multifactorial: chronic pulmonary inflammation, malnutrition, reducer physical activity, delayed pubertal maturation. The aim of this study was to assess bone metabolism markers in patients with cystic fibrosis and compare them with these in healthy controls.

Material and Methods: We examined 44 patients with cystic fibrosis with average age 14,7 years. The control group consist 43 healthy controls with average age 13,8 years. The levels of bone markers- N Mid Osteocalcin and â Cross Laps in the serum were examined by electro-chemiluminescence method- ECLIA; serum alkaline phosphatase (AP)- kinetic method; common and ionized calcium- atomic-absorption method. The statistical processing of data is made with SPSS programme- version 16.0.

Results: It was established a statistically significant and increased levels of the markers of bone formation in patients, probably as a compensative reaction of the organism to the increased bone resorption (comparing the median levels of â Cross Laps in the two groups). This difference stays constant when only men from the two groups were compared, while there is no such difference between women. Only in the group of patients-men there is a significant evaluated levels of â Cross Laps, compared with the mediate levels in patients-women and less increased N Mid Osteocalcine and AP. Increasing the age of patients, the levels of the investigated markers i.e. N Mid Osteocalcine decrease. This is not found out in the group of the controls. N Mid Osteocalcine is in positive relation with AP in the two groups, and that confirms the fact that the growth of AP returns for its bone fraction. The increase of â Cross Laps is related with the increase of N Mid Osteocalcine.

Conclusion: In patients with cystic fibrosis changes in the profile of bone metabolism were observed. The patients should be under a comprehensive medical and nutritional care in order to achieve their optimal peak bone mass.

Key words: cystic fibrosis, bone markers

Cystic Fibrosis (CF) is the most common genetic disease within the Caucasian population. It has a frequency of appearance from 1:2000 to 1:4000 among all newborns. For Europe this frequency is 1:2500 [1].

During the last years scientiests succeeded to throw light upon the sophisticated molecular mechanism of mucoviscidosis. A mutation causes the synthesis of a pathological protein that blocks the chloric channels of the epithelial cell membranes and it is called Cystic Fibrosis Transmembrane Conductance Regulator protein- CFTR. As a result, all exocrin glands in the body are affected. The systems that are most involved in this pathologic process are the respiratory tract, gastrointestinal tract and pancreas with premature respiratory failure.

The life span of these patients has increased from approximately 2 to 32 yr of age over the last three decades because of changes in the clinical management of these patients, including nutritional supplementation, physical therapy and medication regimes [2].

Bone disease has emerged as a common complication in long- term surivors of CF [3]. Bone disease in individuals with CF was first described in 1979. Low bone density and increased fracture rates are now recognized complications of this autosomal recessive disease. The origin of the bone disease in CF appears to be multifactorial [4, 5]. Important contributing factors include: malabsorption of fat- soluble vitamins, poor nutritional status, physical inactivity, glucocorticoid therapy, delayed pubertal maturation. Additionally, chronic pulmonary inflammation increases serum cytokine levels, which, in turn probably stimulates increased bone resorption and decreased bone formation.

The decreased quality and quantity of the bone mineral, as a result of the above mentioned reasons, leads to pathological fractures and kyphosis in patients with CF, earlier than expected in healthy people [6, 7]. Besides pain and weakness they cause, fractures of ribs and vertebri can be associated with a rapid decline in lung function, either through causing a pneumothorax or by inhibiting effective sputum clearance. Vertebral fracture, vertebral deformity and kyphosis can lead to a reduction in forced vital capacity and reduced ventilator efficiency.

Early identification of reduced bone mass values would permit early intervention to prevent the development of osteoporosis. Maintaining pulmonary function, guaranteeing optimal nutritional status, following an adequate program of physical activity and controlling steroid intake could maintain bone mineral density over time [8, 9]

Aim:

The aim of the current project is to assess bone metabolism markers in patients with cystic fibrosis and compare with these in healthy controls. The realization of the goals of this project will give a fillip to the problem for prevention and early treatment of the bone disease. Prevention, early recognition and treatment are the most effective strategies for sustaining bone health to help maintain the quality of life of many individuals with CF.

MATERIAL AND METHODS:

In this project 87 people took part- 44 clinically stable patients with proved desease of CF and 43 age and sex matched controls. All participants in this research were registered exact age in years and gender.The distribution in accordance with the sex in the group of the patients, is as follows: boys N=27 or 61,4% and girls N= 17 or 38,6%. In the group of the controls this distribution is- boys N= 25 or 58,1 % and girls N= 18 or 41,9% (table1). The average age of patients is 14,7 years (SD= 7,4), in controls it is - 13,8 years (SD= 7,9). All of the participants have been taken one time venous blood sample and the values of the common and ionized calcium and â Cross Laps- as markers for the level of bone resorption; common fraction of alkaline phosphatase (AP) and N Mid Osteocalcine- as markers for the level of bone formation were measured.

For the examination of the markers were used some methods as follows: the levels of N Mid Osteocalcine and β Cross Laps in the serum were examined by electro-chemiluminescence method- ECLIA; serum alkaline phosphatase (AP)- kinetic method; common and ionized calcium- atomic-absorption method. The statistical processing of data is made with SPSS programme- version 16.0 and the main methods are descriptive statistics, Kolmogorov- Smirnoff's test, Shapiro- Wilk' s test, a coefficient of Pirson.

RESULTS:

The statistical processing of the obtained laboratory results shows that there is a significant distinction in the levels of AP (p=0,006) and N Mid Osteocalcine (p= 0,000) between the two groups- patients and controls. The values of the above mentioned indicators are higher in the group of patients. But there is no significant difference in the levels of the common (p= 0,587) and ionized (p= 0,256) calcium and β Cross Laps (p= 0,621) between the two groups. There is no significant difference in the age between patients and contols, too- (p=0,070), (table 2). It is important to mention that in the group of girls- patients and controls, there is no difference in none of the indicators that are examined, but this is not the same in the group of boys. The patients of the male gender show higher levels of AP (p= 0,010) and N Mid Osteocalcine (p= 0,000) than controls. This data is shown on table 3 and table 4. A significant difference for the two genders in the levels of AP (p= 0,039), N Mid Osteocalcine (p= 0,045) and β Cross Laps (p=0,004) is observed only in the group of patients. These levels are increased in boys (table 5).

The following correlative subjections are determined for the group of the patients (table 6):

- An inverse correlation of Ca (r=-0,44; p=0,0078), AP (r= -0,45; p=0,0039); N Mid Osteocalcine (r= -0,34; p=0,0226) and β Cross Laps (r= -0,48; p=0,0009) for the age;
- A positive correlation between N Mid Osteocalcine and AP (r= +0,52; p=0,0007);
- A positive correlation between β Cross Laps and N Mid Osteocalcine (r= +0,53; p=0,0002);
- A positive correlation between β Cross Laps and AP (r= +0,48; p=0,0022)

The following correlative subjections are determined for the group of the controls (table 7):

- A negative correlation between the levels of AP (r=-0,59; p=0,0000); β Cross Laps (r=-0,38; p=0,0127) and the age of the participants.
- A positive correlation between β Cross Laps and AP (r= +0,41; p=0,0059).
- A positive correlation between β Cross Laps and N Mid Osteocalcine (r= +0,50; p=0,0006).

In the group of patients, a negative correlation between the levels of AP (r=-0,84; p=0,0002); N Mid Osteocalcine (r=-0,66; p=0,0041) and β Cross Laps (r=-0,63; p=0,0062) with the age of the female patients is determined (table 8). For the female controls the negative correlation between the levels of AP (r=-0,67; p=0,0025); N Mid Osteocalcine (r=-0,47; p=0,0510) and β Cross Laps (r=-0,72; p=0,0008) with the age stays constant (table 9).

While in male patients, there is a possitive correlation between the levels of N Mid Osteocalcine and AP (r=+0,41; p=0,0434); N Mid Osteocalcine and β Cross Laps (r=+0,45; p=0,0199), but a negative dependence of Ca from the age (r=-0,61; p=0,0025) (table 10).

The only one determined dependence in the group of the male controls is the negative correlation between AP and their age (r=-0,46; p=0,0198) (table 11).

DISCUSSION:

The genetic essence of CF as a disease, its multi-systemic character, the increased duration of patients' life in the last decades and many other factors that take part in the pathogenesis of the disease, lead to the revelation of a new problem for the patients with CF- the affection on musculo-skeletal system.

This study proves the existence of a significant difference between the levels of markers for bone formation- N Mid Osteocalcine and AP in two groups - controls and patients with CF. The results show that patients have higher levels of bone formation markers (N Mid Osteocalcine and AP are increased) than controls. There is no significant difference between the levels of the markers for bone resorption (Ca and β Cross Laps) in patients and controls.

Bone disease is more typical for adolescents and adults with CF. Mainly children take part in this study- the average age is 14,7 years. It's important to mention that the process of bone metabolism and the increased levels of bone formation are more typical for one infant organism. Bone diseases are connected with the extend of pulmonary affection, nutritive status of patients, gene mutation type and i.e. That's why younger and clinically stable patients (many of the participants in this study) may have a normal bone density and this may be in a support of our results.

Having in mind that the difference between the marker for bone resorption- β Cross Laps, in the two groups- patients and controls, is not significant, we might say that patients with CF have higher level of it (0,847 ng/ml for patients and 0,796 ng/ml - for controls). We may conclude that patients with cystic fibrosis suffer from increased bone resorption than controls.

In this case the increased levels of bone formation is a consequence- as a compensatory reaction, from the increased bone resorption, if we examine, bone as an organ. The previ-

ously found positive correlative relation, on one hand between β Cross Laps, N Mid Osteocalcine and AP on the other hand, confirm that fact.

The significant difference between N Mid Osteocalcine and AP (markers for bone formation), which is found in boys who participate in the two groups- patients and controls, stays unchanged. In girls, this difference is not significant. In the group of patients with cystic fibrosis, we can say that males are more affected than females are. That is why our conclusion is that boys with CF have higher levels of bone resorption than girls suffering of the same disease do. The low levels of androgens in males have an important role during the period of growing. The androgenous deficit in patients with CF is proved by many researches and it leads to cortical and trabecular osteopenia by means of increasing in bone resorption.

The intensity of bone turnover decreases gradually with age- found negative correlations between measured markers and the age of the patients with CF, mentioned above. The reduction of bone formation in patients with CF, the lack of the same in controls and the reduced levels of marker for bone resorption in patients and controls, shows us a tendency for diminution of the structure of bones in patients with the age.

DEDUCTIONS:

1. Patients with CF who took part in this investigative project show a significantly increased bone formation in comparison to the group of controls. This is probably a compensatory reaction of human body to the increased level of bone resorption (although this is not significant for statistics);

2. Males with CF have an increased bone formation, if we compare them to males who are controls;

3. Females from the group of patients do not show any notable difference in all of the examined markers;

4. The males with cystic fibrosis show a statistically considerable difference of the markers examined in the study, compared to the women with cysic fibrosis. Patients of male gender have increased levels of bone formation and bone resorption than the female patients. Bone resorption is higher than the formation of the bones;

5. The levels of all of the examined markers in patients decrease with age. This shows us that the intensive processes of bone metabolism in early years "calm down" gradually in upper age groups. This fact is more typical for the process of bone formation and it is not registered in the groups of contols;

6. N MID Osteocalcine correlates in a positive way with AP in the two groups and this confirms the fact that the rise of AP-levels is due to its bone fraction.

CONCLUSION:

The realization of the aims of this project- the examination of the markers of bone metabolism in children who suffer from cystic fibrosis compared to children- healthy contols, proves several important assertions: males are more affected than females, there is a statistically considerable increase of bone formation markers in comparison to these of bone resorption in patients and controls, there is a decrease of all these changes with the age of patients.

Table 1. Distribution of the participants**.**

participants	patients	controls
all	44	43
girls	17/ 38,6 %	18 / 41,8%
boys	27/ 61,3 %	25 / 58,1%

Table 2.

marker	group	N	$\overline{X}$	SE	SD	t	df	p
Age	**patients**	44	14,7	1,1	7,4	-1,84	85	0,070
	controls	43	13,8	1,2	7,9			
Ca_all	**patients**	35	2,20	0,02	0,13	0,55	76	0,587
	controls	43	2,22	0,02	0,15			
Ca_i	**patients**	40	1,25	0,01	0,08	-1,14	77	0,256
	controls	39	1,23	0,01	0,08			
AP	**patients**	39	215,3	21,4	133,3	-2,80	80	0,006
	controls	43	152,4	9,1	59,4			
NMidOC	**patients**	44	74,16	7,40	49,06	-3,97	85	0,000
	controls	43	41,85	3,25	21,31			
βCL	**patients**	44	0,847	0,073	0,483	-0,50	85	0,621
	controls	43	0,796	0,074	0,483			

Comparison of the examined indexes between patients and controls.

Table 3. Females

marker	group	N	$\overline{X}$	SE	SD	t	df	p
Age	**patients**	17	17,2	1,9	8,0	-0,72	33	0,478
	controls	18	15,3	1,9	8,1			
Ca_all	**patients**	13	2,23	0,03	0,10	-0,43	29	0,672
	controls	18	2,21	0,03	0,14			
Ca_i	**patients**	16	1,27	0,02	0,09	-0,83	31	0,415
	controls	17	1,24	0,03	0,10			
AP	**patients**	14	156,9	21,3	79,7	-0,78	30	0,442
	controls	18	137,1	15,0	63,8			
NMidOC	**patients**	17	55,63	10,33	42,60	-1,64	33	0,111
	controls	18	36,47	5,87	24,92			
βCL	**patients**	17	0,591	0,080	0,329	0,40	33	0,690
	controls	18	0,645	0,106	0,450			

Comparison of the examined indexes between females-patients and controls.

Table 4. Males

marker	group	N	$\overline{X}$	SE	SD	t	df	p
Age	**patients**	27	13,1	1,3	6,7	-2,15	50	0,037
	controls	25	9,0	1,4	6,8			
Ca_all	**patients**	22	2,18	0,03	0,15	0,87	45	0,389
	controls	25	2,22	0,03	0,16			
Ca_i	**patients**	24	1,24	0,02	0,08	-0,87	44	0,387
	controls	22	1,22	0,01	0,06			
AP	**patients**	25	248,0	29,4	147,0	-2,70	48	0,010
	controls	25	163,4	10,9	54,7			
NMidOC	**patients**	27	85,83	9,62	49,98	-3,79	50	0,000
	controls	25	45,73	3,57	17,83			
βCL	**patients**	27	1,008	0,096	0,499	-0,76	50	0,451
	controls	25	0,904	0,097	0,486			

Comparison of the examined indexes between males-patients and controls.

Table 5.

marker	group	N	$\overline{X}$	SE	SD	t	df	p
Ca_all	**females**	13	2,23	0,03	0,10	-1,11	33	0,274
	males	22	2,18	0,03	0,15			
Ca_i	**females**	16	1,27	0,02	0,09	-1,19	38	0,242
	males	24	1,24	0,02	0,08			
AP	**females**	14	156,9	21,3	79,7	2,14	37	0,039
	males	25	248,0	29,4	147,0			
NMidOC	**females**	17	55,63	10,33	42,60	2,06	42	0,045
	males	27	85,83	9,62	49,98			
βCL	**females**	17	0,591	0,080	0,329	3,05	42	0,004
	males	27	1,008	0,096	0,499			

Comparison of the examined indexes between patients- males and females.

Table 6.

Patients	age	Ca (all)	Ca ++	AP	NMidOC	
	r	-0,44				
Ca (all)	p	0,0078				
	N	35				
	r	0,09	0,26			
Ca ++	p	0,595	0,132			
	N	40	35			
	r	-0,45	-0,04	-0,13		
AP	p	0,0039	0,8143	0,4705		
	N	39	34	35		
	r	-0,34	-0,04	0,01	0,52	
NMidOC	p	0,0226	0,8085	0,9747	0,0007	
	N	44	35	40	39	
	r	-0,48	0,09	-0,17	0,48	0,53
βCL	p	0,0009	0,6225	0,3049	0,0022	0,0002
	N	44	35	40	39	44

Some correlative subjections between the examined indexes in the group of patients.

Table 7.

Controls	age	Ca (all)	Ca ++	AP	NMidOC	
	r	0,03				
Ca (all)	p	0,8606				
	N	43				
	r	0,20	-0,24			
Ca ++	p	0,2241	0,1391			
	N	39	39			
	r	-0,59	-0,05	0,00		
AP	p	0,0000	0,7467	0,9835		
	N	43	43	39		
	r	-0,26	0,17	-0,31	0,26	
NMidOC	p	0,0951	0,2659	0,0519	0,0980	
	N	43	43	39	43	
	r	-0,38	0,18	-0,23	0,41	0,50
βCL	p	0,0127	0,2532	0,1640	0,0059	0,0006
	N	43	43	39	43	43

Some correlative subjections between the examined indexes in the group of controls.

Table 8.

Female Patients	age	Ca (all)	Ca++	AP	NMidOC
	r	-0,48			
Ca (all)	p	0,0967			
	N	13			
	r	-0,04	0,61		
Ca ++	p	0,8694	0,0276		
	N	16	13		

	r	-0,84	0,38	-0,14		
AP	p	0,0002	0,2045	0,6583		
	N	14	13	13		
	r	-0,66	0,14	-0,30	0,73	
NMidOC	p	0,0041	0,6511	0,2569	0,0028	
	N	17	13	16	14	
	r	-0,63	0,25	0,12	0,60	0,53
βCL	p	0,0062	0,4011	0,6660	0,0240	0,0293
	N	17	13	16	14	17

Some correlative subjections between the examined indexes for the group of female patients.

Table 9.

Female Controls	**age**	**Ca (all)**	**Ca ++**	**AP**	**NMidOC**	
	r	0,34				
Ca (all)	p	0,1661				
	N	18				
	r	0,05	-0,40			
Ca ++	p	0,8430	0,1080			
	N	17	17			
	r	-0,67	0,00	-0,04		
AP	p	0,0025	0,9990	0,8923		
	N	18	18	17		
	r	-0,47	0,19	-0,43	0,39	
NMidOC	p	0,0510	0,4398	0,0877	0,1085	
	N	18	18	17	18	
	r	-0,72	0,13	-0,25	0,59	0,69
βCL	p	0,0008	0,6191	0,3243	0,0094	0,0014
	N	18	18	17	18	18

Some correlative subjections between the examined indexes for the group of female controls.

Table 10.

Male- Patients	**age**	**Ca (all)**	**Ca ++**	**AP**	**NMidOC**	
	r	-0,61				
Ca (all)	p	0,0025				
	N	22				
	r	0,09	0,06			
Ca ++	p	0,6678	0,8073			
	N	24	22			
	r	-0,27	0,01	-0,05		
AP	p	0,1982	0,9828	0,8343		
	N	25	21	22		
	r	-0,06	0,00	0,30	0,41	
NMidOC	p	0,7811	0,9825	0,1601	0,0434	
	N	27	22	24	25	
	r	-0,34	0,16	-0,22	0,36	0,45
βCL	p	0,0805	0,4870	0,3089	0,0747	0,0199
	N	27	22	24	25	27

Some correlative subjections between the examined indexes for the group of male patients.

Table 11.

Males- Controls	age	Ca (all)	Ca ++	AP	NMidOC	
Ca (all)	r	-0,17				
	p	0,4264				
	N	25				
Ca ++	r	0,36	-0,02			
	p	0,1035	0,9412			
	N	22	22			
AP	r	-0,46	-0,10	0,13		
	p	0,0198	0,6396	0,5506		
	N	25	25	22		
NMidOC	r	0,13	0,17	-0,04	0,02	
	p	0,5319	0,4296	0,8616	0,9229	
	N	25	25	22	25	
βCL	r	0,01	0,21	-0,17	0,22	0,29
	p	0,9586	0,3222	0,4405	0,3001	0,1638
	N	25	25	22	25	25

Some correlative subjections between the examined indexes for the group of male controls.

REFERENCES:

1. Galeva I., Clinical and genetic characteristics of cystic fibrosis in early childhood. Dissertation, Sofia, 2000. [in Bulgarian]

2. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boylw MP, Elkin SL, et al. Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab.* 2005 Mar; 90(3):1888-96. [PubMed] [CrossRef]

3. Elkin SL, Faimey A, Burnett S, Kemp M, Kyd P, Burgess J, et al. Vertebral deformities and low bone mineral density in adults with cystic fibrosis: a cross sectional study. *Osteoporos Int.* 2001; 12(5):366-72. [PubMed]

4. Sheikh S, Gemma S, Patel A. Factors associated with low bone mineral density in patients with cystic fibrosis. *J Bone Miner Metab.* 2015 Mar; 33(2):180-5. [PubMed] [CrossRef]

5. Donadio MV, Souza GC, Tiecher G, Heinzmana-Fiho JP, Paim TF, Hommerding PX, et al. Bone mineral density, pulmonary function, chronological age and age at diagnosis in children and adolescents with cystic fibrosis. *J Pediatr (Rio J).* 2013 Mar-Apr;89(2):151-7. [PubMed] [CrossRef]

6. Rossini M, Del Marco A, Dal Santo F, Gatti D, Braqqion C, James G, et al. Prevalence and correlates of vertebral fractures in adults with cystic fibrosis. *Bone.* 2004 Sep;35(3):771-6. [PubMed] [CrossRef]

7. Aris RM, Renner JB, Winders AD, Buell HE, Riqqs DB, Lester GE, et al. Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. *Ann Intern Med.* 1998 Feb;128(3):186-93. [PubMed] [CrossRef]

8. Neri AS, Lori I, Festini F, Masi L, Brandi ML, Galici V, et al. Bone mineral density in cystic fibrosis patients under the age of 18 years. *Minerva Pediatr.* 2008 Apr;60(2):147-54. [PubMed]

9. Florescu L, Paduraru DT, Mindres DE, Temneanu OR, Petzariu FD, Matei MC, et al. Epidemiological evaluation regarding the role of cystic fibrosis as a risk factor for child malnutrition. *Rev Med Chir Soc Med Nat Iasi.* 2014 Apr-Jun;118(2):450-6. [PubMed]

Please cite this article as: Naydenova- Stoeva B. Markers of bone metabolism in patients with cystic fibrosis. *J of IMAB.* 2015 Oct-Dec;21(4):901-907. DOI: http://dx.doi.org/10.5272/jimab.2015214.901

Received: 23/09/2015; Published online: 10/11/2015

Address for correspondence:
Dr. Bojana Naydenova- Stoeva
Pediatric Clinic UMHAT "Alexandrovska"
1, G. Sofiysky, Blvd., 1431, Sofia, Bulgaria
e-mail: jani_2005@mail.bg