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Research Article

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Evaluation of Receptor Activator Nuclear Kappa B Ligand and Osteoprotegerin Levels in Patients with Osteoporosis and Bronchiectasis

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Abstract

Objective

Bronchiectasis is a condition characterized by irreversible abnormal dilation and anatomic breakdown of the bronchial tree. In our study, we aimed to understand the causes of osteoporosis at molecular levels and to investigate the usefulness of RANKL and OPG levels as markers in early diagnosis and follow-up of osteoporosis, RANKL inhibitors in the treatment of osteoporosis.

Materials and Methods

30 non-cystic fibrosis bronchiectasis patients were diagnosed with osteoporosis with DEXA and applied to the Pediatrics Chest Diseases Department of Necmettin Erbakan University Faculty of Medicine between June 2015 and June 2016 were included in our study. BMD values were determined by DEXA and QUS in the patient group and only QUS in the control group.

Results

In the patient group 56.6% (n: 17) were male, and 43.4% (n: 13) were. There was no statistically significant difference between RANKL, OPG, RANKL / OPG ratios, BALP, OST, and NTX values between the patient and control group (p> 0.05). The median serum Ca level was 9.51 (IQC: 0.74) in the patient group and 9.75 (IQC: 0.47) in the control group, there was a statistically significant difference between them (p = 0.003). There was a strong positive correlation between QUS z scores and DEXA z scores in the patient group (p = 0.008).

Conclusion

In conclusion, we could not pinpoint the role of RANK / RANKL / OPG in the pathogenesis of osteoporosis in patients with non-cystic fibrosis bronchiectasis. However, we think it is appropriate to conduct studies on a wider series on this topic.

Keywords: Non-Cystic Fibrosis Bronchiectasis, Osteoporosis, RANKL, OPG

Abbreviations

ABPA: Allerjik Bronkopulmoner Aspergillozis
aKMY: Alansal kemik mineral yoğunluğu
ALP: Alkalen Fosfataz
bALP: Bone ALP
BMU: Bone remodelling unit
BO: Bronşiyolitis obliterans
BP: Bifosfonat
CFTR: Cystic fibrosis transmembrane conductance regulator
DEXA: Dual-energy X-ray absorptiometry
ELISA: Enzyme Linked Immunosorbent Assay
FEV1: 1.saniyedeki zorlu ekspiratuar hacim
FVC: Zorlu vital kapasite
GLA: γ-karboksiglutamikasit
GÖR: Gastroözofagiyel reflü
Grb2: Growth factor receptor-bound protein 2
HSV: Herpes Simpleks Virüsü
IFN: İnterferon
IGF-1: İnsulin-like Growth Factor-1
IGFBP-3: IGF binding protein-3
IL: İnterlökin
IJO: İdiyopatik Juvenil Osteoporoz
KBT: Kantitatif bilgisayarlı tomografi
KF: Kistik Fibrozis
KMY: Kemik Mineral Yoğunluğu
LRP5: Low-density lipoprotein receptor-related protein
MAPKs: Mitogen-activated protein kinases
MHC: Major histocompatibility complex
M-CSF: Macrophage-colony stimulating factor
NTX: N-terminal telopeptid
OCIF: Osteoklastogenez inhibitör faktör
ODAR: Osteoclast or osteoclast differentiation and activation receptor
OI: Osteogenesis Imperfecta
OPG: Osteoprotegerin
OPGL: Osteoprotegerin ligand
OSCAR: Osteoclast associated immunoreceptor
P: Fosfor
PEP: Pozitif ekspiratuvar basınç
PGE_2: Prostaglandin E_2
PICP: Tip 1 kolajen C terminal propeptid
PINP: Tip 1 kolajen N terminal polipeptid
PSD: Primer Silyer Diskinezi
QUS: Kantitatif ultrasonografi
RANK: Reseptör Aktivatör Nükleer Kappa B
RANKL: Reseptör Aktivatör Nükleer Kappa B Ligand
RA: Radyografik Absorbsiyometri
SLE: Sistemik Lupus Eritematozus
SP: Serebral Palsi
TGF: Transforming growth factor
TNF-α: Tümör nekrozis faktör alfa
TNFR: TNF reseptör
TRAF: TNFR ilişkili faktör
TRAIL: TNF ilişkili apoptozisi indükleyen ligand
YÇBT: Yüksek çözünürlüklü bilgisayar tomografisi
ZKK: Zirve kemik kütlesi

1. Introduction

Bronchiectasis is characterized by irreversible abnormal dilation and anatomic breakdown of the bronchial tree. Although the frequency of bronchiectasis has been declining due to the improvement in nutrition and sanitation circumstances, increased vaccination rate, and early and frequent use of antibiotics, it still causes severe health problems in developing countries [1].

Pifferi et al [2]. reported the frequency of bronchiectasis as 1/6000 in the general pediatric population in their study in 2004.

The most common cause of bronchiectasis in developed countries is Cystic Fibrosis (CF). Bronchiectasis patients with CF and non-cystic fibrosis have many common features [3].

Osteoporosis and osteopenia are seen in patients with cystic fibrosis and non-cystic fibrosis bronchiectasis. The fact that osteoporosis is seen in patients with cystic fibrosis even at very young ages suggests that this situation may also be related to bone metabolism. Although there are publications on this subject in patients with cystic fibrosis bronchiectasis, there are not many publications in the literature on patients with non-cystic fibrosis bronchiectasis [3].

Osteoporosis is a systemic bone disease characterized by decreased bone mineral density (BMD) and impairment of inner architecture. The amount of mineralization is normal in all forms of osteoporosis, while decreased bone volume in, especially trabecular bone, and decreased trabecular bone cycle are noticed. Osteoporosis is evaluated using BMD, dual-energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT), and quantitative ultrasonography (QUS). Osteoporosis is frequently encountered in chronic diseases, especially with a prolonged hospital stay, inadequate vitamin D intake, and drug use [4].

Many mechanisms are responsible for the development of osteoporosis. Bone resorption secondary to the changes in the RANK-RANKL-OPG system is in the forefront recently [5]. OPG is a member of the receptor TNFR superfamily and is also known as TNFRS11B. Another name for it is osteoclastogenesis inhibitory factor (OCIF). The original OPG molecule is a polypeptide composed of 401 amino acids, and the mature protein composed of 380 amino acids is formed by separating the 21-amino acid propeptide part.

The association between osteoporosis and RANKL/OPG levels has been demonstrated in the literature in patients with CF in whom bronchiectasis is common. However, no study has been published evaluating the association between osteoporosis and RANKL/OPG levels in patients with non-cystic fibrosis bronchiectasis. In this study, by understanding the molecular level causes of osteoporosis developing non-cystic fibrosis bronchiectasis; We aimed to investigate the use of RANKL and OPG levels as a marker in the early diagnosis and follow-up of osteoporosis, and to investigate the usability of RANKL inhibitors in the treatment.

2. Material And Method

Patients between 5-18 years of age and applied to the outpatient clinics of Pediatric Pulmonary Diseases with the diagnosis of non-cystic fibrosis bronchiectasis and osteoporosis were included in the study. When choosing the control group, children in the

same age group who did not have a chronic disease and applied to the healthy pediatric outpatient clinic were included. Children with chronic illness and an active disease such as a base at that time were not included. DEXA measurement was performed to evaluate the bone mass of patients with bronchiectasis.

BMD value measured by DEXA is expressed by Z and T scores. Comparison of the measured bone mass with reference values according to age and gender and definition of it as standard deviation is the Z score [6,7]. The standard deviation of comparison of the bone mass with the mean bone mass of the young adult reference population is the T score. BMD measured in children is evaluated as a Z score. The length of the measured bone, in addition to the height, pubertal phase, skeletal maturation, race, and body composition, is considered to correctly interpret the data obtained by the DEXA method [8]. Another method of evaluation in children is QUS [9].

2.1 Collection and Storage of Samples and Study Method

Blood samples obtained were centrifuged at a cooling centrifugation device with the trademark Hettich Rotina 46R (Hettich Zentrifugen, Tuttlingen, Germany) at 4000 cycles/minute for 10 minutes and the sera were separated. Serum samples were stored in New Brunswick U570 (New Brunswick Scientific, New Jersey, ABD) refrigerator at -80°C. Enzyme Linked Immunosorbent Assay (ELISA) method was used in the serum samples using human RANKL (Biovendor Research and Diagnostic Products Karasek, The Czech Republic), OPG Biovendor Research and Diagnostic Products Karasek, The Czech Republic, bALP (Quidel Corporation, San Diego, USA), OST (Biovendor Research and Diagnostic Products Karasek, The Czech Republic), NTX (Alere Scarborough, Scarborough, USA) kits.

The results were calculated according to the absorbance concentration calibration graphics using Biotek ELX 50 microplate washer (BioTek Instruments, Vermont, USA) and Bio-rad Microplate absorbance reader xMark (Bio-rad Laboratories, California, USA) system.

2.2 Statistical Analysis

All analyzes of the study were performed using SPSS 20.0 package program. Nominal scale (categorical) variables were presented using frequency and percentage; proportional scale (numerical) variables with normal distribution using mean±SS and with non-normal distribution using median (interquartile range) in tables and graphics. All the proportional scale variables were checked for whether they were normally distributed or not using Kolmogorov-Smirnov and Shapiro-Wilk analyses. Age, bALP, Bone US BMD, and Mg values were found to be normally distributed in the groups (p>0,05), and the remaining values were found to be non-normally distributed with a high skewness value. Therefore, the Student t-test was used in an independent two-group comparison for parameters of Age, bALP, Bone US BMD, and Mg since they were parametric variables, and the Mann-Whitney U test was preferred for nonparametric variables. Monte Carlo corrected chi-square analysis was used to determine the significance of the association between categorical variables. Pearson correlation coefficient was used when both parameters were normally distributed to establish the correlation between the numerical variables. Spearman's Rho correlation coefficient was used when any of the parameters was non-normally distributed. Type-1 error level was accepted as 5 % in all the analyzes and p<0,05 was accepted as statistically significant.

3. Results

Among the 30 patients included in the study, 56,6 % (n:17) were boys and 43,3% (n:13) were girls with a mean age of 13,3±3,8 (5-18) years. Among the 40 patients included as the control group, 35 % (n:14) were boys and 65% (n:26) were girls with a mean age of 11,8±3,5 (5-17) years. No significant differences were found in age and gender between the patient and control groups (p>0,05).

When the underlying cause was evaluated in the study group, 13 were found to be immune deficiency, 9 had PSD, 4 had infections, 3 were idiopathic, and 1 was Mac Leod Syndrome.

The median serum RANKL levels in the patient and control groups were 373.9 (IQC:312) and 458.5 (IQC:345.3), respectively, and there was no statistically significant difference between them (p=0.069). The median serum OPG levels in the patient and control groups were 3.694 (IQC: 0.566) and 3.714 (IQC: 0.794), respectively, and the difference between them was not statistically significant (p=0.451). The median values of the RANKL/OPG ratio in the patient and control groups were 98.68 (IQC:112.88) and 117.91 (IQC:84.99), respectively, and no statistically significant difference was found (p=0.065).

The median serum Ca levels in the patient and control groups were 9.51 (IQC:0.74) and 9.75 (IQC:0.47), respectively, and were found to be significantly lower in the patient group (p=0.003). The median urinary Ca/Cr levels in the patient and control groups were 0.074 (IQC:0.092) and 0.029 (IQC:2.0), respectively, and there was a statistically significant difference between them (p=0.012). Similarly, z-score measurements made by QUS in the patient and control groups were -1.88±1.05 and -0.86±0.67, respectively, and were significantly lower in the patient group (p<0.001). The laboratory values of the patients are given in Table 1.

	Patient group (n=30)	Control group (n=40)	p value
RANKL pmol/L	373,9 (312)	458,5 (345,3)	0,069
OPG pmol/L	3,694 (0,566)	3,714 (0,794)	0,451
RANKL/OPG	98,68 (112,88)	117,91 (84,99)	0,065
OST ng/mL	20637 (46821)	27635,5 (48685)	0,222
NTX nM BCE	82,09 (85,9)	64,60 (61,409)	0,549
KMD z score	-2,4 (0,8)		
Ca mg/dL	9,51 (0,74)	9,75 (0,47)	0,003*
ALP u/L	200 (98,5)	196,5 (112,3)	0,943
PTH pg/mL	36,6 (29,5)	39,6 (26,2)	0,962
Vit D ng/mL	15 (15,3)	15,5 (12)	0,938
Urine Ca/Cr	0,074 (0,092)	0,029 (2,0)	0,012*
bALP u/L	89,11 ± 38,15	95,87 ± 41,53	0,488
QUS	-1,88 ± 1,05	-0,86 ± 0,67	<0,001*
P mg/dL	4,35 ± 0,72	4,57 ± 0,62	0,176
Mg mg/dL	1,97 ± 0,23	1,98 ± 0,16	0,835

Table 1: Mean laboratory values in the patient and control groups

**Non parametric values were expressed as median and IQC (in parenthesis) ** Parametric values were expressed as Mean ± Standard Deviation (in parenthesis)*

When a triple comparison was made between the two main disease groups that make up the patient group, immunodeficiency and PSD patients, and the control group, it was observed that there was a significant difference between QUS, Ca, vitamin D, spot urine Ca/Cr values ($p<0.05$).

A significant difference was found between the QUS values in the triple comparison, and it was seen that this difference was due to the difference between the immunodeficiency and PSD patients and the control group ($p<0.001$ and $p=0.003$, respectively). The difference between the Ca values in the triple comparison was due to the difference between the immunodeficiency and PSD group and the immunodeficiency and control groups ($p=0.021$ and $p=0.003$, respectively). In the triple comparison, it was observed that the significant difference between vitamin D levels resulted from the difference between the immunodeficiency and PSD groups ($p=0.038$). It was shown that the statistically significant difference seen in the three-group comparison of Ca/Cr values in spot urine resulted from the difference between the PSD and control groups ($p=0.027$). Comparisons of patient subgroups are given in Table 2.

	Immune deficiency	PSD	Control	P value
Age	13,1(3,91)	15,1(8,25)	12,5(6,02)	0,673
RANKL pmol/L	368,1 (313,4)	432 (303,2)	458,5 (345,3)	0,075
OPG pmol/L	3,743(0,569)	3,344(0,575)	3,714 (0,794)	0,135
OST ng/mL	18390(30165)	22092(40216)	27635,5 (48685)	0,219
NTX nM BCE	72,464(90,087)	88,774(168,77)	64,607 (61,409)	0,919
bALP u/L	95,517(53,696)	73,761(44,127)	93,0405(60,899)	0,28
BMD z score	-2,4(0,8)	-2,3 (0,3)		0,647
QUS	-2 (1,15)	-1,9 (0,75)	-0,8 (0,7)	<0,001*
Ca mg/dL	9,47 (0,85)	9,95 (0,79)	9,755 (0,47)	0,002*
P mg/dL	4,350 (0,85)	4,5 (0,90)	4,56(0,90)	0,288
Mg mg/dL	2,08 (0,27)	2 (0,26)	1,99 (0,17)	0,361
ALP u/L	203 (108)	167 (106)	196,5 (112,3)	0,543
PTH pg/mL	36,5 (29,8)	36 (22,8)	39,6 (26,2)	0,79
D Vit ng/mL	8,5 (5,1)	18 (8,5)	15,5 (12)	0,029*
Spot urine Ca/Cr	0,077 (0,124)	0,072 (0,118)	0,029 (0,043)	0,043*

Table 2: Mean laboratory values in the patient groups and control group

**Non parametric values were expressed as median and IQC (in parenthesis) ** Parametric values were expressed as Mean ± Standard Deviation (in parenthesis)*

When immunodeficiency and PSD patients were compared, Ca values were found to be significantly higher in PSD patients (median values of 9.47 and 9.95, respectively; p=0.014). The median value of vitamin D levels in immunodeficiency patients was 8.5 (IQC:5.1), in PSD patients it was 18 (IQC:8.5) and a statistically significant difference was found between these two patient groups (p=0.015). When immunodeficiency patients and the control group were compared, RANKL values were found to be significantly higher in the control group (368.1 and 458.5, respectively; p=0.026). When immunodeficiency and control groups were compared, Ca and vitamin D levels were found to be significantly higher in the control group (p<0.05). When the PSD and control groups were compared, the median values of spot urinary Ca/Cr were found to be 0.072 and 0.029, respectively, and the Ca excretion was found to be significantly higher in the PSD group (p=0.019). The comparison of the patient groups is given in Table 3.

	RANKL	OPG	OST	NTX	bALP	KMD	QUS	Ca	P	Mg	ALP	PTH	D Vit	Spot Urine Ca/Cr	Δ Bone age
Immune deficiency-PSD	0,357	0,036	0,471	0,845	0,11	0,647	0,209	0,014*	0,324	0,744	0,292	0,556	0,015*	0,744	0,014
Immune deficiency-Control	0,026*	0,796	0,09	0,992	0,82		<0,001*	0,001*	0,121	0,176	0,45	0,694	0,027*	0,126	
PSD-Control	0,408	0,086	0,517	0,675	0,17		<0,001*	0,713	0,829	0,484	0,55	0,62	0,313	0,019*	

Table 3: p values found in the binary comparisons of immune deficiency, PSD and control groups

A statistically significant negative correlation was found between RANKL and ALP in the patient group (rs=-0.391, p=0.04). A positive correlation was found between RANKL levels and QUS z values in the patient and control groups (rs=0.239, p=0.048). A strong negative correlation was found between OPG and OST in the patient group (rs=-0.499; p=0.005).

OST was strongly positively correlated with bALP, ALP, and NTX and positively correlated with Ca (rs=0.565, p=<0.00; rs=0.364, p=0.002; rs=0.546, p<0.001; rs=0.237,p=0.048) in the patient and control groups. When the patient group and the control group were evaluated together, a strong positive correlation was found between NTX and bALP (rs=0.492, p=0.006 and rs=0.599; p<0.001, respectively). A statistically significant positive correlation was found between NTX and spot urine Ca/Cr and bALP ratios in the patient group (rs=0.377; p=0.04 and rs=0.492; p=0.006), respectively. When the patient group and control group were evaluated together, a strong positive correlation was found between NTX and P (Phosphorus) and ALP (rs=0.42; p<0.001 and rs=0.379; p=0.001, respectively).

When the patient and control groups were evaluated together, a strong correlation was found between the BMD values obtained by DEXA and the values obtained by QUS (rs=0.476, p=0.008). When the patient and control group were evaluated together, a positive correlation was found between QUS values and serum Ca values (rs=0.309, p=0.009). Correlations are given in Table 4-5.

		RANKL	Age	OPG	OST	NTX	BALP	BMD	QUS	Ca	P	Mg	ALP	PTH	Vıt D
Age	Rs	-0,21													
	P	0,085													
OPG	Rs	0,124	-0,196												
	P	0,315	0,104												
OST	Rs	-0,054	-0,218	-0,118											
	P	0,661	0,069	0,33											
NTX	Rs	0,039	-0,293	0,146	0,546**										
	P	0,751	0,014	0,228	<,001										
BALP	Rs	0,127	-0,341**	0,176	0,565**	0,599**									
	P	0,303	0,004	0,146	<,001	<,001									
BMD	Rs	0,068	-0,332	-0,059	0,102	0,044	0,01								
	P	0,73	0,073	0,758	0,592	0,819	0,957								
QUS	Rs	0,274*	-0,245*	0,146	-0,084	-0,133	0,037	0,476**							
	P	0,024	0,041	0,228	0,489	0,271	0,761	0,008							
Ca	Rs	0,18	-0,201	0,07	0,237*	0,223	0,228	0,102	0,309**						
	P	0,143	0,095	0,562	0,048	0,064	0,058	0,592	0,009						
P	Rs	0,134	-0,423**	0,103	0,217	0,420**	0,238*	0,458*	0,075	0,076					
	P	0,276	<,001	0,398	0,071	<,001	0,047	0,011	0,537	0,531					
MG	Rs	-0,101	-0,280*	0,065	-0,085	-0,166	0,025	-0,1	0,129	0,047	0,04				

	P	0,412	0,019	0,592	0,485	0,169	0,836	0,597	0,289	0,702	0,74				
ALP	Rs	-0,04	-0,346**	0,169	0,364**	0,379**	0,614**	-0,052	-0,109	0,165	0,171	-0,013			
	P	0,745	0,003	0,163	0,002	0,001	<,001	0,787	0,367	0,172	0,158	0,915			
PTH	Rs	-0,137	0,121	-0,069	0,07	0,073	0,112	-0,238	-0,072	-0,185	-0,049	-0,265*	0,129		
	P	0,265	0,319	0,569	0,563	0,547	0,358	0,206	0,555	0,125	0,689	0,027	0,288		
Vıt D	Rs	0,224	-0,099	0,148	0,128	0,145	0,043	0,301	0,129	0,187	0,23	-0,08	0,06	-0,422**	
	P	0,067	0,414	0,222	0,293	0,232	0,723	0,107	0,289	0,12	0,056	0,509	0,624	<,001	

Table 4: Correlations when comparing patient and control groups (light colored ones r value, others rs value)

		RANKL	OPG	OST	NTX	KMD	Ca	ALP	PTH	D Vit.	Ca/Kre	Age	bALP	QUS	P
OPG	rs	-0,179													
	P	0,362													
OST	rs	-0,151	-0,499**												
	P	0,445	0,005												
NTX	rs	-0,033	0,038	0,383*											
	P	0,866	0,843	0,037											
BMD	rs	0,068	-0,059	0,102	0,044										
	P	0,73	0,758	0,592	0,819										
CA	rs	0,164	-0,326	0,269	0,284	0,102									
	P	0,404	0,079	0,15	0,128	0,592									
ALP	rs	-0,391*	0,04	0,307	0,258	-0,052	-0,135								
	P	0,04	0,834	0,098	0,169	0,787	0,478								
PTH	rs	-0,223	-0,095	0,138	0,016	-0,238	-0,204	0,228							
	P	0,255	0,618	0,467	0,934	0,206	0,279	0,226							
Vıt D	rs	0,285	-0,146	0,176	0,039	0,301	0,206	-0,284	-0,400*						
	P	0,142	0,441	0,353	0,84	0,107	0,275	0,128	0,028						
Ca/Cr	rs	0,249	-0,051	-0,135	0,377*	-0,117	0,103	0,012	-0,009	-0,082					
	P	0,202	0,79	0,477	0,04	0,538	0,589	0,951	0,964	0,665					
Age	rs	-0,023	-0,111	-0,052	-0,364*	-0,332	-0,144	-0,311	-0,009	0,165	-0,156				
	P	0,906	0,56	0,785	0,048	0,073	0,449	0,095	0,961	0,384	0,41				
BALP	rs	0,053	0,039	0,370*	0,492**	0,01	0,103	0,374*	0,279	-0,212	0,208	-0,415*			
	P	0.788	0.838	0.044	0.006	0.957	0.587	0.042	0.136	0.261	0.27	0.023			
QUS	rs	0.216	-0.011	0.246	0.049	0.476**	0.148	-0.333	-0.081	0.263	-0.154	-0.256	0.043		
	P	0.269	0.952	0.189	0.798	0.008	0.435	0.072	0.67	0.16	0.415	0.172	0.82		
P	rs	0.24	0.202	0.029	0.332	0.458*	0.241	-0.056	-0.261	0.255	0.071	-0.359	0.229	0.125	
	P	0.219	0.285	0.878	0.073	0.011	0.199	0.768	0.163	0.174	0.709	0.051	0.224	0.511	
Mg	rs	-0.214	0.29	-0.11	-0.089	-0.1	0.108	0.166	0.045	-0.217	-0.159	-0.483**	0.184	0.192	0.022
	P	0.275	0.12	0.562	0.641	0.597	0.57	0.381	0.813	0.25	0.402	0.007	0.331	0.31	0.909
Bone age	rs	-0.001	-0.182	0.002	-0.296	-0.218	-0.036	-0.297	-0.025	0.26	-0.055		-0.428*	-0.339	-0.480**
	p	0.998	0.337	0.993	0.112	0.248	0.851	0.111	0.895	0.165	0.775		0.018	0.066	0.007

Table 5: Correlations in the patient group (r value and rs value)

Discussion

Bronchiectasis is increasingly being diagnosed with the introduction of high-resolution computed tomography. The primary cause of non-cystic fibrosis bronchiectasis is postinfectious causes worldwide [10,11]. Studies have been published in the literature suggesting the effect of the RANK/RANKL/OPG system on the development of osteoporosis in patients with cystic fibrosis.

OPG is a member of the receptor TNFR superfamily and is also known as TNFRS11B. It is released extracellularly as a soluble glycoprotein and functions as a trap receptor for RANKL (12). Osteoporosis was developed in rats with OPG failure. RANKL is a key regulator in osteoclastogenesis and osteopetrosis is developed in rats without RANKL due to osteoclast deficiency. Presence of RANKL and M-CSF in the environment is required and sufficient for the conversion of osteoclast precursors to mature osteoclasts (11-12). RANKL receptor has been defined as RANK [10,12-14].

RANKL expression is controlled by many factors such as glucocorticoids, vitamin D and IL-1. Low RANKL levels in patients with immune deficiency might be associated with the significantly low levels of vitamin D in these patients compared to the control group [15,16].

OPG levels were lower in the patient group in this present study compared to the control group, though not significant. Median OPG in patients with PSD was found to be lower compared to the controls and patients with immune deficiency, though not statistically significant [12]. We considered that osteoporosis seen in patients with PSD might be due to the decreased expression of OPG in lung tissue.

In the studies performed, the OPG and RANKL levels measured on the 14th day of acute exacerbation and antibiotic treatment in patients with CF were found to be significantly higher compared to the stable period [17,18]. The serum samples were collected during the stable period of the patients and no comparison was made with the acute exacerbation period and this might be the cause of no significant difference in the RANKL, OPG, and RANKL/OPG levels in this present study.

Galluzzi et al [19]. in their study serum OPG levels were found significantly high in patients with Crohn's disease and Type 1 Diabetes Mellitus. Association of the changes in the RANK/RANKL/OPG system with chronic inflammation might be the underlying mechanism of osteoporosis in chronic diseases. We suggest that chronic inflammation might be effective in the pathogenesis of osteoporosis developing in bronchiectasis patients.

A powerful and negative correlation was found between the serum osteocalcin and OPG levels in the patient group. An inverse correlation was found between serum OPG level and BMD and osteocalcin level in a study by Oh et al [20]. In 80 Korean male patients between 42-70 years of age A powerful and positive correlation was found between OPG and osteocalcin in patients with osteoporosis in a study by Fahrleitner-Pammer and found that low OPG levels were associated with vertebral fractures [21].

Vitamin D levels were found to be low in both patient and control groups, with no statistically significant difference between the groups in this present study. Considering the previous studies, low levels of vitamin D in the healthy control group might be attributed to the % 48-80,3 incidence of Vitamin D deficiency and insufficiency in Turkey [22,23]. Vitamin D levels were found to be significantly low in the subgroup of patients with immune deficiency compared to the patients with PSD and the control group. When the immune deficiency and control group was compared, Ca and P levels were found to be significantly high in the control group. We thought that this might be due to the prescription of vitamin D and Ca preparations for patients in the case when osteoporosis was diagnosed subsequent to the required tests for osteoporosis due to the clinical high incidence of osteoporosis and osteopenia in patients with PSD.

The median serum Ca level was significantly different in the patient group and the control group in this present study. Serum Ca level was found to be significantly low in the subgroup with immune deficiency compared to the PSD subgroup and control group. No significant difference was found between the patient and control groups in terms of serum Ca levels in a study by Ambroszkiewicz et al [18]. In patients with CF We suggest that these results in this present study might be due to the low levels of vitamin D in the control group and the subgroup of immune deficiency.

A powerful and positive correlation was found in QUS z scores and DEXA, and BMD z scores in the patient group. According to the literature data, QUS is generally considered not to be a substitution for DEXA, but it could be used as a screening method. Williams et al [23]. Found a powerful correlation between QUS and DEXA in their study of obese patients with CF; however, they concluded that QUS could not be used in place of DEXA, especially in obese patients. Schepper et al [24]. Compared QUS, DEXA, and Perioheral Quantitative CT in adult patients with CF. QUS was deemed not to replace DEXA in that study; but it was stated that it could be used as a screening method in patients with a normal bone mass Similarly, Flohr et al [25]. proved that QUS had no high specificity and sensitivity to replace DEXA. Parallel to the literature findings, a correlation was found in the bone US and DEXA values in the patient group in this present study. This supports the applicability of QUS as a screening test in patients Schepper with bronchiectasis since it is easily applicable compared to DEXA in BMD measurements and includes no radiation exposure.

A powerful and positive correlation was found between NTX and bALP when the patient group was evaluated and with the control group. A positive and statistically significant correlation was found between NTX and spot urine Ca/Cr in the patient group. A positive and statistically significant correlation was found between the serum osteocalcin and bALP, ALP, NTX, and Ca when the patient and control groups were evaluated together. Positive and negative many correlations between bone production and destruction markers were found in this present study in the patient group. This suggests a high bone turnover in patients with bronchiectasis.

Our limitations were determination of serum OPG, and RANKL levels are challenging due to many reasons. The source of RANKL released in circulation is many and might be in many forms. RANKL is present in the serum in free form and most of it is bound to OPG and the two molecules have a circadian rhythm. Therefore, the measured values in the circulation may not completely reflect the effects on the bone micro frame. Serum samples were preferred to be obtained in the morning hours in this present study; however, the study results might be affected by the circadian rhythm.

In conclusion, we could not find the definite role of the RANK/RANKL/OPG system in the pathogenesis of osteoporosis developed in non-cystic fibrosis bronchiectasis patients in this present study. However, future studies in larger series would be appropriate since the number of cases is small in this study.

5. Template for Ethical and Legal Declarations

Ethics board approval was obtained from the Ethics Board Committee dated January 8, 2016, and numbered 2016/399. Informed consent was obtained from all patients included in the study after explaining the aim and extent of the project in detail to each of them.

6. Conflicts of Interest Statement

6.1 The authors declare that they have no conflict of interest.

The authors whose names are listed above, certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affi liations, knowledge or beliefs) in the subject matter or materials discussed in this manuscrip.

7. Highlights

1- The association between osteoporosis and RANKL/OPG levels has been demonstrated in the literature in patients with CF in whom bronchiectasis is common.

2- There are not many publications in the literature about osteoporosis in patients with non-cystic fibrosis bronchiectasis.

3- RANKL, OPG levels can be used as a marker in the early diagnosis and follow-up of osteoporosis.

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