Adolescent Idiopathic Scoliosis and Osteopenia

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ABSTRACT

Introduction: Generalized low bone mass and osteopenia in both axial and peripheral skeleton in adolescent idiopathic scoliosis (AIS) have been reported in literature. However, the exact mechanisms and causes of the bone loss in AIS are not identified yet.

Objective: Therefore, this study examined the relationship between AIS and bone loss represented by: serum concentration of soluble receptor activator of nuclear factor-kB ligand (RANKL), serum level of osteoprotegerin (OPG), serum level of osteocalcin (OST), parathormon (PTH) and BMI.

Materials and methods: We analyzed 15 patients with AIS and compared to the group of 8, age and gender-matched healthy controls. The bone markers in patients with AIS were disturbed compared with than in control individuals. Statistical analysis was performed by using t-test for independent samples.

Results: The mean RANKL and RANKL to OPG ratio in patients with AIS were increased compared with that in control subjects. The RANKL and RANKL to OPG ratios were negatively correlated to serum OPG levels in both groups. These findings mean that the imbalance and the disturbed interaction of RANKL and OPG may be an important cause and pathogenesis in osteopenia in AIS.

Conclusion: Research date showed an important correlation between osteopenia and AIS. The level significant higher of osteocalcin (p<0.01) and RANKL (p<0.01) was observed at adolescents with idiopathic scoliosis comparing with control group. OPG show no differences between the groups of study. RANKL/OPG ratio was significant higher compared with control group. Higher levels of RANKL, in the presence of increased levels of osteocalcin, may induce modification in bone remodelling due to imbalance in RANKL/OPG system.

Keywords: Adolescent idiopathic scoliosis (AIS), RANKL, OPG, OST, bone loss

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INTRODUCTION

Adolescent idiopathic scoliosis (AIS) is a complex three dimensional deformity of the spine occurring mostly in girls between 10 and 14 years old. The aetiology and pathogenesis of idiopathic scoliosis remain unclear despite the large number of studies performed. The cause of scoliosis is believed to be multi-factorial because of the association between the development of scoliosis and growth, hormonal secretion, gravity, etc (1-7).

However, none of these parameters have been conclusively shown to have a causative role in AIS development (5).

Association of osteopenia with idiopathic scoliosis was first reported by Burner and co-workers in 1982. Generalized low bone mass and osteopenia in both the axial and peripheral skeleton in AIS have been reported in literature (7,8). Abnormal histo-morphometric bone cell activity has been found in AIS bone biopsies (9). In addition, low bone mass in AIS patients was likely to persist through to adulthood (3). There is a growing concern that adolescents with idiopathic scoliosis may have a lower peak bone mass (10), thereby increasing the risk of developing osteoporosis and related complications in later life (3,6,8-10).

However, the exact mechanisms and causes of the bone loss in AIS are not identified yet (11-16).

Receptor activator of nuclear factor-kB ligand (RANKL) is a potent stimulator of bone resorption by binding receptor activator of nuclear factor kB (RANK) in the cell membrane of osteoclasts. In contrast, osteoprotegerin (OPG) is a soluble decoy receptor for RANKL which interferes with RANKL/RANK binding and inhibits the maturation and activation of osteoclasts and their precursors (9,11,17-20) (Figure 1).

The balance of RANKL/RANK and OPG has a central role in the regulation of bone remodelling events in diseases such as osteoporosis, glucocorticoid-induced osteoporosis, chronic inflammatory arthritis, hypogonadism, estrogen deficiency, bone marrow transplantation and the osteolytic bony metastasis of malignancies (14-17,18,19,21-31).

However, studies linking RANKL, OPG and bone mass of AIS are lacking. We aimed to study the relationship between serum concentration of soluble RANKL, serum level of OPG and bone mass in AIS girls and compared these to their levels in non-AIS controls. ❑

MATERIALS AND METHODS

Subjects: 15 adolescents diagnosed with AIS (11 girls and 4 boys) and 8 healthy adolescents (6 girls and 2 boys) were hospitalized at the Emergency Hospital Calarasi.

These adolescents, aged between 8-18 years old, were enrolled at the authors' institution.
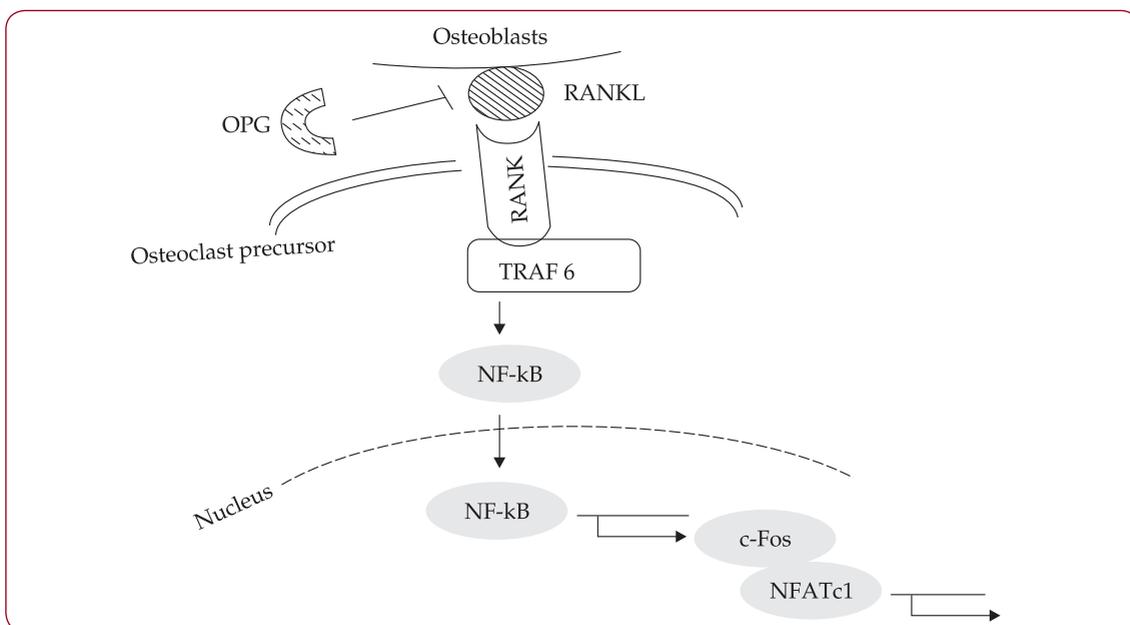

FIGURE 1. Osteoclastogenic genes

Patients receiving any forms of prior treatment for scoliosis were excluded from the study.

All normal controls were also clinically examined to rule out any hidden scoliosis before entering the study.

Subjects with a history of congenital deformities, neuromuscular disease, endocrine disease, skeletal dysplasia, connective tissue abnormalities or mental retardation were excluded from the study. All subjects and their parents gave informed consent before the examination and measurements.

The study was approved by the "C I Parhon – Institute of Endocrinology", Bucharest and by Prof. Dinu Antonescu.

Evaluation of severity of scoliosis was taken for each patient: a standard standing, whole spine, antero-posterior radiograph.

A standard technique was used for the measurement of the Cobb's angle. If more than one curve was found, the more severe curve was selected for measurement. Curves of less than 200 were excluded.

Anthropometric measurement: included body height and body weight. For AIS patients, corrected height was derived from Bjure's formula (Log y = 0.011x – 0.177, where "y" is the loss of trunk height (cm) due to the deformed spine and "x" is the greatest Cobb angle of the primary curve). Body mass index (BMI) was determined by dividing weight (kg) by the square of the uncorrected height (m^2).

Measurement of serum RANKL, OPG, OST, PTH

Blood samples were collected in 4 hours during day and 2 hours during night for 24 houres period.

Serum was obtained from the routinely-drawn blood samples, centrifuged immediately. The samples were kept at – 72°C prior to determination of RANKL, OPG, OST and PTH.

Their levels were measured in serum by a sandwich ELISA (immunodiagnostic).

As a first step, the blood sample and detection antibody were pipette into wells.

After a washing step, which removed all nonspecific bound material, sample was added to the wells, as substrate and the optical density (OD) at 450 nm was read on a standard ELISA plate reader, after an appropriate colour development period, to measure the levels of the marker. The software fits a calibrators-response curve to the OD results of the standards and calculates the markers of unknowns from the standard curve equation. We used five standards and detection limit was for OPG. The assay includes two highly specific antibodies against OPG (derived from goat, immunized with human recombinant)

- for OPG – 0.5 pmol/ml, and the dynamic range was 0.5–3.9 pmol/l;
- for OST – 11 mg/ml and dynamic range 11 mg/ml – 522 mg/ml;
- for RANKL - 0.369 pmol/l and dynamic range 0.369 pmol/l – 10704 pmol/l;
- raport RANKL – OPG: limit detection 2-18278.

Statistical analysis

Statistical analysis was performed with SPSS 11.5 software for Windows. Data were expressed by mean ± standard deviation. Groups (AIS and control) were compared by using the "*t*" test (student). Also, the correlations were presented using Pearson's and Spearman's correlation in each group, as appropriate. A $p < 0.05$ was regarded as statistically significant. ❑

RESULTS

The study shows the results of the patients with AIS and the control individuals the mean age -13.6+/-3.29 vs 13.25 +/- 2.96. The mean weight: 43.9+/-15.8 and height: 149.25+/-25 BMI and corrected BMI in the patients were 19.5+/- 3.1 vs 20.1+/- 2.8 kg/m^2. The mean weight, BMI and corrected BMI were no significant difference between the AIS and the control subjects.

The mean OST, RANKL and RANKL to OPG ratio in patients with AIS were increased compared with that in control subjects, respectively:

- for OST: mg/ml
  - 98.75 +/- 137.58 vs 42.55 +/- 55.77 for girls
  - 144.84 +/- 137.45 vs 88.90 +/- 80.82 for boys
- for RANKL: pmol/l
  - 4483.04 +/- 4156.25 vs 1985.06 +/- 3149.08 for girls
  - 2496.16 +/- 3391 vs 885.96 +/- 319.91 for boys
- for RANKL/OPG: ratio
  - 4480.35 +/- 5493.72 vs 1757 +/- 2764 for girls
  - 1670.95 vs 838.56 for boys.

There was no significant difference in OPG between the AIS and the control subjects (1.30 pmol/l vs 1.26 pmol/l).

The correlations of the parameters are shown in following tables (1 and 2):

The chronological age was positively correlated to OPG and negatively correlated to RANKL and RANKL to OPG ratios in both groups. However, the BMI was not correlated to any parameters in both groups. ❑

DISCUSSION

A generalized low bone mass and osteopenia in both the axial and peripheral skeleton in AIS have been reported in literature. However, the exact mechanisms of bone loss in AIS patients are not fully identified. Reports have shown that osteopenia in children can be affected by factors as: body weight, body height, physical activity, or nutrition status (BMI) (22,26,27).

The relationship between a diagnosis of AIS and low body-weight may indicate disordered eating and well established relationship between eating psychopathology and osteoporosis (18,26,27,31).

No significant difference was found in physical activity between the AIS and the controls (12,13).

The current study revealed the statistically significant differences of loss bone between AIS and controls, but osteopenia was not correlated to any parameter in both groups (such chronological age, weight, body height, and BMI) (10,15).

RANKL is over-expressed and OPG is under-expressed in the tissues of active synovitis in inflammatory arthritis, and there is a general agreement about the fact that the imbalance of these two molecules has an important role in the bone loss and destruction in inflammatory arthritis (15).

There have been many studies performed in regard to the relationship between circulating RANKL-OPG system and osteopenia. Gene therapy with human recombinant OPG has been shown to reverse established osteopenia in growing rats, supporting the role of OPG as the protective action in bone loss (18,23).

However, studies linking RANKL, OPG and bone mass of AIS are lacking. We aimed to study the relationship between serum concentration of soluble RANKL, serum level of OPG, RANKL-OPG and bone mass in AIS girls and compared it with those of healthy non-AIS controls.

In this study, we found out that serum OPG levels in AIS was not so much lower, but RANKL levels and RANKL to OPG ratio were negatively

	AIS	Controls	p
Age	13.6±3.29	13.25±2.96	ns
Height [cm]	149.25±25	-	-
Weight [kg]	43.9±15.8	-	-
Osteocalcine [ng/mL]	111.04 ±138.44	42.77 ±52.26	p<<0.01
RANKL [pmol/L]	3953.20±4048.26	1953.32±2917.27	p<<0.01
Osteoprotegerine pmol/L]	1.17±0.31	1.16±0.23	ns
RANKL/OPG	4024.51±5019.81	1744.46±2476.49	p<<0.01

TABLE 1. Correlation between lots

	AIS		Controls		p
	Girls	Boys	Girls	Boys	
Age	14±3.16	12.75±3.5	13±3.10	16.5±2.12	ns
Height [cm]	150.72±19.77	142.5±40.31	-	-	-
Weight [kg]	44.41±12.62	42.5±25.04	-	-	-
BMI	19.44±3.52	19.66±1.75	-	-	-
Osteocalcine [ng/mL]	98.75±137.58	144.84±137.45	42.55±55.77	88.90±80.82	p<<0.01
RANKL [pmol/L]	4483.04±4156.25	2496.16±3391.08	1985.06±3149.08	885.96±913.91	p<<0.01
Osteoprotegerine [pmol/L]	1.14±0.31	1.28±0.27	1.18±0.26	1.23±0.20	ns
RANKL/OPG	4880.35±5493.72	1670.95±2067.36	1757±2674.20	838.56±865.76	p<<0.01

TABLE 2. Correlation between lots for girls and boys

correlated to the normal group and to the serum OPG levels in both groups.

However, the RANKL, OPG and RANKL to OPG ratios were not correlated to the BMI. These results might impose the assumptions that the loss in AIS may be caused by the changes of the RANKL/OPG system rather than the effects of the weight and BMI loss. These findings mean that the imbalance and the disturbed interaction of RANKL and OPG may be an important cause and pathogenesis in reduced bone mass in AIS. ❑

THE FACTS OF RISK AND CONFUSIONS

Some potential limitations of this study should be considered. Firstly, the number of patients included was relatively small. To reflect the true levels of serum RANKL and OPG in bone, studies with larger patient numbers should be performed. Secondly, it is well known that the serum levels of OPG and RANKL are changed in some disease conditions (it might be difficult to distinguish the AIS from other disease conditions) (28,29). Thirdly, we could not assess the correlation of inflammatory cytokines or bone turnover markers contributory to osteoclastogenesis because we did not measure the serum levels of inflammatory cytokines and bone turnover markers such as interleukin-1, interleukin-6 and alkaline phosphatise and DEXA for BMD. (SLBMD, FN-BMD) measure only OST (20,24, 30).

Further studies with measurement of BMD, cytokines, bone turnover markers (another OST) might support the relationship of these factories OPG, RANKL levels in patients with AIS (31). ❑

CONCLUSION

We attempted to determine the bone loss (osteopenia) to measure the serum levels of OST, RANKL and OPG, and to find the correlation between this and AIS (Figures 2 and 3).

The serum RANKL levels and RANKL to OPG ratios were up-regulated in patients with AIS.

Our data suggest that the imbalance of RANKL and OPG might be one of the mechanisms leading to low bone mass in AIS. When managing patients with AIS, clinicians should pay attention as low bone mass is common in AIS despite young age. Future therapies should be aimed for restoration of the unbalanced RANKL/OPG system in AIS patients. ❑
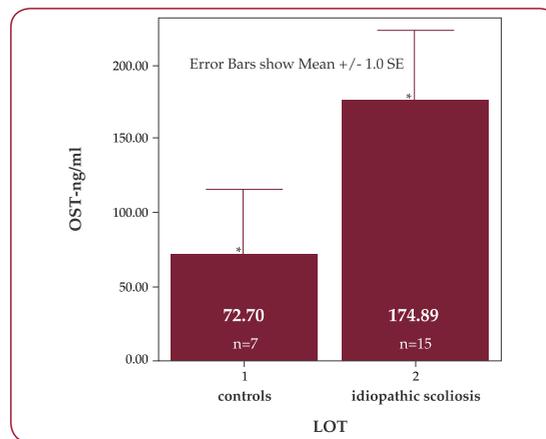
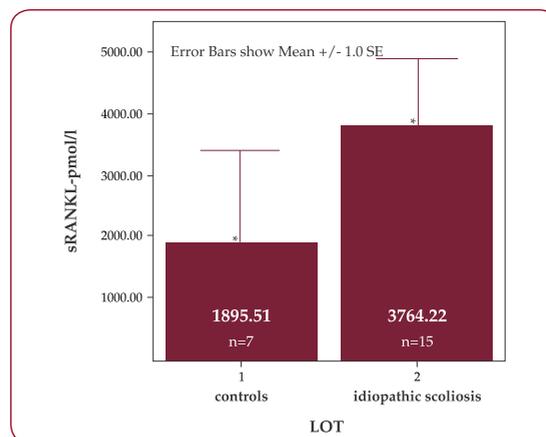**FIGURE 2.** Osteocalcin levels at 8:00 h in idiopathic scoliosis compared with controls

FIGURE 3. sRANKL levels at 8:00 h in idiopathic scoliosis compared with controls

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