Serum RANKL, OPG and Vitamin D in children with systemic lupus erythematosus

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Abstract

Indroduction: SLE is an autoimmune multisystem disease. Glucocorticoid is an irreplaceable medication for SLE. Glucocorticoid and inflammation impact bone remodeling by OPG/RANKL/RANK signal system, which could lead to osteoporosis. Our aim is to clarify the expression of RANKL/OPG in children with SLE, and to preliminarily explore the changes of bone metabolism related indexes in children with SLE.

Methods: Serum RANKL and OPG of 40 children with SLE and healthy children were detected by ELISA, while 25(OH)VitD₃ was detected routinely. Clinical data of children with SLE were recorded, including gender, age, height, weight, BMI, duration of the disease, accumulated dose of glucocorticoid, and correlation analysis was conducted with RANKL, OPG and 25(OH)VitD₃.

Results: Serum RANKL concentration in SLE group was significantly higher than health group (9.82 ± 7.20 vs. 6.80 ± 4.35 pg/ml, $P < 0.05$), and the concentrations of OPG and 25(OH)VitD₃ in serum were significantly lower than health group (156.34 ± 57.33 vs. 189.16 ± 68.70 pg/ml and 43.66 ± 31.27 vs. 59.04 ± 21.56 mmol/L, $P < 0.05$). Serum RANKL in children with SLE was positively correlated with the duration of SLE, accumulated dose of GC ($r = 0.593, 0.727, P < 0.05$). And it was negatively correlated with serum OPG and 25(OH)VitD₃ ($r = -0.601, -0.469, P < 0.05$). In addition, serum OPG and 25(OH)VitD₃ concentrations were inversely correlated with accumulated dose of GC ($r = -0.66, -0.508, P < 0.05$).

Conclusion: Low levels of vitamin D3 and bone metabolic abnormalities were found in children with SLE under the condition of disease remission, while serum RANKL expression was elevated, OPG expression was reduced. These changes associated with the duration of SLE, accumulated dose of GC. In the case of exclusion of disease activity, GC and other factors may be involved in the occurrence and development of abnormal bone metabolism through RANKL/OPG.

Indroduction

Systemic lupus erythematosus (SLE) is an autoimmune multisystem disease with high disability rate and mortality. Glucocorticoid-induced osteoporosis (GIOP) is one of the most common complications of SLE[1]. Bone remodeling is a dynamic equilibrium biological process of the interaction between Osteoblast (OB) and Osteoclast (OC). Receptor activator of nuclear factor- κ B ligand (RANKL) and Osteoprotegerin (OPG) act as a pair of biologic active proteins, which bind to their common receptor activator of nuclear factor- κ B (RANK) to regulate the balance process of bone remodeling. Glucocorticoid(GC) and inflammation impact bone remodeling by OPG/RANKL/RANK signal system[2], which could lead to osteoporosis. In order to define and verify the SLE patients in the presence of RANKL/OPG expression changes and bone metabolic abnormalities, we detected RANKL, OPG and 25(OH)VitD₃ in 40 children with SLE in this study, while correlation between RANKL and other items were analysed, to discuss bone metabolism related changes in SLE condition.

Participants And Methods

Patients and controls

40 children with SLE were enrolled, who were treated and followed up in Shanghai Children's Hospital from January 2019 to July 2020, including 5 males and 35 females. All patients were diagnosed according to 2012 Systemic Lupus International Collaborating(SLICC) classification criteria[3]. Systemic lupus erythematosus disease activity index(SLEDAI) scores[4] of the patients were below 4, which means the disease were in remission. The control group included 40 healthy children who underwent physical examination at the same period, and the age and sex matched. Patients with early onset of lupus-like syndrome due to genetic mutation, SLEDAI scores greater than 4, and severe infection were excluded. The study was approved by the Ethics Committee of Shanghai Children's Hospital(No. 2020R015), and the informed consent was signed by the patient's parents. This study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments.

Experimental detection methods

The whole blood samples of 40 children with SLE and healthy control group were collected and agglutinated for 30 minutes, then centrifuged at 1000 RPM for 10 min. Serum samples were stored at -80°C. RANKL and OPG were detected by Human Trance/TNFSF11/RANKL ELISA Kit and Human Osteoprotegerin/OPG ELISA Kit(Multisciences Biotech, Hangzhou, China). Serum 25(OH)VitD₃ was detected by Roche cobas E601 Analyzer.

Statistical analysis

SPSS 25.0 statistical software package was used for statistics. Serum RANKL, OPG, 25(OH) VitD₃ concentrations, age, duration of SLE, height, weight and accumulated dose of GC were expressed as mean ± standard deviation(SD). The comparisons between two groups were used by t-tests, and Pearson analysis was used to analyze the correlation between two indicators in children with SLE.

Results

General clinical data

In this study, there were 40 children in the SLE group and 40 children in the normal control group, and there was no statistical difference in gender composition and age between the two groups (Table 1).

Table 1
General clinical data of children with SLE group and control group (n or mean ± SD)

	SLE group (n = 40)	Control group (n = 40)	statistics	P value
Sex(male/female)	5/35	6/34	$\chi^2 = 0.3922$	0.5312
Age(years)	10.75 ± 2.56	11.4 ± 1.53	t = 1.378	0.1721

Serum RANKL, OPG and 25(OH) VitD3 concentrations in the SLE group

Serum RANKL concentration in SLE group was significantly higher than that in normal control group (*P < 0.05), and the concentrations of OPG and 25(OH) VitD3 in serum were significantly lower than those in normal control group (*P < 0.05), the difference was statistically significant(Table 2, Fig. 1).

Table 2
Serum concentrations of RANKL, OPG and 25(OH) vitD₃ in SLE and control group (n or mean ± SD)

	SLE group(n = 40)	Control group (n = 40)	Statistics	P value
RANKL(pg/ml)	9.82 ± 7.20	6.80 ± 4.35	t = 2.276	0.0256*
OPG(pg/ml)	156.34 ± 57.33	189.16 ± 68.70	t = 2.319	0.0230*
25(OH)vitD ₃ (mmol/L)	43.66 ± 31.27	59.04 ± 21.56	t = 2.712	0.0082*
*P < 0.05				

Correlation analysis of serum RANKL level and other indicators in SLE group

Serum RANKL concentration in children with SLE was correlated with other indexes, including age, course of disease, weight, height, Body mass index (BMI), GC accumulation, OPG and 25(OH) VitD₃. The results showed that in children with SLE serum RANKL was positively correlated with the duration of SLE and the accumulated dose of GC (*P < 0.05), and was negatively correlated with serum OPG and 25(OH) VitD₃ (*P < 0.05), while height, weight, BMI and age were not correlated (P > 0.05). In addition, serum OPG and 25(OH) VitD₃ concentrations were inversely correlated with GC cumulants (*P < 0.05), the difference was statistically significant(Table 3).

Table 3
Correlation analysis of serum RANKL level and other indexes in children with SLE(n or mean $\pm$ SD)

	SLE group(n = 40)	R $\square$	P $\square$
Age(years)	10.75 $\pm$ 2.56	-0.105	0.519
Duration of SLE(Months)	14.95 $\pm$ 4.94	0.593	< 0.0001*
Weight(kg)	42.33 $\pm$ 9.68	-0.279	0.081
Height(cm)	131.33 $\pm$ 13.92	-0.141	0.385
BMI(kg/m ²)	24.64 $\pm$ 4.88	-0.176	0.278
Accumulated dose of GC (mg/kg)	292.33 $\pm$ 67.93	0.727	< 0.0001*
RANKL(pg/ml)	9.82 $\pm$ 7.20	-	-
OPG(pg/ml)	156.34 $\pm$ 57.33	-0.601/-0.66**	< 0.0001*
25(OH)vitD ₃ (mmol/L)	43.66 $\pm$ 31.27	-0.469/-0.508**	0.002*/-0.001*
*P < 0.05, the difference was statistically significant			
** The correlation with accumulated dose of GC			

Discussion

Bone is a constantly metabolizing organ, and bone remodeling is a dynamic balance process of bone resorption and bone formation. OC absorbs old bone and Osteoblast OB forms new bone, respectively. Bone remodeling relies on the precise coordination of the two kinds of cells to keep in balance. OC can regulate the functions of OB in both positive and negative ways, while OB can adjust OC through OPG/RANKL/RANK signaling system. RANKL and OPG are a pair of important bioactive proteins in regulating the balance of bone metabolism, which are widely expressed in lymphoid tissues. RANKL is a member of tumor necrosis factor(TNF) superfamily, which activates downstream related molecules after binding to its receptor RANK to enhance osteoclast differentiation and activity, then leads to increased bone resorption. RANKL is highly expressed in the thymus gland, lungs and lymph nodes, and low expressed in the spleen and bone marrow[5]. A variety of cytokines, hormones, growth factors can regulate the expression of RANKL. RANK and OPG are also members of TNF Receptor superfamily. RANKL binds to RANK on the surface of osteoclasts to induce the accumulation of TNF receptor-related factor (TRAF6) in osteoclasts and promotes the differentiation and activation of osteoclasts, while the apoptosis of osteoclasts was inhibited. OPG has two forms including monomer and dimer, and is mainly expressed in osteoblasts and vascular cells[6]. It can competitively bind RANK, so the ratio of RANKL/OPG plays a key role in the process of bone remodeling. Our study mainly detected serum RANKL and OPG in children with SLE.

The expression of RANKL, OPG and RANK is regulated by different hormones and cytokines, including Parathormone(PTH), $1,25(\text{OH})_2\text{VitD}_3$, prostaglandins, TNF- α , Interleukin (IL)-6 and M-CSF, etc. The high expression of estrogen could increase the expressions of transforming growth factor (TGF) - β and OPG, which inhibited RANKL signal transduction, and then promoted bone formation. On the contrary, the low expression of estrogen could significantly increase the expressions of IL-1, IL-6, TNF- α and other pro-inflammatory factors, which increased the expression of RANKL and promoted bone resorption[2]. At present, the most commonly used method for bone mineral density(BMD) assessment is dual energy x-ray absorptiometry (DXA). But local DXA is insufficient to reflect the condition, and the whole body DXA is difficult to perform in children because of the high dose of radiation and coordination problem. PTH, $25(\text{OH})\text{VitD}_3$ and osteocalcin and can only represent the body endocrine levels, so that new and simple biomarkers are needed, which are the reflection of bone metabolic state. Fracture risk assessment should be performed on all patients with long-term oral GC and should also be regularly assessed during treatment[7]. RANKL and OPG play a key role in the balance of osteoblasts and osteoclasts, which are very important in the regulation of bone metabolism in vivo and believed to be related to the reduction of bone mineral density in patients with SLE. A number of studies have shown that the RANKL/OPG ratio is decreased in chronic diseases such as type 1 diabetes, juvenile idiopathic arthritis, and nephrotic syndrome[8, 9, 10, 11], which may represent the negative balance of bone remodeling. Therefore, the detection of RANKL and OPG may become new sensitive biomarkers to evaluate bone metabolism in children.

It is reported that annual incidence of SLE in adults ranges from 0.3 to 31.5/100,000[3, 4], and 30 to 70/100,000 in China[12]. The incidence in children ranges from 0.36 to 2.50/ 100,000, and the prevalence ranges from 1.89 to 25.70/100,000[13]. Although the incidence of SLE in children is not high, the vast majority of children with SLE need lifelong treatment, and the mortality is still high in young patients[14]. GC is widely used in patients with inflammatory, autoimmune and allergic diseases, and is one of the most common and irreplaceable drugs for SLE at present. With the application of GC and other immunosuppressants, the mortality of SLE patients has decreased significantly in recent decades. More than 80% of patients with SLE need long-term duration[15, 16] so that GIOP is more common. GC stimulates RANKL and inhibit OPG, which promotes osteoclast differentiation and osteolysis, while it induces osteocyte apoptosis and inhibits osteocyte generation[17]. Studies have shown that bone loss caused by the GC can be roughly divided into two stages, bone mineral density loss fast which is about 6 ~ 12% in the first year of treatment, and about 3% a year later[18]. At the later stages, GC increased apoptosis of osteoblasts and osteocytes, and then the expression of RANKL decreased[19], while the number of osteoclasts decreased by apoptosis and autophagy. In addition, control of inflammation and reduction of GC dosage at the later stage also resulted in less bone loss. A study found that OPG, RANKL and RANKL/OPG were significantly increased in the SLE group, and OPG level was related to the activity of the disease[20]. Studies on children with SLE also showed that RANKL level was not related to disease activity[21]. Therefore our study selected the patients with disease activity controlled to reduce the interference of inflammation. The results showed that serum RANKL and OPG concentrations in inactive state of disease are also significantly different with normal children. It suggested that dynamic balance

regulation of bone metabolism still exists in the remission state of the disease, partially verifying the above conclusion that RANKL is not related to disease activity, but the effect of the active stage of SLE on bone still needs to be further explored and clarified. Studies on lupus nephritis (LN) showed that OPG levels in active LN were increased, and OPG levels were significantly decreased when disease activity was controlled, suggesting that OPG levels may be increased by inflammatory stimulation, but decreased by GC[22]. Our study showed that RANKL expression was increased and OPG expression was decreased in the remission state in children with SLE, and respectively they were positively and negatively correlated with duration of SLE and accumulated dose of GC. It speculated that bone remodeling process could keep active by the action of GC. Since the durations of the disease in our patients are all less than 2 years, longer follow-up and observation are needed. One limitation in this part is absence of patients with other rheumatic diseases on corticosteroid therapy, so we will try to collect more patients, which would have been helpful to better clarify whether or not elevated serum RANKL/OPG ratios are primarily related to disease rather than corticosteroid. Another limitation is that we have not done multivariate analysis because of small sample size, and more patients will be enroll our research in the future.

At present, the correlation between BMD and RANKL and OPG levels is still inconclusive, but the correlation of increased RANKL/OPG ratio and BMD has been confirmed in many childhood diseases[8, 9, 10, 11]. Since SLE may lead to decreased bone mineral density, the effect of RANKL and OPG on bone mineral density in SLE active stage is not clear. In our study, all the 40 children with SLE were in remission, so the chronic inflammation in SLE had little effect on RANKL and OPG. Meanwhile, the correlation between GC accumulation and RANKL/OPG suggested that GC played a very important role in regulating the balance of RANKL and OPG. Considering the amount of radiation and the difficulty of operation, whole-body BMD was not performed in these cases, which is a limitation and the correlation between RANKL/OPG and BMD cannot be determined. So we've done animal and cell study to find out the machanisms of the bone loss by GC in SLE model.

Vitamin D is an important indicator of bone metabolism, which regulates the balance of calcium and phosphorus. It is also associated with a number of non-skeletal diseases, including cardiovascular disease, cancer, autoimmune disease and diabetes. Serum 25(OH)VitD₃ is considered to be the best indicator of vitamin D status. Low vitamin D level is a risk factor of osteoporosis. Our study also found that children in SLE with have low vitamin D level, which is correlated with accumulated dose of GC, RANKL and OPG. It speculated that GC may participate in bone remodeling process in SLE mediated by the transformation of RANKL/OPG, which characterized by low levels of vitamin D status. But the specific regulatory mechanism still needs further research.

Conclusion

Children with SLE had low levels of vitamin D3 and bone metabolic abnormalities. In remission of the disease, serum RANKL expression is elevated, OPG expression is reduced, and these changes are associated with duration of SLE and accumulated dose of GC. It states that GC is involved in the process

of the occurrence and development of bone metabolic abnormalities through RANKL/OPG which rules out other factors such as disease activity.

Abbreviations

SLE

systemic lupus erythematosus

GIOP

glucocorticoid-induced osteoporosis

GC

glucocorticoid

OB

osteoblast

OC

osteoclast

RANKL

receptor activator of nuclear factor- κ B ligand

OPG

osteoprotegerin

RANK

receptor activator of nuclear factor- κ B

SLICC

Systemic Lupus International Collaborating

SLEDAI

systemic lupus erythematosus disease activity index

TNF

tumor necrosis factor

TRAF6

TNF receptor-related factor

TGF

transforming growth factor

PTH

parathormone

LN

lupus nephritis

IL

interleukin

DXA

dual energy x-ray absorptiometry

BMD

bone mineral density.

Declarations

Ethical Approval and Consent to participate

The studies involving human participants were reviewed and approved by The Ethics Committee of Shanghai Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Consent for publication

Each author listed on the manuscript has approved the submission of the manuscript.

Availability of supporting data

Please contact the corresponding author for data requests.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HS and HWY designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. HBX and NXL collected data. ZJ and FD contributed to the detection of RANKL, OPG and VitD3. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Figures

Figure 1

Serum RANKL, OPG, and 25(OH) VitD₃ concentrations in children with SLE and healthy children(control group, CTL). **(A)** Serum RANKL concentration in SLE group was significantly higher than control group (**P*<0.05); **(B)** Serum OPG concentration was significantly lower in the SLE group than control group (**P*<0.05); **(C)** RANKL/OPG ratio in SLE group was significantly higher than control group (**P*<0.05)