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The Correlation between Total Alkaline Phosphatase and Osteocalcin Levels in Systemic Lupus Erythematosus Patients

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ABSTRACT

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease with various complications, including osteoporosis. However, Bone Mineral Density (BMD) examination, a gold standard for diagnosing and monitoring osteoporosis, is static. Alkaline phosphatase (ALP) is a membrane-bound glycoprotein that catalysis the hydrolysis of monoester phosphate. Osteocalcin (OC) is a non-collagenic bone protein that binds calcium and phosphate, which are both dynamic bone formation activity markers. This study analyzes the correlation between total ALP and OC serum levels in SLE patients. A cross-sectional observational analytic study was conducted in the Clinical Pathology Installation of Dr. Moewardi Hospital Surakarta in June 2020. The subjects were SLE patients receiving Methylprednisolone (MEP) therapy ≥1 year. Data distribution normality test by Saphiro-Wilk, comparative analysis with unpaired T-test, degree of correlation strength between research variables by Pearson correlation test. There were 41 female subjects, and comparative analysis of total ALP and serum OC levels were not significantly different in inactive and active SLE (ALP p=0.373, serum OC p=0.700). Total ALP and serum OC was found to have a weak positive correlation in all SLE patients (r=0.337; p=0.031), a moderate positive correlation in active SLE (r=0.426; p=0.043), while in inactive SLE there was no significant correlation (r=0.247; p=0.324). There is a significant moderate positive correlation between total ALP and serum OC in SLE patients. Total ALP and serum OC examinations are necessary for osteoporosis screening in SLE patients with > 1-year glucocorticoid (GC) therapy.

Keywords: Systemic lupus erythematosus, osteoporosis, total alkaline phosphatase, osteocalcin

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease of unknown etiology with a diverse course and prognosis. The etiopathogenesis of SLE is not completely clear. Interactions between genetic, immunological, hormonal, and environmental factors are thought to play a role.[1,2]

One of SLE complications is osteoporosis. Recent studies have found the prevalence of osteoporosis in SLE patient population to be relatively high. A study at Hasan Sadikin Hospital in Bandung reported a 4.12% prevalence among other clinical manifestations.[3] The etiology of bone mass reduction in SLE is multifactorial, including the disease's intrinsic factor and the treatment's effect. Old age, post-menopausal status, smoking, duration of illness, glucocorticoid treatment (GC), renal insufficiency, Raynaud's syndrome, lupus anticoagulants, and decreased Bone Mineral Density (BMD) have been reported as risk factors for osteoporosis and fractures in SLE.[4]

The diagnosis of osteoporosis and fracture risk assessment based on gold standard quantitative analysis of BMD only provides limited information about bone strength (static), so checking dynamic bone markers for an initial evaluation of osteoporosis risk is necessary to provide enough information for diagnosis.[5,6] Bone formation markers examined in this study are total alkaline phosphatase (ALP) and serum osteocalcin (OC).

Alkaline phosphatase is a membrane-bound glycoprotein found in all living cells. ALP's role in bone mineralization is to prepare an alkaline atmosphere in the osteoid tissue so calcium can quickly be deposited. During bone mineralization, the primary function of ALP is to hydrolyze inorganic pyrophosphate to phosphate.[7] Osteocalcin is also known as Bone Gla-Protein (BGP), the most significant non-collagenous protein making up human bones, a small protein of 49 amino acids with a mass molecule of 5.6 kilodaltons (kDa). The production of OC is mainly by osteoblasts, odontoblasts, hypertrophic chondrocyte cells, and a small portion by adipocyte cells.[5] The physiological process of bone remodeling requires OC, which binds calcium and phosphate to form hydroxyapatite and bone matrix.[7]

The previous study on the correlation of total ALP and serum OC is still quite contradictory. Thus this study was conducted to analyze the correlation between total ALP and serum OC levels in SLE patients.

METHODS

This observational analytical study with a cross-sectional design was carried out at the Clinical Pathology Installation of Dr. Moewardi Hospital (RSDM) in Surakarta in June 2020. Subjects were selected using a consecutive sampling technique. The inclusion criteria were SLE patients with more than one year of MEP treatment, aged over 18 years old, and signed informed consent. The American College of Rheumatology (ACR) used the criteria for diagnosing SLE in 1997. Patients with histories of or were suffering from liver disease with increasing SGPT levels from moderate to severe (SGPT >102 IU/L), kidney disease (creatinine >1.3 mg/dL), bone disorders, and fractures, pregnancy, and malignancy were excluded from the study.

Three milliliters of venous blood were taken with a tube without anticoagulants for total ALP and serum OC examination. The samples were centrifuged for 10-15 minutes at a rate of 5,000-6,000 revolutions per minute (rpm). The patients required no special preparation. The method of measuring total ALP was kinetic photometry with the Advia 1800 chemistry analyzer. The total ALP reference value for females and males over 18 years old were 47-119 U/L and 52-171 U/L, respectively.[8] The method of serum OC measurement used a quantitative sandwich enzyme immunoassay system with the COBAS e411 ELISA automatic analyzer machine. The reference values for serum OC with the Enzyme-Linked Immunosorbent Assay (ELISA) method in premenopausal females, post-menopausal females, and males were 11-43 ng/mL, 15-46 ng/mL, and 14-42 ng/mL, respectively.[9] Disease activity was determined based on the Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) scoring system, inactive SLE (score <7), and active SLE (score ≥7).[10] The method of measuring Ca++ was Ion Selective Electrode (ISE), while Erythrocyte Sedimentation Rate (ESR) was calculated by the Westergren method. Vitamin D deficiency was determined according to guidelines from the endocrine society and assessed using electrochemiluminescence immunoassay.[11]

Estimated sample size based on sample size formula for the correlative analysis research design.[12] Case-control studies by Singh *et al.* regarding OC as a marker of primary osteoporosis in post-menopausal females, the correlation between serum OC and total ALP was 0.602.[6] Minimum sample size of 20 people was obtained, so the sample required in this study for each active and inactive MEX-SLEDAI group were 20 samples.

The characteristics of the study subjects on a nominal scale, such as gender and vitamin D group, were presented as frequency and percentage. Continuous variables such as age, Body Mass Index (BMI), MEP dose, and Ca++ ions were presented in mean±SD as they were normally distributed, while the duration of MEP therapy and ESR was in the form of the median (min-max) because they were not normally distributed. Total ALP and OC serum in inactive and active SLE were compared with the unpaired T-test because the data distribution was distributed normally after transformation. A p-value of <0.05 with a 95% confidence interval was considered significant. Correlation analysis between total ALP and serum OC in all SLE populations was done with the Pearson correlation test because the data were distributed normally after data transformation, p was significant <0.05.[13]

The biomedical research Ethics Committee approved this study of Sebelas Maret University, Faculty of Medicine/RSDM in Surakarta, recommendation number 689/V/HREC/2020.

RESULTS AND DISCUSSIONS

The primary characteristics of the study subjects consisted of 41 (100%) female and no male patients. The mean age of all subjects was 34.32±8.71 years. The body mass index average was 23.19±3.99 kg/m^2. The GC therapy duration was 32 (13-222) months and indicated that the average subject received treatment in the early stages of SLE. The mean MEP dose given was 8.03±4.18 mg. The median ESR was 27 (2-105) mm/h. The Ca++ ion was found to be 1.19±0.07 mmol/L. Vitamin D status demonstrated vitamin D deficiency in 6 (14.63%) patients, vitamin D insufficiency in 5 (12.20%) patients, and optimal vitamin D in 30 (73.17%) patients. The baseline characteristics of the study subjects are presented in Table 1.

The comparative analysis of total ALP and serum OC levels did not show statistically significant differences, with p-values of 0.373 for total ALP and 0.700 for serum OC. The mean values of total ALP in all subjects, inactive and active, were 49.06±0.12 U/L, 51.35±0.13 U/L, and 47.33±0.12 U/L, respectively. The serum OC mean values of all subjects, inactive and active were 11.34±0.25 ng/mL, 10.90±0.24 ng/mL, and 11.70±0.27 ng/mL, respectively.

The results of the comparative analysis of study variables are presented in Table 2 and Figure 1.

Table 1. Baseline characteristics of study subjects

Variabel	Number (%)	Mean±SD	Median (min-maks)
Age (years old)*		34.32±8.71	
Gender			
Male	0 (0)		
Female	41 (100)		
Body mass index (kg/m^2)*		23.19±3.99	
Therapy duration (month)**			32 (13-222)
Methylprednisolone dose (mg)*		8.03±4.18	
ESR (mm/h)**			27 (2-105)
Ca^{++} ion (mmol/L)*		1.19±0.07	
Vitamin D			
Deficiency (<20 ng/mL)	6 (14.63)		
Insufficiency (20-29 ng/mL)	5 (12.20)		
Optimal (>30 ng/mL)	30 (73.17)		

Note: SD: Standard Deviation; min: lowest value; max: highest value; mg: milligrams; mm: millimeter per hour; Ca++: calcium; kg/m^2: kilograms per cubic meter; mmol/L: millimoles per liter; ng/mL: nanogram per milliliter.
* Normal data distribution (mean±SD)
** Abnormal data distribution [median (min-max)]

Table 2. The results of comparative analysis of total ALP and serum OC based on disease activity status with MEX-SLEDAI scoring

Variable	Total SLE (n=41)	Inactive SLE	Active SLE	p
		MEX-SLEDAI <7 (n=18)	MEX-SLEDAI ≥7 (n=23)	
Total ALP (U/L)*	49.06±0.12	51.35±0.13	47.33±0.12	0.373
OC serum (ng/mL)*	11.34±0.25	10.90±0.24	11.70±0.27	0.700

Note: SLE: Systemic Lupus Erythematosus; MEX-SLEDAI: Mexican Systemic Lupus Erythematosus Activity Index; n: number of subjects; ALP: alkaline phosphatase; U/L: units per liter; OC: osteocalcin; ng/mL: nanogram per milliliter.
* Normal data distribution after transformation (mean±SD), the results have been transformed back from log data to geometric data, unpaired T-test with significant p-value <0.05.

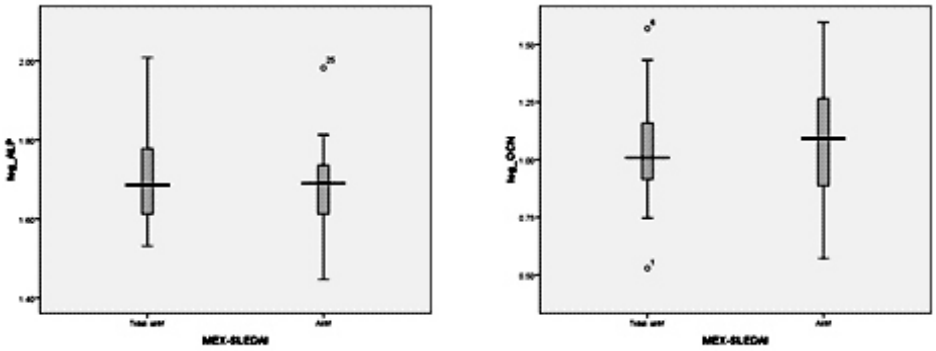

Figure 1. Comparative analysis box plot, A: total ALP in inactive and active SLE subjects; B: Serum OC in inactive and active SLE subjects

Pearson correlation analysis showed a significant weak positive correlation between total ALP levels and serum OC in all SLE subjects (r=0.337; p=0.031). A significant moderate positive correlation was also found between total ALP and serum OC levels in the active SLE patient group (r=0.426; p=0.043), but on the contrary, there was no significant correlation between total ALP levels and serum OC (r=0.247; p=0.324) in the SLE patient group with inactive disease activity.

Table 3. Results of the total ALP correlation analysis with serum OC in total SLE patients and based on the MEX-SLEDAI scoring system

Variable	Total SLE		Inactive SLE MEX-SLEDAI <7		Active SLE MEX-SLEDAI ≥7	
	r	p	r	p	r	p
Total ALP and OC serum*	**0.337****	**0.031****	0.247	0.324	**0.426****	**0.043****

Note: SLE: Systemic Lupus Erythematosus; MEX-SLEDAI: Mexican Systemic Lupus Erythematosus Activity Index; ALP: alkaline phosphatase; OC: osteocalcin
* Pearson correlation test using transformation data
** p significant <0.05

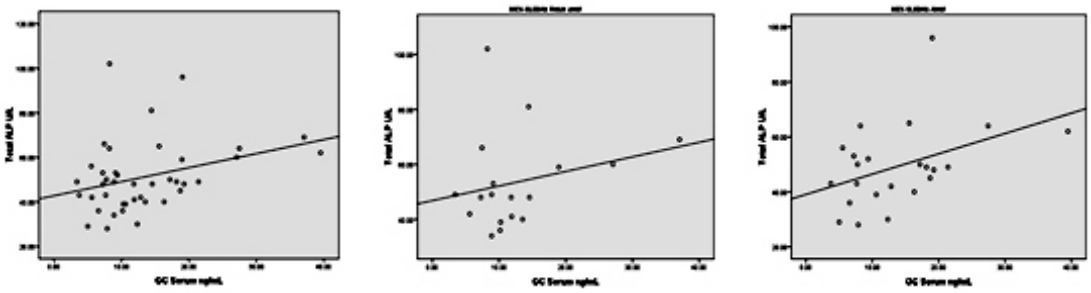

Figure 2. Graph of total ALP and serum OC correlations, A: in all SLE subjects; B: in inactive SLE subjects; C: in active SLE subjects

The results of the Pearson correlation of all subjects, inactive and active SLE subjects, are presented below in Table 3.

The correlation between the total ALP and serum OC levels in SLE patients can be seen in Figure 2.

Long-term complications of SLE have become a primary concern nowadays. Current therapies dramatically improve the survival of SLE patients. Previous studies have shown that patients with SLE have an increased risk of decreased BMD. According to World Health Organization (WHO) criteria, the incidence of osteopenia has been reported from 24% to 74%, while osteoporosis is between 1.4% and 68.7%.[14,15]

The characteristics data of our study subjects showed that the mean age was 34.32±8.71 years old, and the gender was 100% female. Systemic lupus erythematosus is more common in females; this is thought to be related to variants of the X chromosome and estrogen, which are known to have higher immune reactivity in females and contribute to triggering autoimmune diseases, including SLE.[16] The mean BMI in this study was 23.19±3.99 kg/m^2. This is still in the normal BMI range of 18-24 kg/m^2. Body mass index is a risk factor for osteoporosis because of the reduced protective effect of subcutaneous fat tissue on bone density in elderly females.[17]

The median duration of MEP therapy in this study was 32 (13-222) months. Theoretically, GC therapy may put patients at considerable risk for osteoporosis. Rapid bone loss (up to 12%) occurs during the first 6 to 12 months and will decrease with increasing duration of treatment.[18] The effect of GC is related to the time needed for the complete bone remodeling process in one cycle at the Basic Multicellular Unit (BMU), which is 120-200 days (3-5 months).[19,20] The MEP dose in this study was found to be 8.03±4.18 mg, indicating that the use of MEP in the study patients was in the moderate dose range and was at risk for osteoporosis.[18] The main effect of GC on bone is decreased rate of bone formation and the number of osteoblasts, as well as increased osteoclast activity. Decreased osteoblast differentiation comprises induction of adipogenesis transcription factors and suppression of Wnt/beta-catenin signaling pathway signals and osteocyte apoptosis. Glucocorticoid increases Receptor Activator of Nuclear Kappa-Beta Ligand (RANKL) expression and decreases osteoprotegerin expression in stromal cells and osteoblasts; consequently, the longevity of osteoclasts is extended.[19]

This study obtained the median ESR of 27 (2-105) mm/h, showing a slight increase in ESR from the reference value, illustrating an increase in inflammatory processes. However, ESR's role in disease activity assessment is still controversial because of the low ESR specificity value. This is because many factors may influence the value of

ESR.[21] The mean of Ca++ ions in this study was 1.19±0.07 mmol/L, which is in the normal range of Ca++ ion levels in the serum of adult patients. This is related to vitamin D supplementation these patients take, causing maintained optimal levels of vitamin D and disruption of intestinal absorption of Ca++.[22]

Out of all the subjects, 14.17% had vitamin D deficiency, 12.20% had vitamin D insufficiency, 12.20% and 73.17% had optimal vitamin D levels. Subjects with optimal vitamin D status were dominant because they received 400 IU of vitamin D3 supplements daily. There are no specific recommendations for vitamin D supplementation dosage in SLE patients, the American College of Rheumatology (ACR) recommends 800-1000 IU (20-25 μg) for patients using long-term GC.[23]

This study's comparative analysis of total ALP showed no significant difference between subjects with inactive SLE and active SLE (p=0.373). Serum OC levels also did not show any significant difference between inactive SLE and active SLE subjects (p=0.700). The insignificant difference is because the MEP dose of both SLE groups was above 7.5 mg and the MEX-SLEDAI cut-off value used.

This study's findings obtained a significant weak positive correlation between total ALP and serum OC levels in all SLE subjects (r=0.337; p=0.031). A case-control study by Singh *et al.* regarding serum OC levels as diagnostic markers in female osteoporosis found a significantly strong correlation between total ALP and serum OC levels (r=0.602; p=<0.001).[6] In contrast to this finding, a cross-sectional study by Hafez *et al.* regarding the assessment of fracture risk factors in females with SLE in Egypt resulted in a weak negative correlation between total ALP and serum OC (r=-0.28; p=0.017).[24] These findings differ because SLE patients have many osteoporosis risk factors such as BMI, elderly age, post-menopausal state, activity index, and organ damage due to disease. In addition, the side effects of GC therapy are still controversial; references show differences in outcomes based on dosage, method, and route of administration. Other drugs such as immunosuppressive drugs, proton pump inhibitors, anticoagulants, and angiotensin-converting enzyme inhibitors are also associated with decreased BMD and Osteoporosis because they are more widely used in higher doses by patients with severe SLE disease activity.[24]

The correlation of total ALP and serum OC levels in this study was also analyzed separately into two groups based on SLE disease activity using MEX-SLEDAI scoring (inactive SLE score <7 and active SLE score ≥ 7). This study found a significant moderate positive correlation in the active SLE subjects (r=0.426; p=0.043), but no significant correlation was found in the inactive SLE subjects (r=0.247; p=0.324). These findings are consistent with the theory of bone remodeling in SLE, which demonstrates the strong influence of the immune system on bone cells such as osteoblasts, osteoclasts, and osteocytes. Disrupted inflammatory status, mainly caused by Interleukin (IL)-1, IL-6, IL-17, and Tumor Necrosis Alpha (TNF)-α, generally stimulates osteoclast differentiation and inhibits osteoblast activity.[25] Sarkissian *et al.* study about BTM and its relationship with vitamin D in adult SLE patients revealed a moderate negative correlation between serum OC and disease activity (r=-0.350; p=0.034). The higher the disease activity score of SLE patients, the lower the serum OC level.[25] This is due to an increase in the inflammatory process (IL and TNF-α) in active SLE and administration of GC therapy also requires higher doses, which will suppress the proliferation and differentiation of osteoblasts, suppress the process of bone formation and induce osteoblast apoptosis.[26]

This study's correlation of total ALP and serum OC level resulted in a positive correlation, meaning a decrease or increase in total ALP level will always be accompanied by a reduction or increase in serum OC level.[10] This is consistent with the theory of the bone remodeling process, which states that osteoblasts synthesize ALP and OC during the bone formation phase. If the balance of bone remodeling is disrupted, the osteoblastic process will be decreased, leading to reduced levels of ALP and OC in the circulation.[27]

The strengths of this study include the subjects involving SLE patients who received GC therapy for more than one year, the use of the MEX-SLEDAI scoring system that is easily applied with a specificity value of 93% for SLE diagnosis, and the measured serum OC levels are N-MID fragment OC, which has good stability.[28] This study is limited by the cross-sectional study design, excluding healthy controls, and not considering the patient's menopausal status in which menopausal status itself is very much related to the osteoporosis process caused by hormonal disorders and can affect the total levels of ALP and OC measured serum.

CONCLUSIONS AND SUGGESTIONS

In conclusion, there is a significant moderate positive correlation between total ALP and serum OC level in active SLE subjects. Total ALP and serum OC tests need to be performed for osteoporosis

screening in SLE patients with GC therapy for over a year. Further study is required to determine the correlation between total ALP and OC in SLE patients, using different study designs such as case-control or cohorts.

REFERENCES

1. Atmakusuma TD, Lydia A, Gunawan A, Yunihastuti E, Widhani A, *et al*. Rekomendasi Perhimpunan Reumatologi Indonesia untuk diagnosis dan pengelolaan lupus eritematosus sistemik. PRI, 2019; 5-35.
2. Ali A, Sayyed Z, Ameer MA, Arif AW, Kiran F, *et al.* Systemic lupus erythematosus: an overview of the disease pathology and its management. Cureus, 2018; 10(9): e3288.
3. Sutrisno RN, Rahmadi AR, Novita N, Hamijoyo L. Most frequent musculoskeletal manifestation of systemic lupus erythematosus patients in Dr. Hasan Sadikin General Hospital Bandung. Ind. J. of Rheum, 2017; 9(2): 13-17.
4. Elhefny AAM, Hamza SMH, ElSherbiny DAH, Ibraheem RF. Frequency of low bone mineral density and Osteoporosis in a cohort of Egyptian patients with systemic lupus erythematosus. EJHM, 2017; 69(3): 2030-2035.
5. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomarker Research, 2017; 5(18): 1-9.
6. Singh S, Kumar D, Lal AK. Serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women. JCDR, 2015; 9(8): RC04-RC07.
7. Fraser WD. Bone and mineral metabolism. In: Tietz fundamentals of clinical chemistry and molecular diagnostics. 7th Ed., Missouri, Elsevier, 2019; 744-771.
8. Proline. Proline R-90 alkaline phosphatase FS IFCC mod. 370C. Prodia Diagnostic Line, Cikarang, 2017; 1-2.
9. Roche. Elecsys N-MID Osteocalcin kit. Roche Diagnostics GmbH, Mannheim, 2019; 1-4.
10. Castillo JM, Sánchez MAR, Márquez AMB. Prolactin as a marker of active disease in systemic lupus erythematosus. International Journal of Biomedical Engineering and Clinical Science, 2021; 7(4): 73-76.
11. Dewi YP. An overview: Vitamin D. 2019. Available from: https://www.researchgate.net/publication/319997190 (accessed April 5, 2020).
12. Madiyono B, Sastroasmoro S, Budiman I, Purwanto SH. Perkiraan besar sampel dalam dasar-dasar metodologi penelitian klinis. 5th Ed., Jakarta, Sagung Seto, 2018; 352-386.
13. Dahlan MS. Statistik untuk kedokteran dan kesehatan. 6th Ed., Jakarta, Epidemiologi Indonesia, 2017; 223-244.
14. Franco JCP, Henao MPZ, Pineda FL, Pulgarin-Montoya S, Vallejo EO, *et al.* Low bone mass and osteoporosis in patients with systemic lupus erythematosus. Rev. Colomb Reumatol, 2017; 24(1): 4–10.
15. Xia J, Luo R, Guo S, Yang Y, Ge S, Xu G, Zeng R. Prevalence and risk factors of reduced bone mineral density in systemic lupus erythematosus patients: A meta-analysis. BioMed Research International, 2019; 1-10.
16. Nusbaum JS, Mirza I, Shum J, Freilich RW, Cohen RE, *et al.* Sex differences in systemic lupus erythematosus: epidemiology, clinical considerations, and disease pathogenesis. Mayo Clinic Proc, 2019; 95(2): 384-394.
17. Nashirin AK, Zaki A, Nurmilasari, Widjajakusumah D, Fadhilah M. Hubungan nilai indeks massa tubuh dengan nilai risiko fraktur osteoporosis berdasarkan perhitungan Frax tool pada wanita usia ≥ 50 tahun di klub bina lansia Pisangan Ciputat Tahun 2015. JMI, 2015; 12(2); 188-194.
18. Stojan G, Petri M. The risk-benefit ratio of glucocorticoids in SLE: Have things changed over the past 40 years?. Curr Treatm Opt Rheumatol, 2017; 3(3): 164-172.
19. Briot K, Roux C. Glucocorticoid-induced osteoporosis. RMD Open, 2015; 1(e000014): 1-8.
20. Kenkre JS, Bassett JHD. The bone remodelling cycle. An. Clin. Biochem, 2018; 1-20.
21. Dima A, Opris D, Jurcut C, Baicus C. Is there still a place for erythrocyte sedimentation rate and C-reactive protein in systemic lupus erythematosus?. Lupus, 2016; 1-7.
22. Watad A, Tiosano S, Azrielant S, Whitby A. Low levels of calcium or vitamin D, which is more important in systemic lupus erythematosus patients? An extensive data analysis. Clinical and Experimental Rheumatology, 2016; 1-5.
23. Suzan R. Korelasi antara asupan vitamin D dengan kadar 25(Oh)D serum pada pasien lupus eritematosus sistemik perempuan dewasa. JMJ, 2018; 6(1): 56-67.
24. Hafez EA, El Bakry SA, Ibrahim SI, Morad CS, Hamza SA, El Khaliq DMA. Assessment of fracture risk in a cohort of Egyptian female systemic lupus erythematosus patients. The Egyptian Rheumatologist, 2018; 40: 85-91.
25. Adami G, Fassio A, Rossini M, Caimmi C, Giallo A, *et al.* osteoporosis and rheumatic disease. Int. J. Mol. Sci, 2019; 20(5867): 1-23.
26. Sarkissian A, Sivaraman V, Bout-Tabaku S, Ardoin SP, Moore-Clingenpeel M, et al. Bone turnover markers in relation to vitamin D status and disease activity in adults with systemic lupus erythematosus. Lupus, 2018; 1-7.
27. Zoch ML, Clemens TL, Riddle RC. New insights into the biology of osteocalcin. Bone, 2016; 82: 42–49.
28. Klemm KM, Klein MJ. Biochemical markers of bone metabolism. In: Henry's clinical diagnosis and management by laboratory methods. 23th Ed., Missouri, Elsevier, 2017; 188-204.