IHJPAS. 37 (1) 2024
Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407

The Dynamic Role of PD-1, Vitamin D, RANKL, and Sclerostin in Iraqi Patients with Systemic Lupus Erythematosus

[1]Anwar Khalil Ismael* [2] Hazima Mossa Alabassi

[1,2]Department of Biology, College of Education Pure Science Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq.

*Corresponding Author: anwaarkhalil9@gmail.com

Received 27 March 2023, Received 25 April 2023, Accepted 30 April 2023, Published in 20 January 2024

doi.org/10.30526/37.1.3367

Abstract

Systemic lupus erythematosus (SLE) is a chronic, autoimmune disease, with a wide range of clinical symptoms. Some studies have indicated the association between RANKL, Sclerostin, PD-1, and vitamin D concentrations and the pathogenesis of SLE. The current study aimed to evaluate the role of RANKL, Sclerostin, PD-1 and vitamin D in the pathogenesis of SLE. The study included 180 females diagnosed SLE patients and healthy control (60 females as early diagnosed patients without treatment, 60 females as patients under treatment with (prednisolone, and hydroxychloroquine), and 60 females healthy as a control group, with ages ranging from 20 to 45 years. The serum concentration levels of RANKL, Sclerostin, PD-1 and vitamin D were assessed by Enzyme linked immunosorbent assay (ELISA). The results of the current study showed no significant differences in the serum levels of RANKL and Sclerostin in both SLE patients' groups (early diagnosed group and treated) compared with the control group ($p<0.05$). The serum level of PD-1 was significantly higher in both SLE patients' groups compared with the control group ($p<0.05$). The serum level of vitamin D was significantly lower in both SLE patient groups compared with the control group ($p<0.05$). Based on these results, PD-1 may be considered a good therapeutic target for SLE and the level of vitamin D must be sufficient level especially in SLE patients.

Keywords: SLE, RANKL, Sclerostin, PD-1, Vitamin D.

1. Introduction

SLE is a type of chronic inflammatory autoimmune disease caused by autoantibodies [1, 2]. SLE can have an impact on numerous human organs, including the skin, kidneys, joints, blood cells, neurological system, and blood vessels [3]. Also, SLE patients have a higher risk of losing their bone density [4]. This disease is associated with significant morbidity and mortality and affects mainly young females [5, 6]. The main characteristic of this disease is the loss of immunological tolerance, which leads to the production of a wide range of autoantibodies [7]. Programmed cell death protein 1 (PD-1) is a surface protein that is a member of the immunoglobulin superfamily and serves as an immunomodulatory molecule. When PD-1 binds

with its ligand PD-L1, which negatively regulates the immunological response after T cell activation and upholds the equilibrium of immune tolerance, it sends out inhibitory signals [8]. In order to stop autoimmune tissue damage, PD-1 down-regulates T cell activation during immunological responses. Long-term exposure to antigens can cause PD-1 expression in tumors or chronic infections, which can make it harder for the immune system to get rid of damaged cells or pathogens [9]. The PD-1 receptor and its ligands have been associated with SLE development [10]. In SLE, the PD-1 axis plays a role in regulating innate and adaptive immune subsets [11]. Antibodies that target PD-1 receptors to prevent their activation are a well-established treatment for a variety of malignancies [12]. This antibody therapy may also result in clinical symptoms like those of autoimmune diseases such as SLE [13, 12]. Vitamin D, unique among vitamins, can be generated on the skin as a result of sunlight exposure. Vitamin D is involved in regulating calcium and phosphorus metabolism [14]. Many autoimmune diseases, including SLE, scleroderma, and rheumatoid arthritis, are thought to be linked to a deficiency of vitamin D [15]. Although there is evidence linking low vitamin D levels to SLE, it is also critical to take into account the possibility that vitamin D deficiency may be a contributing factor in the etiology of SLE [16]. Many variables may affect vitamin D levels in SLE patients, including photosensitivity, dark skin pigmentation, renal failure, sunscreen use, and medication [17]. On the other hand, individuals with SLE had a significant frequency of low BMD and vertebral fractures, highlighting the fact that osteoporosis is a prevalent feature of SLE [18]. Osteoporosis is a biomechanical phenomenon whereby mechanical stress on the joints results in pathological responses that cause damage to the articular cartilage and alterations in other tissues. These changes often take time to manifest and deteriorate over time [19, 20]. Osteoporosis is characterized by increased bone fragility and low bone mass [21]. Bone remodeling is a dynamic equilibrium biological process that occurs as a result of the interaction of osteoclasts and osteoblasts. When the equilibrium of bone remodeling is disturbed, diseases such as bone loss and osteoporosis develop [22]. Receptor activator of nuclear factor-κB (RANK) is expressed on osteoclastic precursor cells, and when RANK ligand (RANKL) binds to RANK, osteoclast differentiation and activation are facilitated [23]. Moreover, it is crucial for the development of the lymphatic system, the mammary glands, and establishing immunological tolerance [24]. Patients diagnosed with SLE exhibit heightened production of RANKL, which leads to an acceleration in osteoclastogenesis [25]. Elevated concentrations of oxidized low-density lipoproteins (LDL) may potentially cause T-cell activation in SLE, which raises the expression of RANKL and the generation of TNF. They may also have a deleterious impact on bone formation by lowering osteoblast maturation [25, 26]. In the same context, sclerostin is a 190-amino acid glycoprotein that is mainly secreted by adult osteocytes, even though chondrocytes and cementocytes can also produce it [27]. In many rare genetic bone diseases, the deficiency of sclerostin causes osteopetrosis [28]. Serum sclerostin could be used as a useful biomarker for lupus nephritis [29]. The current study aimed to evaluate the role of RANKL, sclerostin, PD-1, and vitamin D in the pathogenesis of SLE in early-diagnosed patients and patients under treatment.

2. Materials and Methods

2.1. Subject Collection

From March 2022 to August 2022, the current study was carried out in the Department of Rheumatology at Al-Yarmouk Teaching Hospital and the Medical City (Baghdad Teaching

Hospital) in Baghdad. There were 180 participants (age arranged between 20 to 45 years) which involved in this study (60 females as early diagnosed patients and 60 patients under treatment with (prednisolone, hydroxychloroquine) in addition to 60 apparently healthy individuals as control). All patients were diagnosed according to clinical examination by a rheumatologist physician and selected based on the SLE revised classification criteria published by the American College of Rheumatology (ACR) [30]. Patients with other diseases (Hypertension, diabetes type I, II, cardiovascular disease) or any other chronic disease including autoimmune diseases were excluded from this study.

2.2. Blood Collection

5 ml of a sample of intravenous blood was withdrawn from all participants (patients and healthy individuals), then centrifuged at 4000 rpm for about 10 minutes and kept at -20 C for subsequent analysis. The practical side was carried out in Hia clinics in Baghdad. The ELISA technique was used to assess serum levels of RANKL, sclerostin, and PD-1. ELISA research kits purchased from the USA company (CLOUD-CLONE CROP) were used for this purpose. ELISA research kits purchased from a German company (Human) were used to assess the serum level of vitamin D.

2.3. Statistics

The data were analyzed using SPSS Statistical software (IBM SPSS 26.0). We used the least significant difference (LSD) test with probability of less than 0.05 ($p<0.05$).

3. Results

The results showed that, serum level of RANKL recorded a non-significant difference among the three understudying groups, the results for SLE early diagnosed group, SLE under treatment group and the control group, the results were (1.203±0.29, 0.921±0.28, 0.870±0.23 pg\mL) respectively**.** Serum level of sclerostin also showed no significant differences among three understudying groups, the results were (0.686±0.09, 0.588±0.09, 0.462±0.0899 ng/mL) respectively, as shown in **Table 1**.

Table 1. Serum levels of RANKL and sclerostin.

Groups	RANKL (pg\mL) (Mean ± SE)	Sclerostin (ng/mL) (Mean ± SE)
Early diagnosed patients	1.203±0.29A	0.686±0.09A
Treated patients	0.921±0.28A	0.588±0.09A
Control	0.870±0.23A	0.462±0.08A
P value	0.106	0.11
LSD(P<0.05)	0.755	0.244
Similar letters denote to the non-significant difference at P<0.05*		

The serum level of PD-1 was significantly higher in the early-diagnosed group and the undertreated group compared with the control (0.611±0.04, 0.579±0.07, and 0.256±0.04 ng\mL, respectively). The serum concentration level of vitamin D was significantly higher in the early-diagnosed group and treated group compared with the healthy control group (56.87±4.45, 56.62±2.08, 46.02±1.57 ng\mL), respectively, as shown in **Table 2**.

Table 2. Serum levels of PD-1 and Vitamin D.

Groups	PD (ng\mL) (Mean ± SE)	Vitamin D (ng\mL) (Mean ± SE)
Early diagnostic patients	0.611±0.04A	56.87±4.45B
Treated patients	0.579±0.07A	56.62±2.08B
Control	0.256±0.04B	46.02±1.57A
P value	0.009	0.001
LSD(P<0.05)	0.153	5.38
*Different letters denote to the significant difference at P<0.05		

4. Discussion

4.1. Serum Level of PD-1

PD-1 receptors and their ligands have been implicated in the pathogenesis of SLE. [11]. The higher level of serum PD-1 in SLE patients may be explained by two theories. The first one is sPD-1, which is produced by alternative splicing and can disrupt the regulatory impact of membrane-bound PD-1 on T cell activation after raising its level in the serum of SLE patients [31]. The second says that there is a noteworthy correlation between increased PD-1 expression in T cells and increased expression in B cells after stimulating hyporesponsive lupus B cells (which exhibit an elevated PD-1 phenotype). These results suggest that PD-1 membrane expression in these cells is reciprocally regulated in SLE [32]. Also, PD-1 may play a role in SLE pathogenesis by other means in addition to its inhibitory function as a negative costimulatory molecule. It may also be associated with disease severity and act as a negative feedback mechanism for preventing potential tissue damage brought on by excessive autoimmune responses in SLE patients [33]. Based on the findings of the study, the increased PD-1 expression in SLE patients may be related to the pathogenesis of the disease and may be helpful in SLE treatment. The current study agrees with the previous study done by Bassiouni *et al.* [34], who discovered that serum PD-1 levels are significantly higher in patients with early-diagnosed SLE compared to healthy individuals. While the present study disagrees with Her *et al.'s* [35] study, which recorded that PD-1 serum levels in patients with SLE were comparable to those in the control group,

4.2. Vitamin D

Evidence has accumulated in recent years on the influence of vitamin D on the immune system. Given that vitamin D supplements are inexpensive and infrequently harmful, it may be advised that all SLE patients take them regularly [36]. It is generally safe, considerably raises serum vitamin D levels in SLE patients, extends life, lowers proteinuria, and improves knee arthritis [37, 38]. Therefore, the current results showed an increase in vitamin D levels in SLE patients compared to the healthy group. In the same context, vitamin D insufficiency is frequent in the general population; an estimated one billion individuals worldwide are thought to suffer from vitamin D deficiency or insufficiency [38, 39]. According to certain research, women are more likely to suffer from vitamin D deficiency than men because they spend less time outdoors and use sunscreen more frequently. This finding may also be related to the Islamic custom of covering the body from head to toe [40; 41]. and this explains the low vitamin D level in the healthy control group. A previous study showed no significant differences in vitamin D levels between SLE

patients taking anti-malarial and corticosteroids and those who were not taking them [37]. The current study disagrees with previous studies conducted by Islam *et al*. [42] and Attar *et al*. [43], who discovered a link between low vitamin D levels in SLE patients and healthy individuals. A study performed by Stockton did not record a difference between patients and controls [44]. It's possible that the literature on SLE exaggerated the vitamin D insufficiency in lupus because the majority of research included healthy hospital-based controls. The healthy controls might not have represented the overall population, and the hospital-based recruiting process could have resulted in selection bias [45]. Therefore, despite the widespread belief that people with SLE have significantly lower levels of vitamin D, differences in assay methods, ethnicity, cut-off values, seasonality, latitude, age, disease duration, and sex all affect the frequency of vitamin D [46], and may all people in the same society have a deficiency in vitamin D level.

4.3. Serum Level of RANKL

The development of RANKL assay has generated significant interest in utilizing them as markers for metabolic bone diseases. Nevertheless, it is worth noting that changes at the cellular level may not necessarily lead to corresponding alterations in serum levels [47]. Because most RANKL is membrane bound, studies using serum RANKL can sometimes be constrained by the significant percentage of individuals with undetectable circulating [48]. Therefore, maybe RANKL is only detectable in vivo by flow cytometry following bone biopsy [49]. In humans, this technique is very impractical [47]. Because only a tiny amount of serum RANKL may enter the systemic circulation and because some of the serum RANKL concentrations may come from non-skeletal sources, serum RANKL may not accurately reflect its level in the bone microenvironment [50], and this may explain why the current result did not record a significancy in serum level of SLE patients compared to healthy individuals. The current study disagrees with previous studies done by Hao et *al*., [51] and Ali *et al*., [52] who showed that RANKL was significantly higher in children with SLE. Another study done by Sandal *et al*., [53] on children with SLE found that RANKL levels were unrelated to disease activity. The current study agrees with a previous study conducted by Carmona-Fernandes *et al*., [48] who found sRANKL levels to be similar between SLE and healthy control women. The reason for the discrepancy in the results of different studies may be attributed to the many factors that influence these measurements, such as differing designs and methodologies.

4.4. Serum Level of Sclerostin

In various autoimmune diseases, sclerostin levels seem to have a heterogeneous pattern [54]. A previous study conducted on rheumatoid arthritis (RA) patients attributed the reason why serum sclerostin levels did not increase in those patients to the fact that increased osteocyte death in bone samples from RA patients may decrease sclerostin synthesis and release from osteocytes into their circulation [55]. Also, sclerostin serum levels may not reflect its tissue concentration or reflect changes in the microenvironment of the joints [56]. and this maybe explains why the current study did not record a significant difference in the sclerostin serum level of SLE patients compared to healthy individuals. The current study agrees with previous studies done by [57] and [58], who observed that sclerostin levels found in SLE patients were comparable to those found in healthy individuals. The current study disagrees with the study done by [59], who recorded a decrease in

serum levels of sclerostin in SLE, and also disagrees with [29], who reported a high sclerostin level in SLE patients compared to healthy controls. The reason for the disparity in the results of different studies may be attributed to the many factors that influence these measurements, such as the number of samples or the examination method.

5. Conclusions

Based on our findings, PD-1 may be considered a good therapeutic target for SLE, and the level of vitamin D must be at a sufficient level, especially in SLE patients.

Acknowledgment

Many thanks to the Department of Biology, College of Education for Pure Sciences, Ibn-AL Haitham, University of Baghdad for facilitating the work of the practice parts in this article .

Conflict of Interest

There are no conflicts of interest.

Funding

There is no funding for the article.

Ethical Clearance

The samples were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health No. 3616 in 24/1/2022.

References

1. Kamil, M.A.; Kadr, Z.H.M.; Alabassi, H.M. Role of CXCL9-CXCR3 AXIS, ANA & DS-DNA ABS in Pathogenicity of SLE in Iraqi Patients. *Pak. J. Med. Health Sci*., **2022***; 16*, 04, 398-398.
2. Abbas, A.H.; Melconian, A.K.; Ad'hiah, A.H. Detection of anti-cytomegalovirus antibodies in sera of Iraqi systemic lupus erythematosus women patients. *Iraqi J. Sci.*, **2016**; 317-320.
3. Ibrahim, N.K.; Ghudhaib, K.K.; Allawi, A.A.D.; Hammoudi, F.A. Evaluation of HE4 and IGFBPs as Novel Biomarkers of Systemic Lupus Erythematosus with Lupus Nephritis. *Indian J. Forensic Med. Toxicol.*, **2020***; 14*, 4124-4130.
4. Gorial, F.I.; Mahmood, Z.A.; Al Obaidi, S. Body composition in Iraqi Women with systemic lupus erythematosus. *Glob. J. Health Sci,* **2019**; *11*, 63-70.
5. Ali, N.S.M.; Zaidan, T.F. Oral manifestations, oral health status and saliva composition changes in a sample of Iraqi systemic lupus erythematosus patients. *J. Baghdad Coll. Dent*., **2012**; *24*, 65.
6. Nasir, H.A.; Sharba, Y.F.; Nasir, N.A. Lupus Nephritis, the therapy and the role of Rituximab in resistant cases. *J. Fac. Med. Baghdad*, **2013***; 55*, 327-332.
7. Abdulridha, R.H., Saud, A.M.; Alosami, M.H. Assessment of miR-146a Gene Polymorphisms in Patients with Systemic Lupus Erythematosus. *Iraqi J. Sci.*, **2023**; 573-582.
8. Fife, B.T.; Pauken, K.E. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann. N. Y. Acad. Sci.*, **2011***;1217*, 45-59.

9. Jubel, J.M.; Barbati, Z.R.; Burger, C.; Wirtz, D.C.; Schildberg, F.A. The role of PD-1 in acute and chronic infection. *Front. Immunol.*, **2020**; *11*, 487.
10. Lee, Y.H.; Woo, J.H.; Choi, S.J.; Ji, J.D.; Song, G.G. Association of programmed cell death 1 polymorphisms and systemic lupus erythematosus: a meta-analysis. *Lupus*, **2009**; *18*, 9-15.
11. Curran, C.S.; Gupta, S.; Sanz, I.; Sharon, E. PD-1 immunobiology in systemic lupus erythematosus. *J. Autoimmun.*, **2019**, *97*, 1-9.
12. Michot, J.M.; Bigenwald, C.; Champiat, S.; Collins, M.; Carbonnel, F.; Postel-Vinay, S.; Berdelou, A.; Varga, A.; Bahleda, R.; Hollebecque, A.; Massard, C. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur. J. Cancer*, **2016***; 54*, 139-148.
13. Kuhn, A.; Bonsmann, G.; Anders, H.J.; Herzer, P.; Tenbrock, K.; Schneider, M. The diagnosis and treatment of systemic lupus erythematosus. *Dtsch. Arztebl. Int.*, **2015**, *112*, 423.
14. Alves, M.; Bastos, M.; Leitão, F.; Marques, G.; Ribeiro, G.; Carrilho, F. Vitamina D–importância da avaliação laboratorial. *Rev. Port. Endocrinol. Diabetes Metabol.*, **2013**, *8*, 32-39.
15. Abo-Shanab, A.M.; Kholoussi, S.; Kandil, R.; Dorgham, D. Cytokines, 25-OH vit D and disease activity in patients with juvenile-onset systemic lupus erythematosus. *Lupus*, **2021**, *30*, 459-464.
16. Hassanalilou, T.; Khalili, L.; Ghavamzadeh, S.; Shokri, A.; Payahoo, L.; Bishak, Y.K. Role of vitamin D deficiency in systemic lupus erythematosus incidence and aggravation. *Autoimmun. Highlights*, **2018**, *9*, 1-10.
17. Bultink, I.E.; Lems, W.F. Systemic lupus erythematosus and fractures. *RMD open*, **2015**, *1*, e000069.
18. Bultink, I.E. *Complications of systemic lupus erythematosus*. Doctoral dissertation. Vrije Universiteit Amsterdam: Amsterdam, **2008**.
19. Abdullah, D.A.; Al-Sharqi, S. A.H.; ALabbassi, H.M.; Swahab, M.; Almishhadany, M.S. Evaluation Pathogenesis Outcomes of Knee and Hip in Patients with Osteoarthritis Based on: Histopathological, Hematological, and Indicators of Inflammation. *J. Res. Med. Dent. Sci*., **2021**, *9*, 178-184.
20. Abdullah, D.A.; ALabassi, H.M.; Al-Sharqi, S.A. and Wahab, M.S. Possible etiological role of cd16 and tgf-β1 in the pathogenesis of osteoarthritis Iraqi patients. *Biochem. Cell. Arch*, **2022**, *22*, 2351-2356.
21. Kadri, Z.H.M. Effect of cigarette and water-pipe smoking on osteocalcin and RANKL serum levels among Iraqi university students. *EurAsian J. Biosci.*, **2020**,*14*, 3025-3030.
22. Ramesh, P.; Jagadeesan, R.; Sekaran, S.; Dhanasekaran, A.; Vimalraj, S. Flavonoids: classification, function, and molecular mechanisms involved in bone remodelling. *Front. Endocrinol.*, **2021**, *12*.
23. Kong, Y.Y.; Yoshida, H.; Sarosi, I.; Tan, H.L.; Timms, E.; Capparelli, C.; Morony, S.; Oliveira-dos-Santos, A.J.; Van, G.; Itie, A.; Khoo, W. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*, **1999**, *397*, 315-323.
24. Holliday, L.S.; Patel, S.S.; Rody Jr, W.J. RANKL and RANK in extracellular vesicles: Surprising new players in bone remodeling. *Extracell. Vesicles Circ. Nucleic Acids*, **2021**, *2*, 18.

25. Amarasekara, D.S.;Yu, J.; Rho, J. Bone loss triggered by the cytokine network in inflammatory autoimmune diseases. *J. Immunol. Res*, **2015**, *2015*, 12.
26. Bultink, I.E. Bone disease in connective tissue disease/systemic lupus erythematosus. *Calcif. Tissue Int.*, **2018**, *102*, 575-591.
27. Moester M.J.; Papapoulos S.E.; Lowik C.W.; van Bezooijen R.L. Sclerostin: current knowledge and future perspectives. *Calcif. Tissue Int*., **2010**, *87*, 99–107.
28. Balemans, W.; Ebeling, M.; Patel, N.; Van Hul, E.; Olson, P.; Dioszegi, M.; Lacza, C.; Wuyts, W.; Van Den Ende, J.; Willems, P.; Paes-Alves, A.FIncreased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum. Mol. Genet.*, **2001**, *10*, 537-544.
29. Fayed, A.; Soliman, A.; Elgohary, R. Measuring Serum Sclerostin in Egyptian Patients With Systemic Lupus Erythematosus and Evaluating Its Effect on Disease Activity: A Case-Control Study. *JCR: J Clin Rheumatol*., **2021**, *27*, 161-167.
30. Hochberg M.C. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*., **1997**, *40*, 1725-1725.
31. Ibrahim, W.S.; Abdelghani, S.E. Programmed cell death 1 gene polymorphism association with activity and severity of systemic lupus erythematosus and rheumatoid arthritis in Egyptian patients. *Tanta Med. J.*, **2018**, *46*, 225.
32. Stefanski, A.L.; Wiedemann, A.; Reiter, K.; Hiepe, F.; Lino, A.C.; Dörner, T. Enhanced Programmed Death 1 and Diminished Programmed Death Ligand 1 Up-Regulation Capacity of Post-Activated Lupus B Cells. *Arthritis Rheumatol*., **2019**, *71*, 1539-1544.
33. Luo, Q; Kong, Y; Fu, B; Li, X; Huang, Q; Huang, Z.; Li, J. Increased TIM-3+ PD-1+ NK cells are associated with the disease activity and severity of systemic lupus erythematosus. *Clin. Exp. Med*., **2022,** *22*, 47-56.
34. Bassiouni, S.A.; Abdeen, H.M., Morsi, H.K.; Zaki, M.E.; Abdelsalam, M.; Gharbia, O.M. Programmed death 1 (PD-1) serum level and gene expression in recent onset systemic lupus erythematosus patients. *Egypt. Rheumatol*. **2021**, *43*, 213-218.
35. Her, M.; Kim, D.; Oh, M.; Jeong, H.; Choi, I. Increased expression of soluble inducible costimulator ligand (ICOSL) in patients with systemic lupus erythematosus. *Lupus*, **2009**, *18*, 501-507.
36. Shoenfeld, Y.; Giacomelli, R.; Azrielant, S.; Berardicurti, O.; Reynolds, J.A.; Bruce, I.N. Vitamin D and systemic lupus erythematosus-The hype and the hope. *Autoimmun. Rev.*, **2018**, *17*, 19-23.
37. Khairallah, M.K.; Makarem, Y.S.; Dahpy, M.A. Vitamin D in active systemic lupus erythematosus and lupus nephritis: a forgotten player. *Egypt. J. Intern. Med*., **2020**, *32*, 1-9.
38. Shoenfeld, N.; Amital, H.; Shoenfeld, Y. The effect of melanism and vitamin D synthesis on the incidence of autoimmune disease. *Nat. Clin. Pract. Rheumatol*., **2009**, *5*, 99-105.
39. Holick, M.F. Vitamin D deficiency. *N. Engl. J. Med*., **2007**, *357*, 266-281.
40. Zargar, A.H.; Ahmad, S.; Masoodi, S.R.; Wani, A.I.; Bashir, M.I.; Laway, B.A.; Shah, Z.A. Vitamin D status in apparently healthy adults in Kashmir Valley of Indian subcontinent. *Postgrad. Med. J.*, **2007**, *83*, 713-716.
41. Hussain, A.N.; Alkhenizan, A.H.; El Shaker, M.; Raef, H.; Gabr, A. Increasing trends and significance of hypovitaminosis D: a population-based study in the Kingdom of Saudi Arabia. *Arch. Osteoporos.*, **2014**, *9*, 1-5.

42. Islam, M.A.; Khandker, S.S.; Alam, S.S.; Kotyla, P.; Hassan, R. Vitamin D status in patients with systemic lupus erythematosus (SLE): A systematic review and meta-analysis. *Autoimmun. Rev.*, **2019**, *18*, 102392.
43. Attar, S.M. and Siddiqui, A.M. Vitamin d deficiency in patients with systemic lupus erythematosus. *Oman Med. J.*, **2013**, *28*, 42.
44. Stockton, K.A.; Kandiah, D.A.; Paratz, J.D.; Bennell, K.L. Fatigue, muscle strength and vitamin D status in women with systemic lupus erythematosus compared with healthy controls. *Lupus*, **2012**, *21*, 271-278.
45. Kavadichanda, C.; Singh, P.; Maurya, S.; Tota, S.; Kiroubagarin, A.; Kounassegarane, D.; Anand, S.; Negi, V.S.; Aggarwal, A. Clinical and serological association of plasma 25-hydroxyvitamin D (25 (OH) D) levels in lupus and the short-term effects of oral vitamin D supplementation. *Arthritis Res. Ther*., **2023**, *25*, 1-10.
46. Nerviani, A.; Mauro, D.; Gilio, M.; Grembiale, R.D.; Lewis, M.J. To supplement or not to supplement? The rationale of vitamin D supplementation in systemic lupus erythematosus. *Open Rheumatol. J*., **2018**, *12*, 226-247.
47. Anastasilakis, A.D.; Goulis, D.G.; Polyzos, S.A.; Gerou, S.; Pavlidou, V.; Koukoulis, G.; Avramidis, A. Acute changes in serum osteoprotegerin and receptor activator for nuclear factor-κB ligand levels in women with established osteoporosis treated with teriparatide. *Eur. J. Endocrinol.*, **2008**, *158*, 411-415.
48. Carmona-Fernandes, D.; Santos, M.J.; Perpétuo, I.P.; Fonseca, J.E.; Canhão, H. Soluble receptor activator of nuclear factor κB ligand/osteoprotegerin ratio is increased in systemic lupus erythematosus patients. *Arthritis Res. Ther.*, **2011**, *13*, 1-6.
49. Eghbali-Fatourechi, G.; Khosla, S.; Sanyal, A.; Boyle, W.J.; Lacey, D.L.; Riggs, B.L. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J. Clin. Investig.*, **2003**, *111*, 1221-1230.
50. Rogers, A.; Eastell, R. Circulating osteoprotegerin and receptor activator for nuclear factor κB ligand: clinical utility in metabolic bone disease assessment. *J. Clin. Endocrinol. Metab*., **2005**, *90*, 6323-6331.
51. Hao, S.; Zhang, J.; Huang, B.; Feng, D.; Niu, X.; Huang, W. Bone remodeling serum markers in children with systemic lupus erythematosus. *Pediatr. Rheumatol.*, **2022**, *20*, 1-6.
52. Ali, R.; Hammad, A.; El-Nahrery, E.; Hamdy, N.; Elhawary, A.K.; Eid, R. Serum RANKL, osteoprotegerin (OPG) and RANKL/OPG ratio in children with systemic lupus erythematosus. *Lupus*, **2019**, *28*, 1233-1242.
53. Sandal, S.; Rawat, A.; Gupta, A.; Singh, S. 141 soluble receptor activator of nuclear factor K b ligand (s rank-l) levels in paediatric onset sle. *Lupus Sci. Med.*, **2017**,4, A64.
54. Fernández-Roldán, C.; Genre, F.; López-Mejías, R.; Ubilla, B.; Mijares, V.; Cano, D.S.; Robles, C.L.; Callejas-Rubio, J.L.; Fernández, R.R.; Ruiz, M.E.; González-Gay, M.Á. Sclerostin serum levels in patients with systemic autoimmune diseases. *BoneKEy Rep.*, **2016**, *5*.
55. Vargas-Muñoz, V.M.; Jimenez-Andrade, M.C.; Villarreal-Salcido, J.C.; Martinez-Martinez, A.; Acosta-Gonzalez, R.I.; Lugo-Zamudio, G.E.; Conde-Mercado, J.M.; Barbosa-Cobos, R.E.; Matias-Morales, F.A.; Ramirez-Rosas, M.B.; Jimenez-Andrade, J.M. Association between sclerostin and bone mineral density in a Mexican sample of women with rheumatoid arthritis: a pilot study. *J. Arthritis*, **2015**,1-6.

56. Fayed, A.; Elgohary, R.; Fawzy, M. Evaluating the role of serum sclerostin as an indicator of activity and damage in Egyptian patients with rheumatoid arthritis: university hospital experience. *Clin. Rheumatol.*, **2020**, *39*, 1121-1130.
57. Fernandez-Roldan, C.; Genre, F.; López-Mejías, R.; Ubilla, B.; Mijares, V.; Cano, D.S.; Robles, C.L.; Callejas-Rubio, J.L.; Fernández, R.R.; Ruiz, M.E.; González-Gay, M.Á. Sclerostin serum levels in patients with systemic autoimmune diseases. *BoneKEy Rep.*, **2016**, *5*.
58. Durosier, C.; van Lierop, A.; Ferrari, S.; Chevalley, T.; Papapoulos, S.; Rizzoli, R. Association of circulating sclerostin with bone mineral mass, microstructure, and turnover biochemical markers in healthy elderly men and women. *J. Clin. Endocrinol. Metab*., **2013**, *98*,3873-3883.
59. Karataş, A.; Ömercikoğlu, Z.; Öz, B.; DAĞLI, A.F.; Catak, O.; Erman, F.; ŞAHİN, K.; Gözel, N.; Koca, S.S. Wnt signaling pathway activities may be altered in primary Sjogren's syndrome. *Turk. J. Med. Sci.*, **2021**, *51*, 2015-2022.