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Relation of Serum Beta 2 Microglobulin Levels to Systemic Lupus Disease Manifestations and Disease Activity

Hossam Marouf Fathy*[1], Rabab Afifi Mohamed[2], Marwa Gamal Korany[1], Mervat Ismail Abd-ElAzeem[1]
Departments of [1]Rheumatology and Rehabilitation and
[2]Clinical Pathology, Faculty of Medicine, Beni-Suef University, Egypt
***Corresponding author:** Hossam Marouf Fathy, **Mobile:** (+20) 01280556463, **E-Mail:** hossamfathy420@gmail.com

ABSTRACT
Background: The autoimmune disease systemic lupus erythematosus (SLE) causes inflammation. Activity evaluation could be conducted using a number of different methods. Biomarkers like β2 is a biomarker, which is found on all nucleated somatic cells and its use as a prognostic is under investigation.
Objective: The study aimed to assess the association between serum levels of β2 microglobulin (B2M) and disease activity.
Patients and Methods: The study included fifty lupus patients and fifty healthy people served as controls. The levels of C-reactive protein (CRP), complement 3, and complement 4, as well as the erythrocyte sedimentation rate (ESR), anti-nuclear antibody (ANA), and anti-double-stranded DNA antibody (dsDNA), were measured. The serum Beta 2-microglobulin (B2M) was measured using an enzyme-linked immunosorbent assay.
Results: The serum B2-microglobulin levels were significantly higher in SLE patients (mean values **27.42± 5.5)** compared with the controls (**6.80± 1.61**) (**P value =0.001**). In contrast to inactive individuals, active patients had a greater mean serum B2-microglobulin concentration.
Conclusion: A good correlation between B2M and SLE disease activity may explain the role of this marker in the inflammatory process.
Keywords: Systemic lupus erythematosus, SLE Activity Index, Beta 2-microglobulin.

INTRODUCTION

The autoimmune disease systemic lupus erythematosus (SLE) primarily strikes teenage and middle-aged women (14–44 years old). It results in multi-organ failure as it produces broad-spectrum antibodies that result in an immune complex deposition **(1).** The influx of pro-inflammatory cytokines mediates the inflammation and produces a variety of acute-phase reactant proteins named inflammatory markers like high-sensitivity C-reactive proteins (hs-CRPs) **(2,3).**

SLE pathogenesis is a complicated condition, T and B cells are activated, apoptosis is impaired and immune complex clearance became insufficient. Excessive autoantibodies resulting from B cell activation unite with chromatin molecules leading to an immune complex that causes inflammation **(4,5).**

SLE activity is assessed in daily practice using disease activity evaluation methods and various serum markers like C3, C4 complements, anti-C1q antibodies and anti-double strand DNA. The look for new activity markers is still on. β2 is the light chain of HLA class I and is located on all nucleated somatic cells like lymphocytes and is used to detect the disease prognosis **(6,7).**

Patients with lymphoproliferative illnesses, renal failure, or other autoimmune disorders have elevated levels of this biomarker because of the activation of both T and B cells **(8-10).**

High levels of β2M in SLE patients have a mysterious origin. The increase in β2M concentrations may be explained by the increased turnover of β2M lymphocytes, which leads to the removal of anti- β2M antibodies by the kidneys **(11).**

The study aimed to assess the association between serum levels of β2 microglobulin (B2M) and disease activity.

PATIENTS AND METHODS

This case-control study was conducted on 50 clinically diagnosed SLE patients (48 females and 2 males) who were presented to the Rheumatology and Rehabilitation Department, Faculty of Medicine, Beni-Suef University and 50 controls. The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria were used to assess the patients.

Patient evaluation:

Full histories as well as clinical and laboratory examinations were performed on all individuals.

All investigations and examinations were performed at department labs.

Laboratory assessment;

1-Routine investigations:

Complete blood picture (CBC), ESR, CRP, anti-deoxyribonucleic acid (Anti-ds-DNA) antibodies, antinuclear antibodies (ANA): by the immune-fluorescence technique**,** serum complement levels (C3, C4)**,** complete urine analysis and 24 hr urinary proteins measurement**,** serum urea and creatinine, anticardiolipin (ACL) antibodies and lupus anticoagulant antibodies, serum uric acid and renal biopsy.

2-Special laboratory tests:

Detection of serum level of serum B2 microglobulin by ELISA technique.

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3-Disease activity assessment:

The Systemic Lupus International Collaborative Clinics/America Collage of Rheumatology (SLICC/ACR) damage index was used to measure the SLE disease activity index (SLEDAI), and the SLE disease severity.

Inclusion criteria: Age of patients (16-54) years, either males or females, and patients were diagnosed according to Systemic Lupus International Collaborating Clinics (SLICC) [12].

Exclusion criteria: Infection, cancer, and severe kidney failure.

Ethical approval:

An approval of the study was obtained from Beni-Suef University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical methods

SPSS, or the Statistical Package for the Social Sciences, version 20 was used for the analysis. Quantitative data were presented as mean and standard deviation (SD) and were compared by the Student t-test. Qualitative data were presented as frequency and percentage and were compared by Chi^2 test. Pearson's correlation was used to calculate the correlation between the studied markers. P-value equal to or less than 0.05 was considered significant.

RESULTS

There was no significant difference between cases and control groups regarding age and sex. The individuals with SLE investigated had the condition for an average of (4.613.6) years (Table 1).

Table (1): Age and sex distribution of the studied population

	Studied Population		
	Cases with SLE N= 50	Healthy Controls N= 50	p-value
Sex; N (%)			
Male	2 (4%)	2 (4%)	1
Female	48 (96%	48 (96%)	
Age; (years)			
Mean ±SD	28.98 ±6	27.10 ±5.6	0.134
Minimum	17	19	
Maximum	42	39	
Disease Duration			
Mean ±SD	4.61 ±3.		
Minimum	1		
Maximum	20		

The most common symptoms and signs were malar rash and photosensitivity. The number of patients with lupus nephritis (as diagnosed by renal biopsy) was 30 patients. Hematologic disorder in the form of leucopenia and thrombocytopenia was found in 10 patients. Serositis in the form of pleurisy, pericarditis and pericardial effusion was found in 24 patients. 1 patient had vasculitis in the form of a skin rash. The neurologic disorder was found in 4 patients 2 of them had seizures and the other 2 patients had psychosis (Table 2).

The SLEDAI and SLICC damage index scores quantified the severity of SLE disease activity. The SLEDAI for the SLE patients evaluated ranged from 2 to 30, with an average of 14.04±8, while the SLICC damage index showed no damage in 41 instances (82%), with damage present in just 9 cases (Table 2).

Table (2): Difference in system involvement and SLEDAI and SLICC scores for SLE assessment; (N= 50)

Clinical Symptoms and Signs		Frequency	Percent
Malar rash	**Yes**	49	98.0
Discoid rash	**Yes**	5	10.0
hotosensitivity	**Yes**	49	98.0
Oral Ulcers	**Yes**	39	78.0
Arthritis	**Yes**	26	52.0
enal Disorder	**Yes**	28	56.0
Hematologic	**Yes**	10	20.0
Serositis	**Yes**	24	48.0
Alopecia	**Yes**	10	20.0
Myositis	**Yes**	0	0.00
Fever	**Yes**	7	14.0
Neurologic	**Yes**	4	8.0
Vasculitis	**Yes**	1	2.0
SLICC	**No Damage**	41	82.00
	Damage	9	18.0
SLEDAI	**ean ±SD**	15.84 ±9.2	
	inimum	2	
	aximum	30	

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, SLICC: Systemic Lupus international collaborating clinics.

The mean values for β2-microglobulin concentration in SLE cases was significantly higher than in control group (Table 3).

Table (3): β2-microglobulin concentration comparison among SLE cases and healthy controls

	Studied Population		Total	p-value
	Cases with SLE (N= 50)	Healthy Controls (N= 50)		
β2-Microglobulin concentration				
Mean ±SD	27.42 ±5.5	6.80 ±1.61	28.04 ±6.3	**0.001***
Minimum	13.90	2.60	17	
Maximum	37.70	17.20	42	

*: Significant

Patients with neuropsychiatric lupus had significantly higher levels of beta 2-microglobulin than those without among the SLE patients. No statistically significant difference could be detected between anti-double stranded DNA (ds-DNA) antibody positive and negative patients regarding serum β2-microglobulin concentration. No detected significant differences in the concentration of β2-microglobulin regarding other prescribed medications (Hydroxychloroquine, cyclophosphamide, azathioprine and mycophenolate mofetil) (Table 4).

Table (4): Relation of β2-microglobulin concentration to clinical symptoms and signs of the studied SLE patients and to drugs used; (N= 50)

Clinical Symptoms and Signs		β2-Microglobulin concentration Mean ±SD	P-value
Malar rash	**No; n=1**	--	0.904
	Yes; n=49	27.41 ±5.6	
Discoid rash	**No; n= 45**	27.83 ±5.5	0.121
	Yes; n=5	23.78 ±5.1	
Photosensitivity	**No; n=1**	--	0.623
	Yes; n=49	27.48 ±5.6	
Oral Ulcers	**No; n=11**	26.70 ±6.7	0.626
	Yes; n=39	27.63 ±5.3	
Arthritis	**No; n=24**	26.88 ±5.1	0.509
	Yes; n=26	27.93 ±5.9	
Renal Disorder	**No; n22**	26.98 ±6.1	0.618
	Yes; n=28	27.78 ±5.1	
Hematologic	**No; n=40**	28.13 ±5.1	0.072
	Yes; n=10	24.62 ±6.7	
Serositis	**No; n=26**	26.04 ±5.7	0.065
	Yes; n=24	28.93 ±5.0	
Alopecia	**No; n=40**	28.00 ±5.1	0.142
	Yes; n=10	25.12 ±6.9	
Myositis	**No; n= 50**	27.43 ±5.5	----
	Yes; n=0	--	
Fever	**No; n=43**	27.07 ±5.8	0.091
	Yes; n=7	29.61 ±2.9	
Neurologic	**No; n=46**	27.06 ±5.6	**0.001***
	Yes; n=4	31.70 ±1.3	
Vasculitis	**No; n=49**	27.35 ±5.6	0.544
	Yes; n=1	--	
Anti ds-DNA	**No; n=19**	27.17 ±5.8	0.793
	Yes; n=31	27.59 ±5.5	
Medications			
Hydroxychloroquine	**Yes; n=47**	27.65 ±5.5	0.259
Cyclophosphamide	**Yes; n=12**	29.57 ±5.9	0.124
Azathioprine	**Yes; n=33**	27.35 ±5.7	0.889
Mycophenolate mofetil	**Yes; n=3**	32.23 ±2.0	0.268

Our study could not detect a statistically significant correlation between patient's age and disease duration with the concentration of β2-Microglobulin among studied SLE disease patients. Concentration of β2-microglobulin showed slight negative correlation with HDL concentration among studied SLE patients. The concentration of β2-microglobulin showed a moderate negative correlation with C3 concentration among studied SLE patients. Patients with SLE investigated revealed a moderate positive connection between β2-Microglobulin concentration and disease activity as measured by SLEDAI score.

Table (5): Correlation between β2-Microglobulin concentration and other parameters

	β2-Microglobulin concentration	
	R	**p-value**
Patients' age (years)	0.136	0.178
Disease duration	-0.110	0.447
ESR (mm/hr)	0.012	0.932
CRP (mg/L)	0.229	0.109
HG (g/dL)	-0.099	0.495
TLC	0.041	0.776
PLT(mcL)	0.314	0.107
ALT (U/L)	-0.191	0.185
Urea (mg/dl)	0.191	0.184
Uric Acid (mg/dl)	-0.071	0.623
TAG	0.143	0.322
Cholesterol (mg/dl)	-0.051	0.723
LDL (mg/dl)	-0.133	0.359
HDL (mg/dL)	-0.280	**0.049***
C3	-0.339	**0.016***
C4	-0.028	0.849
Proteinuria	0.041	0.776
Lymphopenia	0.314	0.107
Hemolytic anemia	0.191	0.184
SLEDAI Score	0.431	**0.002***

There was no significant correlation between SLICC damage index score and 2-microglobulin levels in this sample of individuals with SLE; (p-values =0.848).

Table (6): Relation of SLICC damage index score and β2-microglobulin concentration among SLE Patients; (N= 50)

		β2-Microglobulin concentration	p-value
		Mean ±SD	
SLICC	**No Damage; n=41**	27.50 ±5.5	0.848
	Damage; n=9	27.08 ±6.0	

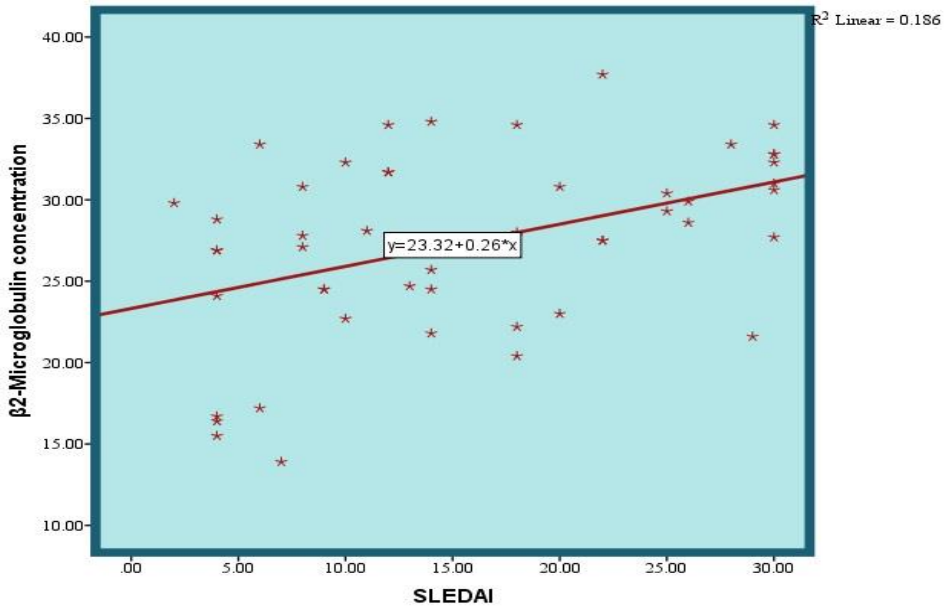

Figure (1): Correlation between β2-microglobulin concentration and SLEDAI Score among studied SLE patients.

DISCUSSION

SLE is an autoimmune disorder that affects the connective tissue. The disease develops in individuals who have an inherited gene in response to exposure to certain environmental factors [13]. The disease is more common in women and the pathogenesis is still uncertain, however, new treatments became available over the last few years [14]. The magnitude of occurrence and severity of the disease differ based on ethnicity [15]. The presentation of the disease differs from one person to another according to the affected organ. Patients may be presented with various symptoms like arthralgia, generalized fatigue, butterfly rash, nephritis, pericarditis, psychological manifestations, or hematological disorders [16-17]. Various presentations make the diagnosis more challenging and might take years before being certain [18,19]. Various activity markers are found like (anti-ds- DNA) anti-double-stranded DNA antibodies, and C3 and C4 complement components however these markers lack specificity [20].

β2-microglobulin (β2M) is known as a low-weight protein with a short half-life in plasma, its level is high in association with lymphoproliferative conditions and autoimmune disorders [21].

Few studies assessed the relationship between serum β2M levels and disease activity [21]. The study aimed to assess this relationship of disease activity in terms of the SLEDAI score and serum level of B2-microglobulin. The main finding was the elevation of the amount of β2M in SLE disease. The presence of a combination of β2M and anti- β2M antibodies, which is removed by the kidney, may account for the increase in lymphocyte turnover seen in lymphoproliferative and autoimmune disease [20]. In mice, the β2M deficiency was associated with cutaneous rather than kidney damage [22].

One hundred participants were enrolled, fifty participants were suffering from SLE and 50 as controls. Of the studied SLE patients, 48 were females and 2 were males with a ratio of 24:1 similar to the finding of **Skare *et al.*** study [23]. This is understandable as the disease is more prominent in females rather than males and the gene CD40, which is located on chromosome X is known to be associated with severe manifestations [19-24].

Serum B2-microglobulin levels were significantly different between patients and controls. The mean serum level of B2-microglobulin was 27.42 ug/mL ±5.5 in the SLE group and 6.80 ug/mL ±1.61 in controls. This is similar to findings by **Kim *et al.*** [11] study, which demonstrated that B2M levels of SLE patients (2.64±0.11 ug/mL) were higher than controls (2.14±0.04 ug/mL) (P=0.001). Another study by **Hermansen *et al.*** [25] found an increase in the median serum levels of β2M between patients and controls (2.8 mg/L, range: 1.1–21.6 and 1.2 mg/L, range: 0.9–1.7, respectively, p<0.001).

In our current study based on the SLEDAI scoring system, the mean B2-microglobulin serum level was higher in active patients compared to the inactive group. This was following findings by **Żychowska *et al.*** [20] study and **Hermansen *et al.*** [25] **study.**

Hermansen *et al.* [25] discovered an association between β2M levels and cytokines such IL-6, IL-10, IL-18, and IFN-alpha (R =0.68, p<0.001). **Kim *et al.*** [11] assumed that serum β2M can be a good biomarker to assess the activity of the disease (P=0.01). **Skare *et al.*** [23] study, which measured the β2M concentration in 129 patients, positively correlated β2M level with SLEDAI (P =0.02). **Choe *et al.*** [21] study reported also an association between urine β2M and SLEDAI (P=0.001). **Wakabayashi *et al.*** [26] showed that immunosuppressive treatment decreases the β2M level.

Patients' age or disease duration were not associated with a concentration of β2M as what **Żychowska *et al.*** [20] reported. Similarly, B2-microglobulin had no association between the presence or absence of Anti-DNA measurement (p=0.099). Similarly **Aghdashi *et al.*** [24] found that β2M values did not correlate with anti-double-stranded DNA (P=0.1). While **Żychowska *et al.*** [20] found that β2M levels correlated significantly with anti-ds- DNA titer (P = 0.001). They correlated B2M with anti-ds-DNA titer, but we compared patients who were Anti-DNA positive and those who were Anti-DNA negative.

Our study found that the concentration of β2-Microglobulin showed a moderate negative correlation with C3 concentration among studied SLE patients; (r= -0.339, p-value= 0.016) and this was in line with various studies [11, 23]. In contrast, **Aghdashi *et al.*** [24] showed no significant relevancy between serum levels of β2M with C3 (P=0.39). Sample size, different demographics, clinical presentation and various therapy may explain the different findings obtained by **Aghdashi *et al.*** [24].

C4 levels and β2-microglobulin levels were not correlated in the SLE illness patients investigated. The same finding was reported by **Aghdashi *et al.*** [24] (P= 0.90) and the opposite was reported by **Żychowska *et al.*** [20] (p= 0.02; r = −0.3).

Similar to the previous study by **Skare *et al.*** [23] the SLICC/ACR score and B2-M showed no statistically significant connection (P-value =0.848).

Results from the current study corroborate those of a study by **Rist *et al.*** [27], which indicated that B2M is an inflammatory marker and is linked to a higher rate of ischemic stroke in women. There was no statistical correlation between CRP and β2-microglobulinas. The C-reactive protein levels of lupus patients with infection were found to be significantly greater than those of lupus patients with only active disease (P=0.2) in a research by **Firooz *et al.*** [28]**.** In this study, and similar to the finding by **Gupta *et al.*** [29] β2-Microglobulin showed a slight negative correlation with HDL concentration; (r= -0.280, p-value= 0.049). There was no statistically significant difference detected in correlating B2-microglobulin with patients treated with prednisolone, hydroxychloroquine, cyclophosphamide, azathioprine and mycophenolate mofetil and this was in line with the finding by **Puchades *et al.* study** [30]**.**

CONCLUSION

The study concluded that B2-microglobulin concentrations were significantly elevated in SLE patients and significantly correlated with the SLEDAI score. This makes β2M assessment helps to determine disease activity.

RECOMMENDATIONS

- Future studies should be undertaken using significantly larger samples of SLE patients.
- Future studies should identify the potential risk factors of the disease.
- Further studies are needed to explore how B2M affect the pathophysiology of lupus.

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REFERENCES

1. **Somers E, Marder W, Cagnoli P *et al.* (2014):** Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance Program. Arthritis Rheumatol., 66(2):369–78.
2. **Umare V, Nadkarni A, Nadkar M *et al.* (2017):** Do high sensitivity C-reactive protein and serum interleukin-6 levels correlate with disease activity in systemic lupus erythematosus patients? Journal of Postgraduate Medicine, 63(2): 92–95.
3. **Devaraj S, Singh U, Jialal I (2009):** The evolving role of C-reactive protein in atherothrombosis. Clin Chem., 55(2):229-38.
4. **Tsokos G, Lo M, Costa Reis P *et al.* (2016):** New insights into the immune pathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol., 2(12):716–30.
5. **Tsantikos E, Quilici C, Harder K *et al.* (2009):** Perturbation of the CD4 T cell compartment and expansion of regulatory T cells in autoimmune-prone Lyn-deficient mice. J Immunol., 183(4):2484–94.
6. **Catalina M, Owen K, Labonte A *et al.* (2020):** The pathogenesis of systemic lupus erythematosus: harnessing big data to understand the molecular basis of lupus. Journal of Autoimmunity, 110: 102359. doi: 10.1016/j.jaut.2019.102359.
7. **Griffiths B, Mosca M, Gordon C (2005):** Assessment of patients with systemic lupus erythematosus and the use of lupus disease activity indices. Best Pract Res Clin Rheumatol., 19:685–708.
8. **Okeke E, Uzonna J (2019):** The pivotal role of regulatory T cells in the regulation of innate immune cells. Frontiers in Immunology, 10: 680. doi: 10.3389/fimmu.2019.00680
9. **Kanne J, Yandow D, Haemel A *et al.* (2011):** Beyond skin deep: thoracic manifestations of systemic disorders affecting the skin. Radiographics, 31: 1651–68.
10. **You T, Lin X, Zhang C *et al.* (2022):** Correlation between serum β2-microglobulin level and systemic lupus erythematosus disease activity. Medicine (Baltimore), 101(39): e30594. doi: 10.1097/MD.0000000000030594
11. **Kim H, Jeon J, Yoon J *et al.* (2010):** Beta2-microglobulin can be a disease activity marker in systemic lupus erythematosus. Am J Med Sci., 339(4):337-40.
12. **Jesus D, Matos A, Henriques C *et al.* (2019):** Derivation and validation of the SLE disease activity score (SLE-DAS): a new SLE continuous measure with high sensitivity for changes in disease activity. Annals of the Rheumatic Diseases, 78(3): 365-371.
13. **Sawda T, Fujimori D, Yamamoto Y (2019):** Systemic lupus erythematosus and immunodeficiency. Immunological Medicine, 42:11-9.
14. **Calabresi E, Zucchi D, Eiefante E (2019):** One year in review: systemic lupus erythematosus. Clin Exp Rheumatol., 37(5):715-22.
15. **Mok C (2018):** Systemic lupus erythematosus: what should family physicians know? Hong Kong Medical Journal, 24(5):501–51.
16. **Vaillant A, Goyal A, Varacallo M (2022):** Systemic lupus erythematous. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK535405/
17. **Solhjoo M, Goyal A, Chauhan K (2022):** Drug-induced Lupus Erythematosus. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK441889/
18. **Gu M, Cheng Q, Wang X *et al.* (2019):** The impact of SLE on health-related quality of life assessed with SF-36: a systemic review and meta-analysis. Lupus, 28(3):371-82.
19. **LeMinh G, Peshkova A, Andrianova I *et al.* (2018):** Impaired contraction of blood clots as a novel prothrombotic mechanism in systemic lupus erythematosus. Clinical Science, 132(2): 243-254.
20. **Żychowska I, Suszek D, Dryglewska M *et al.* (2018):** β2-microglobulin as a marker of systemic lupus erythematosus. activity. Adv Clin Exp Med., 27(3): 379-82.
21. **Choe J, Park S, Kim S (2018):** Urine β2-microglobulin is associated with clinical disease activity and renal involvement in female patients with systemic lupus erythematosus. LUPUS., 23: 1486-93.
22. **Tilstra J, Avery L, Menk A *et al.* (2018):** Kidney-infiltrating T cells in murine lupus nephritis are metabolically and functionally exhausted. The Journal of Clinical Investigation, 128(11): 4884-4897.
23. **Skare T, Ferri K, Santos M (2014):** Systemic lupus erythematosus activity and beta two microglobulin levels. Sao Paulo Med J., 132:239–42.
24. **Aghdashi M, Salami S, Nezhadisalami A (2019):** Evaluation of the serum β2-Microglobulin level in patients with systemic lupus erythematosus and its correlation with disease activity. Biomedicine (Taipei), 9(3): 16-21.
25. **Hermansen M, Hummelshøj L, Lundsgaard D *et al.* (2012):** Increased serum β2-microglobulin is associated with clinical and immunological markers of disease activity in systemic lupus erythematosus patients. Lupus, 21(10):1098-104.
26. **Wakabayashi K, Inokuma S, Matsubara E *et al.* (2013):** Serum β2-microglobulin level is a useful indicator of disease activity and hemophagocytic syndrome complication in systemic lupus erythematosus and adult-onset Still's disease. Clin Rheumatol., 32(7): 999-1005.
27. **Rist P, Jiménez M, Rexrode K (2017):** Neurology Academy prospective association between B2M and ischemic stroke among women. Neurology, 88(23): 2176-2182.
28. **Firooz N, Albert D, Wallace D *et al.* (2011):** High-sensitivity C-reactive protein and erythrocyte sedimentation rate in systemic lupus erythematosus. Lupus, 20(6): 588–97.
29. **Gupta K, Muthukumar T, Dotia A (2003):** A study of tubular dysfunction in Indian patients with lupus nephritis. Hong Kong J Nephrol., 5(2): 90–97.
30. **Puchades R, García-Polo I, Suárez C (2012):** A study on the relationship between serum beta 2-microglobulin levels, underlying chronic kidney disease, and peripheral arterial disease in high-vascular-risk patients. Int Cardiovasc Res J., 6(4):107-12.