ONLINE JOURNAL OF BIOLOGICAL SCIENCES

RESEARCH ARTICLE

suPAR as an Inflammatory and Preclinical Atherosclerotic Marker in Hypovitaminosis D

Shravan Ajith Panchwadkar, Sudha Kuthethur *, Neelam Manjunath Pawar, Sowndarya Kollampare, Reshma Kumarchandra

Department of Biochemistry, Kasturba Medical College Mangalore, Manipal Academy of Higher Education, Manipal, India

*Corresponding Author: sudha.k@manipal.edu

Abstract: The mechanistic link between inflammation and atherosclerosis highlights the critical role of immune modulation in cardiovascular disease. The soluble urokinase plasminogen activator receptor (suPAR), an immune-derived factor, has recently emerged as a non-specific inflammatory marker across chronic diseases and infections. All cells within the atherosclerotic arterial wall can express uPAR and release urokinase, which plays a key role in the etiology of atherosclerosis. Hypovitaminosis D and dyslipidemia are well-recognized risk factors for atherosclerosis; however, the relationship between plasma vitamin D levels and suPAR in apparently healthy individuals has not yet been fully characterized. This study aimed to explore the utility of suPAR as a potential marker of preclinical atherosclerosis and systemic inflammation in the context of hypovitaminosis D. Ninety apparently healthy individuals aged 25–55 years were categorized into three groups (n = 30 each) based on plasma vitamin D levels: sufficient, insufficient, and deficient. The deficient group was further subdivided into moderately and severely deficient subgroups. Lipid profile was assessed spectrophotometrically, C-reactive protein (CRP) and suPAR by enzyme-linked immunosorbent assay (ELISA), and vitamin D by electrochemiluminescence immunoassay (ECLIA). The neutrophil-to-lymphocyte ratio (NLR) was derived from complete blood count data. suPAR levels were significantly elevated in the vitamin D-deficient group compared to the insufficient and sufficient groups ($p < 0.01$ and $p < 0.001$, respectively). Severe vitamin D deficiency was associated with the highest suPAR levels and significant elevations in atherogenic indices, including TC/HDL-C and non-HDL cholesterol. A progressive and significant rise in inflammatory biomarkers, CRP, NLR, and suPAR, was observed across the spectrum from vitamin D sufficiency to deficiency. In conclusion, elevated suPAR levels may serve as an early indicator of vascular inflammation and preclinical atherogenesis in individuals with vitamin D deficiency, supporting its potential utility as a biomarker for subclinical cardiovascular risk stratification.

Keywords: suPAR, Hypovitaminosis D, Preclinical atherosclerosis, Vascular inflammation, Atherogenic index, C-reactive protein, NLR, Cardiovascular risk

Received: 10-06-2025 | **Revised:** 11-08-2025 | **Accepted:** 15-09-2025 | **DOI:** 10.3844/ojbsci.2026.26.01.014

Introduction

Hypovitaminosis D and dyslipidemia are the purported risk factors of atherosclerosis and cardiovascular diseases (CVD) [1]. suPAR is the soluble form of the urokinase plasminogen activator receptor (uPAR), expressed on membranes of various

cells like activated T lymphocytes, macrophages, and endothelial cells. suPAR acts as plasminogen activator and a key regulator of fibrinolysis, being released through proteolytic cleavage of uPAR under inflammatory conditions [2]. Urokinase (uPA) converts plasminogen to plasmin which initiates thrombolytic cascade within the arterial wall. All cell types found in the atherosclerotic artery wall can express surface uPAR and release uPA, thereby contributing to the initiation and advancement of atherosclerotic changes. Accumulating evidence indicates the contribution of urokinase /uPAR pathway in the pathogenesis of atherosclerosis [3]. Systemic inflammation is central to the development of CVD, and elevated suPAR levels have been shown to regulate monocyte activity, promote vascular inflammation, and accelerate atherogenesis. Increased suPAR expression is demonstrated inside the atherosclerotic plaques, where it amplifies the production of inflammatory cytokines [4]. Hence, suPAR may be perceived as an indicator of persistent systemic inflammation in CVD. Vitamin D receptors and 1α-hydroxylase, key enzyme for calcitriol synthesis, are expressed in vascular cells, and their knockout in mice leads to hypertension [5]. Although vitamin D modulates immune reactions and inflammatory pathways, suPAR represents a stable marker of sustained immune activation and low-grade systemic inflammation, particularly elevated in metabolic disorders and chronic infections. Despite these observations, the interrelationship between plasma vitamin D and suPAR in general population remains poorly understood. Therefore, the current study aims to evaluate suPAR as a potential inflammatory biomarker of preclinical atherosclerosis in vitamin D-deficient individuals who are predisposed to atherosclerotic risk.

Materials and Methods

This observational study was conducted after obtaining approval from the Institutional Ethics Committee. Informed consent was taken from all participants prior to enrolment. The study cohort comprised ninety apparently healthy males and females, aged 25 to 55 years. On the basis of plasma vitamin D values, the participants were categorized into three groups of 30 subjects each as vitamin D-sufficient (>30 ng/mL - Group I), vitamin D-insufficient (21-29 ng/mL- Group II), and vitamin D-deficient (≤20 ng/mL -Group III). The vitamin D-deficient group was further subdivided into two subgroups of 15 subjects each as: Group IIIa (moderately deficient) and Group IIIb (severely deficient) with vitamin D ranging between10-20 ng/mL and <10 ng/mL respectively [6]. The exclusion criteria included participants with a history of kidney disease, diabetes mellitus, active infections, or current use of vitamin supplements.

Blood samples collected after overnight fast into heparin vacuum tube were centrifuged at 3000g for ten minutes. The plasma separated was aliquoted and stored at -80℃. Lipid Profile was assessed using spectrophotometric methods [7]. Vitamin D was measured by ECLIA [8]. Atherogenic indices such as total cholesterol/HDL and non-HDL cholesterol were calculated. Sandwich ELISA was used to estimate plasma C reactive protein (CRP) and suPAR [9, 10]. From complete blood count neutrophil lymphocyte ratio (NLR) was calculated.

Data was analyzed using SPSS version 29 (IBM Corp., Armonk, NY, USA). One-way ANOVA was used to compare the groups, followed by post hoc Tukey's test for intergroup analysis. A p-value of <0.05 was considered statistically significant.

Results

Table 1 illustrates the baseline characteristics and demographic details of the study population. Serum suPAR values showed a progressive increase across vitamin D categories- from sufficient to insufficient to deficient groups (1.99 → 2.14 → 2.24 → 2.43 ng/mL). Table 2 indicates that suPAR concentrations were markedly elevated in the vitamin D deficiency compared to both insufficiency ($p < 0.01$) and sufficiency ($p < 0.001$).

Moreover, severely deficient subgroup showed a marked surge in suPAR levels when compared with moderately deficient subgroup ($p < 0.01$) as displayed in Table 3. Triglyceride levels exhibited an apparent increase in insufficient and deficient groups compared with sufficient. Atherogenic lipids, including VLDL and LDL, followed a similar pattern. Though plasma total cholesterol did not alter among the groups, HDL levels were lowest in the vitamin D-deficient group and showed a further decline in those with severe deficiency (Table 3). Mean HDL levels dropped from 61 mg/dl in vitamin D sufficient group to 48 mg/dl in severely deficient group, with a fall of ~15 mg/dl between the moderately and severely deficient strata.

However, atherogenic indices reflected a reverse trend. The TC/HDL ratio showed a significant rise in the vitamin D-insufficient group ($p < 0.01$), whereas non-HDL cholesterol levels were significantly higher in the vitamin D-deficient group ($p < 0.05$) compared with the sufficient group. TC/HDL ratio along with non-HDL cholesterol were elevated markedly in the

severely deficient subgroup relative to the moderately deficient group, paralleling the rise in suPAR levels ($p = 0.004$ and $p < 0.05$, respectively) (Table 3). However, TG/HDL showed no significant change between the groups.

A pronounced rise in plasma suPAR and CRP levels was noted in the vitamin D-deficiency ($p = 0.04$) compared with the sufficiency status, with further elevation in the severely deficient subgroup ($p = 0.05$) (Table 2). Although NLR did not differ significantly between Groups IIIA and IIIB, it demonstrated an upward trend along with suPAR (Table 3). As illustrated in Figure 1, vitamin D levels correlated negatively with suPAR which was statistically significant ($r = - 0.384$ and $p < 0.001$).

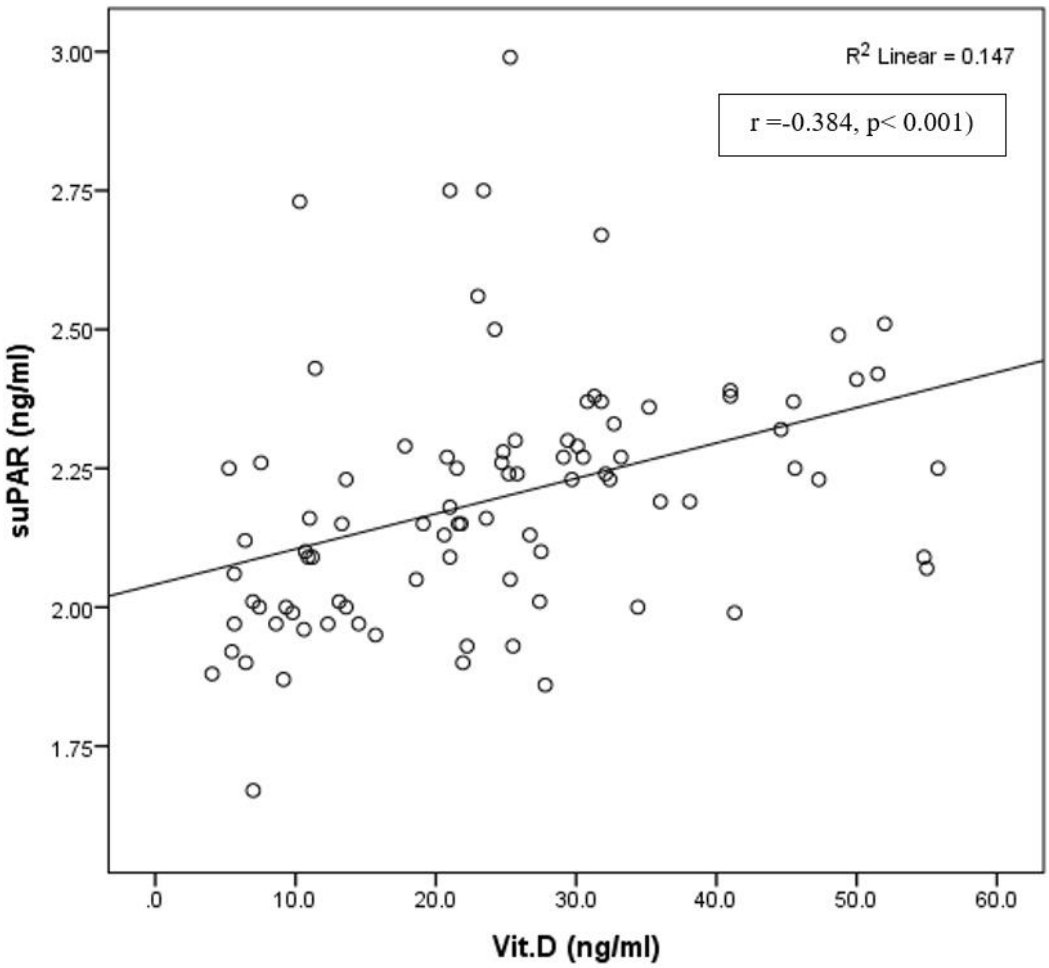

Fig. 1: Correlation between vitamin D and suPAR

Table 1: Demographic and baseline data of the subjects (Mean ± SEM)

	Group I Sufficient group	Group II Insufficient group	Group IIIA Deficient group	Group IIIB Severely Deficient	p value
Age (range) (years)	28-54	26-54	25-50	25-54	NS
Female: Male	17:13	16:14	6:9	8:7	NS
Plasma Vitamin D (ng/ml)	40.53±8.72	24.04 ± 3.36	13.4 ± 0.7	7.0 ± 0.4	0.001
Plasma Creatinine (mg/dl)	0.89 ± 0.14	0.95 ± 0.13	1.13 ± 0.04	1.06 ± 0.05	NS
BMI	23± 1.3	24.9± 1.0	25.2± 1.0	25± 2.1	NS

NS - Not Significant
N = Number of subjects

Table 2: Comparison of atherogenic and inflammatory markers among the 3 groups (Mean ± SEM)

	Group I (Sufficient group) N=30	Group II (Insufficient group) N=30	Group III (Deficient group) N=30
Atherogenic Markers			
Vit.D (ng/ml)	40.53 ± 8.72	24.04 ± 3.36[b]	10.29 ± 4.03[a]
suPAR (ng/ml)	1.99 ± 0.03	2.14 ± 0.05	2.30 ± 0.03 [a ,b]
TG (mg/dl)	113 ± 7	128 ± 12	133 ± 12
TC (mg/dl)	195 ± 9	183 ± 8	192 ± 11
HDL (mg/dl)	61±5	64 ± 3	54±4
TC/HDL	3.09 ± 0.30	3.99 ±0.20[c]	3.59 ± 0.26
TG/HDL	2.82± 1.8	1.92 ± 0.89	2.65± 1.87
LDL (mg/dl)	108 ± 10	124 ± 7	123 ± 10
VLDL (mg/dl)	23 ± 1.38	28 ± 3.24	30 ± 4.22
Non-HDL (mg/dl)	119 ± 7.80	131 ± 11.08	141 ± 9.80[d]
Inflammatory Markers			
CRP	1.54 ± 0.02	3.31 ± 0.03	4.72 ± 0.03[c]
NLR	1.71±0.11	2.29 ± 0.48	2.28 ± 0.29

a - p<0.001 Significantly different from sufficient group
b - p<0.01 Significantly different from insufficient group
c - p<0.04 Significantly different from sufficient group
d - p< 0.05 Significantly different from sufficient group

Table 3: Comparison of atherogenic and inflammatory markers within Vitamin D deficient group III (IIIA and IIIB)

	Group IIIA (Deficient group) Vit D (10-20 ng/ml) N=16	Group IIIB (severely deficient) Vit D (<10 ng/ml) N=14	p value
Atherogenic Markers			
Vit.D (ng/ml)	13.37 ± 0.73	6.97 ± 0.42	**<0.001**
suPAR (ng/ml)	2.24±0.04	2.43 ± 0.05	**0.037**
TG (mg/dl)	125 ± 21	141 ± 13	NS
TC (mg/dl)	184 ± 13	201 ± 19	NS
HDL (mg/dl)	62 ± 8	48 ± 4	**0.01**
TC/HDL	2.90 ±0.32	4.34 ± 0.33	0.004
TG/HDL	2.03± 1.8	3.3 ±1.77	NS
LDL (mg/dl)	110 ± 13	134 ± 15	NS
VLDL (mg/dl)	31 ± 8	28 ± 3	NS
Non HDL (mg/dl)	110 ± 14	153 ± 16	**0.05**
Inflammatory Markers			
CRP	3.86±0.03	5.58±0.04	<0.05
NLR	2.50±0.60	2.16±0.32	NS

N= Number of subjects
NS=Not significant

Discussion

Several laboratory and epidemiological studies have linked vitamin D insufficiency to elevated atherogenic lipids which are the established indicators of early CVD [1]. Current study undertaken on apparently healthy individuals also supports the earlier reports. Vitamin D deficiency may lead to subclinical atherosclerosis in healthy individuals and is a modifiable risk factor of CVD [3]. In a study done on ethnic cohort without known cardiovascular disease, elevated suPAR values strongly correlated with accelerated atherosclerosis and increased incidence of CVD [1]. This finding suggests that, even within the general population, individuals with higher suPAR concentrations are at a higher risk of developing cardiovascular events. The results of our study justify the atherogenic role of suPAR in vitamin D insufficient and deficient groups. In the current study, increased suPAR levels were associated with higher atherogenic lipids like TG, LDL and atherogenic indices like TC/HDL, non-HDL cholesterol, and with lower HDL, especially in vitamin D deficient groups. Elevation of triglycerides and reduction in HDL begins well before the increase in LDL, hence can be considered as the earliest markers of atherosclerosis [11]. Furthermore, non-HDL cholesterol a predictive marker of persistent dyslipidemia was notably high in vitamin D deficient groups where suPAR values were maximum.

In peripheral artery disease, suPAR turns out to be a nonspecific inflammatory marker of prognostic and predictive utility [12]. Upregulation of suPAR in a rodent model of atherosclerosis n increased the plaque size [13] (Drueke TB et al., 2023). Activation of uPAR by soluble form of LDL receptor stimulated lipid uptake by macrophage transforming it to foam cell [14]. Moreover, patients with unstable angina exhibited increased uPAR expression [15].

Elevated suPAR levels contribute to renal inflammation and supresses the activation of vitamin D to calcitriol. Conversely, vitamin D therapy prevents glomerular microthrombus formation and protects renal cells by reducing inflammation and suppressing both local and systemic suPAR production [16]. Additionally, vitamin D treatment downregulated uPAR expression in podocytes in a murine model of chronic kidney disease [17]. These findings justify the inverse association between suPAR and vitamin D observed in this study.

Therefore, it may be hypothesized that hypovitaminosis D may enhance activation of monocytes, macrophages, and T cells, leading to upregulation of uPAR and increased suPAR shedding into the circulation. Therefore, vitamin D deficiency may indirectly drive suPAR elevation via chronic immune stimulation.

Calcitriol supresses vascular inflammatory processes that precede atherosclerosis by inducing the formation of anti-inflammatory molecules and inhibits foam cell formation [18]. Further, in sites of inflammation, macrophages overexpress 1alpha hydroxylase and lead to unregulated activation of vitamin D to calcitriol. suPAR, a stable marker of immune activation, correlates positively with CRP in several infectious diseases [19] and acts as a predictor of deterioration in various inflammatory conditions [20]. This aligns with the findings of our study, where elevated CRP and suPAR levels were observed in subjects with vitamin D insufficiency and deficiency. Compared to CRP, suPAR demonstrates greater in vivo stability, making it a more reliable marker of subclinical immune activation [21]. uPAR is widely expressed on leukocytes involved in inflammatory pathogenesis [22]. In this study, NLR a gold standard inflammatory marker of metabolic diseases was elevated along with suPAR in vitamin D-deficient and insufficient subjects. NLR has been reported as a strong predictor of vitamin D insufficiency and tends to rise in hypovitaminosis D [23, 24]. Vitamin D also suppresses pro-atherogenic T lymphocyte activity [25]. The observed positive association between suPAR and NLR supports suPAR's potential role as a stable marker of low-grade inflammation, reflecting immune activation even in the absence of overt disease.

Conclusion

Overall, it can be concluded that elevated suPAR may be considered as a good marker of preclinical atherogenesis in vitamin D deficiency. suPAR could be a promising candidate that represents initial inflammatory process that precede atherosclerosis.

Our findings warrant prospective longitudinal studies with adequately powered cohorts to elucidate the mechanisms by which vitamin D regulates suPAR levels in atherogenesis.

Acknowledgment

We are grateful to the participants, institutional authorities and associated hospitals, for their support and cooperation in conducting this research.

Author's Contributions

Sudha Kuthethur designed the study, while Shravan Ajith Panchwadkar obtained the ethics approval and conducted the experiments. Sudha Kuthethur drafted the manuscript; Neelam Manjunath Pawar performed the statistical analysis. Reshma Kumarchandra and Sowndarya Kollampare were involved in manuscript review and approval of the final version.

Ethics

Approval for the study was granted by the Institutional Ethics Committee - (IEC- KMCMLR-08/2023/354).

References

1. Haider F, Ghafoor H, Hassan OF, Farooqui K, Bel Khair AOM, Shoaib F (2023). Vitamin D and Cardiovascular Diseases: An Update. *Cureus*,15(11), e49734. DOI: 10.7759/cureus.49734.
2. Benucci M, Damiani A, Russo E, Guiducci S, Li Gobbi F, Fusi P, et al (2022). The Association of uPA, uPAR, and suPAR System with Inflammation and Joint Damage in Rheumatoid Arthritis: suPAR as a Biomarker in the Light of a Personalized Medicine Perspective. *J Pers Med*, 12(12):1984. DOI: 10.3390/jpm12121984.
3. Goodchild TT, Li Z, Lefer DJ (2022). Soluble urokinase plasminogen activator receptor: from biomarker to active participant in atherosclerosis and cardiovascular disease. *J Clin Invest*, 132(24):e165868. DOI:10.1172/JCI165868.
4. Hindy G, Tyrrell DJ, Vasbinder A, Wei C, Presswalla F, Wang H, et al (2022). Increased soluble urokinase plasminogen activator levels modulate monocyte function to promote atherosclerosis. *Journal of Clinical Investigation*, 132(24):1-14. DOI: 10.1172/jci158788.
5. Su H, Liu N, Zhang Y, Kong J (2021). Vitamin D/VDR regulates peripheral energy homeostasis via central renin-angiotensin system. *J Adv Res*, 33:69-80. DOI: 10.1016/j.jare.2021.01.011.
6. Gani L, How C (2015). Vitamin D deficiency. *Singapore Medical Journal*, 56(08): 433-437. doi.org/10.11622/smedj.2015119.
7. Kim MR, Jeong SJ (2019). Relationship between Vitamin D Level and Lipid Profile in Non-Obese Children. *Metabolites*, 9(7):125. DOI: 10.3390/metabo9070125.
8. Shin HR, Lee YJ, Ly SY (2023). Optimal Serum 25(OH)D Levels and Vitamin D Intake in Korean Postmenopausal Women. *Nutrients*, 15(8):1856. DOI: 10.3390/nu15081856.
9. Kumar P, Kakar A, Gogia A, Waziri N (2019). Evaluation of soluble urokinase-type plasminogen activator receptor (suPAR) quick test for triage in the emergency department. *Journal of family medicine and primary care*, 8(12): 3871-3875. DOI: 10.4103/jfmpc.jfmpc_116_19.
10. Ghule A, Kamble TK, Talwar D, Kumar S, Acharya S, Wanjari A, et al (2021). Association of Serum High Sensitivity C-Reactive Protein With Pre-diabetes in Rural Population: A Two-Year Cross-Sectional Study. Cureus,13(10):e19088. DOI: 10.7759/cureus.19088.
11. Zhang BH, Yin F, Qiao YN, Guo SD (2022). Triglyceride and Triglyceride-Rich Lipoproteins in Atherosclerosis. *Front Mol Biosci*, 9:909151. DOI: 10.3389/fmolb.2022.909151.
12. Rotbain Curovic V, Theilade S, Winther SA, Tofte N, Eugen-Olsen J, Persson F, et al (2019). Soluble Urokinase Plasminogen Activator Receptor Predicts Cardiovascular Events, Kidney Function Decline, and Mortality in Patients With Type 1 Diabetes. *Diabetes Care*, 42(6):1112-1119. DOI: 10.2337/dc18-1427.
13. Drueke TB, Massy ZA (2023). Soluble urokinase plasminogen activator receptor (suPAR) promotes atherosclerosis. *Kidney Int*, 103(3):451-454. DOI: 10.1016/j.kint.2022.12.006.

14. Fuhrman B (2012). The urokinase system in the pathogenesis of atherosclerosis. *Atherosclerosis*, 222(1):8-14. DOI: 10.1016/j.atherosclerosis.2011.10.044.
15. Reisinger AC, Niedrist T, Posch F, Hatzl S, Hackl G, Prattes J,et al (2021). Soluble urokinase plasminogen activator receptor (suPAR) predicts critical illness and kidney failure in patients admitted to the intensive care unit. *Sci Rep*. 11(1):17476. DOI: 10.1038/s41598-021-96352-1.
16. Liao TH, Wu HC, Liao MT, Hu WC, Tsai KW, Lin CC, et al (2022). The Perspective of Vitamin D on suPAR-Related AKI in COVID-19. *Int J Mol Sci* , 23(18):10725. DOI: 10.3390/ijms231810725.
17. Sudhini YR, Wei C, Reiser J (2022). suPAR: An Inflammatory Mediator for Kidneys. *Kidney Dis (Basel)*, 8(4):265-274. DOI: 10.1159/000524965.
18. Pop TL, Sîrbe C, Benţa G, Mititelu A, Grama A (2022). The Role of Vitamin D and Vitamin D Binding Protein in Chronic Liver Diseases. Int J Mol Sci. 23(18):10705. DOI:10.3390/ijms231810705.
19. Enocsson H, Idoff C, Gustafsson A, et al (2021). Soluble Urokinase Plasminogen Activator Receptor (suPAR) Independently Predicts Severity and Length of Hospitalisation in Patients With COVID-19. Front Med (Lausanne), 8:791716. DOI:10.3389/fmed.2021.791716.
20. Rasmussen LJH, Petersen JEV, Eugen-Olsen J (2021). Soluble Urokinase Plasminogen Activator Receptor (suPAR) as a Biomarker of Systemic Chronic Inflammation. Front Immunol, 12:780641. DOI: 10.3389/fimmu.2021.780641.
21. Özdirik B, Maibier M, Scherf M, Nicklaus JM, Frohme J, Puengel T, (2022). Soluble Urokinase Plasminogen Activator Receptor Levels Are Associated with Severity of Fibrosis in Patients with Primary Sclerosing Cholangitis. J Clin Med,11(9):2479. DOI: 10.3390/jcm11092479.
22. Hamada M, Varkoly KS, Riyadh O, Beladi R, Munuswamy-Ramanujam G, Rawls A, et al (2024). Urokinase-Type Plasminogen Activator Receptor (uPAR) in Inflammation and Disease: A Unique Inflammatory Pathway Activator. Biomedicines. 12(6):1167. DOI:10.3390/biomedicines12061167.
23. Wang SY, Shen TT, Xi BL, Shen Z, Zhang X (2021). Vitamin D affects the neutrophil-to-lymphocyte ratio in patients with type 2 diabetes mellitus. J Diabetes Investig, 12(2):254-265. DOI: 10.1111/jdi.13338.
24. Okuyan O, Dumur S, Elgormus N, Uzun H (2024). The Relationship between Vitamin D, Inflammatory Markers, and Insulin Resistance in Children. Nutrients, 16(17):3005. DOI: 10.3390/nu16173005.
25. Alissa EM (2024). Vitamin D and cardiovascular diseases: A narrative review. J Family Med Prim Care,13(4):1191-1199. DOI: 10.4103/jfmpc.jfmpc_1481_23.