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Evaluation of Vascular Cell Adhesion Molecule-1 (VCAM-1) as a Marker of Microvascular Dysfunction in Patients with Diabetic Nephropathy

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ABSTRACT

Background: Diabetic nephropathy is a clinical syndrome characterized by a set of structural and functional kidney abnormalities in patients with diabetes mellitus and is the leading cause of end stage renal failure.

Objective: This study aimed to evaluate VCAM-1 as a marker of microvascular dysfunction in patients with diabetic nephropathy. **Methods:** The present study was conducted through the duration between December 2022 and July 2023. The patients were selected from Diabetes and the Nephrology Outpatient Clinics and inpatients of the Internal Medicine Department, at Menoufia University Hospitals. This study included 36 diabetic patients divided into 3 groups according to urinary albumin excretion: Group 1 included 15 diabetic patients with normoalbuminuria, group 2 included 11 diabetic patients with microalbuminuria and group 3 included 10 diabetic patients with macroalbuminuria. In addition to fourteen apparent healthy subjects as a control group.

Results: The mean VCAM-1 levels were higher in diabetics compared to controls. There was a highly significant difference between the three diabetic subgroups as regards serum VCAM-l. There was a highly positive correlation between VCAM-1 and serum creatinine. Also, there was a highly significant positive correlation between VCAM-1and urinary albumin excretion in whole diabetic patients as well as patients with diabetic nephropathy. Receiver operating characteristic (Roc) graph revealed that the best cut off of serum VCAM-1 to differentiate between diabetics and control subjects was 646.5ng/ml as the diagnostic sensitivity was 80.6%, specificity was 64.3%, and diagnostic accuracy was 76%. The area under the ROC curve was 0.789. The best cut off of serum VCAM-1to detect diabetic nephropathy was 792.5 ng/ml as the diagnostic sensitivity was 100 %, specificity was 80%, and diagnostic accuracy was 91.7%. The area under the Roc curve was 0.917.

Conclusion: VCAM-1 could potentially serve as a marker for diabetic nephropathy.

Keywords: Vascular cell adhesion molecule-1 (VCAM-1), Microvascular dysfunction, Diabetic nephropathy.

INTRODUCTION

Diabetes mellitus, a collection of metabolic disorders, is distinguished by its persistent hyperglycemia. These disorders are the result of impairments in insulin secretion, insulin efficacy, or a combination of both. Progressive and irreversible injury, malfunction, and eventual failure of the eyes, kidneys, peripheral nerves, cardiovascular system, and vasculature can result from prolonged elevations in blood glucose levels in diabetes [1]. According to WHO statistics, diabetics around the world were estimated to be 171 million in the year 2000 and are expected to reach 366 million by 2030. In Egypt, it is estimated as 2.623,000 by the year of 2000 and 6,726,000 by the year of 2030 [2]. Diabetic nephropathy represents a clinical condition typified by an array of both structural and functional renal impairments in individuals with prolonged diabetes, and it stands as the primary contributor to end-stage renal disease [3]. A hallmark of diabetes is endothelial dysfunction, which is frequently accompanied by inflammation and elevated levels of soluble adhesion molecules in the bloodstream, notably serum vascular cell adhesion molecule [4].

VCAM-1 is a transmembrane glycoprotein classified within the immunoglobulin gene superfamily. Endothelial cells are the primary site of its expression in response to pro-inflammatory cytokines, including IL-1 and TNF. Beyond endothelial cells, VCAM-1 can also be expressed by bone marrow fibroblasts, tissue-resident macrophages, dendritic cells, and myeloblasts [5]. This immune-associated membrane protein facilitates the adhesion of leukocytes to endothelial cells and is implicated in signal transduction processes. It is believed to be crucial in modulating inflammatory and immune responses [6]. VCAM-1 also exists in a soluble form detectable within the circulatory system, making its plasma concentration a potential marker of endothelial activation, injury, or cellular turnover [7]. Recent accumulating evidence suggests that elevated plasma levels of VCAM-1 might not only serve as a diagnostic marker for diabetic microangiopathy but could also actively contribute to its underlying pathophysiological mechanisms [8].

This investigation was designed to evaluate the utility of VCAM-1 as an indicator of microvascular dysfunction in individuals diagnosed with diabetic nephropathy.

PATIENTS AND METHODS

This investigation was conducted at the Clinical Pathology Department, Faculty of Medicine, Menoufia University, from December 2022 to July 2023. The patients were selected from the Diabetes and Nephrology Outpatient Clinics, as well as the inpatients of the

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Internal Medicine Department, Menoufia University Hospitals.

Subjects: The study included 36 patients with type 1 and type 2 diabetes mellitus, with a mean age of 49.89 ± 11.0 years (range 18-68). The cohort consisted of 21 males and 15 females. Additionally, 14 apparently healthy subjects, matched for gender, age, and BMI, served as the control group. The control group had a mean age of 44.21 ± 7.37 years (range 32-57), with an equal gender distribution of 7 males and 7 females.

The study subjects were categorized into four groups based on urinary albumin excretion (UAE):

- **Group I:** 15 diabetic patients without micro- or macro-albuminuria (7 males and 8 females with mean age of 48 ± 6.79 years and range of 32-60).
- **Group II:** 11 diabetic patients with micro-albuminuria (7 males and 4 females, mean age 51.82 ± 17.41 years, range 18-68).
- **Group III:** 10 diabetic patients with macro-albuminuria (7 males and 3 females with mean age of 50.6 ± 7.17 years and range of 39-65).
- **Group IV:** 14 apparently healthy subjects matched by age to the previous three groups as a control group.

For all individuals, the following assessments were performed:

1. History and clinical examination.
    - Laboratory investigations that included 2-hour postprandial blood glucose, glycated hemoglobin (HbA1c) level, renal function tests (serum urea and serum creatinine), Micro-albumin levels and serum vascular cell adhesion molecule-1 (sVCAM-1).

II. Analytical Methods: Biochemical tests for detecting blood glucose, serum urea, and serum and urinary creatinine were performed using the Synchron Cx 5 autoanalyzer with kits supplied by Beckman. Micro-albumin was measured with the nephlometric ARRAY 360 system, also using Beckman kits. Glycated hemoglobin was measured by column chromatography (Stanbio Laboratory, Inc.), and sVCAM-1 was measured using an ELISA test.

1. **Determination of blood glucose (2-Hour Postprandial):** Blood glucose levels were measured two hours after meals.
2. **Estimation of glycated hemoglobin (HbA1c):** Glycated hemoglobin levels were measured to assess long-term glucose control.
3. **Estimation of serum urea:** Serum urea levels were determined as part of renal function assessment.
4. **Estimation of creatinine in serum and urine:** Both serum and urinary creatinine levels were measured to evaluate kidney function.
5. **Estimation of micro-albumin in urine:** Micro-albumin levels were determined to assess kidney damage.
6. **VCAM-1 Assay:** The sVCAM-1 levels were measured using the solid-phase sandwich ELISA technique. A monoclonal antibody specific to human VCAM-1 was pre-coated onto microplate wells, where standards, controls, and test specimens were added. A biotinylated secondary antibody was introduced, and a streptavidin-peroxidase enzyme was bound to form a sandwich complex. After incubation, a colorimetric signal was generated using a substrate solution, and the absorbance was measured at 450 nm.

Procedure:

1. Samples were diluted 1:50 with standard diluent buffer.
2. 100 µL of the standard diluent was added to the blank well.
3. 100 µL of standards, samples, and controls were added to the appropriate wells.
4. 50 µL of biotinylated anti-VCAM-1 solution was added to all wells and incubated at 37°C.
5. Wells were washed five times with working wash buffer.
6. 100 µL of streptavidin-HRP working solution was added and incubated at room temperature for 30 minutes.
7. Wells were washed four times to remove unbound substances.
8. 100 µL of stabilized chromogen was added and incubated for 30 minutes at room temperature in the dark.
9. 100 µL of stop solution was added.
10. Absorbance was read at 450 nm within 2 hours, and results were calculated accordingly.

Ethical considerations: The study was done after being accepted by The Research Ethics Committee, Menoufia University. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. 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 analysis

The data were compiled, organized into tables, and subjected to statistical analysis using a personal computer and the SPSS software package, version 10, for comprehensive evaluation (IBM, Armonk, New York, United States). Statistics were carried out in two distinct categories: Descriptive statistics, including percentage (%), mean (X), and standard deviation (SD), and analytic statistics. The analytic methods included the Chi-square test (X^2), used to study associations between two qualitative variables. The Mann-Whitney test, a non-parametric test for comparing two groups with quantitative variables that are not normally distributed. The one-way analysis of variance (ANOVA), which compares the quantitative variables of three or more

normally distributed groups, followed by the LSD (least significant difference) post-hoc test for pair-wise comparisons. The Student's t-test was employed to determine the significance between two normally distributed quantitative variables. The Kruskall-Wallis test, a non-parametric method, was used to compare three or more non-normally distributed groups with quantitative variables. Spearman's correlation coefficient (r) was utilized to quantify the correlation between two variables, with values interpreted as follows: $r < 0.3$ indicates no correlation, $r = 0.3$-0.5 represents mild correlation, $r = 0.5$-0.7 signifies moderate correlation, and $r > 0.7$ suggests strong correlation. The level of significance was set at a p-value ≤ 0.05. Diagnostic measures were also assessed, including diagnostic sensitivity, which refers to the percentage of patients diagnosed as positive for a disease, combining true positive (TP) and false negative (FN) results, diagnostic specificity representing the proportion of disease-free patients reported as negative and diagnostic efficacy, which reflects the total number of diseased and healthy individuals. Additional parameters included positive predictive value (PPV), which reflects the percentage of patients who test positive and have the disease, and negative predictive value (NPV), which represents the percentage of patients who test negative but have the disease. Finally, the receiver operator characteristic (ROC) curve was utilized to visualize the relationship between sensitivity and specificity across various diagnostic cut-off points.

RESULTS

In whole diabetic patients, serum VCAM-1 level ranged from 397 to 3435 ng/mL with a mean of 1121.25 ± 670.11 ng/mL and in controls the level ranged from 465.5 to 807 ng/mL with a mean of 630.71 ± 127.28 ng/mL. Comparing serum VCAM-1 level between 2 groups, it was found that there was highly significant difference between 2 groups as regards serum VCAM-1 as p value < 0.001 (Table 1).

Table (1): Comparison between diabetic patients and controlsregarding VCAM-1

	Diabetic patients (n=36)	Controls (n=14)	U	P. value
VCAM-1 ng/mL				
Mean ± SD	1121.25 ± 670.11	630.71 ± 127.28	98.0	<0.001
Range	397 – 3435	465.5 – 807.0		

U = Mann Whitney test SD = standard deviation n = Number P < 0.001= highly significant.

There was statistically highly significant difference in the meanvalue of VCAM-1 between group 1 (diabetics with normoalbuminuria) and group 2 (diabetic with microalbuminuria) as P1 < 0.001. Group 1 (diabetics with normoalbuminuria) and group 3 (diabetics with macroalbuminuria) as P2 < 0.001. Group 2 (diabetics with microalbuminuria) and group 3 (diabetics with macroalbuminuria) as P3 <0.001 (Table 2).

Table (2): Comparison between diabetic patients subgroups regarding VCAM-1

	Diabetics with normoalbuminuria (n = 15)	Diabetics with microalbuminuria (n = 11)	Diabetics with macroalbuminuria (n = 10)	*K*	P. value
	Mean ± SD	Mean ± SD	Mean ± SD		
VCAM-1 (ng/ml)	698.79 ± 32.04	955.09 ± 74.46	1937.45 ± 72.49	19.26	P_1 <0.001 P_2 < 0.001 P_3 < 0.001

K= Kruskall Wallis test SD = standard deviation n = Number P < 0.001 = highly significantP1 = I VS II, P2 = I VS III, P3 = II VS III.

VCAM-1 showed highly statistical significant positive correlation with ACR (r=0.82, p<0.001), S. creatinine (r=0.93, p < 0.001) and S.urea (r 0.84, p<0.001). Also, there was significant positive correlation between VCAM-1 and HbA1c (r= 0.35, <0.05). On the other hand, sVCAM-1 showed no significant correlation with 2 hour post prandial blood glucose (Table 3).

Table (3): Pearson correlation between VCAM1 and different laboratory parameters among diabetic patients

	Vcam1	
Parameters	**r**	**p**
ACR (mg /g creat.)	0.82	<0.001
2h pp (mg/dl)	0.29	>0.05
S. urea (mg/dl)	0.84	<0.001
S. creatinine (mg/ dl)	0.93	<0.001
HbA1c (%)	0.35	<0.05

r = Spearman Correlation coefficient test ACR=Albumin creatinine ratio 2hpp=2 hour post prandial blood glucose

HbA_1c = glycated hemoglobin P<0.001 = highly significant P<0.05 = statistically significant P > 0.05= non-significant.

Patients with diabetic nephropathy: VCAM-1 showed high significant correlation regarding ACR (r=0.97, p<0.001), s. Creatinine (r=0.96, p<0.001) and serum urea (r=085, p<0.001). Also, there was a significant positive correlation between VCAM-1 and both glycated haemoglobin and 2 hour post prandial blood glucose (Table 4).

Controls and diabetics without nephropathy: VACM-1 showed high significant positive correlation with ACR(r= 0.49.p<0.001). Also, there was positive correlation between VCAM-1 and creatinine (r= 0.45, p<0.05). However, no correlation was found between VCAM-1 and other parameters (Table 4).

Table (4): Correlation of VCAM-1with other laboratory measures in patients with diabetic nephropathy versus controls and diabetics without nephropathy

Parameters	VCAM-1 controls and diabetics without nephropathy (n=29)		VCAM-1 Cases with diabeticnephropathy (n=21)	
	r	p	r	P
ACR (mg/g creat.)	0.49	<0.001	0.97	<0.001
2hpp (mg/dl)	0.06	>0.05	0.48	<0.05
S.urea (mg/dl)	0.09	<0.05	0.85	<0.001
S. creatinine (mg/dl)	0.45	<0.05	0.96	<0.001
HbA_1c (%)	0.06	>0.05	0.53	<0.05

r= Spearman correlation coefficient. ACR= Albumin creatinine ratio. HbA_1c= glycated hemoglobin.2hpp =2hour postprandial blood glucose. n= Number. P< 0.001= highly significant P< 0.05 = statistically significantP> 0.05= non-significant.

Figure (1) illustrated the ROC graph analysis of sVCAM-1, which was implemented to evaluate and quantify its diagnostic efficacy in distinguishing between diabetics and non-diabetics. It was discovered that the optimal cut-off value for sVCAM-1 was 646.5 ng/mL, with a diagnostic sensitivity of 80.6%, specificity of 64.3%, positive predictive value of 85.3%, negative predictive value of 56.3 %, and diagnostic accuracy of 76%. Under the ROC curve, the area was 0.798.

Figure (1): Receiver operating characteristic (ROC) curve for sVCAM-1 in diabetic patients and control subjects for detection of diabetes.

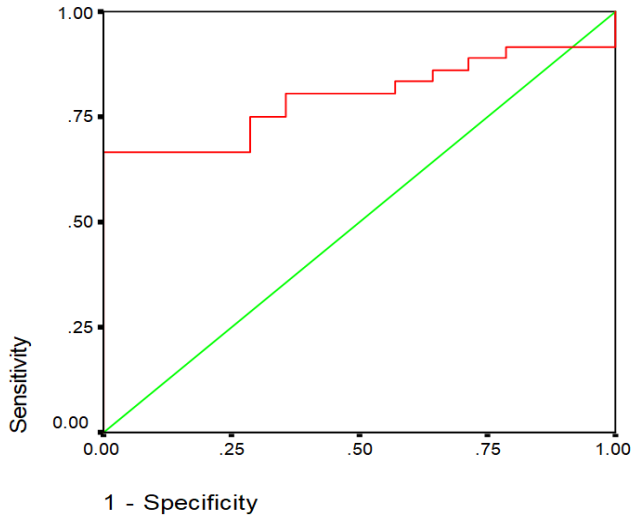

Figure (2) ROC graph analysis for sVCAM-1 was performed in order to quantify and evaluate its diagnostic performance in the differentiation of diabetics with and without nephropathy. The diagnostic specificity was 80%, sensitivity was 100%, NPV was 100%, PPV was 87.5%, and diagnostic accuracy was 91.7%. The optimal cut-off value for sVCAM-1 was 792.5 ng/mL. The area under the ROC curve is 0.917.

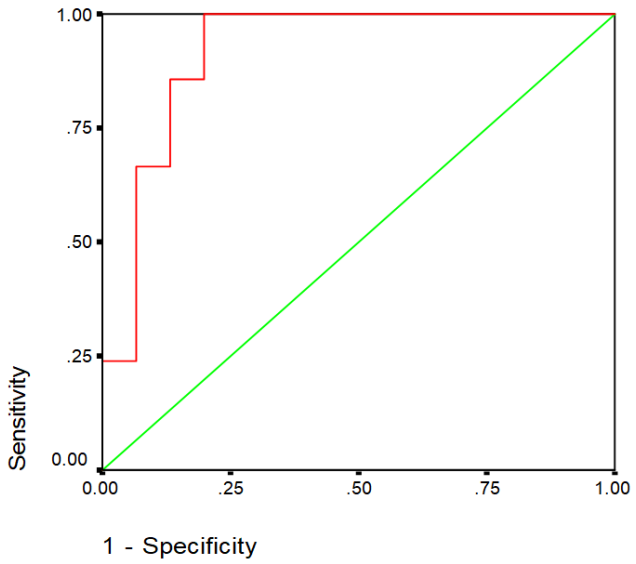

Figure (2): ROC curve for sVCAM-1 in diabetics with nephropathy and diabetics without nephropathy for detection of diabetic nephropathy

DISCUSSION

In current work, the mean sVCAM-1 was found to be significantly higher in diabetic patients (1121.25 ± 670.11) compared to control subjects (630.91 ± 127.28) P<0.001. This result agrees with those reported by **Rubio-Guerra *et al.*** [9], **Seo *et al.*** [10] and **Murakami *et al.*** [11] who found that sVCAM-1 was significantly higher in diabetics than in controls P<0.001. This result supports the suggestion that in poorly controlled diabetic patients, the enhanced non-enzymatic glycation of plasma proteins cause an enhanced oxidative-stress through generation of oxygen free radicals resulting in endothelial dysfunction. Reduction of endothelial function stimulates inflammation and subsequent increase in sVCAM-1 levels [6].

In current work, the mean serum VCAM-1 level was found to be not only significantly higher in macroalbuminuria patients (group 3) (1937.45 ± 712.48) compared to microalbuminuria patients (group2) (955.09 ± 74.46) (P3<0.001). Also, in micro- and macro-albuminuria patients (group 2, group 3) compared to diabetics with normoalbuminuria (group 1) (698.97±322.04) (P1<0.001, P2<0.001). These results are in accordance with **Bruno *et al.*** [4], **Murakami *et al.*** [11] and **Clausen *et al.*** [13] who showed high levels of serum VCAM-1 were in diabetic nephropathy patients.

The current results showed among 36 diabetic patients, VCAM-1 showed high significant positive correlation with s.creatinine (r=0.93), s. urea (r=0.48) P<0.001 in both. Also, there was significant correlation between VCAM-1 and HbA1c (r=0.35, P<0.05) but no significant correlation was found with 2h pp (r=0.29, P>0.05). These results agree with those reported by **Clausen *et al.*** [12] who showed that there was statistically significant positive correlation between VCAM-1 and both s.creatinine (r=0.83, P<0.001), HbA_1c (r=0.4, P<0.05) in diabetic patients.

Murakami *et al.* [11] reported that there was no correlation between VCAM-1 and HbA1c in diabetics. This discrepancy may be attributed to the different number of patients in both studies, duration of study and effect of therapy to control glycemic state. It seems that the only correct way to accurately demonstrate a possible relation between sVCAM-1 and glycemic control is to study these parameters in the same patients before and after good glycemic control.

The present work showed a high positive correlation of sVCAM- 1 with ACR among whole diabetic patients (r=0.82, P<0.001). These results are in accordance with **Rubio-Guerra *et al.*** [10] who found significant positive correlation between VCAM-1 and 24-h albuminuria in diabetics (r=0.7, P<0.001). However, this was quiet different with that reported by **Lin *et al.*** [13] who showed that there was correlation between VCAM-1 and ACR in diabetics but did not reach the statistic significant level (P>0.05). This difference may be related to different number of patients and duration of both studies, renal impairment and other pathological conditions known to affect sVCAM-1 levels e.g. atherosclerosis, cancer, autoimmune and inflammatory diseases that may be excluded from the study and more accurate and precise instruments used for collection of specimens and interpretation of results.

In the present study, analysis of 21 diabetic patients with nephropathy revealed a highly significant positive correlation between VCAM-1 levels and albumin-to-creatinine ratio (ACR) (r=0.97, P<0.001), serum creatinine (r=0.96, P<0.001), and serum urea (r=0.85, P<0.001). Additionally, VCAM-1 demonstrated a significant positive association with both 2-hour postprandial glucose (2hpp) (r=0.48, P<0.05) and HbA1c (r=0.53, P<0.05). These findings align with the results of ***Seo et al.*** [11] who reported that in patients with diabetic nephropathy, sVCAM-1 was positively correlated with serum creatinine (r=0.34, P<0.001) and

24-hour urinary protein (r=0.26, P<0.05). The observed relationship between sVCAM-1 and ACR may be linked to diabetes-induced endothelial dysfunction, which contributes to both albuminuria and elevated sVCAM-1 expression [(4)]. This supports the notion that elevated sVCAM-1 levels may be indicative of more advanced stages of renal impairment. The study results revealed that the optimal threshold for sVCAM-1 to distinguish diabetic individuals from healthy controls was 646.5 ng/mL, with a diagnostic sensitivity of 80.6%, specificity of 64.3%, a positive predictive value of 85.3%, a negative predictive value of 56.3%, and an overall diagnostic accuracy of 76%. Furthermore, the findings indicated that the ideal sVCAM-1 cut-off for identifying early-stage diabetic nephropathy was 792.5 ng/mL, yielding a diagnostic sensitivity of 100%, specificity of 80%, a NPV of 100%, PPV of 87.5%, and a diagnostic accuracy of 91.7%.

CONCLUSION

VCAM-1 concentrations were significantly higher in diabetics compared to controls, with elevated levels observed in macro- and micro-albuminuria diabetic patients relative to those with normoalbuminuria. A notable positive correlation was found between VCAM-1 levels and urinary albumin excretion in patients with micro- and macro-albuminuria. These results suggest that VCAM-1 could potentially serve as a marker for diabetic nephropathy. However, further cost-benefit analysis is necessary to determine the practicality and effectiveness of using VCAM-1 as a tool for early detection and prevention of vascular complications in diabetic patients.

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REFERENCES

1. **Antar S, Ashour N, Sharaky M *et al.* (2023):** Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomed Pharmacother. ,168: 115734.
2. **Abouzid M, Ali K, Elkhawas I *et al.* (2022):** An Overview of Diabetes Mellitus in Egypt and the Significance of Integrating Preventive Cardiology in Diabetes Management. Cureus, 14: e27066.
3. **Samsu N (2021):** Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int., 8: 1497449.
4. **Bruno C, Valenti M, Bertino G *et al.* (2008):** Plasma ICAM-1 and VCAM-1 levels in type 2 diabetic patients with and without microalbuminuria. Minerva Med., 99: 1-5.
5. **Singh V, Kaur R, Kumari P *et al.* (2023):** ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders. Clin Chim Acta, 548: 117487.
6. **Ivetic A, Hoskins Green H, Hart S (2019):** L-selectin: A Major Regulator of Leukocyte Adhesion, Migration and Signaling. Front Immunol., 10: 1068.
7. **Taylor W, Aizenstein H, Alexopoulos G (2013):** The vascular depression hypothesis: mechanisms linking vascular disease with depression. Mol Psychiatry, 18: 963-74.
8. **Siddiqui K, George TP, Mujammami M *et al.* (2023):** The association of cell adhesion molecules and selectins (VCAM-1, ICAM-1, E-selectin, L-selectin, and P-selectin) with microvascular complications in patients with type 2 diabetes: A follow-up study. Front Endocrinol (Lausanne), 14: 1072288.
9. **Rubio-Guerra A, Vargas-Robles H, Ayala G *et al.* (2007):** Correlation between circulating adhesion molecule levels and albuminuria in type 2 diabetic normotensive patients. Med Sci Monit., 13: Cr349-52.
10. **Seo J, Min W, Kim Y (2003):** Patients with Type 2 Diabetes Mellitus with Overt Nephropathy. 대한신장학회지 제, 22: 294-302.
11. **Murakami H, Tamasawa N, Matsui J *et al.* (2001):** Plasma levels of soluble vascular adhesion molecule-1 and cholesterol oxidation product in type 2 diabetic patients with nephropathy. J Atheroscler Thromb., 8: 21-4.
12. **Clausen P, Jacobsen P, Rossing K *et al.* (2000):** Plasma concentrations of VCAM-1 and ICAM-1 are elevated in patients with Type 1 diabetes mellitus with microalbuminuria and overt nephropathy. Diabet Med., 17: 644-9.
13. **Lin J, Glynn R, Rifai N *et al.* (2008):** Inflammation and progressive nephropathy in type 1 diabetes in the diabetes control and complications trial. Diabetes Care, 31: 2338-43.