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Association of Plasma Sialic Acid concentrations with Diabetic Nephropathy in Syrian Individuals with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) is a significant global health concern, often leading to microvascular complications such as Diabetic Nephropathy, a major cause of chronic kidney disease. The objective of this study was to assess the association between plasma Sialic Acid (SA) levels and C-Reactive Protein (CRP), along with other laboratory indicators of the Diabetic Nephropathy in Syrian patients.

Materials and Methods: The study included 115 participants divided into 3 groups: 33 T2DM patients without complications, 52 with Diabetic Nephropathy, and 30 healthy controls. Parameters like plasma Glucose, Urea, Creatinine, C-Reactive Protein (CRP), and Urine Microalbumin were measured using available kits. SA levels were determined using Ehrlich's method, and eGFR was calculated using the Cockcroft–Gault formula.

Results: T2DM patients, especially those with Nephropathy, showed significant increasing in plasma Glucose, SA, CRP, Creatinine, Urea, and Urine Microalbumin compared to control. A positive correlation was observed between SA levels and these parameters, and a notable negative correlation with eGFR.

Conclusion: Plasma SA levels are significantly elevated in Syrian T2DM patients with Nephropathy. These findings suggest that SA could be a potential biomarker for Diabetic Nephropathy in Syrian patients, offering a predictive tool for early diagnosis and preventive strategies for managing this complication.

1. Introduction:

Type 2 Diabetes Mellitus (T2DM) is a global health concern characterized by impaired insulin production and reduced tissue response to insulin, leading to hyperglycemia.[1] This metabolic disorder is often linked with various microvascular complications, including Diabetic Nephropathy. [2] The latest statistics from the 10th edition of the IDF Diabetes Atlas reveal that there are approximately 537 million adults globally diagnosed with diabetes. [3] T2DM makes up about 90% of these diabetes cases. [2] Diabetic Nephropathy stands as a critical complication of Diabetes Mellitus, and is recognized as a primary cause of chronic kidney disease.

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This condition significantly contributes to the increased prevalence of morbidity and mortality Growing research supports the vital role of inflammation in the pathophysiology of T2DM and its related complications. [5] C-Reactive Protein [CRP], a standard biomarker for inflammation, is synthesized in the liver and secreted following inflammatory responses and tissue injuries. [5] Furthermore, Sialic Acid (SA) can act as a marker for assessing the acute phase response, as it forms the terminal sugar in the structure of numerous immune glycoproteins. [4]

Sialic acid [SA], an acidic sugar appertains to neuraminic acid derivative, the most familiar member of this family is the N-acetylated derivative of neuraminic acid (NANA). SA is a part of the structural component of cellular membranes being an end terminal of glycoproteins and glycolipids. [6]

Higher levels of SA might indicate severe damage to cell membranes, particularly to the cells in the smallest blood vessels, like those found in the brain, heart, retina, and kidneys, such damage can give rise to conditions such as Neuropathy, Retinopathy, and Nephropathy. [6]

In patients with Diabetic Nephropathy, there is a significant elevation in SA concentrations, attributed to damage to the renal vascular endothelial cells. Recent studies suggest that this increased Sialic Acid serves as a potential biomarker for the progression of Diabetic Nephropathy. Evaluating SA levels may provide predictive value and contribute to strategies for preventing microvascular complications in individuals diagnosed with T2DM. [6]

Some studies [7,8] indicate a positive correlation between Sialic Acid and CRP, whereas other studies [9] have not found any significant relationship. Therefore, the exact association between SA and CRP levels remains uncertain.

The objective of this study was to assess the association between plasma Sialic Acid (SA) levels and C-Reactive Protein (CRP), along with other laboratory indicators of Diabetic Nephropathy in Syrian patients, and to assess if it could be utilized as a potential biomarker for Diabetic Nephropathy.

2. Materials and Methods:

2.1. Subjects and study design:

This study included 115 participants, 56 males and 59 females, ages (45-85) years, were classified into three groups: 33 were identified as T2DM without any complications, 52 were T2DM with Diabetic Nephropathy, and 30 healthy Control.

The Faculty of Pharmacy's Ethics Committee at Aleppo University approved this study. All individuals participating in the study gave their voluntary consent.

All participants were chosen from patients who attending the National Diabetes Center in Ashrafieh in Aleppo during the period between October 2022 and October 2023.

A comprehensive history and clinical examination were conducted for each patient, with particular emphasis on sex, age, Body Mass Index (BMI), type of diabetes, and any complications.

The study groups exclude participants with histories of cardiac diseases, smoking, alcohol consumption, pregnancy, cancer, or any inflammatory disorders.

2.2. Samples collections:

The Collection of fasting blood samples was carried out using lithium-heparin-coated tubes. Urine specimens were also gathered in dry and sterile containers.

2.3. Determination of studied parameters:

Plasma Glucose was measured using a colorimetric enzymatic assay, Creatinine through a Jaffe colorimetric kinetic assay, Urea via a colorimetric enzymatic assay, CRP using a turbidimetric assay, and Urine Microalbumin with a colorimetric assay. The assays were performed using the following Kits: (Coral Clinical Systems, Cat. No.1102113150), (Coral Clinical Systems, Cat. No. 1101070275), (Biosystem, Cat. No. 11537), (AMS, Cat. No. GD842701), Medichem, Cat. No. 12570) respectively.

A Cockcroft–Gault formula was utilized to calculate estimated Glomerular Filtration Rate (eGFR) [10]

Ehrlich's method was utilized to determine plasma SA levels [11,12]: 500 µl of plasma was treated with 2 ml of 5% perchloric acid for 5 min at 100 °C and centrifuged at 4000 RPM for 5 min. The supernatant (1 ml) was mixed with 200 µl Ehrlich reagent (5 g p-dimethylaminobenzaldehyde/50 ml, HCl/50 ml distilled water). After incubation at 100 °C for 15 min, a spectrophotometer was used to read the

absorbance at 525 nm, Sialic Acid concentration was calculated depending on Sialic Acid standard curve as follow:

1. stock solution: 25 mg of solid Sialic Acid in 25 ml of distilled water. The resulting solution had a 100 mg/dl concentration.
2. Serial dilution was used to create standard samples in various concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 mg/dl) from the stock solution (100 mg/dl)
3. The standard samples were then analyzed using Ehrlich's method and absorbance was measured by spectrophotometer at 525 nm.
4. Using the Microsoft Excel 2019 program, a standard curve was created between the standard sialic acid concentration on the x-axis and the standard sample's absorbance on the y-axis.
5. The following curve was produced with y = 0.0131x - 0.0488 equation and $R^2 = 0.9925$. (Fig 1)

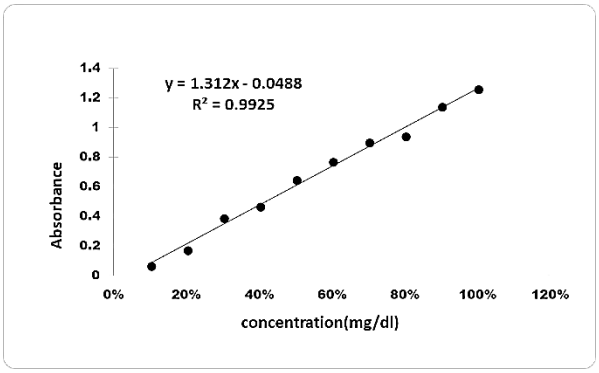

***Fig 1**. Sialic Acid standard curve*

6. SA concentration of study subjects' samples was determined using the following equation: X= (Y+ 0.0488)/0.0131 Where, X represents the concentration of the test samples and Y represents their absorbance.

2.4. Statistical analysis:

The data underwent processing utilizing SPSS software, version 24.

One-way ANOVA was employed to determine significant differences between groups at p-value <0.05.

Independent sample t-test was employed to determine the mean difference between the two groups. The p-value of less than 0.05 is considered as significant at 95% confidence level.

Pearson correlation coefficient (r) was employed to identify significant correlations among the quantitative parameters for each group at a significance level of $p < 0.05$.

3. Results:

The participants in this study were aged between 45 and 85 years, with a median age of 53 years. The gender distribution included 58 males and 57 females. The Body BMI ranged from 18 to 52 kg/ m2.

This study showed a significant difference in BMI in both types of T2DM (with and without nephropathy) in comparison to the control. [Table1]

The one-way ANOVA test showed a significant increase in plasma Glucose, SA, CRP, Creatinine, Urea, and Urine Microalbumin levels in both types of T2DM (with and without nephropathy) in comparison to the control. Additionally, there was a significant decrease in eGFR in both types of T2DM (with and without nephropathy) in comparison to the control. [Table2]

Table 1. Demographic characteristics in study groups.

Items	Controls	T2DM	T2DM+ Nephropathy	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age(years)	56.70 ± 8.59	60.24 ± 7.94	58.92 ± 9.13	0.568
BMI(KG/m^2)	23.67 ± 5.02	29.88 ± 4.02	29.79 ± 6.7	0.000
Sex	N	N	N	
Male Female	14 16	19 14	25 27	0.619

The comparison results using an independent sample t-test showed a significant increase in plasma Glucose, SA, CRP, Urea, and Urine microalbumin in T2DM with Nephropathy in comparison to those without any complications, and no significant difference in plasma Creatinine and eGFR. [Table3]

The Pearson correlation test showed a significant positive correlation between Sialic Acid levels and studied parameters (Sugar, Creatinine, Urea, CRP, and Urine Microalbumin) in both groups. Additionally, the analysis indicated a significant negative correlation between SA levels and eGFR. [Table4, Fig 2a,b]

Table 2 a. Comparison of the studied parameters between subject groups. p = < 0.05 significant

Parameters	Controls	T2DM	T2DM+ nephropathy	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Glucose(mg/dl)	73.97 ±10.762	173.70 ± 51.456	224.38±.91.17	0.000
Sialic acid (mg/dl)	54.40 ± 6.946	72.22 ± 6.842	75.40 ± 12.4270	0.000
CRP (mg/l)	1.283 ± 1.02	2.93 ± 1.896	3.349±3.728	0.007
Creatinine (mg/dL)	0.723± 0.113	1.204 ± 0.133	1.3 ± 0.294	0.000
Urea (mg/dL)	24.13 ± 8.905	34.26± 6.383	37.25 ±14.839	0.000
urine microalbumin (mg\g)	7.83±5.059	21.38± 8.737	440.47 ± 719.39	0.038
eGFR (mL/min/1.73 m2)	120.83±30.28	72.52 ± 20.71	66.02 ± 23.72	0.000

Table 2 b. Comparison of the studied parameters between control and T2DM. p = < 0.05 significant

Parameters	control	T2DM	p-value
	Mean ± SD	Mean ± SD	
Glucose(mg/dl)	73.97 ±10.762	173.70 ± 51.456	0.000
Sialic acid (mg/dl)	54.40 ± 6.946	72.22 ± 6.842	0.004
CRP(mg/l)	1.283 ± 1.02	2.93 ± 1.896	0.031
Creatinine (mg/dL)	0.723± 0.113	1.204 ± 0.133	0.000
Urea (mg/dL)	24.13 ± 8.905	34.26± 6.383	0.001
Urine microalbumin (mg/g)	7.83±5.059	21.38± 8.737	0.922
eGFR (mL/min/1.73 m2)	120.83±30.28	72.52 ± 20.71	0.000

Table 2 c. Comparison of the studied parameters between control and T2DM + nephropathy. p = < 0.05 significant

Parameters	control	T2DM+ nephropathy	p-value
	Mean ± SD	Mean ± SD	
Glucose(mg/dl)	73.97 ±10.762	224.38±.91.17	0.000
Sialic acid (mg/dl)	54.40 ± 6.946	75.40 ± 12.4270	0.000
CRP (mg/l)	1.283 ± 1.02	3.349±3.728	0.001
Creatinine (mg/dL)	0.723± 0.113	1.3 ± 0.294	0.000
Urea (mg/dL)	24.13 ± 8.905	37.25 ±14.839	0.000
Urine microalbumin (mg/g)	7.83±5.059	440.47 ± 719.39	0.000
eGFR (mL/min/1.73 m2)	120.83±30.28	66.02 ± 23.72	0.000

Table 3. Comparison of the studied parameters between T2DM group and T2DM with Nephropathy. p = < 0.05 significant

Parameters	T2DM	T2DM+ nephropathy	p-value
	Mean ± SD	Mean ± SD	
Glucose(mg/dl)	173.70 ± 51.456	224.38± 117.73	0.008
Sialic acid (mg/dl)	72.22 ± 6.842	75.40 ± 12.4270	0.004
CRP(mg/l)	2.93 ± 1.896	3.349 ±3.728	0.037
Creatinine (mg/dL)	1.204 ± 0.133	1.3 ± 0.294	0.085
Urea (mg/dL)	34.26± 6.383	37.25 ±14.839	0.007
Urine microalbumine(mg/g)	21.38± 8.737	440.47 ± 719.39	0.009
eGFR((mL/min/1.73 m2)	72.52 ± 20.71	66.02 ± 23.72	0.933

Table 4. Correlation between Sialic Acid and studied parameters within T2DM and T2DM with nephropathy. p = < 0.05 significant.

Parameters correlated	T2DM		T2DM+ nephropathy	
	r-value	p-value	r-value	p-value
Sialic Acid and Glucose	0.642**	0.000	0.45**	0.000
Sialic Acid and Creatinine	0.747**	0.000	0.516**	0.000
Sialic Acid and Urea	0.405**	0.000	0.247*	0.026
Sialic Acid and GFR	-0.499**	0.000	-0.448**	0.000
Sialic Acid and Urine Microalbumine	0.559**	0.000	0.332**	0.002
Sialic Acid and CRP	0.536**	0.000	0.366**	0.001

4. Discussion:

Diabetes Mellitus ranks among the most prevalent health issues globally, including in Syria. Inflammation is a key factor in the pathogenesis of Type 2 Diabetes Mellitus and its complications, such as Nephropathy. Therefore, inflammatory markers, like SA, might serve as significant indicators of disease severity, potentially acting as predictors of the diabetic condition. However, further researches are needed to comprehensively understand this role.

This study evaluated SA concentrations in T2DM Syrian patients with and without Nephropathy. There was a significant increase in plasma Sialic Acid levels in both types of T2DM (with and without nephropathy) in comparison to the control. Moreover, the SA levels showed a significant elevation in T2DM patients with nephropathy compared to those without complications. These findings align with comparable research conducted in Egypt in 2021 by El Badawy and colleagues. [13]

Normally vascular endothelium is rich in SA. Several studies indicate that Sialic Acid levels rise during pathological conditions characterized by tissue damage, proliferation, and inflammation. Consequently, the widespread microvascular damage seen in Diabetes Mellitus may result in the release of Sialic Acid into the circulation, resulting in elevated SA concentrations. [4]

Tissue damage resulting from diabetic vascular complications such as Nephropathy, stimulates the release of local Cytokine from cellular infiltrates like macrophages and endothelial cells. This initiates an acute phase response, characterized by the release of glycoproteins rich in SA into the circulatory system, thereby leading to elevated SA concentrations in patients with Diabetic Nephropathy. [4]

CRP, a marker of acute phase inflammation, was also assessed in This research.

The findings showed a significant increase in CRP levels among the study groups, with CRP being notably higher in patients with Diabetic Nephropathy. These findings align with comparable research conducted in India in 2016 by Varma and colleagues. [8] Indicating that CRP may play a role in the pathogenesis of T2DM and its complications. Additionally, the increased damage to the vascular cells of the kidneys in T2DM patients could lead to a more active inflammatory state, further elevating CRP levels. [8]

Our study demonstrated a positive association between SA and CRP in both T2DM and T2DM with Nephropathy groups. This correlation was also observed in other similar studies. [7,8] It can be interpreted that SA serves as a combined marker for various acute-phase proteins, reflecting the general acute-phase response. In contrast, CRP is a specific acute-phase protein. Consequently, the increase in Sialic Acid levels is associated with an elevation in CRP levels, indicating a comprehensive inflammatory response. [7]

Furthermore, the correlation between SA and fasting Glucose has been investigated, the result showed a positive correlation in both types of T2DM (with and without nephropathy) which was similar to other studies. [13,14] Consequently, SA may be regarded as a potential early marker of Type 2 Diabetes Mellitus.

Blood Urea, Creatinine, Urine Microalbumin, and eGFR measurements have been widely used as indicators of kidney function.

Our study showed a significant increase in Blood Urea and Urine Microalbumin in T2DM with Nephropathy compared to those without any complications. This result aligns with the outcomes of other comparable studies. [4,9]

The higher levels of these parameters in T2DM patients with nephropathy, compared to those without complications, indicate a more severe impact of the disease on kidney function and overall metabolism. The elevation in urine albumin in the diabetics can be viewed as an early indication of kidney damage in those patients. The elevation in urine albumin observed in Diabetic Nephropathy can be explained by glomerular basement membrane degradation and hypertension, both manifestations of Diabetic Nephropathy. [4]

Urine Microalbumin is indicative of endothelial dysfunction, often triggered by the release of cytokines and various inflammatory agents during

intense inflammatory reactions, typically observed in serious illnesses. This dysfunction compromises the endothelial barriers, leading to alterations in glomerular permeability and consequently, an increased leakage of albumin into the glomerular ultrafiltrate. When the tubular mechanism responsible for reabsorbing albumin from the ultrafiltrate exceeds its maximum capacity, it results in a higher release of albumin into the urine. [4]

Our study showed that there is no significant difference in plasma Creatinine and eGFR between T2DM with Nephropathy and those without any complications. This result aligns with the outcomes of other comparable studies. [15,16] suggesting that while these markers are indicative of renal impairment, they might not be sensitive enough to detect early Nephropathy.[15] Therefore, SA with its significant elevation, may act as an indicator of early Diabetic Nephropathy.

Our study showed a positive correlation between SA levels and Blood Urea, Creatinine, and Urine Microalbumin in both types of T2DM (with and without nephropathy). This result aligns with the outcomes of other comparable studies. [4,13] suggesting that SA may play a significant role in contributing to renal damage.

Furthermore, a negative correlation was observed between SA levels and eGFR in both groups. This implies that as Sialic Acid increases, eGFR decreases, aligning with the findings of El Badawy and colleagues.[13] This indicates that SA levels rise with the worsening of diabetic renal complications.

5. Conclusions:

Plasma SA levels are significantly elevated in Syrian T2DM patients with Nephropathy. These findings suggest that SA could be a potential biomarker for Diabetic Nephropathy in Syrian patients, offering a predictive tool for early diagnosis and preventive strategies for managing this complication.

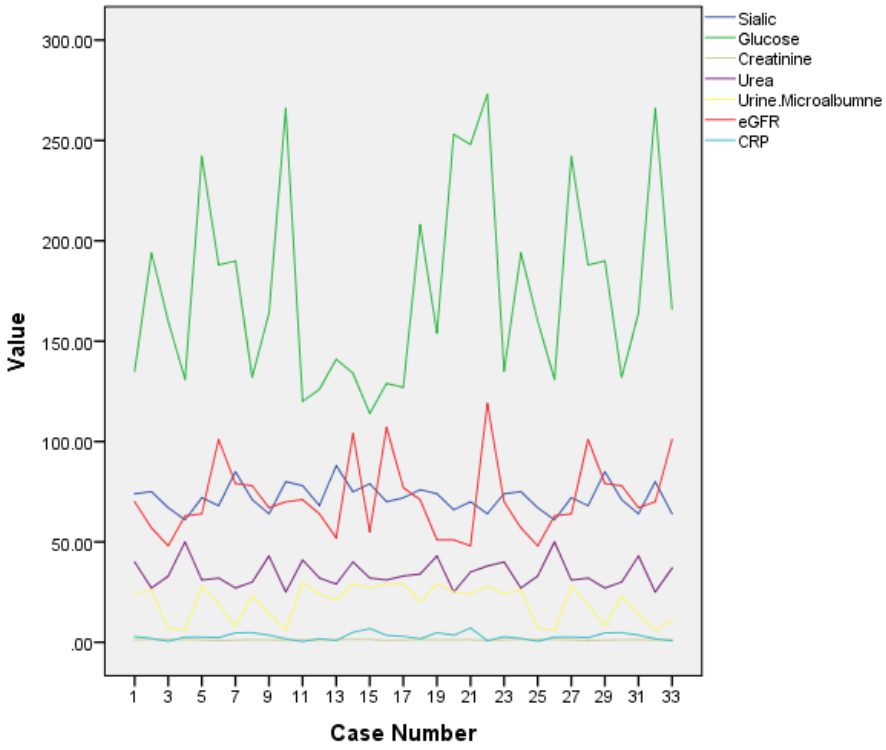

Fig 2a. Correlation between Sialic Acid and studied parameters

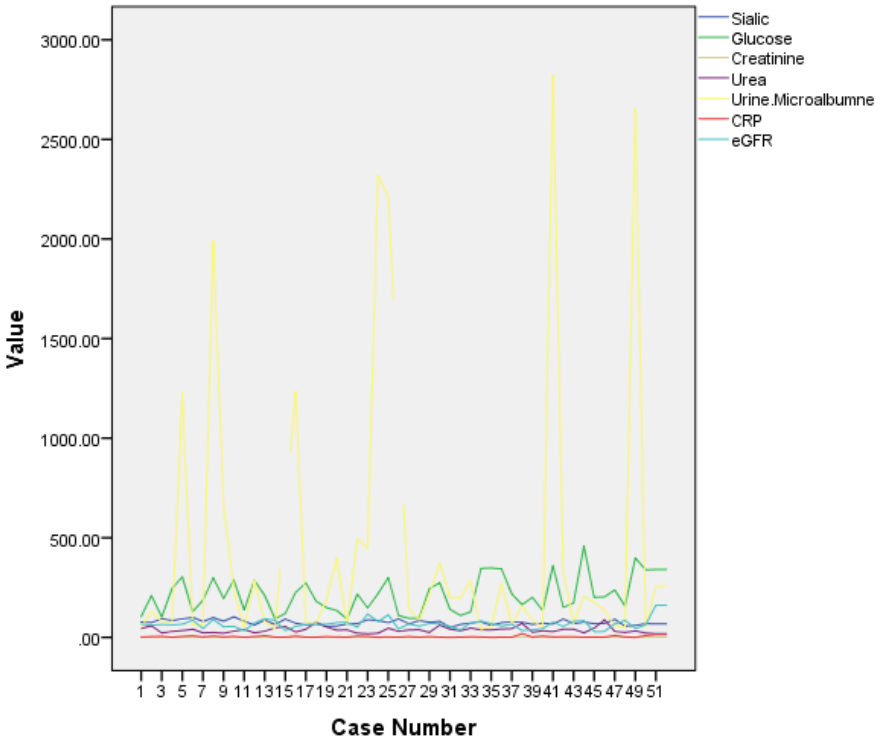

Fig 2b. Correlation between Sialic Acid and studied parameters

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship

Each author listed in the manuscript has approved the submission of this version of the manuscript and takes full responsibility for it.

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References:

[1] Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21(17):6275. Published 2020 Aug 30. doi:10.3390/ijms21176275

[2] Jafri SA, Rehman KU, Ilyas N, Imran AB, Qasim M, Zahra S. Evaluation of serum sialic acid and other risk factors in diabetes mellitus. African Journal of Biotechnology. 2017;16(50):2326-2330. doi:10.5897/ajb2017.16049

[3] IDF Diabetes Atlas | Tenth Edition. Copyright © IDF Diabetes Atlas 2023. All Rights Reserved. https://diabetesatlas.org/%20.

[4] Pal M, Ghosh J, Datta S. Role of sialic acid in prediction of diabetic nephropathy. ResearchGate. January 2016. https://www.researchgate.net/publication/297715459_Role_of_sialic_acid_in_prediction_of_diabetic_nephropathy.

[5] Stanimirovic J, Radovanovic J, Banjac K, et al. Role of C-Reactive Protein in Diabetic Inflammation. Mediators Inflamm. 2022;2022:3706508. Published 2022 May 17. doi:10.1155/2022/3706508

[6] K P, Kumar J A, Rai S, et al. Predictive value of serum sialic Acid in type-2 diabetes mellitus and its complication (nephropathy). J Clin Diagn Res. 2013;7(11):2435-2437. doi:10.7860/JCDR/2013/6210.3567 7

[7] Keti DB, Muhtaroğlu S, Ünlühızarcı K, Çetin İ. The association of sialic acid levels with nitric oxide and C-reactive protein in patients with diabetic foot ulcer / Diyabetik ayak ülseri olan hastalarda sialik asit düzeylerinin nitrik oksit ve C-reaktif protein ile ilişkisi. Türk Biyokimya Dergisi. 2016;41(4). doi:10.1515/tjb-2016-0037

[8] Varma V, Varma M, Varma A, Kumar R, Bharosay A, Vyas S. Serum Total Sialic Acid and Highly Sensitive C-reactive Protein: Prognostic Markers for the Diabetic Nephropathy. J Lab Physicians. 2016;8(1):25-29. doi:10.4103/0974-2727.176230

[9] Nayak BS, Roberts L. Relationship between inflammatory markers, metabolic and anthropometric variables in the Caribbean type 2 diabetic patients with and without microvascular complications. J Inflamm (Lond). 2006;3:17. Published 2006 Dec 22. doi:10.1186/1476-9255-3-17

[10] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41. doi:10.1159/000180580

[11] Sydow G. A simplified quick method for determination of sialic acid in serum. Biomed Biochim Acta. 1985;44(11-12):1721-1723.

[12] Crook M. The determination of plasma or serum sialic acid. Clin Biochem. 1993;26(1):31-38. doi:10.1016/0009-9120(93)90014-w

[13] El Badawy AM, Mansour A, Elsayed MS, Abdelmoniem RO. Relationship between serum sialic acid concentration and diabetic nephropathy in Egyptian patients with type 2 diabetes mellitus. Journal of Diabetology: Official Journal of Diabetes in Asia Study Group. 2021;12(1):70. doi:10.4103/jod.jod_12_20

[14] Singh YP, Nandkeoliar MK, Punia VPS, Rai G. Evaluation of sialic acid levels in type-2 diabetes mellitus patients in NCR, Delhi. International Journal of Clinical Biochemistry and Research. 2020;7(2):197-200. doi:10.18231/j.ijcbr.2020.042

[15] Sapkota S, Khatiwada S, Shrestha S, et al. Diagnostic Accuracy of Serum Cystatin C for Early Recognition of Nephropathy in Type 2 Diabetes Mellitus. Int J Nephrol. 2021;2021:8884126. Published 2021 Apr 26. doi:10.1155/2021/8884126

[16] Chae HW, Shin JI, Kwon AR, Kim HS, Kim DH. Spot urine albumin to creatinine ratio and serum cystatin C are effective for detection of diabetic nephropathy in childhood diabetic patients. J Korean Med Sci. 2012;27(7):784-787. doi:10.3346/jkms.2012