Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Assessment Of Serum Fetuin-A Levels In Diabetic Nephropathy. A Case Control Study.

Deepali S. Malgavkar[1*] , Nilima Dongre[2], S M. Goornavar[3], J G. Ambekar[4] , Sanjeev Ratna[5] , Manjula R[6].

1. PhD Scholar, Department of Biochemistry, Shri B M Patil Medical College Hospital and Research Centre BLDE(DU) Vijaypura, Vijaypura, IND
2. Professor, Department of Biochemistry, Shri B M Patil Medical College Hospital and Research Center BLDE(DU) Vijaypura, Vijaypura, IND
3. Professor, Department of Medicine, S Nijalingappa Medical College, H.S.K Hospital and Research Center, Bagalkot, IND
4. Professor, Department of Biochemistry, Shri B M Patil Medical College Hospital and Research Centre BLDE (DU) Vijaypura, Vijaypura, IND
5. Professor, Department of Biochemistry, S Nijalingappa Medical College and H.S.K Hospital and Research Center, Bagalkot, IND

6. Professor, Department of Community Medicine, S Nijalingappa Medical College, H.S.K Hospital and Research Center, Bagalkot, IND

Corresponding Author: Deepali S. Malgavkar,
Email I'd - malgavkardeepali@yahoo.in.

Cite this paper as: Deepali S. Malgavkar, Nilima Dongre, S M. Goornavar, J G. Ambekar, Sanjeev Ratna, Manjula R. (2024) Assessment Of Serum Fetuin-A Levels In Diabetic Nephropathy. A Case Control Study.*Frontiers in Health Informatics, 13* (3), 10358-10367

Abstract

Introduction: *Diabetic Nephropathy (DN) is a most common vascular complication of Type 2 diabetes. Fetuin-A, after binding with Tyrosine kinase, acts as endogenous inhibitor of insulin receptor in diabetic subjects, causing insulin resistance (IR).*

Aim and objective: *This study was aimed to estimate the serum concentration of fetuin-A levels in the subjects having diabetic nephropathy and healthy controls. To determine cut off value of fetuin-A levels to prevent severity of renal damage in Diabetic Nephropathy subjects.*

Methods: *30 subjects having diabetic nephropathy and 30 healthy controls (age group 35 to 65 years) were selected from the medicine OPD of S. N. Medical College and HSK Hospital, Bagalkot. Based on the presence of microalbuminuria and eGFR levels, 30 subjects of nephropathy secondary to diabetes were selected. Cases having other systemic illnesses like hypertension, thyroid disorders and cardiac disorders were excluded from the study. The serum fetuin-A levels were estimated by the*

ELISA method in the cases and controls. The statistical analysis was done using SPSS software version 19, utilizing unpaired "t" test and Pearson's correlation tests for quantitative data.

Results: *The estimated serum fetuin-A levels in the cases were found to be higher as compared with control subjects and were found to be highly significant (p=0.001). We found the best cut-off value of serum fetuin-A was ≤ 36.66 ng/ml in the subjects having diabetic nephropathy. The area under the curve (AUC) was 0.515 with a specificity of 75 % and sensitivity of 41%.*

Conclusion: *Serum fetuin-A levels were higher in cases compared to controls, which could be used as a diagnostic marker for the severity of renal damage with a cut-off value of ≤ 36.66 ng/ml and could be used to control the nephropathy secondary to the patients suffering with diabetes.*

Categories: *Diabetes*

Keywords: *Type 2 diabetes Mellitus (T2DM), diabetic nephropathy (DN), Fetuin-A-cut-off value, microalbuminuria (MA), eGFR.*

Introduction

The most common cause of End-Stage Renal Disease (ESRD) is diabetic nephropathy (DN) which has become a major global predictor of mortality and morbidity in patients with diabetes [1,2]. The severity of renal damage along with cardio vascular disease linked to type 2 Diabetes mellitus (T2DM) can be reduced by early detection of patients at raised risk for microalbuminuria (MA) and by its treatment. As a renal indicator of widespread vascular endothelial damage, increased MA might be a useful early marker of atherosclerosis and cardiovascular death. Therefore, more precise and targeted biomarkers are required to detect MA early for enhance clinical care and enable timely intervention to prevent further complications. Diabetic kidney disease (DKD) is a syndrome characterized by a gradual rise in the excretion of albumin in urine linked to glomerular lesions, which ultimately results in the loss of glomerular filtration and ESRD. About 20-40% of diabetic patients have DKD which is a major global cause of ESRD [3,4]. Despite being just one component of renal excretory function, GFR is considered the best overall indicator of kidney function in chronic kidney disease (CKD) due to its tendency to decrease with extensive renal structural damage [5].

Fetuin-A, AHSG (α-2 Heremans schmid glycoprotein), has been a multifunctional glycoprotein which causes insulin resistance (IR). Fetuin-A after binding with tyrosine kinase, functions as main hepatokine and endogenous inhibitor of the insulin receptor in the skeletal muscle and liver, causing insulin resistance [6].

The human fetuin-A gene is situated on the chromosome 3q27, which also co-exists genetic susceptibility loci for T2DM as well as metabolic syndrome. Fetuin-A, prevents phosphorylation of insulin receptors in the muscle and liver, lowers insulin signaling and IR. Consequently, elevated levels of fetuin-A have been linked to the onset of T2DM and IR. Insulin resistance is a pathological mechanism of T2DM that may contribute to its onset and consequences [7,8].

Many research workers have depicted that fetuin-A levels influence the severity of diseases, such as diabetes and its consequences. Therefore, the current study was planned to investigate the relationship between microvascular issues like diabetic nephropathy and fetuin-A levels in individuals having T2DM.

Materials and Methods

Patient selection

The current study was carried out at the Medicine and Biochemistry Department of S N Medical College and HSK Hospital and Research Centre, a tertiary care hospital in Bagalkot, Karnataka, India. An institutional ethical committee approvals and certificates were obtained (SNMC/IECHSR/2020/A-71/1.1) & (BLDE(DU)/IEC/399/2020) from S. N. Medical College and BLDE(Deemed to be University), respectively. Before collecting blood samples, the study's purpose and procedures were thoroughly explained to the patients and their families, ensuring informed and written consent, taken from all the participants of the study, conducted in between January - October 2022.

Inclusion Criteria

Type 2 diabetic nephropathy patients characterized by the presence of microalbuminuria and eGFR, within the age group of 35-65 years, attending medicine OPD, were included in the study. Subjects in the same age group without diabetes were referred to as healthy controls.

Exclusion Criteria

Patients having other systemic diseases such as hypothyroidism or hyperthyroidism, cardiovascular diseases, pregnancy, malignancy and systemic drug or alcohol abuse were excluded from the study.

Sample Size calculation

In the present study, sample size was calculated to 60 (30 cases and 30 controls) using the study by Karajibani et. al [9]. The correlation coefficient of serum fetuin-A with serum creatinine was r= -0.61, the sample size calculated using the formula, $N= ([Z\alpha+Z_\beta]/C) + 3$. Where $C = 0.5^* \ln ([1+ r] /[1-r])$. At 99% confidence level $\alpha= Z\alpha = 2.54$, 90% power of study $\beta = Z_\beta = 1.64$.The sample size calculated was = 31, which is round off to 30.

Clinical sample collection

5ml Fasting blood sample was collected under aseptic precautions after overnight fast, out of which 1ml was transferred in EDTA containing vacutainers for HbA1c and 1ml was transferred in fluoride containing vacutainers for fasting blood sugar estimation, 3ml in plain vacutainers for estimation of other biochemical parameters. 2ml blood sample was collected, 2 hours after the meals, in fluoride containing vacutainers under aseptic precautions for PPBS estimation. After centrifugation of all the

vacutainers, at 3000 rpm for 20 minutes, plasma/serum was obtained. The separated serum was used to estimate fetuin-A levels and other biochemical parameters.

At the same time, 10 ml of urine sample was collected after overnight fast from the same patients in a sterile container and estimated for microalbumin within 2 hours. Biosystem kits [10,11] were used to estimate serum glucose, urea, creatinine, uric acid and insulin by auto analyzer BA 400 Biosystem. The serum fetuin-A level was determined using the ELISA method (Robotic) using Bioassay Technologies kits.

Statistical Analysis

The data was analyzed using the mean ± standard deviation for age (years), Microalbumin (mg/dl), FBS (mg/dl), PPBS (mg/dl), Insulin (µIU/l), HOMA-IR, Urea(mg/dl), Creatinine (mg/dl), eGFR (ml/min/1.73m^2), Uric acid (mg/dl) and Fetuin-A (ng/ml). The ANOVA, the unpaired "t" test and Pearson's correlation tests were used for quantitative data and statistical analysis. The SPSS software version 19 was used for Receiver operating curve (ROC curve) analysis. The validity tests such as sensitivity, specificity and diagnostic accuracy of serum fetuin-A were used to determine the best cut-off value for assessing the severity of diabetic nephropathy.

Results

This study included 60 participants classified into two groups. Group 1: Age and sex matched healthy controls. Group 2: Diabetic nephropathy Subjects. Demographic data of studied group is shown in Table 1, which is not statistically significant.

Table 1: Demographic data of study groups.

	Group 1 (n=30) **Mean ± SD**	**Group 2 (n=30)** **Mean ± SD**	**p Value**
Age (years)	51.34 ± 8.73	53.41 ± 10.17	0.176

Table 2: Comparison of glycemic status & insulin levels between the study groups

Parameters	**Healthy controls (n=30) Mean ±SD**	**DN Cases (n=30)** **Mean ± SD**	**t value**	**p value**

FBS (mg/dl)	101.68± 6.03	114.60 ± 31.03	3.086	0.003*
PPBS (mg/dl)	124.76 ± 9.20	165.55 ± 39.02	-5.478	0.001**
HbA1c (%)	5.31± 0.45	8.02 ± 0.58	-19.479	0.000**
Insulin (µIU/ml)	9.64 ± 1.63	27.62 ± 1.41	2.187	0.001*
HOMA-IR	2.39 ± 0.36	11.1 ± 1.27	3.164	0.001*

FBS, PPBS, HbA1c, Insulin and HOMA-IR were significantly increased in DN cases when compared to healthy controls which is highly significant ($p<0.001$).

Table 3: Serum fetuin-A levels in DN cases and healthy controls

Parameters	**Healthy controls** **Mean ± SD**	**DN Cases** **Mean ± SD**	**t value**	**p Value**
Fetuin–A (ng/ml)	38.66 ± 4.77	85.72 ± 5.93	-33.267	0.001**

Fetuin A level was significantly elevated in diabetic nephropathy cases compared to healthy controls.

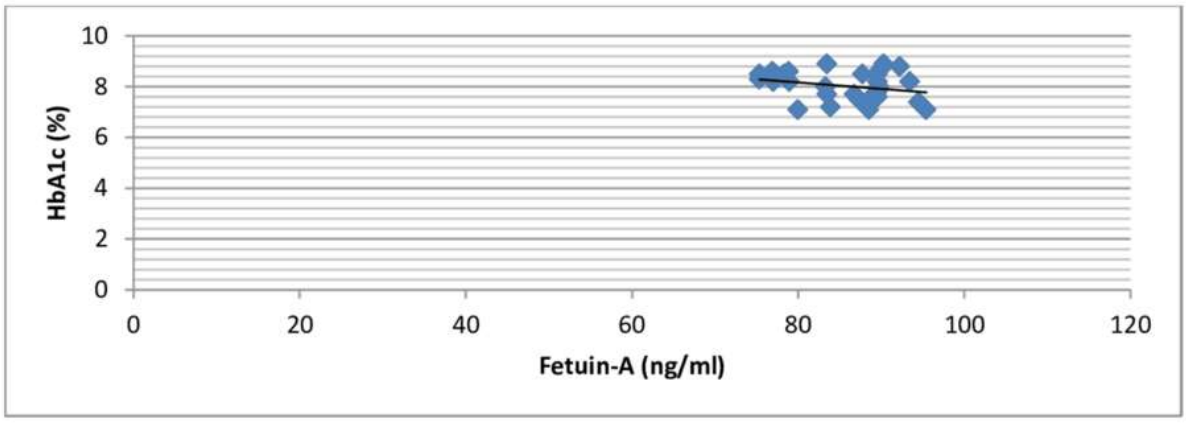
Table 4: Shows comparison between renal parameters and fetuin-A.

Parameters	**Healthy controls (n=30) Mean ±SD**	**DN Cases (n=30)** **Mean ± SD**	**t value**	**p value**
Urea (mg/dl)	47.13 ± 10.61	38.11 ± 8.26	3.612	0.001*
Creatinine (mg/dl)	0.74 ± 0.11	1.39 ± 0.29	-11.036	0.000**
eGFR (ml/min/1.73m^2)	100.48 ± 10.59	50.93 ± 7.74	20.336	0.000**
Uric acid (mg/dl)	4.90 ± 1.21	4.33 ± 1.32	1.703	0.094

Microalbumin (mg/dl)	24.41 ± 4.73	136.94 ± 52.98	-11.393	0.000**

*significant, ** highly sign

Figure 1: Correlation between serum fetuin-A (ng/ml) and HbA1c (%).



Positive correlation was seen between the HbA1c and serum fetuin-A levels in cases.

Figure 2: Correlation between serum fetuin-A (ng/ml) and microalbumin (mg/dl)

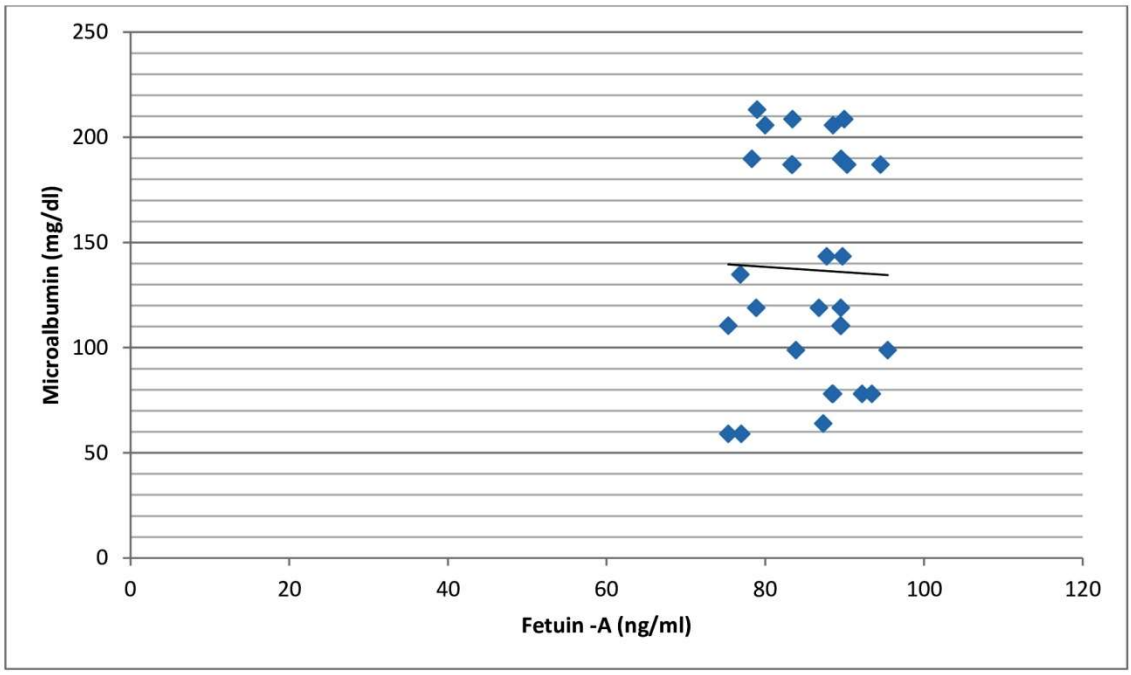
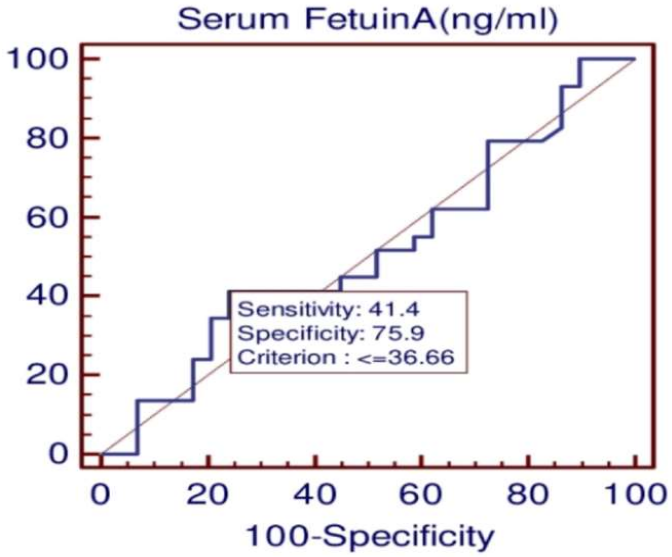


Figure 3: ROC curve analysis for fetuin-



ROC curve analysis for fetuin-A levels showed AUC= 0.515 ($p<0.001$), sensitivity 41.4 % and specificity 75.9% in diabetic nephropathy cases. With this curve fetuin-A cut off value was found to be ≤ 36.66 ng/ml in diabetic nephropathy cases.

Discussion

The most frequent consequences of diabetes that causes renal failure and other issues, in adults, is diabetic nephropathy. Early detection and prevention can decrease the severity of the complications caused by T2DM. The objective of the study was to ascertain fetuin-A levels as a preliminary diagnostic indicator for severity of diabetic nephropathy.

The primary discovery of our research was a noteworthy distinction in fetuin-A concentrations between the DN cases and healthy controls. Fetuin-A has been observed to have a highly significant positive association with FBS, PPBS, HbA1c, Insulin and HOMA-IR.

These results are in accordance with the research conducted by Atef Farouk et al. who also reported increased fetuin-A levels before hemodialysis and after hemodialysis [12].

Nonetheless, our analysis revealed a statistically significant distinction between the levels of fetuin-A and microalbumin. El-Batch et al. found that patients having microalbuminuria had significantly higher serum fetuin-A levels than both the normoalbuminuria patients and the control group [13]. The connection between fetuin-A and aberrant albuminuria may be explained by the function it plays in mediating IR, abnormalities of lipid profile and dysfunction of endothelium. According to Mitkees et al., diabetic patients with microalbuminuria had significantly higher serum fetuin-A levels than those in the control group. This may be because the healthy control group was less likely to experience vascular complications from fetuin-A than that of diabetic patients [14].

Al-Said et al.'s research supports our findings, demonstrating that the group of diabetics with nephropathy had significantly higher mean levels of fetuin-A, HOMA-IR, and insulin than the other groups. There has been a strong positive correlation observed among fetuin-A and renal parameters [15], which also supports our findings. The mechanism that fetuin-A plays a role in the pathophysiology of T2DM was supported by the outcomes of the study conducted by Ahn M B et al. In this study, which led to IR, they demonstrated elevated levels of fetuin-A in obese adolescents with the T2DM [16]. A conflict of results was observed by Sherif et al., who discovered that low levels of fetuin-A appeared to be linked to common micro and macrovascular problems in T2DM [17].

The study limitation is measurement of fetuin-A levels at a single point in time, which may not be accurately reflect its long-term levels. Furthermore, the absence of crucial data regarding the family history of T2DM introduces the possibility of residual confounding factors. Further studies correlating fetuin-A, with the severity of DN and other complications of the disease, are required with the larger sample size.

Conclusion

In diabetic patients with nephropathy, elevated fetuin-A levels may cause endothelial dysfunction and microvascular complications. Fetuin-A may therefore can be used as predictor for the diagnosis and management of diabetic nephropathy. Additional research is necessary to clarify the role of fetuin-A in T2DM using a larger sample size and at various stages of diabetic nephropathy

References:

1. Hou Y, Gao Y, Zhang Y, Lin Si, Yu Y, Yang Liu: Interaction between ELMO1 gene polymorphisms and environment factors on susceptibility to diabetic nephropathy in Chinese Han population. Diabetol Metab Syndr. 2019, 97:1-7. 10.1186/s13098_019_0492_0
2. Morsy EY, Saad NL, Elghoneimy HA, Smeid AA, Ismail AA: Fetuin-A gene polymorphism and its serum level association with atherosclerotic vascular disease in type 2 diabetes patients with early diabetic kidney disease. BMRAT. 2020,11:4122-31. 10.15419/bmrat.v7i11.649
3. Lim AK: Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014, 7:361–81. 10.2147/IJNRD.S40172
4. Tuttle KR, Bakris GL, Bilous RW, et al.: Diabetic Kidney Disease: A Report from an ADA Consensus Conference. AJKD.2014,4:510-33. 10.1053/j.ajkd.2014.08.001
5. Furuichi K, Shimizu M, Yamanouchi M, et al.: Clinico-pathological features of fast eGFR decliners among patients with diabetic nephropathy. BMJ open diabetes res care. 2020,8:e001157. 10.1136/bmjdrc-2019-001157
6. Shim YS, Kang MJ, Oh YJ, Baek JW, Yang S, Hwang IT: Fetuin-A as an Alternative Marker for Insulin Resistance and Cardiovascular Risk in Prepubertal Children. Journal of atherosclerosis and thrombosis[JAT]. 2017, 24: 1031–38. 10.5551/jat.38323
7. El-Batch MM, Hamouda HE, Hassan SA, Mohammed WF, Horany EE: Preliminary study of biochemical role of serum fetuin-A level and its gene polymorphism in diabetic patients with microalbuminuria. JDMDC.2015,5:158-64. 10.15406/jdmdc.2015.02.00053
8. Birukov A, Polemiti E, Jäger S, Stefan N, Schulze BM: Fetuin-A and risk of diabetes-related vascular complications: A prospective study. Cardiovasc. Diabetol.2022,21:6. 10.1186/s12933-021-01439-8
9. Karajibani M, Montazerifar F, Sadeghi MB , Keikhaie MA, Dashipour A: Serum Fetuin-A and Adipsin Levels in Type II Diabetes Patients. Int J High Risk Behav. Addict. 2019, 8:e91963. 10.5812/ijhrba.91963
10. Trinder P: Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Ann.clin.Biochem.1969,6:24-27. 10.1177/000456326900600108.
11. Michael JF: Microvascular and Macrovascular Complications of Diabetes. Clin Diabetes. 2008, 2: 77–82. 10.2337/diaclin.26.2.77

12. Akad AF, Tawfeik HM, Kamel HM, Mahmoud EM: Serum fetuin-A in diabetic and nondiabetic hemodialysis patients. Minia Journal of Medical Research[MJMR]. 2023, 34: 72-77. 10.21608/mjmr.2023.187092.1300
13. Huddam B, Azak A, Koçak G. Bayraktar N, Sezer S: The relationship between serum fetuin-A, cystatin-C levels, and microalbuminuria in patients with metabolic syndrome. Journal J. Clin .Lab. Anal. 2013, 27: 317–22. 10.1002/jcla.21605
14. Mitkees NM, El-Sayed HM, Gad AM, Mansour HM, Mohamed NA: Fetuin a as an early marker of diabetic nephropathy in patients with type 2 diabetes mellitus. AIMJ/Al-Azhar Medical Journal. 2020, 492: 785-796. 10.21608/amj.2020.82592
15. Nehal AH, Taha MF, Abdel-Aziz GM, Tawab MA : Fetuin-A level in type 2 diabetic patients: relation to microvascular complications. Egypt J Intern Med[EJIM]. 2018, 30:121–130. 10.4103/ejim.ejim2418
16. Ahn MB, Kim SK, Kim SH, et al.: Clinical Significance of the Fetuin-A-to-Adiponectin Ratio in Obese Children and Adolescents with Diabetes Mellitus. Children (Basel). 2021,12:1155. 10.3390/children8121155
17. Sherif W, Ahwal LE, Elsawy AA, Attia M, Kotb NA, Eissa A: Serum level of Fetuin-A as a biomarker for vascular complications and severity of insulin resistance in individuals with type 2 diabetes. Int.J.Adv.Res.Med. 2024,2:62-70. 10.22271/27069567.2024.v6.i2a.560