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Evaluation of Serum Chromogranin A as an Early Marker of Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetic nephropathy (DN) is a significant complication of type 2 diabetes mellitus (T2DM), resulting in end-stage renal failure and chronic kidney disease. Current diagnostic methods, primarily based on microalbuminuria, have limitations, as renal function may decline before its onset. Chromogranin A (CgA), a neuroendocrine protein cleared by the kidney, was proposed as a potential early biomarker for DN. **Objective:** This research evaluates serum CgA levels in relation to different stages of albuminuria and its diagnostic utility in initial identification of DN. **Subjects and methods:** This case-control research comprised 80 patients split up into 4 groups: T2DM with normoalbuminuria (n=20), microalbuminuria (n=20), macroalbuminuria (n=20), and healthy controls (n=20). Serum CgA was determined utilizing enzyme-linked immunosorbent assay (ELISA). Other parameters comprised eGFR, serum creatinine, lipid profile, HbA1c, and fasting plasma glucose (FPG). Diagnostic accuracy was ascertained utilizing receiver operating characteristic (ROC) curve analysis. **Results:** Serum CgA levels were significantly elevated in macroalbuminuria (1606.8 ± 868.9 ng/L), microalbuminuria (706.7 ± 213.2 ng/L), and normoalbuminuria (557.6 ± 94.1 ng/L) compared to controls (500.5 ± 56.3 ng/L) ($p<0.001$). CgA positively connected to albumin-creatinine ratio ($r = 0.507$, $p < 0.001$) and negatively with eGFR ($r = -0.317$, $p=0.005$). ROC analysis showed CgA had a sensitivity of 85.0% and specificity of 75.0% at a cutoff of 610 ng/L for detecting albuminuria (AUC=0.853, $p<0.001$). **Conclusions:** Serum CgA is a promising biomarker for initial identification of DN, showing significant associations with albuminuria and renal function decline. Incorporating CgA into clinical practice may enhance early diagnosis and intervention in DN.

Keywords: Chromogranin A, Diabetic nephropathy, Type 2 diabetes mellitus, Biomarker, Renal function.

INTRODUCTION

Diabetes mellitus (DM) is a diverse collection of conditions marked by hyperglycemia brought on by a total or relative insufficiency of insulin action or production [1, 2]. The retina, kidneys, neurological system, heart, and blood vessels are among the end organs that are impacted by chronic hyperglycemia in DM [1].Diabetic nephropathy (DN) is a prevalent and severe consequence of DM that affects 25–40% of patients [3]. It significantly contributes to end-stage renal disease and chronic kidney disease (CKD) [4]. Furthermore, a significant portion of the excess mortality risk in diabetic patients is connected to presence of DN [5].

Currently, clinical diagnosis of diabetic nephropathy primarily is dependent upon detecting urine microalbuminuria. While, microalbuminuria is thought of as the most reliable method for initial identifying DN, it has a limited capacity for prediction. Before microalbuminuria appears, type 2 diabetes mellitus (T2DM) patients may occasionally have been steadily declining in renal function [6, 7]. Non-albuminuric DN is a well-established concept. There is increasing evidence that a significant number of patients with DM may experience renal function decline without overt proteinuria or even with normoalbuminuria [8, 9]. In recent decades, substantial efforts have been created to identify and confirm substitute biomarkers, as eGFR and albuminuria have limits in early diagnosis of DN [10]. Chromogranin A (CgA) an acidic protein that is composed of 439 amino acids and has a molecular weight of 48 kDa. It is expressed by a variety of both normal and neoplastic cells in diffuse endocrine and neuroendocrine systems, and by certain cancer cells that are undergoing neuroendocrine differentiation [11]. Nowadays, plasma serum CgA is frequently utilised as a prognostic and diagnostic marker, and to track how well pharmacotherapeutic treatments are working in conditions such endocrine malignancies, heart failure, hypertension, and neurodegenerative and neuropsychiatric illnesses [12]. Kidney is the primary site for CgA removal, and its levels increase in the serum as renal function declines [13]. Like other proteins with a low molecular weight eliminated by the kidney, CgA is closely associated with GFR. However, serum CgA levels rise more significantly in renal failure than creatinine levels [14]. CgA was demonstrated to be elevated in diabetic patients [15], however its connection to DN is not yet well-established, and only limited research has been conducted on this topic. Therefore, this study aimed to estimate serum CgA levels, analyze their association with different grades of albuminuria, and determine this biomarker's sensitivity and specificity for DN early detection.

PATIENTS AND METHODS

This was a case-control research that was performed on 80 subjects older than 18 years, both sexes, recruited from wards of Internal Medicine Department in Tanta University Hospitals throughout the time frame from February 2023 to July 2023. American Diabetes Association's criteria were utilized to diagnose T2DM.

Subjects were further subdivided into four equal groups: Group (I): 20 patients with T2DM with normoalbuminuria, Group (II): 20 patients with T2DM with microalbuminuria, Group (III): 20 patients with

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T2DM patients with macroalbuminuria and Group (VI): non-diabetic apparently healthy subjects.

Exclusion criteria: Pregnant women, patients with known neuroendocrinal and other malignancies, rheumatoid arthritis and systemic lupus erythematosus patients, patients with cirrhosis and chronic hepatitis, patients with inflammatory bowel diseases, patients with chronic heart failure or other renal conditions like glomerulonephritis, and patients on histamine type-2 receptor antagonists or proton pump medications.

All subjects underwent thorough history taking, complete clinical examination, and laboratory tests comprising fasting plasma glucose (FPG), two hours postprandial glucose (2HPP), glycated haemoglobin (HbA1c), lipid profile (including serum cholesterol, serum triglycerides, LDL and HDL), serum creatinine and serum urea, Albumin/creatinine ratio (ACR), estimated glomerular filtration rate (eGFR) and serum chromogranin A (CgA).

Blood sampling

Quality control and safety procedures for sample collection: Venous blood samples (7 mL) were collected in plain vacutainer tubes under strict quality control and safety protocols. Of this, 2 mL was added to an EDTA tube for glycosylated hemoglobin percentage (HbA1c%) measurement. The remaining 5 mL was used for other laboratory investigations, including CgA assessment. Serum was separated from blood by centrifuging it for 10 minutes at 3000 rpm, which was stored in Eppendorf tubes at −20°C until analysis.

Serum samples were transported to laboratory within two hours of collection for analysis. Routine investigations included FPG, 2h postprandial glucose, serum creatinine, serum urea, and lipid profile (serum cholesterol, serum triglycerides, LDL, and HDL), which were measured using the Konelab PRIME 60i. Hemoglobin A1c (HbA1c) was analyzed utilizing SIEMENS Dimension system, and eGFR was measured utilizing CKD-EPI creatinine equation [16]: GFR = 141 * min (Scr/κ, 1) α * max (Scr/κ, 1)-1.209 * 0.993Age * 1.018 [if female] * 1.159 [if black]. Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, and "min" and "max" indicate minimum or maximum values of Scr/κ or 1, respectively.

Urine sample collection and analysis: Morning urine samples (30 mL) were collected in aseptic plastic containers and centrifuged at 1000 × g for 20 minutes to eliminate particles. A 15 mL aliquot was used immediately to measure the albumin-creatinine ratio (ACR) using the Konelab PRIME 60i.

Measurement of human chromogranin A (CgA):

CgA levels were measured employing a double-antibody sandwich ELISA (Catalogue No. 201-12-1731). The test kit provided a standard reagent that was diluted in compliance with the manufacturer's guidelines. Each sample was prepared based on the required quantity.

Procedure:

- **Blank wells:** CgA antibody labeled with biotin and Streptavidin-HRP were not included; only Stop Solution and Chromogen solutions A and B were used.
- **Standard wells:** 50 µL of the standard was mixed with 50 µL of Streptavidin-HRP (pre-combined with biotin antibody).
- **Test wells:** 40 µL of sample was mixed with 10 µL of CgA antibody and 50 µL of Streptavidin-HRP.

All wells were sealed with a membrane, incubated for 60 minutes at 37°C after being gently shaken.

Washing and color development: Distilled water was used to dilute the 30× washing concentration. After gently removing the membrane, the liquid was drained and any leftover water was shaken away. Each well was filled with 50 µL of chromogen solutions A and B, which were then carefully mixed and allowed to sit at 37 °C for 10 minutes in dark. Following adding 50 µL of stop solution to each well, the color instantly altered from blue to yellow.

Measurement and analysis: After injecting stop solution, optical density (OD) was determined at 450 nm within 15 minutes. Utilizing the standard concentrations and matching OD values as a basis, linear regression was used to create the standard curve. CgA concentrations were computed using the regression equation and sample OD values.

Ethical considerations: After being approved by Tanta University Hospitals' Research Ethics Committee, the study was executed (Approval code: 36264MS5/1/23). Prior to enrolment, all subjects provided written informed permissions. The consent form explicitly detailed their agreement to take part in the research and for the publication of data, while ensuring the confidentiality and privacy of their personal information. Ethical guidelines provided in Declaration of Helsinki were followed in the conduct of this study, guaranteeing compliance with global norms for research involving humans.

***Statistical Analysis*:** Data analysis was done with IBM SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Histograms and the Shapiro-Wilks test were utilized to determine normality. Tukey's post hoc analysis and ANOVA tests were utilized to analyse parametric quantitative data, which were displayed as mean ± standard deviation (SD). Utilizing Kruskall-Wallis test, non-parametric quantitative data were analysed and displayed as median and interquartile range (IQR). Chi-square analysis was employed to examine qualitative data, which were displayed as percentages and frequencies. To evaluate correlations between variables, Pearson's correlation coefficient was utilized. To determine diagnostic performance, receiver operating characteristic (ROC) curve analysis was employed to identify negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity. A statistically significant value was obtained when two-tailed P-value was ≤ 0.05.

RESULTS

No significant variation was existed between both groups concerning age, sex distribution and BMI (P = 0.455, 0.810 and 0.274 respectively). However, statistically significant difference was noted between studied groups regarding disease duration, SBP and DBP (P = 0.007, 0.023 and 0.039 respectively) (Table 1).

Table (1): Comparison between all groups under study concerning demographic and clinical data

		Groups				P-Value
		Group I (n=20)	Group II (n=20)	Group III (n=20)	Group VI (n=20)	
Age (Years)		55.70±11.5	55.95±10.7	59.4±8.9	54.40±9.5	0.455
Sex	**Male**	11 (55%)	11 (55%)	13 (65%)	10 (50%)	0.810
	Female	9 (45%)	9 (45%)	7 (35%)	10 (50%)	
BMI (Kg/m^2)		29±2.5	30.1±2.1	30.4±2.7	29.4±2.6	0.274
disease duration (Years)		10.4±4.9	12.0±7.5	16.9±7.0	-	0.007*****
SBP (mmHg)		126.0±13.1	130.0±12.6	130.5±18.5	118.0±11.1	0.023*****
DBP (mmHg)		82.0±9.5	83.0±12.2	86.0±15	75.6±8.3	0.039*****

Data are presented as mean ± SD or frequency (%), BMI: Body Mass Index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, *: statistically significant result.

Regarding FPG, 2HPP and HBA1c, mean fasting blood glucose was statistically significantly larger in microalbuminuria & macroalbuminuria patients in contrast to control & normoalbuminuria groups (P value < 0.001). Mean 2-h postprandial blood glucose was statistically significantly larger in microalbuminuria & macroalbuminuria patients as opposed to control & normoalbuminuria groups (P value < 0.001).

Mean HbA1c was statistically significantly larger in macroalbuminuria patients in contrast to control, normoalbuminuria & microalbuminuria groups (P value <0.001).

Concerning renal function tests, mean blood urea was statistically significantly greater in macroalbuminuria patients in contrast to control, normoalbuminuria and microalbuminuria groups. Also, mean urea was statistically significantly greater in microalbuminuria patients in contrast to control and normoalbuminuria group (P value <0.001).

Mean serum creatinine was statistically significantly larger in patients with macroalbuminuria in contrast to control, normoalbuminuria & microalbuminuria groups.

Also, mean serum creatinine was statistically significantly larger in microalbuminuria patients in contrast to control & normoalbuminuria groups (P value <0.001). Mean eGFR was statistically significantly reduced in macroalbuminuria patients in contrast to control, normoalbuminuria & microalbuminuria groups. Also, mean eGFR was statistically significantly reduced in microalbuminuria patients in contrast to control & normoalbuminuria groups (P value <0.001). Concerning mean ACR, it was statistically significantly bigger in macroalbuminuria patients in contrast to control, normoalbuminuria & microalbuminuria groups. Also, mean ACR was statistically significantly greater in microalbuminuria patients in contrast to control & normoalbuminuria groups.

Mean ACR was statistically significantly larger in macroalbuminuria patients in contrast to control, normoalbuminuria & microalbuminuria groups. Also, mean ACR was statistically significantly larger in microalbuminuria patients in contrast to control & normoalbuminuria groups (P value <0.001).

Regarding mean serum chromogranin A, its level was statistically significantly bigger in macroalbuminuria patients in contrast to control, normoalbuminuria & microalbuminuria groups.

Also, mean serum chromogranin A level was statistically significantly larger in microalbuminuria patients in contrast to control and normoalbuminuria groups. Moreover, it was statistically significantly larger in normoalbuminuria group in contrast to control group (P value <0.001) (Table 2).

Table (2): Comparison between all groups under study concerning laboratory findings

	Group I (n=20)	Group II (n=20)	Group III (n=20)	Group VI (n=20)	Test	P-value
FPG (mg/dl)	158.9±40.9	232.6± 58.9	209.7±6.5	86.7±6.9	36.318(a)	**0.001***
2HPP (mg/dl)	189.7±38.9	252.4±53.7	243.0±57.6	127.6±7.4	34.050(a)	**0.001***
HBA1c (%)	8.0±0.8	9.9±1.6	10.9±1.5	5.1±0.3	18.992(a)	**0.001***
Cholesterol (mg/dl)	188.8±19.2	193.1±28.0	218.0±19.5	158.7±31.7	18.595(a)	**0.001***
LDL (mg/dl)	125.0±24.5	137.1±31.4	170.1±22.9	97.1±24.9	26.778(a)	**0.001***
HDL (mg/dl)	46.3±8.2	43.6±8.2	38.9±7.8	56.4±6.7	18.143(a)	**0.001***
TG (mg/dl)	135.8±29.2	152.8±6.6	166.1±30.7	107.4±22.7	8.400(a)	**0.001***
Urea (mg/dl)	44.7±5.6	55.2±9.4	59.4±8.9	27.7±5.02	36.637(a)	**0.001***
Creatinine (mg/dl)	0.9±0.2	1.2±0.2	1.7±0.2	0.8±0.1	34.682(a)	**0.001***
eGFR (ml/min/1.73m^2)	83.3±18.3	59.5±5.6	40.4±10.1	91.0±10.9	52.146(a)	**0.001***
ACR (mg albumin/g Cr)	20.9±4.7	116.9±5.1	473.7±57.3	17.1±3.7	136.165(a)	**0.001***
Chromogranin A (ng/l)	557.6±94.1	706.7±13.2	1606.8±68.9	500.5±56.3	45.857(b)	**0.001***

Data were displayed as mean ± SD or median (IQR). *Significant P value <0.05. FPG: Fasting plasma glucose, 2HPP: Two hours post prandial, HBA1c: Glycated hemoglobin, LDL: Low-density Lipoprotein Cholesterol, HDL: High-density Lipoprotein Cholesterol, TG: Triglycerides, ACR: albumin creatinine ratio, eGFR: estimated glomerular filtration rate, (a): ANOVA Test, (b): Kruskal-Wallis H test, *: statistically significant result.

In diabetic patients, statistically significant positive connection existed between serum Chromogranin A level with duration of diabetes, SBP, DBP, FBG, 2hPP, HbA1c, total cholesterol, LDL and creatinine. There was negative correlation with eGFR. Also, no connection between serum Chromogranin A level and BMI, TG, HDL and urea (Table 3).

Table 3: Connection between Chromogranin A and other parameters

Variables	Chromogranin A	
	r_s	P
Disease duration	0.305**	0.007*
BMI	-0.193	0.090
SBP	0.251*	0.026*
DBP	0.255*	0.024*
FBS	0.306	0.006*
2 hours post prandial	0.299	0.008*
HbA1c	0.382	0.001*
Cholesterol	0.293	0.009*
TG	0.123	0.284
LDL	0.314	0.005*
HDL	-0.145	0.205
Urea	0.126	0.273
Creatinine	0.354	0.001*
eGFR	-0.317	0.005*
ACR	0.507	0.001*

*Significant P value <0.05. rs: spearman correlation, BMI: Body Mass Index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure FPS: Fasting plasma glucose, 2HPP: two hours post prandial: HbA1c: Glycated haemoglobin, TG: Triglycerides, LDL: Low-density Lipoprotein Cholesterol, HDL: High-density Lipoprotein Cholesterol, eGFR: estimated glomerular filtration rate, ACR: albumin creatinine ratio.

Binary logistic regression revealed that factors that significantly increased the presence of diabetic nephropathy even after adjustment were chromogranin A (OR: 1.803 & p=0.001), eGFR (OR: 6.359 & p=0.032), and creatinine (OR: 1.275 & p=0.009) (Table 4).

Table (4): Binary logistic regression analysis for factors determining diabetic nephropathy among the study patients

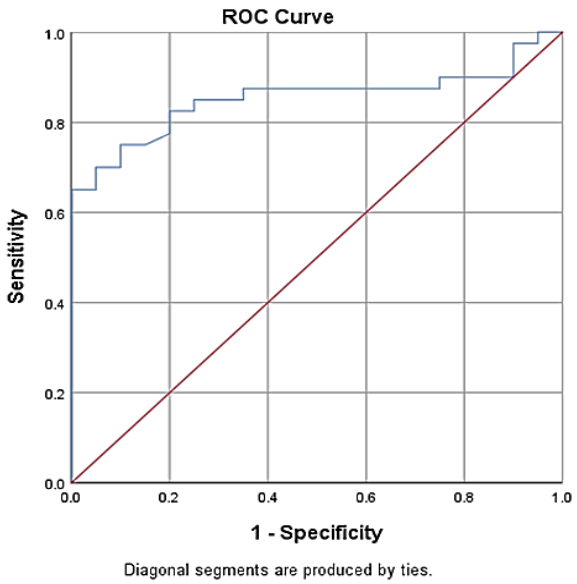
Variables	B	S.E.	Sig.	OR	95% CI	
					UL	LL
Chromo-granin A	-0.589	0.172	0.001*	1.803	1.286	2.527
FBS	-0.151	0.128	0.238	1.164	0.905	1.497
2HPP	-0.059	0.063	0.352	1.060	0.937	1.200
HbA1c	-0.084	0.053	0.111	1.087	0.981	1.205
Urea	-1.223	1.036	0.238	0.294	0.039	2.244
Creatinine	-0.243	0.094	0.009*	1.275	1.062	1.532
eGFR	-1.850	0.864	0.032*	6.359	1.169	34.590
ACR	0.001	0.002	0.549	0.999	0.995	1.003

B: Beta coefficient, S.E.: Standard Error, Sig.: Significance, OR: Odds Ratio, CI: Confidence Interval, UL: Upper Limit, LL: Lower Limit, FBS: Fasting Blood Sugar, 2HPP: Two-Hour Postprandial Glucose, HbA1c: Glycosylated Hemoglobin, eGFR: Estimated Glomerular Filtration Rate, ACR: Albumin-Creatinine Ratio.

Validity of Chromogranin A for initial identification of microalbuminuria and macroalbuminuria in diabetic patients: To investigate the diagnostic ability of CgA for detection of microalbuminuria in patients with DN, ROC curve analysis of CgA levels in type II DM patients was performed. As shown in table (5) and figure (1), AUC exhibited a sensitivity of 85.0% and a specificity of 75.0% for albuminuria identification.

Table (5): Agreement sensitivity, specificity of Chromogranin A for detecting albuminuria.

	Cut-off	AUC	P	Sensitivity	Specificity
Chromo-granin A	610.0	0.853	<0.001	85.0	75.0



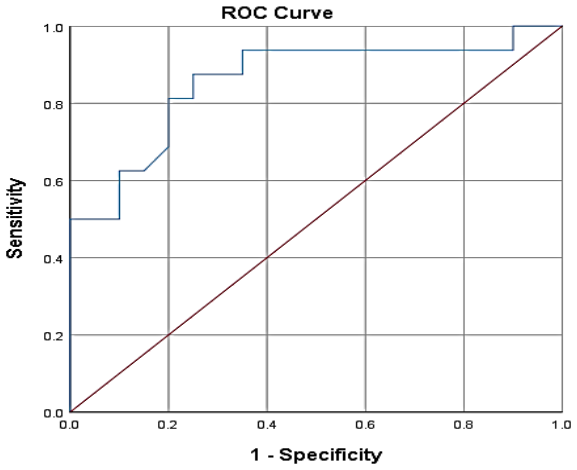
AUC: area under the curve.

Figure (1): Receiver operating characteristic (ROC) curve analysis of Chromogranin A levels for differentiating (macro and microalbuminuria) from normoalbuminuria.

As shown in table (6) and figure (2), AUC exhibited a sensitivity of 93.8% and a specificity of 70.0% for differentiating microalbuminuria and normoalbuminuria.

Table (6): Agreement sensitivity, specificity of Chromogranin A for differentiating microalbuminuria and normoalbumiura.

	Cutoff	AUC	P	Sensitivity	Specificity
Chromo-granin A	575.5	0.858	<0.001	93.8	70.0



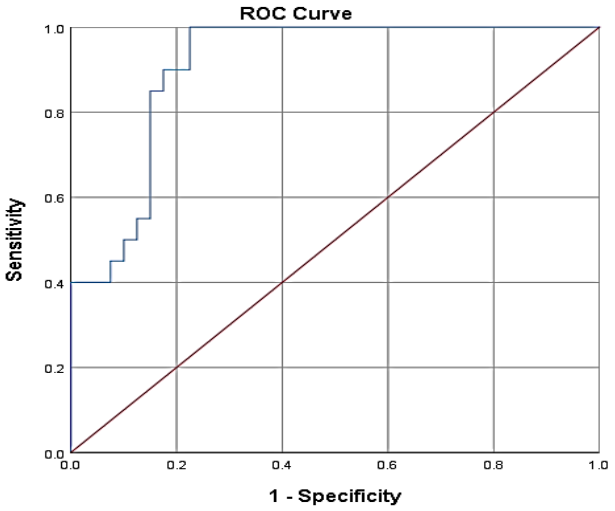
AUC: area under the curve.

Figure (2): Receiver operating characteristic (ROC) curve analysis of Chromogranin A levels for differentiating microalbuminuria and normoalbuminuria.

As shown in table (7) and figure (3), AUC exhibited a sensitivity of 95.0% and a specificity of 90.0% for differentiating microalbuminuria and macroalbuminuria.

Table (7): Agreement sensitivity, specificity of Chromogranin A for differentiating microalbuminuria and macroalbuminuria.

	Cutoff	AUC	P	Sensitivity	Specificity
Chromo-granin A	789.5	0.900 *	0.001*	95.0%	90.0%



AUC: area under the curve.

Figure 3: Receiver operating characteristic (ROC) curve analysis of Chromogranin A levels for differentiating microalbuminuria and macroalbuminuria.

DISCUSSION

Diabetes mellitus is a chronic illness that is a significant public medical issue globally. Its prevalence continues to rise in both numbers and significance across nearly all countries. Concerning International Diabetes Federation (IDF), 10.5% of adults between 20 and 79 years had diabetes in 2021, and nearly half of those individuals were unaware that they had the disease. T2DM affects more than 90% of diabetics. Concerning IDF forecasts, 1 in 8 adults will have DM by 2045, a 46% rise [17].

Diabetic nephropathy is a significant clinical and public health challenge. It is the primary reason for CKD in the developed areas and a frequent side effect of diabetes. About 40% of those with T2DM go on to develop DN [18]. A prolonged increase in urine albumin excretion (albuminuria), a low eGFR, or other signs of kidney impairment are used to diagnose DN [19]. The hallmark of DN is usually the development of proteinuria, which is followed by a progressive deterioration in renal function. But without proteinuria, some diabetic patients have vascular problems and impaired renal function [20]. Additionally, it has been noted that a significant amount of renal damage happens prior to the development of microalbuminuria [21]. Recently, 20–40% of people with T2DM may have deteriorating kidney function without albuminuria [22]. Moreover, diabetic patients can exhibit advanced diabetic glomerular changes on biopsies even in the absence of proteinuria [23]. As a result, recent studies have concentrated on finding and assessing biomarkers for diabetic kidney damage. Since early management might slow the progression of renal function loss and reduce bad outcomes, diagnostic indicators are essential for initial detection of DN.

Numerous endocrine, neuroendocrine, and neuronal cells express numerous acidic soluble proteins that make up the granin family [11]. Furthermore, chromaffin cells of adrenal medulla, granins are abundantly expressed in enterochromaffin cells, intestinal neuroendocrine cells, pancreatic islet cells, anterior pituitary cells, parathyroid chief cells, neurons of central and peripheral nervous systems and thyroid C cells [24, 25]. Chromogranin A is a secretory protein made up of 439 amino acids and having a molecular weight of 48 kDa. It was first discovered in the adrenal medulla, hence its name [11]. Human CgA gene is 12,192 base pairs long, with 8 exons and 7 introns, and it is found on chromosome 14q32.12. It produces a transcript with 2,041 base pairs that codes for a mature protein with 439 amino acids [26].

Nowadays, plasma CgA is frequently employed as a predictive and diagnostic marker as well as to track how well pharmacotherapeutic treatments are working for a number of illnesses, such as heart failure, endocrine tumors, neuropsychiatric disorders, neurodegenerative diseases, and hypertension [11, 12]. CgA is mostly cleared by the kidney, and as renal function deteriorates, its serum levels rise. Furthermore, there is a substantial correlation between serum CgA concentrations and eGFR [27].

In our research, a statistically significant variation was existed in mean urea levels among the studied groups with the highest mean urea level observed in diabetic patients with macroalbuminuria. Similarly, a significant difference in mean creatinine levels was noted with the highest mean creatinine level found in diabetics with macroalbuminuria. Furthermore, mean eGFR levels significantly differed between the groups with the lowest mean eGFR level recorded in diabetics with macroalbuminuria. Serum CgA levels in our study were positively correlated with creatinine and negatively connected to eGFR. These findings are supported by **Bech *et al.*** [28], who investigated how plasma levels of neuroendocrine neoplasia biomarkers, such as CgA, were affected by renal impairment. Their study revealed that median CgA concentrations were greater in CKD patients than in healthy controls. CgA concentrations were elevated as eGFR decreased, with the greatest median concentrations observed in patients with eGFR values < 15 mL/min/1.73 m^2.

Our study also demonstrated that serum CgA levels were positively connected to urinary albumin-to-creatinine ratio (UACR). Mean serum CgA levels were substantially greater in macroalbuminuria patients in contrast to those with normoalbuminuria, microalbuminuria, and the control groups. Similarly, mean serum CgA levels were substantially greater in microalbuminuria group in contrast to control and normoalbuminuria groups. Moreover, serum CgA levels in normoalbuminuria group were substantially greater than those in control group. These outcomes are in line with those of **Hui Yu *et al.*** [29], whose study revealed that serum CgA levels were greater in T2DM patients in contrast to healthy cases. Their study also showed significant differences in serum CgA levels across diabetic nephropathy (DN) groups, with levels increasing in tandem with the severity of DN. Additionally, they reported a positive connection between serum CgA levels, diabetes duration, and UACR, as observed in our study.

Our findings are further supported by **Ghaffar *et al.*** [30], whose results indicated that serum CgA levels were greater in diabetic patients in contrast to healthy cases, with levels increasing in proportion to the degree of DN. Their research also revealed a strong positive connection between serum CgA levels and HbA1c, as well as UACR, while showing a negative connection with eGFR in diabetic patients. Similarly, our study aligns with the recent findings of **Morsy *et al.*** [31], whose research demonstrated that serum CgA levels were elevated in diabetic kidney disease patients (UACR >30 mg/g) compared to diabetics without kidney disorder (UACR <30 mg/g), indicating that higher serum CgA levels serve as a sensitive predictor to develop diabetic kidney disease.

Limitations: The findings of our research support the possibility of CgA as an initial biomarker for identification of DN. However, our research has some limitations, comprising a relatively small sample size, its cross-sectional design, and its conduction at a single center.

CONCLUSION

Serum CgA is a promising biomarker for initial identification of DN, showing significant associations with albuminuria and renal function decline. Incorporating CgA into clinical practice may enhance early diagnosis and intervention in DN.

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