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Original Article

Assessment of Serum C-Terminal Agrin Fragment in Chronic Kidney Disease Patients

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ABSTRACT

Background: Chronic kidney disease (CKD) is a prevalent and progressive condition associated with significant morbidity and mortality. Early detection is essential for improving patient outcomes. C-terminal Agrin fragment (C-TAF), a degradation product of Agrin, has emerged as a potential biomarker reflecting glomerular and renal injury. Our study aimed to investigate and evaluate serum (C-TAF) levels in CKD patients to evaluate its role in early prediction and better management of disease progression. **Methods:** This case control study was carried out on 85 individuals recruited from the outpatient clinics and inpatient wards of the Department of Nephrology, Zagazig University Hospitals. The participants were divided into two main groups; 17 healthy individuals (Control group) and 68 age- and gender-matched patients with known CKD, further subdivided into four subgroups, each comprising 17 patients, according to their disease stage based on the KDIGO classification (Cases group). Using the Modification of Diet in Renal Disease (MDRD) formula, serum (C-TAF) by ELISA, the estimated glomerular filtration rate (eGFR) was determined for each patient. **Results:** Compared to healthy controls, CKD patients had significantly higher serum (C-TAF) levels ($p<0.001$), and these levels increased as CKD stages progressed. The best cutoff value for predicting CKD was ≥207.1 pg/ml (AUC=0.898, sensitivity=82.4%, specificity=88.2%). C-TAF showed high diagnostic performance in identifying early-stage CKD, particularly stage 1 at a cutoff ≥124.85 pg/ml (AUC=0.73, sensitivity=100%). **Conclusion:** that C-TAF level increased with progression of kidney impairment stages so it could serve as a promising non-invasive biomarker for prediction of progression of CKD, potentially aiding in diagnosis and management of the disease.

Keywords: Chronic Kidney Disease; C-terminal agrin fragment; eGFR

INTRODUCTION

Chronic kidney disease (CKD) is a rapidly growing health issue worldwide , and is defined as persistant structural or functional renal damage for more than three months that could progress to end stage renal disease requiring renal replacement therapy (either dialysis or kidney transplantation) with its negative impact on patient morbidity and mortality [1].

The most common risk factors for chronic kidney disease are hypertension and diabetes mel litus, while other less common causes include glomerulonephritis, interstitial nephritis, polycystic kidney, reflux nephropathy, obstructive uropathy and certain medications as chemotherapy [2]. Chronic renal disease is a progressive and non curable disease, thus early diagnosis is crucial to slowdown the disease progression and avert

complications specially end stage renal disease (ESRD) [3].

Agrin is a large proteoglycan encoded by the AGRN gene and named for its role in acetylcholine receptor aggregation during sysynaptogenesis). It plays a part in the establishment of the neuromuscular junction during embryogenesis [4]. In addition to being an important part of the glomerular basement membrane's proteoglycans and could be involved in cell-matrix interactions and renal filtration. In the kidney, Agrin is highly expressed and plays a significant role in the creation of the glomerular basement membrane, connection with podocyte and subsequently regulates the filter acting capacity of renal golmeruli [5].

Aim of the work

We aimed to evaluate the role of serum c-terminal a grin fragment as an early predictor of CKD progression in non dialysis CKD patients.

METHODS

This case controll study included 17 healthy individuals as controls and 68 patients with established chronic kidney disease (CKD), recruited from the outpatient clinics and inpatient wards of the Department of Nephrology. The estimated glomerular filtration rate (eGFR) was calculated for all patients using the computerized Modification of Diet in Renal Disease (MDRD) equation. Based on eGFR values, patients were classified into CKD stages as follows: stage I (eGFR ≥ 90 mL/min), stage II (60–89 mL/min), stage III (30–59 mL/min), and stage IV (15–29 mL/min). Patients undergoing regular hemodialysis were excluded from the study.

Inclusion criteria were both male and female participants aged 18 years or older with a documented history of renal impairment lasting more than three months. Exclusion criteria included patients with sepsis, acute kidney injury (AKI), post-myocardial infarction, muscle wasting diseases, recent administration of nephrotoxic drugs, or chronic liver diseases. In addition, ESRD patients on dialysis, individuals with malignancies, and pregnant women were excluded from the study.

Every participant underwent a thorough physical examination, a detailed history taking, and standard laboratory testing, such as serum electrolytes, kidney and liver function tests, and complete blood counts (CBCs) urine analysis, urinary albumin-to-creatinine ratio (UACR), lipid profile, random and fasting blood glucose levels were among the calculated parameters. In addition, a 24-hour urine protein test and renal biopsy were performed in selected cases when clinically indicated.

Each patient's estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula as follows:

eGFR = 186 × (Pcr) -1.154 × (Age in years) -0.203 [6]

For female participants, the result was multiplied by **0.**742, and for Black individuals, it was multiplied by **1.21**. Plasma creatinine (Pcr) was expressed in mg/dL.

Estimation of Serum C-Terminal Agrin Fragment (C TAF)

Serum levels of C-terminal a grin fragment (C TAF) were assessed through the use of the enzyme-linked immunosorbent assay (ELISA) method. Anti-CAF antibodies were pre-coated onto 96-well plates as part of the sandwich ELISA approach that underpinned the experiment. The detecting antibodies were biotin-conjugated anti-CAF antibodies. Following the sequential addition of standards, test samples, and biotin-conjugated detection antibodies, the wells were cleaned using wash buffer. Unbound conjugates were removed by further washing following the addition of HRP-Streptavidin. The enzymatic reaction was seen using the TMB (tetramethylbenzidine) substrate, which HRP catalyzed to provide a blue result. When an acidic stop solution was applied, the color changed to yellow. A microplate reader was used to measure the optical density (O.D.) at 450 nm in order to calculate the C TAF concentrations in the samples.

Ethical approval:

The Institutional Review Board (IRB#11088) at Zagazig University's Faculty of Medicine gave its approval to the study (IRB). Every study procedure complied with the Declaration of Helsinki's and its

modifications' ethical guidelines. Prior to enrollment, all individuals provided written informed consent.

Statistical analysis

The statistical package for the social sciences, or SPSS, version 28 was used to analyze the data. The chi square test and, when applicable, Monte Carlo tests were used to compare categorical variables, which were presented using their absolute frequencies. Ordinal data between two groups was examined using the chi square trend test. The Kolmogorov-Smirnov test was used to confirm the assumptions for parametric testing. To compare quantitative data between more than two groups, the Kruskal Wallis test (for non-normally distributed data) and the one-way ANOVA test (for normally distributed data) were used Fisher LSD and pairwise comparison were used to identify differences between each of the two individual groups when the difference was significant. The degree of association and the Spearman rank correlation coefficient (for non-normally distributed data) and Pearson correlation (for regularly distributed variables) were used to evaluate the correlation between two continuous variables. When diagnosing a certain health issue, the ROC curve was utilized to establish the best cutoff of a particular quantitative parameter. To measure related independent components for the dependent factor, linear regression analysis was used. To find independent risk factors linked to specific health issues, binary logistic regression was employed. The criterion for statistical significance was set at $P<0.05$. If $p\leq0.001$, there was a highly significant difference.

RESULTS

This study included 85 participants divided into five groups; control group and four patients with different stage of chronic kidney disease we found that there was statistically significance regarding BMI, systolic and diastolic blood pressure but no significant regarding sex, diabetes or smoking as showing in (Table 1) .

Hemoglobin, total cholesterol, calcium, phosphorus, ptassium, creatinine, urea, UACR, and eGFR were all statistically significant in our investigation, however TLC, platelets, AST, and ALT were not, as shown in Table 2.

Table 3; demonstrated that the groups under study differed statistically significantly regarding Serum C-Terminal Agrin Fragment (C-TAF). On doing posthoc test, difference is significant between stage 4 CKD and each other group. The difference is significant between stage 2 and each other group except stage 3 CKD. The difference is significant between control group and each other group except group 1 CKD. Increasing C-TAF significantly increases risk of CKD (versus control) by 1.02 folds ($p<0.001$). Increasing C-TAF significantly increased risk of stage 1 by 1.01 folds (stage I versus control), stage 2 by 1.04 folds (versus stage I and control), stage 3 by 1.02 folds (stage 3 versus control, stage 1 and 2 CKD) and 1.02 folds (stage 4 versus control, stage 1, 2 and 3 CKD).

The best cutoff of C-TAF in prediction of chronic kidney disease is ≥207.1 pg/ml with area under curve 0.898, sensitivity 82.4%, specificity 88.2%, positive predictive value 96.6%, negative predictive value 55.6% and overall accuracy 83.5% ($p<0.001$) as showing in (Figure 1).

The best cutoff of C-TAF in prediction of stage 4 chronic kidney disease is ≥309.25 pg/ml with area under curve 0.92, sensitivity 88.2%, specificity 80.9%, positive predictive value 53.6%, negative predictive value 96.5% and overall accuracy 82.4% ($p<0.001$). as shown in table (4).

There is statistically non-significant relation between C-TAF and either gender, diabetes or smoking (non-significantly higher among males, diabetics and smokers as showing in (Table 5)

There is statistically non-significant relation between C-TAF and either gender, diabetes or smoking (non-significantly higher among males, diabetics and smokers.

Among all patients with CKD, (figure 2) there was statistically significant positive correlation between C-TAF and age, potassium, phosphorus, UACR, serum urea and creatinine. There is statistically significant negative correlation between C-TAF and BMI, hemoglobin, platelet count, calcium and sodium.

Table (1): Comparison between the studied groups regarding demographic data

	Control group	Chronic kidney disease				χ^2	P
		Stage I CKD	Stage 2 CKD	Stage 3 CKD	Stage 4 CKD		
Sex Female Male	**14 (82.4%)** **3 (17.6%)**	**5 (29.4%)** **12(70.6%)**	**8 (47.1%)** **9 (52.9%)**	**5 (29.4%)** **12(70.6%)**	**9 (52.9%)** **8 (47.1%)**	**3.665**	**0.056**
Smoking No Yes	**13 (76.5%)** **4 (23.5%)**	**11(64.7%)** **6 (35.3%)**	**11(64.7%)** **6 (35.3%)**	**10(58.8%)** **7 (41.2%)**	**14(82.4%)** **3 (17.6%)**	**0.027**	**0.869**
Diabetes No Yes	**4 (23.5%)** **13 (76.5%)**	**4 (23.5%)** **13(76.5%)**	**5 (29.4%)** **12(70.6%)**	**3 (17.6%)** **14(82.4%)**	**4 (23.5%)** **13(76.5%)**	**0.032**	**0.857**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**F**	**P**
Age (year)	**46.59±7.58**	**44.65±11.9**	**49.41±11.01**	**52.53±12.25**	**53.67±12.79**	**1.989**	**0.104**
BMI(kg/ m^2)	**20.9±2.3**	**24.9±3.95**	**27.07±4.23**	**23.92±4.36**	**23.11±2.78**	**6.68**	**<0.001****
Systolic blood pressure	**113.53±9.96**	**134.71±20.95**	**124.12±10.04**	**141.18±27.36**	**130.59±14.78**	**5.907**	**<0.001****
Diastolic blood pressure	**70.59± 8.27**	**84.71±11.25**	**77.06±18.29**	**88.24±12.89**	**82.94±9.85**	**5.188**	**<0.001****

Table (2): Comparison between the studied groups regarding basic lab:

	Control group[1]	Chronic kidney disease				F	P
		Stage I CKD	Stage 2 CKD	Stage 3 CKD	Stage 4 CKD		
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
TLC (10^3/mm^3)	**7.94±1.64**	**6.54±1.84**	**6.97±2.42**	**6.4±1.8**	**6.05±2.37**	**2.173**	**0.079**
Hemoglobin (g/dl)	**12.22 ± 1.18**	**13.59±1.49**	**11.47±1.3**	**9.17±1.06**	**8.19 ± 1.42**	**49.965**	**<0.001 ****
Platelet (10^3/mm^3)	**353.71±67.02**	**368.65±63.92**	**318.53±82.68**	**325.61±96.19**	**292.35 ± 90.06**	**2.189**	**0.078**
Total cholesterol	**203.24±18.11**	**210.12±57**	**247.4±67.5**	**249.88±41.44**	**309.35±53.58**	**11.904**	**<0.001 ****
Calcium	**9.14 ± 0.62**	**9.21±0.87**	**9.28±0.72**	**7.97±1.02**	**7.32±0.7**	**21.051**	**<0.001 ****
Phosphorus	**2.24 ± 0.48**	**2.67±0.89**	**2.65±0.65**	**4.76±1.66**	**5.71±1.51**	**30.784**	**<0.001 ****
Potassium	**4.02 ± 0.48**	**3.74± 0.4**	**3.81±0.39**	**4.44±0.79**	**4.83±1.12**	**7.466**	**<0.001 ****
Creatinine (mg/dl)	**0.7 ± 0.05**	**0.74±0.09**	**1.01±0.12**	**1.67±0.33**	**2.63±0.24**	**281.85**	**<0.001 ****
Urea (mg/dl)	**26 ± 4**	**27.12±4.83**	**33.76±8.48**	**49.76±10.53**	**62.41±10.88**	**62.279**	**<0.001 ****
eGFR (ml/min/1.73 m^3)	**118.12±9.84**	**95.12±6.51**	**72.82±8.68**	**44.94±8.67**	**22.82±4.26**	**400.868**	**<0.001 ****
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**KW**	**P**

UACR	**15 (10-20)**	**190 (116 – 340)**	**422 (333 – 555)**	**1320 (559.5–2337.5)**	**2323 (1626.5– 4322.5)**	**62.363**	**<0.001****
ALT (u/l)	**18 (14.5 – 23)**	**18(12–33.5)**	**22(12.5–31.5)**	**16(11.5– 25.5)**	**19(13.5 – 23)**	**1.394**	**0.845**
AST (u/l)	**16(14.5 – 24)**	**22(14–33.5)**	**22(15–28.5)**	**18(13–18.5)**	**22(15.5 – 28)**	**1.309**	**0.86**

F one way ANOVA test KW Kruskal Wallis test **p≤0.001 is statistically highly significant *p<0.05 is statistically significant

Table (3): Comparison between the studied groups regarding C-TAF:

pg/ml	**Control group[1]**	**Chronic kidney disease**				**F**	**P**
		Stage I CKD	**Stage 2 CKD**	**Stage 3 CKD**	**Stage 4 CKD**		
	Mean ± SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**		
C-TAF	**145.91±74.19**	**194.6±46.6**	**284.23±70.5**	**330.78±66.92**	**466.14±109.95**	**45.411**	**<0.001****
COR (95% CI)	**For CKD**	**1.02 (1.01 – 1.04)**					**<0.001****
		For stage 1	**For stage 2**	**For stage 3**	**For stage 4**		
COR (95% CI)		**1.01 (1 – 1.03)**	**1.04 (1.0 –1.06)**	**1.02 (1.01 – 1.03)**	**1.02 (1.01 – 1.03)**		
		P=0.04*	**<0.001****	**<0.001****	**<0.001****		

F one way ANOVA test **p≤0.001 is statistically highly significant
*p<0.05 is statistically significant

Table (4): Performance of serum C-terminal Agrin fragment in prediction of different stages of chronic kidney disease

Groups	**Cutoff**	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**P**
Control	**≥207.1 pg/ml**	**0.898**	**82.4%**	**88.2%**	**96.6%**	**55.6%**	**83.5%**	**<0.001****
Stage 1	**≥124.85 pg/ml**	**0.73**	**100%**	**58.8%**	**70.8%**	**100%**	**79.4%**	**0.022***
Stage 2	**≥231.3 pg/ml**	**0.908**	**94.1%**	**85.3%**	**76.2%**	**96.7%**	**88.2%**	**<0.001****
Stage 3	**≥261.85 pg/ml**	**0.872**	**82.4%**	**76.5%**	**53.8%**	**92.9%**	**77.9%**	**<0.001****
Stage 4	**≥309.25 pg/ml**	**0.92**	**88.2%**	**80.9%**	**53.6%**	**96.5%**	**82.4%**	**<0.001****

AUC area under curve, PPV positive predictive value, NPV negative predictive value **p≤0.001 is statistically highly significant

Table (5): Relation between C-TAF and gender, diabetes, smoking among patients with CKD

	Median (IQR)	**Z**	**P**
Gender **Female** **Male**	 **281.5(234.5 – 466.9)** **284.9(218.15 – 389.9)**	**-0.445**	**0.656**
Diabetes **No** **Yes**	 **285.9(231.25 – 393.93)** **281.9(222.53 – 444.08)**	**-0.022**	**0.983**
Smoking **No** **Yes**	 **275.4(231.35 – 437.43)** **291.55(212.83 – 388.58)**	**-0.033**	**0.974**

Z Mann Whitney test IQR interquartile range

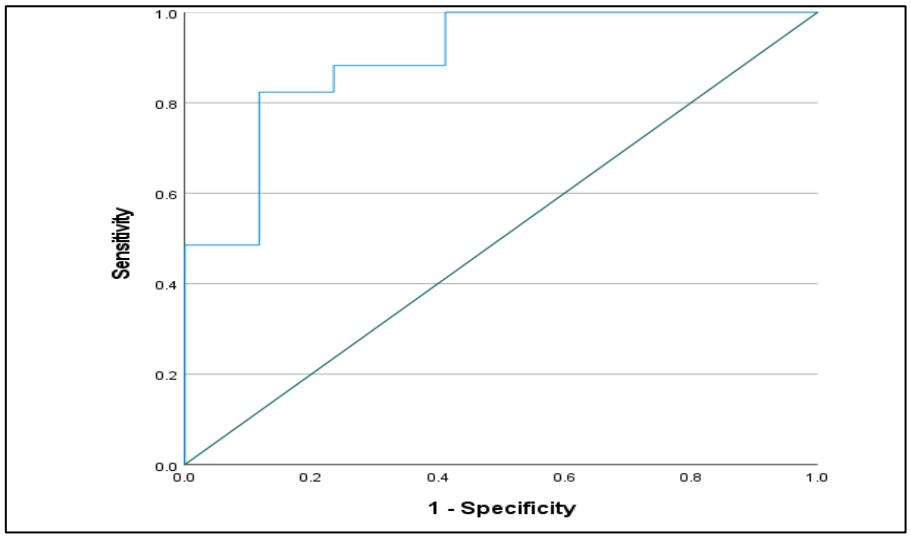

Figure (1): ROC curve showing performance of serum C-terminal Agrin fragment in prediction of chronic kidney disease

Figure (2): Scatter dot plot showing significant negative correlation between eGFR and C-TAF

DISCUSSION

With a rising prevalence of Type II diabetes mellitus, hypertension, and aging populations worldwide, chronic kidney disease (CKD) is a significant and expanding concern for health care systems. The accumulation of metabolic waste products in the blood, irregularities in electrolytes, anemia, and problems with minerals and bones are the hallmarks of chronic kidney disease (CKD). Although CKD is detectable in its early stages, it is typically asymptomatic. There is evidence that treatment can delay or prevent the CKD progression, reduce or prevent the development of complications [7]; thus finding new biomarkers that can detect early progression of CKD stages can improve disease prognosis.

With a core protein of roughly 220 kDa, Agrin is a heparin sulfate proteoglycan (HSPG) that is thought to be a crucial organizer of postsynaptic differentiation at Neuromuscular Junctions (NMJs). The kidneys' glomerular basement membranes exhibit the highest expression of Agrin, which may play a role in membrane permeability and filtration. So, this study aimed to evaluate plasma level of C-agrin fragment in relation to kidney function in patients with different stages of CKD to throw more light on its clinico-pathological significance in such cases [8] and to evaluate Agrin in early detection of CKD progression, we include 17 healthy subjects as control group and cases group included 68 patients in different stages of CKD, but not on dialysis.

We found that increasing C-TAF significantly increased risk of stage 1 by 1.01 folds (stage I versus control), stage 2 by 1.04 folds (versus stage I and control), stage 3 by 1.02 folds (stage 3 versus control, stage 1 and 2 CKD) and 1.02 folds (stage 4 versus control, stage 1, 2 and 3 CKD).

(C-TAF) levels increased progressively with worsening CKD stages, indicating its potential role as a biomarker of declining kidney function. The significant rise in C-TAF, especially in later stages, may reflect glomerular and endothelial injury, supporting its utility as a biomarker for early detection and progression monitoring in CKD [9, 10].

Supporting our study with Gehan et al. study revealed that, in comparison to the control group, the plasma levels of (C-TAF) increased gradually over the course of chronic kidney disease (CKD). The mean C-TAF level was lowest in the control group, and it gradually increased in stages I and II. This increase became more pronounced in stages III, with a sharp elevation observed in stages IV and V patients had the highest CAF levels, suggesting a clear correlation between advanced renal disease and excessive C-TAF levels [11].

In our study, (C-TAF) demonstrated strong diagnostic performance in predicting chronic kidney disease (CKD) and its individual stages. For overall CKD prediction, a C-TAF cutoff value of ≥207.1 pg/ml showed high diagnostic accuracy, with excellent sensitivity and specificity, and a robust area under the ROC curve (AUC). Stage-specific analysis revealed progressively increasing C-TAF thresholds corresponding to advancing CKD severity.

For stage 1 CKD, a lower cutoff (≥124.85 pg/ml) was associated with perfect sensitivity but moderate specificity. As disease severity increased, higher cutoff values were required ranging from ≥231.3 pg/ml for stage 2 to ≥309.25 pg/ml for stage 4 with consistently high AUCs, sensitivities, and specificities.

(C-TAF) showed strong potential as a diagnostic biomarker for chronic kidney disease (CKD), with its levels correlating closely with disease progression. As kidney function declines, C-TAF accumulates in the circulation, likely due to reduced renal clearance and increased extracellular matrix remodeling. Diagnostic analysis demonstrated that specific C-TAF cutoff values could effectively differentiate between CKD stages.

For early-stage CKD, lower thresholds were sufficient to detect subtle renal impairment, while higher cutoffs were needed for more advanced stages. The progressively rising thresholds and consistently high sensitivity and specificity values across stages underscore the role of C-TAF in both early detection and accurate staging of CKD, could indicate its pathophysiological involvement in renal tissue damage and filtration dysfunction [12].

According to Gehan et al., C-TAF levels have 89% sensitivity and 90% specificity in relation to CKD prognosis, which is consistent with our findings. Additionally, the study mentioned that C-TAF predicts CKD with 90% accuracy [11].

On the contrary, Arampatzis et al. mentioned a study investigating C-TAF's role in predicting kidney injury found low sensitivity (37%) and moderate specificity (85%), suggesting limited utility as a predictive biomarker [13].

In our research, there was slightly increase in level of C-TAF in correlation with smoking, diabetic status, and gender; but this increase was statically not significant.

Among patients with CKD, C-TAF levels showed significant positive correlations with markers of worsening renal function, such as elevated serum potassium, phosphorus, urea, creatinine, and urinary albumin-to-creatinine ratio (UACR). As well as statistically significant negative correlation with hemoglobin and calcium as anemia and Hypocalcemia are more predominant and severe with advanced CKD stages.

Conversely, C-TAF levels were significantly inversely correlated with body mass index (BMI), findings that further correlates C TAF with deteriorating nutritional status in advanced CKD [14].

CONCLUSIONS

That C-TAF level increased with progression of kidney impairment stages so it could serve as a promising non-invasive biomarker for prediction of progression of CKD, potentially aiding in diagnosis and management of the disease.

Conflict of Interest: The authors declare that they have no competing interest.

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Availability of the data: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors contribution: The authors were responsible for data collection and analysis, as well as writing and preparing the manuscript for publication. All authors reviewed and approved the final version.

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Citation

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