

Diagnostic Performance of Urinary Clusterin for Early Detection of Diabetic Kidney Disease in Pediatric Type 1 Diabetes Mellitus: A Case-Control Analysis

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Abstract

Background: Urinary Clusterin, a glycoprotein linked to oxidative stress and tubular injury, is being evaluated as a more sensitive biomarker for diabetic kidney disease (DKD). This study aims to assess the role of Clusterin in the early detection and prognostication of diabetic kidney disease in children with Type 1 Diabetes Mellitus (T1DM), addressing a critical gap in pediatric clinical data.

Methods: This case-control study at Beni-Suef University compared 50 children with T1DM (duration ≥ 5 years) to 40 healthy controls. Assessments included Body Mass Index (BMI), glycated hemoglobin (HbA1c), and lipid profiles. Urinary Clusterin was measured using Enzyme Linked Immunosorbent Assay (ELISA). A Receiver Operating Characteristic (ROC) curve analysis was employed to determine the diagnostic performance of urinary Clusterin in detecting diabetic nephropathy.

Results: Diabetic cases exhibited significantly higher levels of AST, cholesterol, triglycerides, and serum creatinine compared to the control group. The urinary profile revealed significantly elevated urinary creatinine, albumin, and albumin-to-creatinine ratios in cases, with urinary clusterin levels being significantly higher in children with diabetes (49.7 vs. 32.3 pg/mL, $p < 0.001$) than in healthy controls. Within the case group, urinary clusterin significantly correlated with ALT, serum creatinine, and triglycerides, although no significant associations were found with age, BMI, or HbA1c. At a cutoff value of 37.6 pg/mL, urinary clusterin is a highly effective marker for early detection of diabetic nephropathy with an 80% sensitivity, and 90% specificity.

Conclusion: Urinary clusterin is a potential non-invasive biomarker for early diabetic nephropathy in children with T1DM, outperforming traditional measures and offering a promising tool for earlier screening and improved clinical management.

Keywords: Urinary Clusterin, Apolipoprotein J, Type 1 Diabetes Mellitus, Diabetic Kidney Disease.

What is already known on this topic?

Microalbuminuria is the standard screening tool for diabetic kidney disease in T1DM, but it may not detect early renal injury. Urinary clusterin has been proposed as a biomarker of tubular and oxidative renal damage, although pediatric evidence remains limited.

What this study adds?

Urinary clusterin was significantly elevated in children with T1DM and showed high diagnostic accuracy for diabetic kidney involvement. These findings support urinary clusterin as a promising non-invasive biomarker for early renal injury in pediatric diabetes.

Introduction

Type 1 diabetes mellitus (T1DM) is one of the most common chronic diseases impacting pediatric populations, with 15-20% of newly diagnosed children being below 5 years of age (1, 2). Recent studies suggest that early-onset T1DM is an independent factor for the development of diabetic nephropathy (3). To prevent renal affection in children with T1DM, research has been focused on early detection. Although clinical screening traditionally relies on detecting microalbuminuria (30 to 300 mg/g) (4), evidence indicates that significant pathology, such as glomerular basement membrane thickening and nephromegaly, begins shortly after a diabetes diagnosis, often preceding detectable albuminuria (5, 6). In addition, the ability of albuminuria to predict disease progression is sometimes limited, as some diabetic patients with albuminuria experience spontaneous remission, while others progress to kidney failure (7).

Recent research examined several biomarkers elevated in the serum and urine of pediatric T1DM patients compared to healthy children, which may reflect underlying early-stage pathophysiological processes (10, 11). Many biomarkers have been explored, such as MCP-1, activin-A, and Clusterin (8). Clusterin, also known as apolipoprotein J, is a ubiquitous glycoprotein found in a myriad of tissues of the body, being noticeably upregulated in the event of kidney injury (9, 10). Data have shown concurrent upregulation of urinary clusterin and albuminuria, which may reflect renal injury (11). These findings align with in vitro increases in Clusterin levels in experimental models of insulin resistance, with positive correlations between clusterin and urinary proteins, as well as negative correlation with creatinine clearance, reflecting kidney injury (12).

The primary isoform relevant to urinary biomarkers is secretory clusterin (sCLU). sCLU acts as an extracellular chaperone, regulating the folding process of secreted proteins and signaling oxidative stress (13, 14). Moreover, it can interact with macrophage phosphoinositide 3-kinase (PI3K), an insulin signaling mediator, and the proinflammatory nuclear factor kappa B (NFkB), leading to inflammation and insulin resistance (15). In T1DM, Clusterin has shown statistically significant positive correlations with HbA1c and negative correlations with eGFR, indicating that it is a potential prognostic biomarker (16).

Although the current literature is not entirely lacking in assessing the role of Clusterin in T1DM (8, 16), a large portion of the available evidence stems from either experimental models (17), or patients with type 2 DM (T2DM) (18). Moreover, the scarcity of prospective data makes it difficult to firmly draw causal inferences between T1DM-related diabetic nephropathy and Clusterin. In this case-control study, we assess the association between urinary Clusterin and diabetic nephropathy in children with longstanding T1DM, aiming to provide data concerning the potential of urinary Clusterin in early detection and prognostication of diabetic nephropathy.

Methods

Study Design and Population

In this case-control study, conducted at the Pediatric Department of Beni-Suef University Hospital from July 2023 to January 2024, our aim was to evaluate the utility of urinary Clusterin as a diagnostic and prognostic marker of diabetic nephropathy in children with T1DM. The study population consisted of 90 participants divided into two groups:

- Patient Group: 50 children diagnosed with T1DM.
- Control Group: 40 healthy children, age- and sex-matched to the patient group.

Our patients were recruited based on predefined eligibility criteria. We included children aged 5-18 years who had an established diagnosis of T1DM for a duration of at least 5 years. Conversely, we excluded those with a history of chronic kidney disease (CKD), kidney transplant, immunotherapy within six months of the recruitment date, or refusal to provide consent.

Clinical Assessment

A comprehensive medical history was obtained for each participant, including age at disease onset, disease duration, hospital admission history, and family history of T1DM. A thorough physical examination was performed, including anthropometric measurements, such as weight, height, and BMI. BMI values were converted to z-scores based on the Egyptian growth charts. In addition, a systemic assessment of vital signs and a detailed abdominal examination were conducted.

Laboratory Investigations

Five milliliters of whole blood were collected under aseptic conditions from each subject for complete blood count (CBC), performed using a Diatron hematology analyzer. Additionally, HbA1c was measured via quantitative colorimetric ion-exchange resin chromatography (Stan Bio Laboratory), standardized to the DCCT assay and NGSP certified. Fasting blood glucose (FBG) was determined using the Trinder method on a Beckman Coulter 480 after 6–8 hours of fasting. A biochemical profile was also obtained, including serum creatinine, total cholesterol, triglycerides, HDL, and LDL, which were measured using the Beckman Coulter 480 equipment.

Renal Function Calculations

The estimated glomerular filtration rate (eGFR) was calculated using the revised Schwartz equation:

$$eGFR = 0.413 \times \left(\frac{\text{Height (cm)}}{\text{Serum Creatinine } \left(\frac{mg}{dL} \right)} \right)$$

Urine Analysis and Albumin-to-Creatinine Ratio (ACR)

Urine samples were collected for the determination of microalbuminuria, measured by quantitative turbidimetry (Biosystem kit). Albuminuria was categorized as normal (<30 mg/g), microalbuminuria (30–300 mg/g), or clinical albuminuria (>300 mg/g). Urinary creatinine was measured according to the Young method.

Urinary Clusterin (CLU) Determination

Urinary Clusterin levels were quantified using a double-antibody sandwich ELISA. Urine samples were centrifuged at 2000–3000 rpm for 20 minutes to remove precipitation and stored at -20°C. Subsequently, 40 µl of the sample was added to wells pre-coated with monoclonal anti-human clusterin antibodies, followed by 10 µl of biotin-labeled CLU antibodies and 50 µl of Streptavidin-HRP. After 60 minutes of incubation at 37°C and subsequent washing, Chromogen solutions A and B were added. The reaction was halted using a Stop Solution (shifting color from blue to yellow). The optical density (OD) was measured at 450 nm within 15 minutes. Final concentrations were determined via linear regression against a standard curve.

Statistical Analysis

Data processing was performed using SPSS version 25. Continuous variables were expressed as Mean and standard deviation (SD) or Median (IQR). Independent t-tests were used for normally distributed data, while the Mann-Whitney U test was applied for non-normal distributions. Chi-square tests were utilized for categorical comparisons. Relationships between Clusterin, HbA1c, and other parameters were assessed using Pearson or Spearman correlation coefficients. A ROC curve was constructed to evaluate the sensitivity, specificity, and Area Under the Curve (AUC) of Clusterin as a biomarker for diabetic nephropathy.

Ethical Considerations

The study was approved by the Beni-Suef University Faculty of Medicine Research Ethics Committee (Approval No: FMBSUREC/09072023/Hasan). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participants after a clear explanation of the study procedures.

Results

Our sample consisted of 90 children divided into two groups. Fifty cases with T1DM with a mean disease duration 5.12 years and 40 age- (p=0.570) and sex-matched healthy children (p=0.850). The BMI of both groups was statistically comparable (19.68 vs. 19.85 kg/m²; p=0.700).

Laboratory findings of the studied participants.

Cases exhibited significantly higher AST (p<0.001), cholesterol (p<0.001), WBCs (p=0.001), serum creatinine (p=0.010), and triglycerides (p<0.001) compared to controls. On the other hand, platelet counts (p=0.040) were significantly lower in the cases (**Table 1**).

The urinary profile indicated that the cases had significantly higher creatinine (p=0.001), albumin (p=0.016), and albumin/creatinine ratio (p=0.001). Furthermore, urinary clusterin was significantly elevated in cases compared to controls (49.7 vs. 32.3 pg/ml, p<0.001). While 100% of controls were normoalbuminuric, only 60% of cases were, with the remainder showing micro- or macroalbuminuria (p<0.001; **Table 2**).

Albuminuria was classified using the urinary albumin/creatinine ratio (ACR) as A1 (<30 mg/g), A2 (30-300 mg/g), and A3 (>300 mg/g). No participant had an ACR exactly equal to 300 mg/g, so the boundary convention does not affect the observed grouping. Clusterin concentration did not differ significantly among the three albuminuria categories by the Kruskal-Wallis test (H = 3.279, p = 0.194; **Table 3**).

Correlation between urinary parameters and Clusterin

Correlation analysis within the cases demonstrated no significant relationship between disease duration, HbA1c, or BMI and levels of Clusterin (Table 4).

Expanded correlation analysis identified that Clusterin significantly correlated with ALT ($r=-0.307$, $p=0.030$), serum creatinine ($p=0.049$), and triglycerides ($p=0.044$). (Table 4).

Predictive data

The diagnostic performance of Clusterin diabetic nephropathy was evaluated in Table 5 and Figs. 1 & 2. Our analysis revealed that urinary clusterin is a robust marker for predicting diabetic nephropathy, with an area under the curve (AUC) of 0.916 ($p<0.001$). Using a cut-off value of 37.6 pg/ml, the marker demonstrated 80% sensitivity, 90% specificity, a PPV of 90.9%, and an NPV of 78.3%.

For discrimination of A2/A3 albuminuria from A1, lower clusterin values were associated with positive status. The AUC was 0.597 (95% CI 0.435-0.759; $p=0.243$). The Youden-based criterion was clusterin <52.85 , providing 80.0% sensitivity and 46.7% specificity. For discrimination of A3 albuminuria from A1/A2, the AUC was 0.531 (95% CI 0.341-0.722; $p=0.746$). The Youden-based criterion was clusterin >45.95 , with 72.7% sensitivity and 46.2% specificity (Table 5 and Figs 2).

Discussion

In this case-control study, we aimed to shed light on the association between urinary clusterin and diabetic nephropathy in 50 children with T1DM in comparison to 40 healthy controls. We investigated our participants for urinary clusterin levels and found them to be significantly higher in cases compared to controls (49.7 vs. 32.3 pg/ml; $p<0.001$). Corroborating our findings, Fadel et al. relayed data which showed a considerably higher urinary clusterin in diabetic patients compared to controls (114.9 vs. 1.60 ng/mL; $p<0.001$) (18). He et al. observed evident glomerular clusterin upregulation in patients with diabetic nephropathy, and to further amplify their evidence, they tested the expression of clusterin with methylglyoxal-induced oxidative stress and they found that clusterin levels were expressed at higher rate on podocytes under oxidative conditions. Methylglyoxal is a precursor in the formation of glycated end products and increases ROS production, which simulates diabetic conditions (19).

Reports by Kostopoulou et al. were at odds with ours, as they inferred that there were no significant differences regarding clusterin levels between patients with T1DM when compared with healthy controls ($p>0.05$). However, their study addressed clusterin levels in the serum while ours focused on urinary clusterin levels, which might explain this discrepancy (8). Musial et al. showed that the levels of urinary clusterin rose consistently with the progression of kidney injury following stem cell transplantation in children, and this was statistically significant for both oncological and non-oncological patients compared with the healthy controls ($p<0.05$) (20). Under a divergent protocol, Abd El-Khalik et al. aimed to explore the role of urinary clusterin in renal damage in children with beta-thalassemia major and they found that levels were significantly higher in cases than in healthy controls (667 vs. 324 ug/mL; $p=0.007$) (21).

We hypothesized that this overexpression of clusterin in diabetic subjects could be a means for the body to protect itself from oxidative stress. Studies have shown that clusterin has many isoforms, one of them being intracellular and binds to the endoplasmic reticulum to function as an inhibitor to the formation of the Bax-Bak complex, a pro-apoptotic complex, thus preventing apoptosis of cells. Moreover, it could cut short the arrival of Bax to the mitochondria by directly stabilizing Ku70-Bax complex in the cytoplasm. These mechanisms are meant to prevent cellular damage by oxidative stress, such as those seen under diabetic conditions (22-25). Although most studies described clusterin as a protective molecule, other studies have shown its direct implication in systemic inflammation and the development of insulin resistance through the binding to LPR2 which promotes gluconeogenesis, inhibits insulin signaling, and leads to the development of diabetes, obesity, and cardiovascular diseases (26, 27).

We attempted to draw correlation between age and clusterin levels as well as HbA1c and noted that the only significance was a positive correlation between age and HbA1c ($r=0.318$, $p=0.024$). Kostopoulou et al. contrasted these findings as they observed a positive correlation between clusterin levels and age ($r=0.268$, $p=0.045$). It is worth noting that their study focused on serum clusterin, not urinary clusterin, which could be correlated with other disease entities as it is present in many tissues in the body in addition to the kidney (8). Abd El-Khalik et al. found no significant correlation between urinary clusterin and the age of children ($p=0.059$) (21). No statistically significant correlations were noted between either clusterin or IgM and disease duration, HbA1c, or BMI. Kostopoulou et al. endorsed these findings as they found that patients with T1DM had similar clusterin levels despite having different disease durations. Moreover, their data highlighted no substantial alteration in clusterin levels with glycemic control, as patients who had HbA1c levels below 7% had comparable clusterin levels to those who did not. Lastly, they observed no significant variation in clusterin levels in obese patients, underlining no correlation with the BMI of patients (8).

Zeng et al. reported in agreement with our findings, after a thorough analysis of the temporal relation between urinary clusterin and diabetic nephropathy, they found that urinary clusterin levels did not differ significantly with advancing stages of the disease, neither were they different between patients with diabetes without renal affection and those with diabetic nephropathy. Moreover, they noted that patients with HbA1c of less than 7% and those above 7% did not show any variation in terms of urinary clusterin ($p=0.925$) (28). Marchovecchio et al. contrasted our data, indicating that they found a significant positive correlation between HbA1c and clusterin in children with T1DM ($p<0.002$) (16).

We addressed the correlation between thyroid functions and clusterin, and HbA1c, and while we found no substantial associations with either clusterin nor HbA1c with TSH and fT4. There is a broadly recognized correlation between diabetes and hypothyroidism with many studies underlining hypothyroidism as an independent risk factor for the development of diabetic nephropathy in diabetics (29). Relevant to our findings, Silva et al. hypothesized a correlation between hypothyroidism and clusterin, and showed that in hypothyroid rat models, there was an overexpression of clusterin, intriguingly in a nuclear pattern rather than the cytoplasmic localization which was found in control rats. This emphasized a delay in physiologic apoptosis in hypothyroid experimental models. However, this correlation is yet to be elucidated in human models within the context of diabetes (30).

We established a negative correlation between serum creatinine and clusterin concentration ($r=-0.280$, $p=0.049$), meaning that with rising serum creatinine, urinary clusterin concentration falls. Kostopoulou et al. contrasted our findings, revealing a negative correlation with eGFR ($r=-0.391$, $p=0.025$), and considering that eGFR is inversely correlated with serum creatinine, these results were deemed discrepant from ours (8). We attempted to explain the negative correlation between serum creatinine and clusterin, evident in our study, by highlighting the fact that our sample consisted of children with T1DM, at a mean age of 13.65, 48% of whom were females, and given that serum creatinine is essentially a metabolite of creatine, which is almost entirely located in skeletal muscles, it would not be surprising for our subjects to have lower serum creatinine levels than those found in older populations, such as those sampled in the aforementioned study, due to their significantly lower muscle mass (31, 32). Likewise, there was a statistically significant negative correlation between clusterin and ALT ($r=-0.307$, $p=0.030$). This demonstrated that with lower clusterin levels were associated with elevated ALT, a marker of liver affection. In a study by Deesophon et al., conducted on 112 patients with HIV, no significant correlation could be detected between plasma clusterin and ALT ($r=-0.118$, $p=0.241$). Conversely, they reported a statistically significant negative correlation with AST ($r=-0.218$, $p=0.021$). To contextualize these findings, it must be noted that in their cohort, plasma clusterin levels were significantly lower in individuals with HIV-related liver fibrosis compared to controls ($p<0.05$) (33). In an experimental study by Wang et al., models with liver fibrosis and clusterin gene deletion demonstrated exacerbated disease progression,

highlighting that clusterin may be a protective protein in the event of liver fibrosis. However, in models without the clusterin gene, markers of inflammation and liver enzymes were not significantly altered, indicating that while clusterin may act as an anti-fibrotic agent, it functions independently of inflammatory processes or hepatocellular injury. Furthermore, no alterations in serum AST, ALT or alkaline phosphatase could be noted after the administration of high doses of CLUtide, a peptide mimicking clusterin, further emphasizing that clusterin functions are independent from liver injury markers (34).

Bradley et al. reported that adipocyte-derived clusterin was highly expressed in hepatic steatosis, showing positive correlations with insulin resistance in the adjusted multivariate analysis (Beta 0.49, $p=0.009$). Furthermore, they found that clusterin levels positively correlated with triglycerides, total cholesterol, and lower HDL cholesterol, all adding up to a higher risk of metabolic syndrome. In the experimental model, they found that recombinant clusterin treatment attenuates insulin signaling in human hepatocytes (HepG2 cells), specifically reducing Akt phosphorylation. Notably, clusterin did not inhibit insulin action in skeletal muscle cells or adipocytes, suggesting the liver is the primary target. These hepatic effects are mediated by **LRP2**, a receptor highly expressed in the liver. Knocking down LRP2 abolishes the ability of clusterin to inhibit insulin signaling (35). Liu et al. supported these findings, noting that clusterin (apolipoprotein J) accelerates liver fibrosis via the STAT3 signaling pathway. In addition, mice models without hepatic apolipoprotein J showed significantly lower ALT and AST levels, reflecting lower hepatocellular injury (36).

Additionally, clusterin concentrations were shown to be positively correlated with serum TG ($r=0.286$, $p=0.044$). However, no significant association with cholesterol could be extrapolated ($p=0.711$). In discordance with our findings, Kostopoulou et al. reported a significant correlation between clusterin levels and total cholesterol ($r=0.312$, $p=0.039$). However, it must be pointed out that their sample included obese patients in whom clusterin levels were addressed along with those who had T1DM, so this correlation was noted in all patients combined, not only in those with T1DM, which affected the comparability to our findings (8). Bradely et al. found that clusterin treatment decreases the expression of APOA1 (a major component of HDL) and SREBP-1, which explains the clinical observation of reduced HDL levels in patients with high clusterin. Increases in adipocyte clusterin expression paralleled the progressive accumulation of liver fat, inflammation, and fibrosis. Adipocyte clusterin expression positively correlated with the percentage of liver fat and the severity of non-alcoholic hepatic steatosis (35). We presented the urinary profile of the cases and controls and highlighted considerable differences concerning multiple parameters, such as significant elevations in urinary creatinine, urinary albumin, and albumin/creatinine ratio in the cases compared to the controls ($p=0.001$, $p=0.016$, $p=0.001$). Furthermore, none of the controls were shown to have micro- or macro-albuminuria, whereas 18% of the cases had microalbuminuria and 22% had macroalbuminuria ($p<0.001$). However, uCLU showed non-significant correlations with urinary creatinine, albuminuria, or albumin/creatinine ratio. In contrast, Zeng et al. demonstrated a significant positive correlation between uCLU and albumin/creatinine ratio ($r=0.549$, $p=0.000$). Furthermore, they noted a prominent negative correlation between uCLU and the eGFR of patients with diabetic nephropathy ($r=-0.489$, $p=0.000$) (28). Fadel et al. addressed the influence of uCLU levels on albuminuria and deduced that there was a positive correlation between the stage of albuminuria in diabetics and uCLU levels ($p<0.001$). These studies, however, were conducted on adult diabetic populations, where age-related decline in kidney function is a known factor, which may explain the divergent results (3, 18). We evaluated the diagnostic performance of clusterin and found it to be appreciably predictive of diabetic nephropathy at a cutoff of 37.6 pg/ml (AUC=0.916; $p<0.001$). At this cutoff value, clusterin had a sensitivity of 80%, a specificity of 90%, a PPV of 90.9%, and a NPV of 78.3%. Fadel et al. concurred with our findings and reported that at a cutoff of 16.9ng/dL, clusterin impressively predicted diabetic nephropathy with a sensitivity and specificity of 97.8%, and a PPV and NPV of 97.8% (AUC=1.000; $p<0.05$) (18). Zeng et al. inferred that urinary clusterin predicted the diagnosis of diabetic nephropathy, at a cutoff of 636.9ng/mL, with a sensitivity of 64.3% and a specificity of 84.6% (AUC=0.775; $p<0.05$). Additionally, they found that it performed well in the prediction of macroalbuminuria in diabetic patients, at a cutoff of 695.4ng/mL, with a sensitivity of 68% and a specificity of 82.6% (AUC=0.795, $p<0.05$) (28). Abd El-Khalik et al., although under a different protocol, addressed the role of urinary clusterin in the diagnosis of early renal disease in children, and they showed that at a cutoff value of >470.8ug/mL, urinary clusterin predicted early nephropathy with a sensitivity of 90% and a specificity of 76.6% (AUC=0.930), and they showed that this performance was superior to serum creatinine, eGFR, and albumin/creatinine ratio (21).

Although our study provided significant data in an area where research is relatively lacking, some limitations, such as the small sample size and the lack of follow-up data, emphasized the need for further longitudinal evaluation of the correlation between urinary Clusterin and renal damage in pediatric T1DM patients.

Conclusions

In conclusion, this study demonstrates that urinary clusterin is significantly elevated in children with T1DM and serves as a highly effective, non-invasive biomarker for the early detection of diabetic nephropathy, independent of disease duration or glycemic control. With an impressive diagnostic performance, urinary clusterin proves to be a more decisive indicator of early renal involvement in pediatric populations than traditional measures like serum creatinine or albuminuria. These findings suggest that clusterin upregulation may reflect a critical protective response against diabetes-induced oxidative stress and tubular apoptosis, offering a robust tool for clinical screening that could facilitate earlier intervention and improved management of renal complications in pediatric diabetic patients.

References

1. Dabelea D, Bell RA, D'Agostino RB, Imperatore G, Johansen JM, Linder B, et al. Incidence of Diabetes in Youth in the United States. *Jama*. 2007;297(24):2716. <https://doi.org/10.1001/jama.297.24.2716>
2. Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, et al. Prevalence of Diabetes in U.S. Youth in 2009: The SEARCH for Diabetes in Youth Study. 2014;37(2):402-8. <https://doi.org/10.2337/dc13-1838>
3. Baek JH, Lee WJ, Lee BW, Kim SK, Kim G, Jin SM, et al. Age at Diagnosis and the Risk of Diabetic Nephropathy in Young Patients with Type 1 Diabetes Mellitus. *Diabetes Metab J*. 2021;45(1):46-54. <https://doi.org/10.4093/dmj.2019.0134>
4. Mamilly L, Mastrandrea LD, Mosquera Vasquez C, Klammer B, Kallash M, Aldughiem A. Evidence of Early Diabetic Nephropathy in Pediatric Type 1 Diabetes. *Frontiers in Endocrinology*. 2021;Volume 12 - 2021. <https://doi.org/10.3389/fendo.2021.669954>
5. Drummond K, Mauer M. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. *Diabetes*. 2002;51(5):1580-7. <https://doi.org/10.2337/diabetes.51.5.1580>
6. Afkarian M. Diabetic kidney disease in children and adolescents. *Pediatr Nephrol*. 2015;30(1):65-74; quiz 0-1. <https://doi.org/10.1007/s00467-014-2796-5>
7. de Boer IH, Rue TC, Cleary PA, Lachin JM, Molitch ME, Steffes MW, et al. Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort. *Arch Intern Med*. 2011;171(5):412-20. doi:10.1001/archinternmed.2011.16

8. Kostopoulou E, Kalavrizioti D, Davoulou P, Papachristou E, Sinopidis X, Fouzas S, et al. Monocyte Chemoattractant Protein-1 (MCP-1), Activin-A and Clusterin in Children and Adolescents with Obesity or Type-1 Diabetes Mellitus. *Diagnostics*. 2024;14(4):450. <https://doi.org/10.3390/diagnostics14040450>
9. Vaidya VS, Ferguson M, Bonventre JV. Biomarkers of Acute Kidney Injury. *The Annual Review of Pharmacology and Toxicology*. 2008;48(1):463-93. <https://doi.org/10.1146/annurev.pharmtox.48.113006.094615>
10. Trougakos IP, Gonos ES. Clusterin/Apolipoprotein J in Human Aging and Cancer. *The International Journal of Biochemistry & Cell Biology*. 2002;34(11):1430-48. [https://doi.org/10.1016/S1357-2725\(02\)00041-9](https://doi.org/10.1016/S1357-2725(02)00041-9)
11. Kim SS, Song SH, Kim JH, Jeon YK, Kim BH, Kang MC, et al. Urine clusterin/apolipoprotein J is linked to tubular damage and renal outcomes in patients with type 2 diabetes mellitus. *Clin Endocrinol (Oxf)*. 2017;87(2):156-64. <https://doi.org/10.1111/cen.13360>
12. Laping NJ, Olson BA, Day JR, Brickson BM, Contino LC, Short BG, et al. The age-related increase in renal clusterin mRNA is accelerated in obese Zucker rats. *J Am Soc Nephrol*. 1998;9(1):38-45. DOI: 10.1681/ASN.V9138
13. Wilson MR, Easterbrook-Smith SB. Clusterin is a secreted mammalian chaperone. *Trends in biochemical sciences*. 2000;25(3):95-8. [https://doi.org/10.1016/S0968-0004\(99\)01534-0](https://doi.org/10.1016/S0968-0004(99)01534-0)
14. Antonelou MH, Kriebardis AG, Stamoulis KE, Trougakos IP, Papassideri IS. Apolipoprotein J/Clusterin is a novel structural component of human erythrocytes and a biomarker of cellular stress and senescence. *PLoS One*. 2011;6(10):e26032. <https://doi.org/10.1371/journal.pone.0026032>
15. Shim YJ, Kang BH, Jeon HS, Park IS, Lee KU, Lee IK, et al. Clusterin induces matrix metalloproteinase-9 expression via ERK1/2 and PI3K/Akt/NF-κB pathways in monocytes/macrophages. *J Leukoc Biol*. 2011;90(4):761-9. <https://doi.org/10.1189/jlb.0311110>
16. Marcovecchio ML, Colombo M, Dalton RN, Benitez-Aguirre P, Cameron F, Chiesa ST, et al. Biomarkers Associated With Early Stages of Kidney Disease in Adolescents With Type 1 Diabetes. *Pediatric Diabetes*. 2020;21(7):1322-32. <https://doi.org/10.1111/pedi.13095>
17. Heo J-Y, Kim J-E, Dan Y, Kim Y-W, Kim J-Y, Cho KH, et al. Clusterin deficiency induces lipid accumulation and tissue damage in kidney. *Journal of Endocrinology*. 2018;237(2):175-91. <https://doi.org/10.1530/JOE-17-0453>
18. Fadel N, Abd El-Sattar E, Abdel Fattah Ouda I, Taha Morsi F. Performance of Urinary Clusterin as a Biomarker for Diagnosis of Early Diabetic Nephropathy in Type 2 Diabetes. *Medicine Updates*. 2023;15(15):60-8. <https://doi.org/10.21608/muj.2023.234857.1147>
19. He J, Dijkstra KL, Bakker K, Bus P, Bruijn JA, Scharpfenecker M, et al. Glomerular clusterin expression is increased in diabetic nephropathy and protects against oxidative stress-induced apoptosis in podocytes. *Scientific Reports*. 2020;10(1):14888. <https://doi.org/10.1038/s41598-020-71629-z>
20. Musiał K, Augustynowicz M, Miśkiewicz-Migoń I, Kałwak K, Ussowicz M, Zwolińska D. Clusterin as a New Marker of Kidney Injury in Children Undergoing Allogeneic Hematopoietic Stem Cell Transplantation—A Pilot Study. *Journal of Clinical Medicine*. 2020;9(8):2599. <https://doi.org/10.3390/jcm9082599>
21. El-Khalik SRA, Sharaby RM, Nasif E, Hamza MB, Ibrahim RR. Netrin-1 and Clusterin: Innovative Potential Diagnostic Biomarkers for Early Renal Damage in B-thalassemia Major Children. *Iubmb Life*. 2021;73(5):800-10. <https://doi.org/10.1002/iub.2464>
22. Lin JH, Walter P, Yen TSB. Endoplasmic Reticulum Stress in Disease Pathogenesis. *Annual Review of Pathology: Mechanisms of Disease*. 2008;3(Volume 3, 2008):399-425. <https://doi.org/10.1146/annurev.pathmechdis.3.121806.151434>
23. Graves TK, Patel S, Dannies PS, Hinkle PM. Misfolded growth hormone causes fragmentation of the Golgi apparatus and disrupts endoplasmic reticulum-to-Golgi traffic. *Journal of Cell Science*. 2001;114(20):3685-94. <https://doi.org/10.1242/jcs.114.20.3685>
24. Li N, Zoubeidi A, Beraldi E, Gleave ME. GRP78 regulates clusterin stability, retrotranslocation and mitochondrial localization under ER stress in prostate cancer. *Oncogene*. 2013;32(15):1933-42. <https://doi.org/10.1038/onc.2012.212>
25. Trougakos IP, Gonos ES. Regulation of clusterin/apolipoprotein J, a functional homologue to the small heat shock proteins, by oxidative stress in ageing and age-related diseases. *Free Radical Research*. 2006;40(12):1324-34. <https://doi.org/10.1080/10715760600902310>
26. Ha J, Moon MK, Kim H, Park M, Cho SY, Lee J, et al. Plasma Clusterin as a Potential Link Between Diabetes and Alzheimer Disease. *The Journal of Clinical Endocrinology & Metabolism*. 2020;105(9):3058-68. <https://doi.org/10.1210/clinem/dgaa378>
27. Mechanick JJ, Zhao S, Garvey WT. Leptin, An Adipokine With Central Importance in the Global Obesity Problem. *gh*. 2018;13(2):113. <https://doi.org/10.1016/j.ghheart.2017.10.003>
28. Zeng XF, Lu DX, Li JM, Tan Y, Li Z, Zhou L, et al. Performance of urinary neutrophil gelatinase-associated lipocalin, clusterin, and cystatin C in predicting diabetic kidney disease and diabetic microalbuminuria: a consecutive cohort study. *BMC Nephrol*. 2017;18(1):233. <https://doi.org/10.1186/s12882-017-0620-8>
29. Vemula SL, Aramada S, Mannam R, Sankara Narayanan R, Bansal A, Yanamaladoddi VR, et al. The Impact of Hypothyroidism on Diabetes Mellitus and Its Complications: A Comprehensive Review. *Cureus*. 2023;15(6):e40447. <https://doi.org/10.7759/cureus.40447>
30. Silva D, Lizama C, Tapia V, Moreno RD. Propylthiouracil-induced hypothyroidism delays apoptosis during the first wave of spermatogenesis. *Biological Research*. 2011;44(2):181-8. <http://dx.doi.org/10.4067/S0716-97602011000200010>
31. Dabla PK. Renal function in diabetic nephropathy. *World J Diabetes*. 2010;1(2):48-56. <https://doi.org/10.4239/wjd.v1.i2.48>
32. Molitoris BA. Acute kidney injury. *Goldman's Cecil Medicine: Elsevier*; 2012. p. 756-61.
33. Deesophon N, Khattiya J, Sretapunya W, Akeawatchai C. Correlations between plasma clusterin levels and liver fibrosis in people living with HIV. *Journal of Associated Medical Sciences*. 2025;58(3):147-55. <https://he01.tci-thaijo.org/index.php/bulletinAMS/article/view/277702>
34. Wang M, Chen F, Gao J, Liu Q, Li Z, Kong Q, et al. Clusterin Drives Fiber Endocytosis by Mesothelial Cells to Resolve Liver Fibrosis. *Gastroenterology*. 2025. <https://doi.org/10.1053/j.gastro.2025.08.022>
35. Bradley D, Blaszcak A, Yin Z, Liu J, Joseph JJ, Wright V, et al. Clusterin Impairs Hepatic Insulin Sensitivity and Adipocyte Clusterin Associates With Cardiometabolic Risk. *Diabetes Care*. 2019;42(3):466-75. <https://doi.org/10.2337/dc18-0870>
36. Liu L, Mo Z, Qin N, Pi J, Mao L, Deng G, et al. Hepatocyte Apolipoprotein J Accelerates Injury-induced Liver Fibrosis by Activation Signal Transducer and Activator of Transcription 3 Through Ranbp2 Mediated-SUMOylation. *Cellular and Molecular Gastroenterology and Hepatology*. 2025;19(10):101556. <https://doi.org/10.1016/j.jcmgh.2025.101556>

Table 1: Laboratory Findings of the Studied Groups

Items	Cases (N.=50)	Controls (N. =40)	P-value
TSH (μIU/mL) (mean±SD)	2.5±1.6	2.1±0.8	0.175
Free T4 (ng/dL) (mean±SD)	1.2±0.2	1.2±0.2	0.269
Hb (g/dL)	12.4±1.1	12.5±0.4	0.809
PLT (×10⁹/μL)	295.9±73.8	311.7±28.5	0.040* (MW)

WBCs ($\times 10^3/\mu\text{L}$)	6.9 $\pm$ 1.6	5.9 $\pm$ 0.9	0.001* (MW)
Urea (mg/dL)	26.2 $\pm$ 5.8	25.3 $\pm$ 2.2	0.337
Creatinine (mg/dL)	0.8 $\pm$ 0.2	0.7 $\pm$ 0.2	0.010*
ALT (U/L)	23.8 $\pm$ 11.3	20.3 $\pm$ 3.7	0.066
AST (U/L)	29.8 $\pm$ 10.3	20.7 $\pm$ 3.5	<0.001*
Cholesterol (mg/dL)	192.7 $\pm$ 32.5	151.1 $\pm$ 19.7	<0.001*
TG (mg/dL) (mean $\pm$ SD) Median (IQR)	176.9 $\pm$ 153.6 98.0(73.0,237.0)	79.80 $\pm$ 15.79 81.0(70.0,90.0)	<0.001* (MW)
*p-value is significant TSH, thyroid-stimulating hormone; FT4, free thyroxine; Hb, hemoglobin; PLT, platelet count; WBCs, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglycerides			

Table 2: Urinary profile of the studied groups

Items	Cases (N. =50)	Controls (N. =40)	P-value (MW)
Creatinin in urine (mg/dl) (mean $\pm$ SD) Median (IQR)	91.8 $\pm$ 78.3 79.0(22.0,113.7)	17.95 $\pm$ 5.78 17.5(13.3,21.7)	0.001*
Albumin in urine (mg/dl) (mean $\pm$ SD) Median (IQR)	62.44 $\pm$ 156.91 4.9(1.9,36.0)	1.68 $\pm$ 1.31 1.4(0.7,1.9)	0.016*
ACR (mg/g) (mean $\pm$ SD) Median (IQR)	175.56 $\pm$ 315.86 17.3(5.1,123.8)	10.37 $\pm$ 6.19 9.2(5.2,14.6)	0.001*
Albuminuria categories Normo-albuminuria Micro-albuminuria Macro-albuminuria	30(60.0%) 9(18.0%) 11(22.0%)	40(100%) 0(0%) 0(0%)	<0.001*
Clusterin (pg/mL) (mean $\pm$ SD) Median (IQR)	49.7 $\pm$ 12.9 47.9(39.5,58.8)	32.3 $\pm$ 4.6 32.3(29.3,35.1)	<0.001*
*p-value is significant ACR, albumin-to-creatinine ratio; IQR, interquartile range; SD, standard deviation; MW, Mann–Whitney U test; IgM, immunoglobulin M.			

Table 3: Clusterin concentration according to albuminuria category

Albuminuria category	n	Mean $\pm$ SD	Median (IQR)	P-value (KW)
A1 (≤ 30 mg/g)	30	52.17 $\pm$ 13.75	50.55 (42.55-60.20)	0.194
A2 (30-300 mg/g)	9	42.96 $\pm$ 9.90	41.00 (37.10-47.60)	
A3 (>300 mg/g)	11	50.86 $\pm$ 12.76	49.30 (42.90-55.85)	

Table 4: Correlations with Clusterin and HbA1c, in cases

Variables		Clusterin (pg/mL)	HbA1C (%)
Age	r	0.067	0.318
	P-value	0.642	0.024
TSH ($\mu\text{IU/mL}$)	r	.035	0.008
	P-value	.809	0.956
FT4 (ng/dL)	r	.229	-0.106
	P-value	.110	0.462
Hb (g/dL)	r	-0.006	0.177
	P-value	0.968	0.218
PLT ($\times 10^3/\mu\text{L}$)	r	-0.042	0.001
	P-value	0.772	0.997
WBCs ($\times 10^3/\mu\text{L}$)	r	-0.251	-0.064
	P-value	0.079	0.657
ALT (U/L)	r	-0.307	-0.047
	P-value	0.030*	0.745
AST (U/L)	r	-0.079	-0.139
	P-value	0.584	0.337
Urea (mg/dL)	r	-.0100	.082
	P-value	0.487	.572
Creatinine (mg/dL)	r	-0.280	.219
	P-value	0.049*	.126
Cholesterol (mg/dL)	r	0.054	0.008
	P-value	0.711	0.957
Triglycerides (mg/dL)	r	0.286*	0.059
	P-value	0.044*	0.683
Disease duration	r	-0.121	-----

	P-value	0.402	-----
HbA1c (%)	r	-0.046	-----
	P-value	0.752	-----
BMI (kg/m²)	r	0.106	-----
	P-value	0.465	-----
*P-value is significant (highlighted in orange) HbA1c, glycated hemoglobin; BMI, body mass index; TSH, thyroid-stimulating hormone; FT4, free thyroxine; Hb, hemoglobin; PLT, platelet count; WBCs, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; r, correlation coefficient.			

Table 5: Sensitivity, specificity, positive predictive value, and negative predictive value of clusterin in prediction of diabetic nephropathy in children with T1DM	
Item	Values
Cut off	37.6
P-value	<0.001*
AUC	0.916
Sensitivity	80(66.3 - 90.0)
Specificity	90(76.3 - 97.2)
positive predictive value (PPV)	90.9(79.6 - 96.2)
negative predictive value (NPV)	78.3(67.2 - 86.4)
T1DM, type 1 diabetes mellitus; AUC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.	

Table 6: ROC performance of clusterin concentration for albuminuria-category comparisons							
Comparison	Positive group	n (+/-)	AUC (95% CI)	p	Criterion	Sensitivity % (95% CI)	Specificity % (95% CI)
A2/A3 vs A1	A2/A3	50 (20/30)	0.597 (0.435-0.759)	0.243	<52.85	80.0 (56.3-94.3)	46.7 (28.3-65.7)
A3 vs A1/A2	A3	50 (11/39)	0.531 (0.341-0.722)	0.746	>45.95	72.7 (39.0-94.0)	46.2 (30.1-62.8)
A2 vs A1	A2	39 (9/30)	0.696 (0.509-0.884)	0.040	<47.95	77.8 (40.0-97.2)	60.0 (40.6-77.3)
A3 vs A1	A3	41 (11/30)	0.515 (0.315-0.716)	0.882	<52.85	72.7 (39.0-94.0)	46.7 (28.3-65.7)
A3 vs A2	A3	20 (11/9)	0.687 (0.442-0.932)	0.135	>44.55	72.7 (39.0-94.0)	66.7 (29.9-92.5)

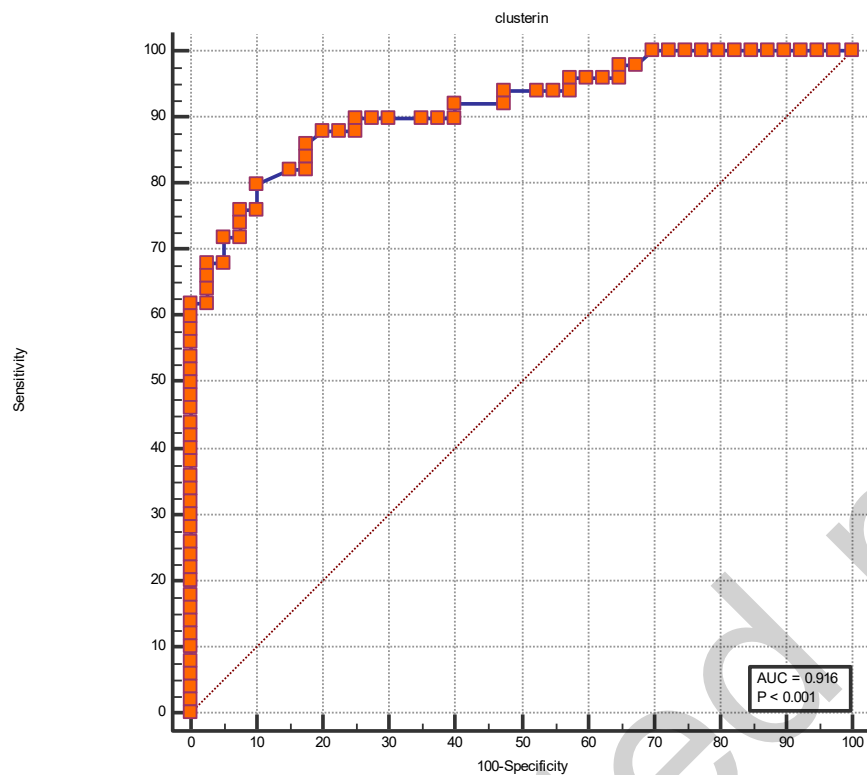


Figure 1. ROC curve analysis for the role of clusterin in prediction of diabetic nephropathy in children with type 1 Diabetes Mellitus.

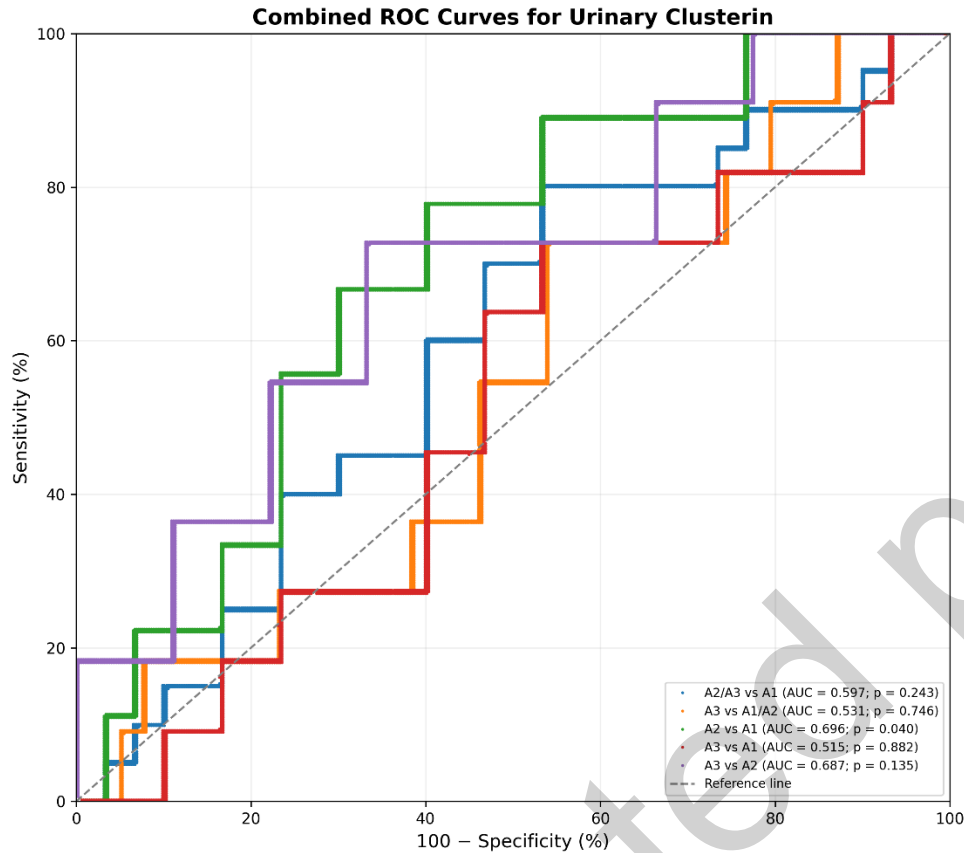


Figure 2. ROC curve of clusterin concentration for albuminuria-category comparisons

A2/A3 vs A1. AUC = 0.597 (95% CI 0.435-0.759), $p = 0.243$. Positive cases: 20; negative cases: 30. Lower values indicate positive status.

Optimal criterion: clusterin <52.85; sensitivity 80.0% (95% CI 56.3-94.3%); specificity 46.7% (95% CI 28.3-65.7%).

A3 vs A1/A2. AUC = 0.531 (95% CI 0.341-0.722), $p = 0.746$. Positive cases: 11; negative cases: 39. Higher values indicate positive status.

Optimal criterion: clusterin >45.95; sensitivity 72.7% (95% CI 39.0-94.0%); specificity 46.2% (95% CI 30.1-62.8%).

A2 vs A1. AUC = 0.696 (95% CI 0.509-0.884), $p = 0.040$. Positive cases: 9; negative cases: 30. Lower values indicate positive status. Optimal criterion: clusterin <47.95; sensitivity 77.8% (95% CI 40.0-97.2%); specificity 60.0% (95% CI 40.6-77.3%).

A3 vs A1. AUC = 0.515 (95% CI 0.315-0.716), $p = 0.882$.

Positive cases: 11; negative cases: 30. Lower values indicate positive status. Optimal criterion: clusterin <52.85; sensitivity 72.7% (95% CI 39.0-94.0%); specificity 46.7% (95% CI 28.3-65.7%).

A3 vs A2. AUC = 0.687 (95% CI 0.442-0.932), $p = 0.135$. Positive cases: 11; negative cases: 9. Higher values indicate positive status. Optimal criterion: clusterin >44.55; sensitivity 72.7% (95% CI 39.0-94.0%); specificity 66.7% (95% CI 29.9-92.5%).