



EARLY DETECTION OF RENAL TUBULAR DISORDERS USING BIOCHEMICAL MARKERS IN CHILDREN BELOW AGE OF 12 YEARS IN SOUTHERN PUNJAB, PAKISTAN

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ABSTRACT

Objective: To assess the diagnostic potential of serum tissue cystatin-associated factor (t-CAF) and a panel of urinary biomarkers netrin-1, α -glutathione S-transferase (α -GST), π -glutathione S-transferase (π -GST), calbindin-D28K, and calprotectin (S100A8/A9) in the early identification of renal tubular disorders among pediatric populations under 12 years of age in Southern Punjab.

Materials and methods: This cross-sectional study enrolled 120 children under age 12 years exhibiting clinical indications of renal tubular dysfunction and 31 sex-matched healthy controls. All participants were normoalbuminuric and excluded for known metabolic or glomerular diseases. The patient cohort was further categorized into two subgroups based on clinical severity indices. Serum t-CAF and urinary biomarker levels were measured using validated enzyme-linked immunosorbent assay (ELISA) kits under standardized laboratory conditions. Comparative statistical analyses were performed to evaluate the association of these markers with early tubular injury. Data analysis was done using SPSS 23 with p-value <0.05 considered as significant.

Results: Serum t-CAF levels were significantly elevated in children with suspected tubular dysfunction compared to healthy controls ($p < 0.05$). Similarly, urinary concentrations of netrin- 1, α -GST, π -GST, and calprotectin were markedly increased in the patient group, indicating tubular stress or damage. In contrast, no significant difference was observed in urinary calbindin levels between the groups. Furthermore, biomarker expression did not differ substantially between the severity-based subgroups, suggesting early and consistent release irrespective of clinical progression.

Conclusion: Serum t-CAF and specific urinary biomarkers namely netrin-1, α -GST, π -GST, and calprotectin demonstrates potential as non-invasive, sensitive indicators of early renal tubular injury in children. Their detection prior to the onset of overt renal dysfunction supports their utility in preclinical screening, facilitating earlier diagnosis and timely clinical intervention in at-risk pediatric populations.

Keywords: Renal Tubular Disorders, Biochemical Markers, Children <12 years

Introduction

Over the past several decades, there has been a marked rise in the prevalence of childhood and adolescent obesity, accompanied by an increasing burden of associated comorbidities, including kidney disease [1]. Obesity-related kidney disease (ORKD) arises from a complex interplay of structural, hemodynamic, and metabolic alterations mediated by excess adipose tissue, which exert detrimental effects on renal function. These include glomerular hyperfiltration, increased intraglomerular pressure, podocyte stress, and lipotoxicity, ultimately promoting glomerular hypertrophy and progressive renal injury [2,3,4].

In the majority of cases, ORKD remains clinically silent in its early stages, often going undetected for prolonged periods. This subclinical progression may eventually culminate in a decline in glomerular filtration rate and the development of chronic kidney disease (CKD). It is estimated that 15–30% of individuals with overweight or obesity will develop CKD during their lifetime [5]. Notably, a large cohort study involving 1.2 million adolescents with a median follow-up of 25 years demonstrated that those with obesity had a 3.4-fold higher risk of progressing to end-stage renal disease (ESRD) due to non-diabetic nephropathy compared to their normal-weight peers [6].

Given the insidious onset and long-term renal consequences, early detection of kidney injury in obese pediatric populations is critical. There is a compelling need to identify and validate sensitive, non-invasive biomarkers capable of detecting subclinical renal damage before irreversible structural changes occur, enabling timely intervention and improved long-term outcomes.

In routine clinical practice, assessment of renal function in children predominantly relies on serum creatinine levels and the estimated glomerular filtration rate (eGFR) derived from the updated Schwartz formula [7]. However, creatinine is widely recognized as an imperfect biomarker due to several well-documented limitations, including its dependence on muscle mass, dietary protein intake, and tubular secretion, which can lead to inaccurate estimation of renal function, particularly in pediatric populations.

Cystatin C has emerged as a more specific and reliable alternative, as it is freely filtered at the glomerulus, entirely reabsorbed, and catabolized by proximal tubular epithelial cells, with minimal extrarenal influence. Unlike creatinine, serum cystatin C concentrations are less affected by body composition or nutritional status. Nevertheless, recent evidence indicates a potential confounding factor: a threefold upregulation of cystatin C mRNA expression has been observed in adipose tissue of obese adults, suggesting adipocyte-derived overproduction and a possible role in adipogenesis and metabolic dysregulation [8]. This ectopic production may elevate circulating cystatin C levels independently of renal function, thereby limiting its accuracy as a filtration marker in individuals with obesity.

Microalbuminuria remains a widely accepted indicator of early glomerular injury. As early as 2005, Csernus et al. reported significantly higher levels of microalbuminuria in obese children compared to their non-obese counterparts, highlighting the presence of subclinical renal damage in this population [9].

Beyond glomerular markers, several tubular injury biomarkers have been investigated for their potential in detecting early kidney damage, particularly in the context of obesity-related renal stress. These include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), N-acetyl- β -D-glucosaminidase (NAG), and osteopontin molecules associated with tubular inflammation and dysfunction [10,11]. More recently, serum alpha-1 acid glycoprotein

(orosomuroid) has been identified as a potential early marker of glomerular alteration in children with simple obesity, with elevated levels observed even prior to the development of microalbuminuria, suggesting its utility in identifying incipient renal involvement [12]. Emerging evidence highlights the C-terminal agrin fragment (t-CAF) as a promising and more specific biomarker of early kidney dysfunction. Agrin, a heparan sulfate proteoglycan with a molecular weight of approximately 250 kDa, is predominantly known for its role in the central nervous system and neuromuscular junctions. However, it is also a key structural component of the glomerular basement membrane (GBM), renal tubular basement membranes, and the renal extracellular matrix [13]. Physiologically, agrin is cleaved by the serine protease neurotrypsin, generating a 22 kDa C-terminal fragment (t-CAF), which is freely filtered by the glomerulus and almost entirely reabsorbed and degraded by proximal tubular epithelial cells under normal conditions [14]. Consequently, elevated levels of t-CAF in urine or serum reflect impaired proximal tubular reabsorptive capacity, indicating early tubular injury.

The diagnostic potential of t-CAF as a marker of renal damage was first demonstrated by Steubl et al. in adult populations, including patients with chronic kidney disease (CKD) and renal transplant recipients, where it showed high sensitivity for detecting subclinical tubular dysfunction [15,16]. Despite these advances, t-CAF has not yet been investigated in pediatric populations with obesity, a group at increased risk for early renal injury.

Similarly, other markers of tubular damage such as glutathione S-transferases (GSTs), netrin-1, calbindin-D28K, and calprotectin (S100A8/A9) remain understudied in obese children. GSTs are cytosolic enzymes involved in cellular detoxification, with tissue-specific isoforms: α -GST is predominantly expressed in the proximal tubules, while π -GST is localized in the distal tubules and collecting ducts. Tubular cell injury leads to the release of these enzymes into the tubular lumen, resulting in increased urinary excretion. The presence of distinct isoforms allows for topographic localization of tubular damage.

Elevated urinary levels of α -GST and π -GST have been documented in early acute kidney injury (AKI) associated with nephrotoxic exposure [17], as well as in chronic conditions such as diabetic nephropathy and proteinuric glomerulopathies [18,19], underscoring their sensitivity to structural and functional tubular impairment.

The aim of our study is to assess the diagnostic potential of serum tissue cystatin-associated factor (t-CAF) and a panel of urinary biomarkers netrin-1, α -glutathione S-transferase (α -GST), π -glutathione S-transferase (π -GST), calbindin-D28K, and calprotectin (S100A8/A9) in the early identification of renal tubular disorders among pediatric populations under 12 years of age.

Materials and methods

A cross-sectional analytical study was conducted at the Department of Pediatrics, a tertiary care referral center in Southern Punjab, Pakistan, between January 2024 and December 2024. The study aimed to evaluate the diagnostic performance of serum tissue cystatin-associated factor (t-CAF) and a panel of urinary tubular injury biomarkers netrin-1, α -glutathione S-transferase (α -GST), π -glutathione S-transferase (π -GST), calbindin-D28K, and calprotectin (S100A8/A9) in the early detection of renal tubular dysfunction in children under 12 years of age.

The study was conducted after approved by the Institutional Review Board (IRB). Written informed consent was obtained from the parents or legal guardians of all participants, and assent was obtained from children aged 7 years and above. Participant confidentiality was maintained through anonymized data coding and secure storage. The sample size was calculated using the formula for comparing two independent means, based on preliminary data from a pilot study and published literature on urinary biomarkers in pediatric renal disorders. Assuming a medium effect size (Cohen's $d = 0.6$), a power of 80%, and a significance level of $\alpha = 0.05$, a minimum of 116 participants (88 patients and 28 controls) were required. To account for potential data loss and enhance statistical robustness, a total of 151 participants were enrolled 120 patients and 31 controls providing a power of 87% to detect clinically meaningful differences. A total of 151 children aged 0–12 years were

enrolled. The patient group (n = 120) consisted of children presenting with clinical features suggestive of renal tubular dysfunction, including polyuria, polydipsia, growth retardation, recurrent dehydration, or unexplained electrolyte imbalances (e.g., hypokalemia, metabolic acidosis). The control group (n = 31) comprised healthy children recruited from routine pediatric check-ups, matched to patients by age (± 6 months) and sex. Controls had no history of renal, metabolic, or chronic diseases and exhibited normal growth parameters and urinalysis. Inclusion Criteria: Age < 12 years, for patients: clinical suspicion of tubular dysfunction supported by laboratory findings. Normoalbuminuria, defined as urine albumin-to-creatinine ratio (UACR) <30 mg/g creatinine in a spot urine sample. Exclusion Criteria: Participants were excluded if they had: Known glomerular disease (e.g., nephrotic syndrome, hematuria, UACR ≥ 30 mg/g), Inborn errors of metabolism (e.g., cystinosis, Lowe syndrome), Active urinary tract infection (positive urine culture $> 10^5$ CFU/mL with corresponding pyuria), Acute illness or systemic infection within the past four weeks, Recent use of nephrotoxic agents (e.g., aminoglycosides, NSAIDs) or antibiotics within the preceding 14 days, Chronic conditions such as diabetes mellitus, congenital heart disease, or malignancy. The patient cohort was stratified into two subgroups based on a composite clinical severity score incorporating symptom duration, number of abnormal laboratory parameters, presence of growth failure, and degree of electrolyte disturbance: Group I (Mild-to-Moderate): Children with ≤ 2 abnormal lab values and mild or intermittent symptoms (n = 68), Group II (Severe): Children with ≥ 3 abnormal parameters, persistent symptoms, or documented growth failure (n = 52). Blood samples: 3 mL of venous blood was drawn into serum separator tubes, allowed to clot for 30 minutes, and centrifuged at 3000 rpm for 10 minutes. Serum was aliquoted and stored at -80°C until analysis. Urine samples: Midstream spot urine specimens were collected in sterile containers. Within 1 hour of collection, samples were centrifuged (2000 rpm, 10 min) to remove cellular debris, supplemented with protease inhibitors, and stored at -80°C . All biomarkers were quantified using commercially available, validated enzyme-linked immunosorbent assay (ELISA) kits, following manufacturer protocols. Serum t-CAF: Measured using a human t-CAF ELISA kit (MyBioSource), with a detection range of 0.5–20 ng/mL and intra- and inter-assay coefficients of variation (CV) $< 8\%$ and $< 10\%$, respectively. Urinary biomarkers: Netrin-1: ELISA kit (MyBioSource), detection range: 0.156–10 ng/mL, α -GST and π -GST: Specific ELISA kits (MyBioSource), detection limits: 0.3 ng/mL and 0.2 ng/mL, respectively. Calbindin-D28K: ELISA (Biomatik), range: 0.78–50 ng/mL, Calprotectin (S100A8/A9): ELISA (Biocompare), range: 0.312–20 ng/mL. All assays were performed in duplicate to ensure reproducibility. Urinary biomarker concentrations were normalized to urinary creatinine (mg/dL) and expressed as ng/mg creatinine to correct for urine dilution. Where available, age-adjusted reference ranges from published pediatric studies were used for comparison: Urinary α -GST: < 1.5 ng/mg creatinine, Urinary π -GST: < 0.8 ng/mg creatinine, Urinary netrin-1: < 2.0 ng/mg creatinine, Serum t-CAF: < 3.0 ng/mL (based on preliminary data in healthy children). Data were analyzed using IBM SPSS Statistics, Version 23. Normality was assessed using the Shapiro-Wilk test and Q-Q plots. Normally distributed data were expressed as mean $\pm$ standard deviation (SD); non-normally distributed variables were presented as median (interquartile range, IQR). Group comparisons were performed using the independent samples t-test (for normally distributed data) or the Mann–Whitney U test (for non-parametric data). One-way ANOVA or Kruskal-Wallis test was used for comparisons across three groups (e.g., Controls, Group I, Group II), with post-hoc testing as appropriate. Correlation between biomarkers and clinical parameters was assessed using Pearson or Spearman correlation coefficients. A p-value < 0.05 was considered statistically significant. All tests were two-tailed.

Results

A total of 151 children were included in the study: 120 with suspected renal tubular dysfunction (Group I: n = 68, mild-to-moderate; Group II: n = 52, severe) and 31 healthy controls. The mean age of the total cohort was 7.4 ± 2.9 years, with no significant difference in age or sex distribution between

groups ($p > 0.05$). All participants were normoalbuminuric, with urine albumin-to-creatinine ratio (UACR) below 30 mg/g creatinine.

Biomarker Levels Across Study Groups

Serum and urinary biomarker levels are summarized in **Table 1**. Serum t-CAF and several urinary markers netrin-1, α -GST, π -GST, and calprotectin were significantly elevated in children with tubular dysfunction compared to controls ($p < 0.001$). In contrast, urinary calbindin levels did not differ significantly between patients and controls ($p = 0.21$).

Table 1. Comparison of biomarker levels between patients and healthy controls

BIOMARKER	CONTROLS (N = 31)	PATIENTS (N = 120)	P-VALUE
Serum t-CAF (ng/mL)	2.31 $\pm$ 0.42	4.67 $\pm$ 1.35	<0.001
Urinary netrin-1 (ng/mg Cr)	1.24 (0.98–1.62)	3.85 (2.76–5.41)	<0.001
Urinary α -GST (ng/mg Cr)	0.92 (0.75–1.10)	3.05 (2.10–4.60)	<0.001
Urinary π -GST (ng/mg Cr)	0.58 (0.45–0.72)	2.40 (1.80–3.30)	<0.001
Urinary calbindin (ng/mg Cr)	1.05 (0.88–1.30)	1.18 (0.95–1.50)	0.21
Urinary calprotectin (ng/mg Cr)	0.82 (0.65–1.05)	2.90 (2.10–4.20)	<0.001

When comparing biomarker levels between severity subgroups (**Table 2**), no statistically significant differences were observed for serum t-CAF, netrin-1, α -GST, or π -GST. Calprotectin showed a moderate increase in Group II but did not reach statistical significance ($p = 0.07$).

Table 2. Biomarker levels by clinical severity subgroup

BIOMARKER	GROUP I (N = 68)	GROUP II (N = 52)	P-VALUE
Serum t-CAF (ng/mL)	4.52 $\pm$ 1.28	4.86 $\pm$ 1.42	0.14
Urinary netrin-1 (ng/mg Cr)	3.60 (2.50–5.10)	4.15 (3.00–5.80)	0.11
Urinary α -GST (ng/mg Cr)	2.90 (2.00–4.30)	3.25 (2.30–4.90)	0.19
Urinary π -GST (ng/mg Cr)	2.30 (1.70–3.10)	2.55 (1.90–3.50)	0.23
Urinary calprotectin (ng/mg Cr)	2.65 (1.90–3.90)	3.20 (2.40–4.60)	0.07

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of each biomarker in distinguishing children with tubular dysfunction from healthy controls. As shown in **Table 3**, serum t-CAF demonstrated the highest area under the curve (AUC = 0.94),

followed by urinary α -GST (AUC = 0.91) and netrin-1 (AUC = 0.89). All three markers exhibited high sensitivity and specificity.

Table 3. Diagnostic performance of biomarkers for detecting renal tubular dysfunction

BIOMARKER	AUC (95% CI)	OPTIMAL CUT-OFF	SENSITIVITY (%)	SPECIFICITY (%)
Serum t-CAF	0.94 (0.90–0.97)	3.1 ng/mL	89.2	87.1
Urinary α -GST	0.91 (0.86–0.94)	1.8 ng/mg Cr	86.7	83.9
Urinary netrin-1	0.89 (0.84–0.93)	2.4 ng/mg Cr	84.2	80.6
Urinary π -GST	0.85 (0.79–0.89)	1.5 ng/mg Cr	80.8	77.4
Urinary calprotectin	0.87 (0.82–0.92)	1.8 ng/mg Cr	82.5	77.4

Spearman's correlation analysis revealed moderate to strong positive correlations between serum t-CAF and all significantly elevated urinary biomarkers (Table 4). The strongest correlation was observed between serum t-CAF and urinary α -GST ($r = 0.72$, $p < 0.001$), suggesting coordinated tubular injury.

Table 4. Correlation between serum t-CAF and urinary biomarkers (Spearman's rho)

URINARY BIOMARKER	CORRELATION COEFFICIENT (R)	P-VALUE
Netrin-1	0.65	<0.001
α -GST	0.72	<0.001
π -GST	0.61	<0.001
Calprotectin	0.68	<0.001
Calbindin	0.18	0.32

The findings demonstrate that serum t-CAF, along with urinary netrin-1, α -GST, π -GST, and calprotectin, is significantly elevated in children with early renal tubular dysfunction, even before clinical severity manifests. These biomarkers show strong diagnostic accuracy, with serum t-CAF exhibiting the highest discriminatory power. The lack of significant differences between mild and severe subgroups suggests early and consistent biomarker release, highlighting their potential for pre-symptomatic detection. Strong correlations among markers further support their biological relevance. Overall, serum t-CAF and selected urinary biomarkers may serve as sensitive, non-invasive tools for early identification of tubular injury in pediatric populations.

Discussion

Given the well-documented nephrotoxic effects of obesity, there has been growing interest in identifying sensitive and reliable biomarkers of early renal injury in pediatric populations. However,

evidence in children remains limited, and there is currently no consensus on the optimal markers for detecting subclinical kidney damage in the context of obesity. While microalbuminuria and cystatin C have emerged as promising indicators of early glomerular dysfunction in obese individuals, their specificity and interpretability particularly in relation to adipose-derived influences remain under investigation [20]. Several urinary markers of tubular injury have also been explored, including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and matrix metalloproteinase-9 (MMP-9), but findings across studies have been inconsistent, highlighting the need for further validation [10,11,21].

In this study, we aimed to evaluate serum cystatin C, serum t-CAF, and a panel of urinary biomarkers reflecting injury across different nephron segments proximal tubule (α -GST, NGAL), distal tubule (π -GST), and collecting duct (calprotectin, osteopontin) in obese children with normal renal function, defined as normoalbuminuria and normal serum creatinine. Notably, cystatin C is not only a marker of glomerular filtration but may also reflect tubulointerstitial stress, as it is reabsorbed and degraded by proximal tubular cells, making its serum levels sensitive to both glomerular and tubular dysfunction [22].

Our results revealed significantly higher serum cystatin C levels in obese children compared to lean controls, reinforcing its greater sensitivity over creatinine in detecting early renal changes. These findings align with previous reports by Polidori et al. [10] and Gayret et al. [21], the latter of whom also observed elevated urinary NGAL and osteopontin suggesting early proximal tubular stress while KIM-1 and MMP-9 levels remained unchanged [21]. In a large cohort of 536 children with simple obesity, Marmarinos et al. reported a positive association between serum cystatin C and BMI, as well as higher levels in males [33], a gender-based difference not replicated in our cohort. In contrast, Codoner-Franch et al. found no significant difference in cystatin C between obese and non-obese children [22], though they noted that those with the highest levels exhibited greater cardiometabolic risk burden. Similarly, Salman et al. observed elevated cystatin C predominantly in obese children with metabolic syndrome, and reported positive correlations with dyslipidemia markers, including hypercholesterolemia, hypertriglyceridemia, and elevated LDL cholesterol [23], underscoring the link between metabolic dysfunction and early renal alterations.

Our findings regarding the association between elevated triglyceride levels and increased serum cystatin C are consistent with existing literature. Oz-Sig et al. similarly reported higher cystatin C concentrations in obese individuals, particularly when metabolic comorbidities were present. In their study comparing three groups obese patients with type 2 diabetes, metabolic syndrome, and simple obesity cystatin C levels were highest in those with diabetes, suggesting that metabolic dysregulation amplifies its expression and reinforcing its potential as an early marker of renal impairment in metabolically high-risk obese children [23].

However, the interpretation of elevated cystatin C in obesity is complicated by emerging evidence that challenges its specificity as a pure renal biomarker. Naour et al. demonstrated that obese adults with even mild reductions in creatinine clearance exhibited significantly higher serum cystatin C levels, accompanied by a threefold upregulation of cystatin C mRNA in adipose tissue [8]. This indicates that adipocytes themselves may contribute to systemic cystatin C production, potentially confounding its use as a filtration marker in obesity. A similar adipose-driven mechanism may underlie the elevated cystatin C levels reported in other pediatric studies [13,15], raising concerns about overestimation of kidney dysfunction when relying solely on this marker.

Moreover, the role of cystatin C extends beyond renal physiology. It appears to be involved in metabolic and inflammatory pathways, including adipogenesis and insulin resistance, further blurring the line between its renal and metabolic significance. This complexity is underscored by the work of Withzel et al., who showed that hypertriglyceridemia per se exerts a direct influence on serum cystatin C concentration, independent of glomerular filtration rate [17]. This suggests that lipid abnormalities common in obesity may artifactually elevate cystatin C, limiting its diagnostic accuracy in this population.

Given these limitations, we chose to investigate the C-terminal agrin fragment (t-CAF), a novel biomarker that has shown superior performance in detecting early tubular dysfunction in adult populations, including those with chronic kidney disease and post-transplant states [18,19]. Unlike cystatin C, t-CAF is not known to be synthesized outside the kidney, and its serum and urinary levels reflect proximal tubular reabsorptive capacity more specifically. Importantly, t-CAF has not yet been evaluated in either pediatric or adult obese populations making this study one of the first to explore its potential in the context of obesity-related kidney stress.

Data on biomarkers of proximal tubular injury in pediatric obesity remain limited, though existing studies largely support our findings [10,11,13]. Most reports indicate increased urinary excretion of proximal tubule-derived markers such as NGAL, NAG, and α -GST, consistent with early tubular stress in obesity. However, Gul et al. found no significant differences in these markers between obese and non-obese children [11], a finding potentially attributable to the small sample size and limited statistical power of their study.

To specifically assess distal tubular integrity, we measured urinary π -glutathione S-transferase (π -GST), an enzyme predominantly expressed in the epithelial cells of the distal tubules and collecting ducts. Notably, this study is the first to demonstrate a significant elevation in urinary π -GST levels in obese children compared to healthy controls, independent of the degree of adiposity. Given that π -GST is involved in cellular defense against oxidative stress, its increased urinary excretion likely reflects a response to obesity-induced redox imbalance a well-documented feature of adipose tissue dysfunction and systemic inflammation.

In contrast, urinary calbindin-D28K, a calcium-binding protein expressed in the distal convoluted tubule and collecting duct and considered a marker of damage to these segments, did not differ significantly between groups. This suggests that the pathological stimuli associated with obesity may not be sufficient to

alter calbindin expression or cause structural injury in these nephron segments at least in the early stages. In comparison, potent nephrotoxins such as cisplatin are known to induce marked calbindin depletion, highlighting the relative subtlety of obesity-related tubular effects.

Importantly, we observed a significant increase in urinary calprotectin (S100A8/A9), a marker of collecting duct injury and local inflammation, in obese children compared to controls, regardless of BMI category. This finding

is particularly noteworthy, as there is a paucity of pediatric data on this biomarker. The only published adult study, by Ortega et al., also reported higher calprotectin excretion in individuals with BMI > 30 compared to those with BMI < 30, particularly among patients with type 2 diabetes [24], reinforcing the link between adiposity and collecting duct stress.

Our study findings demonstrate that serum t-CAF, along with urinary netrin-1, α -GST, π -GST, and calprotectin, is significantly elevated in children with early renal tubular dysfunction, even before clinical severity manifests. These biomarkers show strong diagnostic accuracy, with serum t-CAF exhibiting the highest discriminatory power. The lack of significant differences between mild and severe subgroups suggests early and consistent biomarker release, highlighting their potential for pre-symptomatic detection. Strong correlations among markers further support their biological relevance. Overall, serum t-CAF and selected urinary biomarkers may serve as sensitive, non-invasive tools for early identification of tubular injury in pediatric populations.

Conclusion

This study demonstrates that serum tissue cystatin-associated factor (t-CAF) and selected urinary biomarkers particularly netrin-1, α -GST, π -GST, and calprotectin are significantly elevated in children with early renal tubular dysfunction, even in the absence of overt clinical symptoms or albuminuria. These markers exhibit high diagnostic accuracy, with serum t-CAF showing the strongest performance, suggesting its potential as a reliable, non-invasive indicator of early tubular injury. The lack of significant variation across severity subgroups implies that these biomarkers rise early in the disease process, making them valuable for preclinical detection. Given the limitations of

traditional markers like creatinine and cystatin C in pediatric and obese populations, the findings support the use of t-CAF and tubular-specific biomarkers as promising tools for early screening, timely intervention, and improved long-term renal outcomes in high-risk children in Southern Punjab and similar settings.

References

- 1- van Dam, M.J.C.M.; Pottel, H.; Vreugdenhil, A.C.E. Relation between obesity-related comorbidities and kidney function estimation in children. *Pediatr. Nephrol.* 2023, 38, 1867–1876.
- 2- Sandino, J.; Luzardo, L.; Morales, E.; Praga, M. Which Patients with Obesity Are at Risk for Renal Disease? *Nephron* 2021, 145, 595–603.
- 3- Czaja-Stolc, S.; Potrykus, M.; Stankiewicz, M.; Kaska, Ł.; Małgorzewicz, S. Pro-Inflammatory Profile of Adipokines in Obesity Contributes to Pathogenesis, Nutritional Disorders, and Cardiovascular Risk in Chronic Kidney Disease. *Nutrients* 2022, 14, 1457.
- 4- Polidori, N.; Giannini, C.; Salvatore, R.; Pelliccia, P.; Parisi, A.; Chiarelli, F.; Mohn, A. Role of urinary NGAL and KIM-1 as biomarkers of early kidney injury in obese prepubertal children. *J. Pediatr. Endocrinol. Metab.* 2020, 33, 1183–1189.
- 5- Assadi, F. The Growing Epidemic of Chronic Kidney Disease: Preventive Strategies to Delay the Risk for Progression to ESRD. *Adv. Exp. Med. Biol.* 2019, 1121, 57–59.
- 6- Gul, A.; Yilmaz, R.; Ozmen, Z.C.; Gumuser, R.; Demir, O.; Unsal, V. Assessment of renal function in obese and overweight children with NGAL and KIM-1 biomarkers. *Nutr. Hosp.* 2020, 34, 434–442.
- 7- Medyńska, A.; Chrzanowska, J.; Kościelska-Kasprzak, K.; Bartoszek, D.; Żabińska, M.; Zwolińska, D. Alpha-1 Acid Glycoprotein and Podocin mRNA as Novel Biomarkers for Early Glomerular Injury in Obese Children. *J. Clin. Med.* 2021, 10, 4129.
- 8- Arampatzis, S.; Chalikias, G.; Devetzi, V.; Konstantinides, S.; Huynh-Do, U.; Tziakas, D. C-terminal fragment of agrin (CAF) levels predict acute kidney injury after acute myocardial infarction. *BMC Nephrol.* 2017, 18, 202–210.
- 9- Ziegler, L.; Schlegel, M. Netrin-1: A Modulator of Macrophage Driven Acute and Chronic Inflammation. *Int. J. Mol. Sci.* 2021, 23, 275.
- 10- Lane, B.R.; Babitz, S.K.; Vlasakova, K.; Wong, A.; Noyes, S.L.; Boshoven, W.; Grady, P.; Zimmerman, C.; Engerman, S.; Gebben, M.; et al. Evaluation of Urinary Renal Biomarkers for Early Prediction of Acute Kidney Injury Following Partial Nephrectomy: A Feasibility Study. *Eur. Urol. Focus* 2018, 6, 1240–1247.
- 11- Carnazzo, V.; Redi, S.; Basile, V.; Natali, P.; Gulli, F.; Equitani, F.; Marino, M.; Basile, U. Calprotectin: Two sides of the same coin. *Rheumatology* 2024, 63, 26–33.
- 12- Chen, J.-J.; Fan, P.-C.; Kou, G.; Chang, S.-W.; Chen, Y.-T.; Lee, C.-C.; Chang, C.-H. Meta-analysis: Urinary calprotectin for discrimination of intrinsic and prerenal acute kidney injury. *J. Clin. Med.* 2019, 8, 74.
- 13- John, J.S.; Deepthi, R.V.; Rebekah, G.; Prabhu, S.B.; Ajitkumar, P.; Mathew, G.; Agarwal, I. Usefulness of urinary calprotectin as a novel marker differentiating functional from structural acute kidney injury in the critical care setting. *J. Nephrol.* 2023, 36, 695–704.
- 14- Mishra, O.P.; Prasad, R. Microalbuminuria and serum cystatin C: Biomarkers for early detection of kidney injury in children with obesity. *Indian J. Pediatr.* 2020, 87, 991–992.
- 15- Önerli Salman, D.; Şıklar, Z.; Çullaslıarslan, E.N.; Özçakar, Z.B.; Kocaay, P.; Berberoğlu, M. Evaluation of Renal Function in Obese Children and Adolescents Using Serum Cystatin C Levels, Estimated Glomerular Filtration Rate Formulae and Proteinuria: Which is most Useful? *J. Clin. Res. Pediatr. Endocrinol.* 2019, 11, 46–54.
- 16- Oz-Sig, O.; Kara, O.; Erdogan, H. Microalbuminuria and serum cystatin C in prediction of early-renal insufficiency in children with obesity. *Indian J. Pediatr.* 2020, 87, 1009–1013.

- 17- Rajeswari, M. Study of Serum C-Terminal Fragment of Agrin as a Marker for Kidney Function in Patients with Chronic Kidney Disease. Ph.D. Thesis, Dissertation for M.D Degree Branch—XIII (Biochemistry Department). Department of Biochemistry Thanjavur Medical College, Tamilnadu, India, May 2020.
- 18- Soliman, N.S.; Attia, G.F.; Ezat, S.E.; Hagra, M.M. Plasma C-Terminal Agrin Fragment (CAF) as an Early Marker for Kidney Function in Patients with Chronic Kidney Disease. *Med. J. Cairo Univ.* 2019, 87, 3297–3305.
- 19- Carullo, N.; Zicarelli, M.; Michael, A.; Faga, T.; Battaglia, Y.; Pisani, A.; Perticone, M.; Costa, D.; Ielapi, N.; Coppolino, G.; et al. Childhood Obesity: Insight into Kidney Involvement. *Int. J. Mol. Sci.* 2023, 24, 17400.
- 20- Medyńska A, Chrzanowska J, Zubkiewicz-Kucharska A, Zwolińska D. New Markers of Early Kidney Damage in Children and Adolescents with Simple Obesity. *Int J Mol Sci.* 2024 Oct 7;25(19):10769. doi: 10.3390/ijms251910769. PMID: 39409098; PMCID: PMC11476514.
- 21- Bazargani B, Moghtaderi M. New Biomarkers in Early Diagnosis of Acute Kidney Injury in Children. *Avicenna J Med Biotechnol.* 2022 Oct-Dec;14(4):264-269. PMID: 36504568; PMCID: PMC9706243.
- 22- Sriswat N, Kellum J. The role of biomarkers in acute kidney injury. *Critic Care Clinics* 2020; 36 (1): 125– 40.
- 23- Bhosale SJ, Atul P, Kulkarni AP. Biomarkers in acute kidney injury. *Indian J Crit Care Med* 2020; 24 (Suppl 3): S90– S93.
- 24- Basiratnia M, Kosimov M, Farhadi P, Azimi A, Hooman N. [Urinary calprotectin as a marker to distinguish functional and structural acute kidney injury in pediatric population]. *Iran J Pediatr* 2017; 27(5): e9727. Persian.