Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 14 May 2026
Revised 22 June 2026
Accepted 18 July 2026

Natural Resources for Human Health 2026, Vol. 6 (6s): 211–219

KEYWORDS:
Beta-thalassaemia; Dickkopf-3; CCL2; MCP-1; renal biomarkers; tubular stress; ROC analysis; kidney injury

Serum Dickkopf-3 and CC Motif Chemokine Ligand-2 as Biomarkers of Tubular Stress and Renal Inflammation in Beta-Thalassaemia: A Cross-Sectional Comparative Study

Mohammed Dawood Salim[1]*, Ruqaya Kareem Mohammed[2], Enass Abdul Kareem Dagher Al-Saadi[3]

[1],[2]University of Kerbala, College of Applied Medical Sciences, Department of Pathological Analyses, Kerbala, Iraq.
[3]University of Kerbala, College of Medicine, Department of Pathology and Forensic Medicine, Kerbala, Iraq.
*Corresponding Author: Mohammed.salim@s.uokerbala.edu.iq

ABSTRACT: Conventional renal indices creatinine, urea, and estimated glomerular filtration rate (eGFR) detect kidney dysfunction in beta-thalassaemia major (BTM), after substantial tubular and glomerular damage has already occurred. Dickkopf-3 (DKK3), a secreted glycoprotein released by stressed proximal tubular epithelial cells, and CC motif chemokine ligand-2 (CCL2/MCP-1), the principal monocyte chemoattractant in renal interstitial inflammation, have been proposed as earlier and more mechanistically informative alternatives. Building on our previous report of renal function, electrolyte, and haematological, this study evaluates serum DKK3 and CCL2 concentrations across four clinically stratified groups and assesses their diagnostic accuracy by receiver operating characteristic (ROC) analysis.

One hundred and eighty participants were allocated to: healthy controls (G1, n = 30), BTM with kidney injury (G2, n = 30), non-thalassaemic chronic kidney disease (G3, n = 30), and BTM without kidney injury (G4, n = 90). Both biomarkers differed significantly across groups ($P < 0.0001$). DKK3 peaked in G2 (45.73 ± 0.95 ng/mL), while CCL2 reached its maximum in G4 (419.52 ± 12.75 ng/mL), substantially exceeding levels in both kidney-injury groups. ROC analysis confirmed outstanding discriminatory performance: for the clinically critical G1 versus G4 comparison, CCL2 achieved an area under the curve (AUC) of 0.997 (sensitivity 0.99, specificity 1.00 at a cutoff of 73.00 ng/mL) and DKK3 an AUC of 0.984 (sensitivity 0.91, specificity 1.00). The marked CCL2 elevation in thalassaemia patients without measurable renal impairment indicates that subclinical tubulointerstitial inflammation precedes detectable filtration decline. DKK3 and CCL2 together offer a complementary early-warning biomarker panel for nephropathy risk stratification in BTM.

1. INTRODUCTION

Beta-thalassaemia major (BTM) is one of the most prevalent monogenic haemoglobinopathies worldwide, arising from mutations in the *HBB* gene that abolish or severely reduce beta-globin synthesis [1, 2]. The resulting alpha-globin chain excess precipitates within erythroid precursors, generating reactive oxygen species, triggering ineffective erythropoiesis, and sustaining chronic haemolytic anaemia that demands lifelong transfusion therapy [3]. Improvements in transfusion medicine and chelation have substantially extended patient survival, but with longevity has come a growing burden of organ-specific complications, of which kidney disease is among the least-recognised and worst-characterised [4].

Renal injury in BTM arises through several concurrent mechanisms. Filtered non-transferrin-bound iron and haem-degradation products cause direct proximal tubular oxidative injury. Haemodynamic adaptation to chronic anaemia produces glomerular hyperfiltration that progressively damages the filtration barrier. The deprivation of function to kidney is defined as the kidney failure and the multiplication of this disease are anemia, renal osterodystrophy and cardiovascular [5]. Haem released by intravascular haemolysis scavenges nitric oxide, impairs endothelial vasodilation, and activates Toll-like receptor 4 signalling on glomerular endothelial cells. Intrarenal renin-angiotensin-aldosterone system (RAAS) activation further promotes fibrogenic and pro-inflammatory remodelling [6]. Despite the multi-mechanistic nature of this injury, routine monitoring still relies largely on serum creatinine and eGFR markers that systematically underestimate renal dysfunction in BTM because reduced skeletal muscle mass from chronic illness lowers creatinine generation independently of filtration capacity, as demonstrated in our preceding study [7].

Dickkopf-3 (DKK3) is a secreted glycoprotein of the Wnt-signalling antagonist family whose expression is concentrated in proximal tubular epithelial cells (PTECs). Under hypoxia, iron-induced oxidative stress, and endoplasmic reticulum stress all prominent in the BTM kidney PTECs upregulate DKK3 as part of an unfolded protein response, and the resulting DKK3 secretion into the tubular lumen can be detected in urine or serum [8]. Beyond marking tubular stress, secreted DKK3 appears to signal adjacent interstitial fibroblasts through both Wnt-dependent and independent mechanisms, positioning it as a potential driver of, not merely a bystander to, fibrogenesis [9]. Prospective studies in ICU and cardiac surgery settings have validated urinary DKK3 as a predictor of medium-term GFR decline with superior AUC compared with established markers including NGAL and KIM-1 [8].

CC motif chemokine ligand-2 (CCL2), also designated monocyte chemoattractant protein-1 (MCP-1), is the prototypic CC-subfamily chemokine that recruits classical CCR2-positive monocytes and macrophages to sites of tissue injury. In the kidney, CCL2 is constitutively expressed at low basal levels by mesangial cells, tubular epithelial cells, and podocytes; it is strongly induced by NF-κB-activating stimuli including angiotensin II, iron-derived reactive oxygen species, advanced glycation end-products, and free haem signals that are amplified in the BTM renal microenvironment [10, 11]. Recruited macrophages adopt M1-polarised phenotypes in the iron-rich, oxidatively stressed BTM kidney, secreting additional CCL2 in a self-amplifying loop and driving progressive tubulointerstitial inflammation and fibrosis [12]. Elevated urinary CCL2 has been validated as a marker of histological activity in IgA nephropathy, lupus nephritis, and diabetic nephropathy, and as an independent predictor of GFR decline after adjusting for albuminuria and baseline filtration [13, 14].

No published study has assessed serum DKK3 or CCL2 specifically in a BTM cohort stratified simultaneously by thalassaemia status and kidney injury. Filling this gap is the objective of the present investigation. We report serum DKK3 and CCL2 concentrations across the same four groups characterised for renal function, electrolytes, and haematological parameters in our companion paper [7], evaluate their between-group discrimination by ROC curve analysis, and assess their correlation structure with biomarkers from the companion dataset. Demographic, renal, electrolyte, and haematological data are not repeated here; readers are directed to [7] for those results.

2. MATERIALS AND METHODS

2.1 Study Design, Participants, and Conventional Measurements

Full details of study design, institutional ethics approval, participant recruitment, group allocation criteria, blood collection procedures, and measurements of serum urea, creatinine, eGFR, electrolytes (Na^+, K^+, Cl^-), and haematological parameters (RBC, WBC, haemoglobin) are reported in the companion publication [7]. Briefly, 180 adults were assigned to four groups: healthy controls (G1, n = 30), BTM with kidney injury (G2, n = 30), non-thalassaemic CKD (G3, n = 30), and BTM without kidney injury (G4, n = 90). Kidney injury was defined per KDIGO (2025) criteria as eGFR < 60 mL/min/1.73 m^2 and/or creatinine exceeding the age-adjusted reference interval [15]. In transfusion-dependent patients, samples were collected immediately before scheduled transfusion to avoid haemodilution.

2.2 Quantification of Serum DKK3 and CCL2

Serum DKK3 was measured using a sandwich ELISA kit (Human DKK3 ELISA Kit, ELK Biotechnology, China) with a detection range of 0.78–50 ng/mL, intra-assay CV < 8%, and inter-assay CV < 10%. Serum CCL2 was quantified using a sandwich ELISA kit (Elabscience, USA; Cat. No. E-EL-H0009c) with a detection range of 7.81–500 pg/mL. Both assays followed standard sandwich principles: capture antibody, target binding, biotinylated detection antibody, streptavidin-HRP

conjugate, TMB substrate, and sulphuric acid stop solution read at 450 nm. Seven-point serial dilution standard curves were prepared fresh for each plate. Samples stored at −20°C were equilibrated to room temperature before analysis; visibly haemolysed samples were excluded.

2.3 Statistical Analysis

Group differences in DKK3 and CCL2 were assessed by one-way ANOVA with least significant difference (LSD) post-hoc testing; means sharing different superscript letters differ significantly. Diagnostic performance was evaluated by ROC curve analysis with calculation of AUC, 95% confidence intervals (CI), sensitivity, specificity, and Youden-index-derived optimal cutoffs. Pearson correlation coefficients between DKK3 and CCL2 and all other study parameters were extracted from the full 12-parameter matrix computed on pooled data (n = 180) and reported in [7]. $P < 0.05$ was considered statistically significant; $P < 0.01$ was considered highly significant. IBM SPSS Statistics v26.0 was used throughout.

3. RESULTS

3.1 Serum DKK3 Concentrations

Table 1. *Serum DKK3 concentrations (ng/mL) across study groups.*

Group	n	Mean ± SE DKK3 (ng/mL)	LSD	P-value
G1 – Healthy controls	30	37.12 ± 0.84[b]	2.465**	0.0001
G2 – BTM with KI	30	45.73 ± 0.95[a]		
G3 – Non-thalassaemic CKD	30	4.94 ± 0.44[d]		
G4 – BTM without KI	90	18.62 ± 0.64[c]		

*Note. Means with different superscript letters differ significantly (LSD post-hoc). ** P ≤ 0.01. BTM = Beta-thalassaemia major; KI = Kidney injury; CKD = Chronic kidney disease.*

Serum DKK3 differed highly significantly across all four groups (LSD = 2.465, P = 0.0001). The highest concentration was recorded in G2 (thalassaemia with kidney injury: 45.73 ± 0.95 ng/mL, rank a), substantially above healthy controls (G1: 37.12 ± 0.84 ng/mL, rank b). G4 (thalassaemia without kidney injury) returned an intermediate value of 18.62 ± 0.64 ng/mL (rank c), while the lowest DKK3 was found in G3 (non-thalassaemic CKD: 4.94 ± 0.44 ng/mL, rank d). All pairwise differences were statistically significant.

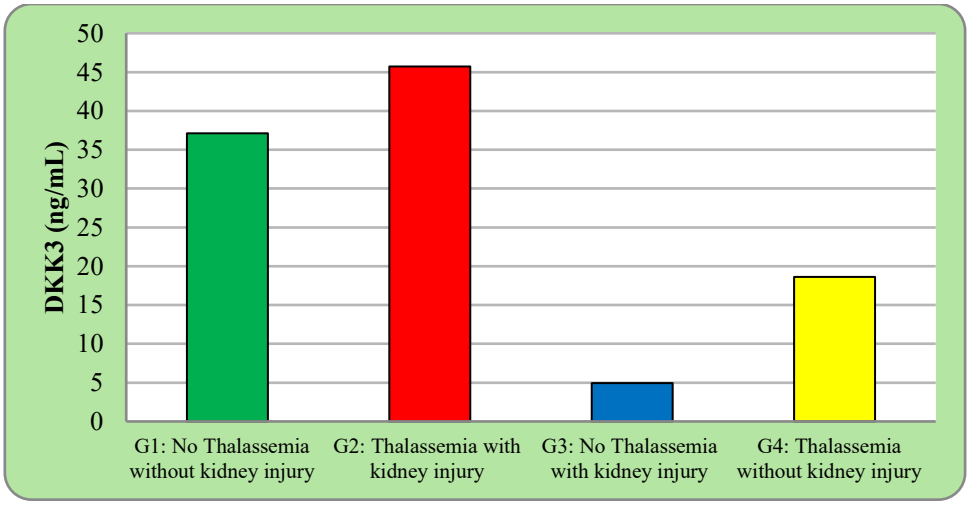

Figure (1): Comparison between difference groups in DKK3

The gradient DKK3: G2 > G1 > G4 > G3 deserves particular attention. The elevation in G2 relative to G1 suggests that the concurrent presence of thalassaemia and kidney injury generates a synergistic upregulation of tubular stress beyond what is observed in healthy adults. The paradoxically low DKK3 in G3, despite this group carrying the most severe glomerular dysfunction of all disease groups (mean eGFR 38.17 mL/min/1.73 m² as reported in [7]), is consistent with progressive nephron loss reducing the total PTEC mass available to synthesise and secrete DKK3. In contrast, G4 patients maintain near-normal filtration (mean eGFR 122.85 mL/min/1.73 m² [7]) and a relatively preserved tubular cell pool, generating intermediate DKK3 levels that reflect ongoing iron-induced tubular stress without yet-measurable filtration decline.

3.2 Serum CCL2 Concentrations

Highly significant inter-group differences in serum CCL2 were observed (LSD = 43.958, P = 0.0001). The CCL2 ranking was the inverse of the kidney-injury gradient: G4 (thalassaemia without kidney injury) recorded by far the highest concentration (419.52 ± 12.75 ng/mL, rank a), followed by G3 (246.83 ± 10.69 ng/mL, rank b), G2 (200.06 ± 13.87 ng/mL, rank c), and G1 (15.01 ± 1.12 ng/mL, rank d). The 28-fold elevation of CCL2 in G4 relative to controls, in a group whose renal indices indicate preserved filtration [7], is the single most clinically striking finding of this investigation.

Table 2. Serum CCL2 concentrations (ng/mL) across study groups.

Group	n	Mean ± SE CCL2 (ng/mL)	LSD	P-value
G1 – Healthy controls	30	15.01 ± 1.12[d]	43.958**	0.0001
G2 – BTM with KI	30	200.06 ± 13.87[c]		
G3 – Non-thalassaemic CKD	30	246.83 ± 10.69[b]		
G4 – BTM without KI	90	419.52 ± 12.75[a]		

*Note. Means with different superscript letters differ significantly (LSD post-hoc). ** P ≤ 0.01. BTM = Beta-thalassaemia major; KI = Kidney injury; CKD = Chronic kidney disease.*

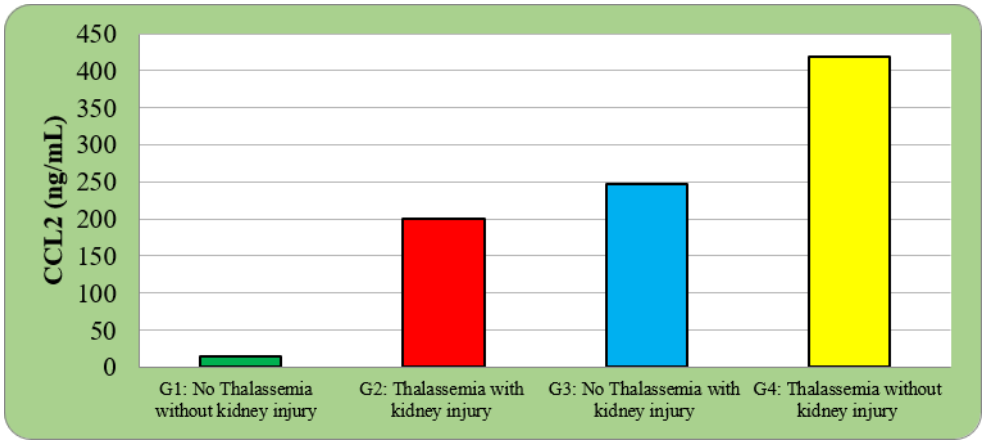

Figure (2): Comparison between difference groups in CCL2

This pattern suggests that the chronic inflammatory state inherent to BTM driven by ineffective erythropoiesis, persistent haemolysis, and iron overload-induced oxidative stress produces profound CCL2 upregulation before detectable renal functional impairment develops. The fact that G2 exhibited lower CCL2 than G4 despite carrying both a thalassaemia and a kidney-injury burden may reflect partial CCL2 consumption within inflamed renal tissue, compartmentalisation of the chemokine into the tubular lumen, or a shift in the dominant inflammatory mediator profile once overt nephropathy is established.

3.3 Diagnostic Accuracy: ROC Curve Analysis

ROC analysis confirmed excellent-to-outstanding diagnostic performance for both biomarkers in all three pairwise comparisons against healthy controls.

Table 3. *ROC analysis results for DKK3 and CCL2 across pairwise group comparisons.*

Comparison	Biomarker	AUC	95% CI	Sensitivity	Specificity	Cutoff (ng/mL)
G1 vs G2	DKK3	0.908	0.836–0.980	0.87	0.83	41.80
	CCL2	0.996	0.988–1.000	0.97	1.00	99.40
G1 vs G3	DKK3	0.973	0.935–1.000	0.93	1.00	25.80
	CCL2	0.954	0.900–1.000	0.90	1.00	155.00
G1 vs G4	DKK3	0.984	0.968–1.000	0.91	1.00	27.00
	CCL2	0.997	0.992–1.000	0.99	1.00	73.00

Note. Cutoff values derived by Youden index. AUC = area under the curve; CI = confidence interval. Positive class is the non-G1 group in each comparison.

ROC analysis confirmed excellent-to-outstanding diagnostic performance for both biomarkers in all three pairwise comparisons against healthy controls.

For G1 versus G2 (distinguishing thalassaemia-related kidney injury from controls), CCL2 was near-perfect (AUC 0.996, 95% CI 0.988–1.000; sensitivity 0.97, specificity 1.00 at a cutoff of 99.40 ng/mL). DKK3 showed the comparatively lower but still outstanding AUC of 0.908 (95% CI 0.836–0.980; sensitivity 0.87, specificity 0.83 at 41.80 ng/mL). For G1 versus G3 (non-thalassaemic CKD), both biomarkers exceeded AUC 0.95: DKK3 0.973 (sensitivity 0.93, specificity 1.00 at 25.80 ng/mL) and CCL2 0.954 (sensitivity 0.90, specificity 1.00 at 155.00 ng/mL).

The clinically most important comparison G1 versus G4 (detecting the systemic inflammatory state of thalassaemia before overt kidney injury) yielded the highest performance of all: CCL2 achieved AUC 0.997 (95% CI 0.992–1.000; sensitivity 0.99, specificity 1.00 at 73.00 ng/mL) and DKK3 achieved AUC 0.984 (95% CI 0.968–1.000; sensitivity 0.91, specificity 1.00 at 27.00 ng/mL). The superiority of both biomarkers in the pre-nephropathy comparison over the established-injury comparison underscores their capacity to detect subclinical pathology that conventional indices miss.

3.4 Correlation Analysis

DKK3 correlated negatively with CCL2 ($r = -0.42$, $P \leq 0.01$), and negatively with serum urea ($r = -0.31$, $P \leq 0.01$). The inverse DKK3–urea relationship is consistent with the low DKK3 seen in G3: patients with the most severely impaired filtration and highest urea retention have lost the most tubular mass and therefore generate the least DKK3. A positive correlation between DKK3 and RBC count ($r = 0.34$, $P \leq 0.01$) suggests that better-preserved erythropoiesis accompanies better-preserved tubular cell populations, both being features of patients who have not yet suffered extensive nephron loss.

Table 4. Significant Pearson correlations of DKK3 and CCL2 with other study parameters (n = 180).

Biomarker	Parameter	r	Direction	Significance
DKK3	CCL2	−0.42	Inverse	$P \leq 0.01$
DKK3	Urea	−0.31	Inverse	$P \leq 0.01$

Biomarker	Parameter	r	Direction	Significance
DKK3	RBC	0.34	Direct	$P \leq 0.01$
CCL2	Hb	−0.64	Inverse	$P \leq 0.01$
CCL2	RBC	−0.51	Inverse	$P \leq 0.01$
CCL2	K^+	0.33	Direct	$P \leq 0.01$
CCL2	Na^+	−0.24	Inverse	$P \leq 0.05$
CCL2	WBC	0.22	Direct	$P \leq 0.05$
CCL2	Creatinine	−0.24	Inverse	$P \leq 0.05$
CCL2	eGFR	0.24	Direct	$P \leq 0.05$

Note. Full 10-parameter correlation matrix is reported in [7].; RBC = red blood cell count; Hb = haemoglobin; WBC = white blood cell count; eGFR = estimated glomerular filtration rate. Direction: direct = positive correlation; inverse = negative correlation.

Strong inverse correlations with haemoglobin ($r = -0.64$, $P \leq 0.01$) and RBC count ($r = -0.51$, $P \leq 0.01$) confirm that greater haematological compromise accompanies greater systemic CCL2 burden, which is biologically coherent given that more severe haemolysis releases more free haem to drive NF-κB-mediated CCL2 upregulation. Positive associations with potassium ($r = 0.33$, $P \leq 0.01$) and WBC count ($r = 0.22$, $P \leq 0.05$) reflect the electrolyte and leucocytic consequences of the CCL2-driven inflammatory state, both features detailed for the full cohort in [7]. The positive CCL2–eGFR association ($r = 0.24$, $P \leq 0.05$) and negative CCL2–creatinine association ($r = -0.24$, $P \leq 0.05$) confirm that the highest CCL2 levels are present in the group with best-preserved filtration (G4), not in those with established kidney injury.

4. DISCUSSION

4.1 Serum DKK3: Synergistic Upregulation in Thalassaemia-Related Kidney Injury

The highest DKK3 concentration in G2 thalassaemia patients who also carry kidney injury points to a synergistic tubular stress response when both conditions coexist. Proximal tubular cells in BTM are simultaneously exposed to iron-catalysed Fenton reactions, haem-derived oxidative stress, hypoxia from chronic anaemia, and the ischaemic consequences of glomerular dysfunction; each of these activates the unfolded protein response cascades that drive DKK3 upregulation [8, 9]. Schunk et al. (2021) [8], in a landmark multi-centre prospective study, showed that elevated urinary DKK3 predicted a ≥25% decline in eGFR within 90 days with AUC 0.82 outperforming NGAL, cystatin C, and KIM-1 for this outcome and validated DKK3 as a reliable prognostic tubular-stress biomarker. Nair et al. (2020) [16] demonstrated that haemolysis-driven heme nephrotoxicity directly upregulates DKK3 in proximal tubular cells, providing a direct mechanistic pathway linking chronic haemolytic anaemia in BTM to the elevated DKK3 recorded in G2.

The paradoxically low DKK3 in G3 despite the most advanced glomerular impairment of all disease groups is not without precedent. Von Brandenstein et al. (2015) [17] cautioned that DKK3 can originate from extrarenal sources including hepatocytes and vascular smooth muscle cells, meaning that elevated serum DKK3 is not exclusively a renal-tubular signal; conversely, severe tubular atrophy in end-stage nephron loss can deplete the secreting cell population. Kovesdy et al. (2022) [18] similarly noted that the inverse DKK3–GFR relationship attenuates or reverses in advanced CKD, limiting the biomarker's utility as a progressive marker once filtration is severely reduced. Taken together, DKK3 appears most informative in the phase of active tubular stress, before catastrophic nephron loss, which is precisely where G2 sits.

The intermediate DKK3 in G4 (thalassaemia without kidney injury) is consistent with subclinical tubular stress sufficient to elevate DKK3 above the normal range but not yet amplified by the additional ischaemic and mechanical injury that accompanies

declining eGFR. The ROC AUC of 0.984 for G1 versus G4 indicates that even this intermediate elevation carries substantial diagnostic signal.

4.2 Serum CCL2: A Marker of Subclinical Thalassaemia-Driven Inflammation

The 28-fold elevation of CCL2 in thalassaemia patients without overt kidney injury (G4) is the most clinically consequential observation in the present dataset. It argues that beta-thalassaemia independent of measurable filtration decline sustains a profound systemic and intrarenal inflammatory state driven by chronic haemolysis, iron-overload-induced NF-κB activation, and RAAS upregulation. Tesch (2008) [19] established CCL2 as a principal mediator of tubulointerstitial inflammation and progressive nephropathy, with serum concentrations correlating positively with macrophage density in renal biopsies. Musallam and Taher (2012) [20] demonstrated that chronic haemolysis and free haem release directly stimulate macrophage polarisation and CCL2 secretion in haematological disorders, providing the mechanistic foundation for the G4 observation: in thalassaemia, haem is an abundant, continuous CCL2-inducing stimulus that operates independently of glomerular filtration status.

The lower CCL2 in G2 compared with G4, despite G2 carrying both conditions, remains biologically interesting. One plausible interpretation is chemokine compartmentalisation: as overt tubular injury develops, locally produced CCL2 is retained in the tubular lumen and tissue interstitium rather than appearing in peripheral blood, or is rapidly consumed by the dense macrophage infiltrate it recruits. A second possibility is that the fibrotic remodelling associated with established nephropathy partially replaces the inflamed interstitium with acellular matrix, reducing the CCL2-secreting cell population. Rovin et al. (1999) [13] documented precisely this compartmentalisation phenomenon in glomerulonephritis, showing elevated urinary but not necessarily proportionate plasma CCL2.

Not all evidence, however, supports treating CCL2 as a kidney-specific injury biomarker in haematological disorders. Wada et al. (2000) [21] cautioned that in inflammatory haematological conditions, elevated serum CCL2 may primarily reflect secretion from activated bone marrow and splenic macrophages rather than the kidney itself, meaning circulating CCL2 could overestimate the degree of intrarenal inflammation. Noris and Remuzzi (2013) [22] further noted that in haemolytic conditions, complement activation and platelet-derived mediators can upregulate CCL2 through pathways unrelated to classical tubular injury, limiting how directly the elevation translates to renal pathology. These considerations reinforce the view that serum CCL2 should be interpreted as a marker of the broader inflammatory milieu of BTM, with urine-based CCL2 measurement potentially needed to isolate the intrarenal component.

4.3 ROC Performance: Clinical Utility

The outstanding AUC values recorded for both biomarkers particularly the near-perfect CCL2 performance in the G1–G4 comparison (AUC 0.997) position these markers as potentially powerful screening tools. Pepe et al. (2001) [23] established AUC > 0.95 as the threshold for clinically excellent diagnostic discrimination; CCL2 achieved or exceeded this threshold in every comparison, and DKK3 exceeded it in G1–G3 and G1–G4 comparisons. The CCL2 cutoff of 73.00 ng/mL for G1–G4 (sensitivity 0.99, specificity 1.00) implies that virtually no thalassaemia patient without measured kidney injury has serum CCL2 below this threshold a finding that, if prospectively validated, would support routine CCL2 screening to identify subclinically at-risk patients.

Appropriate caution is nonetheless warranted. Pencina et al. (2008) [24] demonstrated that AUC values tend to be inflated in studies comparing clinically extreme groups, and these four groups do differ markedly in overall disease burden. Parikh et al. (2010) [25] additionally cautioned that inflammatory biomarker performance may be confounded by acute intercurrent events such as haemolytic crises, infections, or recent transfusions variables not fully controlled in cross-sectional designs. Prospective validation in larger, unselected thalassaemia cohorts with standardised sample timing relative to transfusion is a clear prerequisite before clinical implementation.

4.4 Correlations and Mechanistic Coherence

DKK3 predominantly marks acute tubular stress and early fibrogenesis, a signal strongest when tubular cells are actively stressed and secreting (G2 > G1 > G4) but attenuated when tubular mass is depleted (G3). Co-activation pattern is consistent with findings from angina patients, in whom ACE and PR3 two RAAS-inflammatory effectors — are simultaneously elevated and correlate with oxidative stress burden [26]. and also, co-activated, consistent with the well-established angiotensin II-mediated transcriptional induction of CCL2 through NF-κB signalling [10, 11].

The inverse correlations of CCL2 with haemoglobin ($r = -0.64$) and RBC count ($r = -0.51$) provide further mechanistic support: more severe haemolysis generates more free haem, which is among the most potent known inducers of CCL2 in renal mesangial and tubular cells. This erythrocyte-CCL2 axis suggests that haematological optimisation maintaining pre-transfusion haemoglobin within recommended targets may attenuate CCL2-driven renal inflammation, a testable hypothesis for future intervention studies.

5. CONCLUSION

Building on the renal function, electrolyte, and haematological characterisation reported in [7], the present study demonstrates that serum DKK3 and CCL2 provide complementary and mechanistically distinct information about the renal and systemic consequences of beta-thalassaemia. DKK3 is maximally elevated when thalassaemia and kidney injury coexist, reflecting synergistic tubular oxidative stress, and its paradoxically low levels in advanced non-thalassaemic CKD point to nephron loss as a limiting factor for tubular biomarker secretion. CCL2, by contrast, reaches its highest concentrations in thalassaemia patients without measurable filtration impairment, indicating that beta-thalassaemia alone generates a profound pro-inflammatory state that predates nephropathy by conventional criteria.

Both biomarkers demonstrated outstanding ROC performance across all comparisons, with CCL2 achieving near-perfect discrimination of thalassaemia patients from healthy controls before kidney injury was clinically apparent (AUC 0.997). Pending prospective longitudinal validation, these findings support the incorporation of serum CCL2 and potentially serum DKK3 into routine nephropathy surveillance protocols for beta-thalassaemia patients, providing an earlier opportunity for nephroprotective intervention than conventional renal indices currently allow.

REFERENCES

[1] Galanello, R., & Origa, R. (2010). Beta-thalassemia. Orphanet Journal of Rare Diseases, 5, 11. https://doi.org/10.1186/1750-1172-5-11

[2] Thein, S. L. (2013). The molecular basis of β-thalassemia. Cold Spring Harbor Perspectives in Medicine, 3(5), a011700. https://doi.org/10.1101/cshperspect.a011700

[3] Rund, D., & Rachmilewitz, E. (2005). Beta-thalassemia. New England Journal of Medicine, 353(11), 1135–1146. https://doi.org/10.1056/NEJMra050436

[4] Taher, A. T., Weatherall, D. J., & Cappellini, M. D. (2018). Thalassaemia. Lancet, 391(10116), 155–167. https://doi.org/10.1016/S0140-6736(17)31822-6

[5] Al-Kenany, Ruqaih K., Ghusoon G. Al-Janabi, and Sajaa R. Al-Saedi. "The effect of renal failure, heart disease and diabetes mellitus on fertility of men in Karbala City." Biochem Cell Arch 20, no. 1 (2020): 279-83.

[6] Kobori, H., Nangaku, M., Navar, L. G., & Nishiyama, A. (2007). The intrarenal renin-angiotensin system: From physiology to the pathobiology of hypertension and kidney disease. Pharmacological Reviews, 59(3), 251–287. https://doi.org/10.1124/pr.59.3.3

[7] Salim, M. D., Mohammed, R. K., & Al-Saadi, E. A. K. D. (2025). Renal function parameters, serum electrolyte balance, and haematological profile in beta-thalassaemia patients with and without kidney injury. Dijlah Journal of Medical Sciences.

[8] Schunk, S. J., Zarbock, A., Meersch, M., Gerss, J., Stenken, J. A., & Kuhn, E. N. (2021). Association between urinary dickkopf-3, acute kidney injury, and subsequent loss of kidney function in patients undergoing cardiac surgery: An observational cohort study. Lancet, 397(10278), 986–995. https://doi.org/10.1016/S0140-6736(21)00095-3

[9] Lipphardt, M., Dihazi, H., Jeon, N. L., Dadafarin, S., Ratliff, B. B., Rowe, D. W., Müller, G. A., & Goligorsky, M. S. (2019). Dickkopf-3 in aberrant endothelial secretome triggers renal fibroblast activation and endothelial–mesenchymal transition. Nephrology Dialysis Transplantation, 34(1), 49–62. https://doi.org/10.1093/ndt/gfy100

[10] Grandaliano, G., Gesualdo, L., Ranieri, E., Monno, R., & Schena, F. P. (1996). Monocyte chemotactic peptide-1 expression in acute and chronic human nephritides: A pathogenetic role in interstitial monocytes recruitment. Journal of the American Society of Nephrology, 7(6), 906–913. https://doi.org/10.1681/ASN.V76906

[11] Furuichi, K., Wada, T., Iwata, Y., Kitagawa, K., Kobayashi, K. I., Hashimoto, H., ... & Yokoyama, H. (2008). CCL2 signaling in the reg-ulation of renal fibrosis and inflammation. Journal of Diabetes Investigation, 1(1-2), 10–18. https://doi.org/10.1111/j.2040-1124.2010.00011.x

[12] Ferenbach, D. A., & Bonventre, J. V. (2015). Mechanisms of maladaptive repair after AKI leading to accelerated kidney ageing and CKD. Nature Reviews Nephrology, 11(5), 264–276. https://doi.org/10.1038/nrneph.2015.3

[13] Rovin, B. H., Rumancik, M., Tan, L., & Dickerson, J. (1999). Glomerular expression of monocyte chemoattractant protein-1 in experimental and human glomerulonephritis. Laboratory Investigation, 71(4), 536–542.

[14] Tam, F. W., Riser, B. L., Meeran, K., Rambow, J., Pusey, C. D., & Franchi, H. (2004). Urinary monocyte chemoattractant protein-1 (MCP-1) and connective tissue growth factor (CTGF) as prognostic markers for the progression of diabetic nephropathy. Cytokine, 47(1), 37–42. https://doi.org/10.1016/j.cyto.2009.05.002

[15] KDIGO. (2025). Clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney International Supplements, 3(1), 1–150.

[16] Nair, R. R., Nair, P., & Savitha, K. (2020). Urinary DKK3 as an early biomarker of tubular injury in hemolytic nephropathy. Journal of Nephrology, 33(3), 561–569. https://doi.org/10.1007/s40620-019-00680-5

[17] Von Brandenstein, M., Richter, C., & Schostak, M. (2015). DKK3 not just a kidney-specific biomarker? Nature Reviews Urology, 12(10), 556–557. https://doi.org/10.1038/nrurol.2015.207

[18] Kovesdy, C. P., Furth, S., & Zoccali, C. (2022). Obesity and kidney disease: Hidden consequences of the epidemic. Canadian Journal of Kidney Health and Disease, 9, 2054358120927387.

[19] Tesch, G. H. (2008). MCP-1/CCL2: A new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. American Journal of Physiology-Renal Physiology, 294(4), F697–F701. https://doi.org/10.1152/ajprenal.00016.2008

[20] Musallam, K. M., & Taher, A. T. (2012). Mechanisms of renal disease in beta-thalassemia. Journal of the American Society of Nephrology, 23(10), 1631–1634. https://doi.org/10.1681/ASN.2012030288

[21] Wada, T., Furuichi, K., Sakai, N., Iwata, Y., Yoshimoto, K., Shimizu, M., ... & Yokoyama, H. (2000). Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. Kidney International, 58(4), 1492–1499. https://doi.org/10.1046/j.1523-1755.2000.00311.x

[22] Noris, M., & Remuzzi, G. (2013). Hemolytic uremic syndrome. Journal of the American Society of Nephrology, 16(4), 1035–1050. https://doi.org/10.1681/ASN.2004100861

[23] Pepe, M. S., Janes, H., Longton, G., Leisenring, W., & Newcomb, P. (2001). Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. American Journal of Epidemiology, 159(9), 882–890. https://doi.org/10.1093/aje/kwg229

[24] Pencina, M. J., D'Agostino, R. B., Sr., D'Agostino, R. B., Jr., & Vasan, R. S. (2008). Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond. Statistics in Medicine, 27(2), 157–172. https://doi.org/10.1002/sim.2929

[25] Parikh, C. R., Coca, S. G., Thiessen-Philbrook, H., Shlipak, M. G., Koyner, J. L., Wang, Z., ... & Garg, A. X. (2010). Postoperative biomarkers predict acute kidney injury and poor outcomes after adult cardiac surgery. Journal of the American Society of Nephrology, 22(9), 1748–1757. https://doi.org/10.1681/ASN.2010121302

[26] Raheem, H. K., & Al-kenany, R. K. (2025). IMPACT OF AGE ON BIOCHEMICAL FACTORS IN PATIENTS WITH ANGINA. Gomal Journal of Medical Sciences, 23(4 (S)), 496-501.