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ORIGINAL ARTICLE

# DIAGNOSTIC IMPORTANCE OF LIVER-TYPE FATTY ACID-BINDING PROTEIN AND DICKKOPF-RELATED PROTEIN 3 IN THE PROGRESSION OF CHRONIC KIDNEY DISEASE

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## ABSTRACT

**Background:** Chronic kidney disease (CKD) is a growing global health issue throughout the world. Serum biomarkers can improve CKD diagnostics by revealing early kidney damage. Therefore, study aimed to evaluates the potential of L-FABP and DKK3 biomarkers for early detection of CKD.

**Materials & Methods:** A case-control study was organized among 200 participants in the Nishtar and Ibn-e-Siena Hospitals Multan, Pakistan from January to December 2022. L-FABP and DKK3 biomarkers were assessed via sandwich enzyme-linked immunosorbent assay (ELISA). Their diagnostic performance was evaluated by receiver–operator curve (ROC) and correlations were analyzed using Pearson coefficient (r). Data were statistically analyzed by one-way analysis of variance (ANOVA).

**Results:** The present study encompassed a total of 200 participants, comprising 130 individuals diagnosed with kidney disease and 70 healthy control subjects without any prevalent medical conditions. Serum L-FABP was significantly ($p<0.0001$) higher among AKI (299 ng/L±103), as that of CKD patients (152 ng/L±40), while DKK3 was higher ($p<0.0001$) in AKI (21 ng/ml±9.8), and CKD (6.6 ng/ml±2.3), compared to controls. ROC shows excellent CKD detection in both biomarkers (AUC > 0.99). Among CKD stages highest level of serum L-FABP and DKK3 was seen in stage-4 and 5 compared to stage-2 and 3. Correlation results found L-FABP shows a strong positive correlation with DKK3 among cases.

**Conclusion:** Serum L-FABP and DKK3 are effective biomarkers for early CKD detection, which may help in slowing CKD progression.

**KEY WORDS:** Biomarkers; Chronic Kidney Disease; Diseases; Enzyme-Linked Immunosorbent Assay; Kidney.

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## INTRODUCTION

Chronic kidney disease (CKD) is an increasingly serious public health problem throughout the world.[1-3] It is one of the non-contagious diseases that pose a significant global health threat due to consistently reduced kidney function over 3 months.[1] Globally, about 850 million people have been suffered from CKD.[2] In Pakistan, CKD affects 21.2% of the population, especially women over the age of 50 years.[3] Estimated glomerular filtration rate (eGFR) of <60 ml/min/per 1.73 $m^2$ and albuminuria of 30 mg/24 h are the main characteristics of CKD detection.[4] It is extremely challenging to measure renal tubular health using traditional techniques.[5] CKD is commonly identified late when kidney damage is severe, because traditional diagnostic

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tools, such as serum creatinine and eGFR, are not sensitive enough to detect early-stage kidney injury.[6] This late diagnosis hampers timely intervention, worsening disease progression and mortality risk, highlighting the necessity for a reliable, non-invasive detection method for early CKD. Serum biomarkers can improve CKD diagnostics by revealing kidney damage and dysfunction at a molecular level before noticeable impairment.[7] Urinary L-FABP, also known as u-L-FABP, is primarily thought to be a urine tubular biomarker linked to kidney injury. L-FABP is expressed in the proximal epithelial cells of kidney and can bind to free fatty acids (FFAs).[8] Elevated levels of u-LFABP have been reported to be strongly associated with kidney tubulointerstitial damage because of the excessive reabsorption of FFAs into the proximal region of kidney tubules.[9] Similarly, DKK-3 is a glycoprotein released by kidney cells in stressed tubular epithelium via the Wnt signaling pathway, which primarily encourages kidney tubular epithelial cells to produce fibrogenic molecules.[10] DKK-3 expression in the developing kidney ceases after maturation but reactivates upon kidney damage. This multifunctional protein influences various cellular functions, including proliferation, differentiation, and apoptosis. Elevated urine DKK3 levels has been reported to may signal individuals at high risk for rapid renal function decline in CKD.[11] The study aimed to detects CKD using L-FABP and DKK3 biomarkers in Pakistani cohort, differentiates it with acute kidney injury (AKI) patients, and finds correlations between detected biomarkers. Thus, early diagnosis through such biomarkers enabling early diagnosis, intervention, risk stratification, and improved therapeutic monitoring.

## MATERIALS AND METHODS

A case-control study was carried out among 200 participants in nephrology outpatient departments of Nishtar and Ibn-e-Siena Hospitals Multan, Pakistan from January to December 2022. The sample size of 200 was determined with 95% confidence interval and 5% absolute precision. The study protocol was approved by the Ethical Review Committee (ERC No. CRiMM/23/Research/39; October, 2023), The University of Lahore (UOL) and Institution Review Board of Nishtar (IRB No.13323/NMU) and Ibn-e-Siena Hospital (IRB No. C-18-921). Each participant was given informed consent following the Declaration of Helsinki. The study included the participants after fulfilling the inclusion and exclusion criteria. The patients having diabetes, hypertensive, cardiovascular, arthritis and chronic glomerulonephritis were included in the study, while patients with any type of cancer, uterine fibroid, steroid therapy, specific immune therapy systemic lupus erythematosus (SLE), rheumatoid arthritis, acute infection, septic shock, hypotensive, and corona virus were not included in this study. All participants' vital signs, such as temperature, heart rate, and blood pressure, were measured using standard tools such as a thermometer, stethoscope, and B.P. apparatus, respectively. For additional evaluations, blood samples were drawn from all participants and kept at 4 °C. CKD patients were sub-grouped by stages of the disease and staging was based on the Modification of Diet in Renal Disease (MDRD) equation as described by Romagnani et al.[12] Specifically designed human ELISA Kit provided by Bioassay Technology Laboratories, was used for human serum analysis. Fasting blood was centrifuged immediately after sampling at 4 °C with a speed of 1000× g for 15-30 min. Serum was tested using the L-FABP ELISA Kit (catalogue number E2159Hu) and the DKK3 ELISA Kit (catalogue number E2065Hu) via sandwich ELISA. The standard curves followed kit guidelines for biomarker quantification. Assay sensitivity was defined as per instructions. Optical density readings were taken at 450nm with a microplate reader for each well. Data were organized in Microsoft Excel and analyzed using one-way ANOVA and Tukey multiple comparison test in GraphPad Prism. "ns" indicates non-significant results ($p > 0.05$), while, asterisks(*) denote increasing significance levels. The areas under the curves (AUCs) compared diagnostic performance, calculated using receiver–operator curve (ROC) analysis in GraphPad Prism. The correlations were measured using the Pearson correlation coefficient (r) and are accompanied by their corresponding p-values, providing a comprehensive understanding of the associations among the biomarkers, and the correlation between biomarkers was assessed using a matrix scatter plot.

## RESULTS

The present study encompassed a total of 200 participants, comprising 130 individuals diagnosed with kidney disease and 70 healthy control subjects without any prevalent medical conditions. Of 130 patients, 17 had AKI and 113 had CKD. Among kidney disease patients, 63.8% were male and 36.2% were female, while the control group comprised 60% males and 40% females. The mean age in the CKD group was 46 years ±15, compared to 42 years ±15 for healthy controls.

L-FABP is a valuable biomarker for diagnosing renal disorders like CKD and AKI. Serum L-FABP levels were evaluated using the human L-FABP ELISA Kit (E2159Hu), revealing significant differences across CKD, AKI, and control groups (Table 1). AKI patients had the highest mean L-FABP level (299 ng/L±103), followed by CKD patients (152 ng/L±40), while controls showed the lowest level (26 ng/L±2.3) (**Figure 1a**). The study also highlighted significant variation in L-FABP levels across different CKD stages, with p-value < 0.0001. Mean serum L-FABP levels in CKD stages were 49 ng/L±20.4 for stage-2,

137 ng/L±8 for stage-3, 148 ng/L±24 for stage-4, and 171 ng/L±22 for stage-5. Thus, L-FABP serves as an early indicator for stages 4 and 5 of CKD (**Figure 1b**). Therefore, L-FABP is associated with CKD.

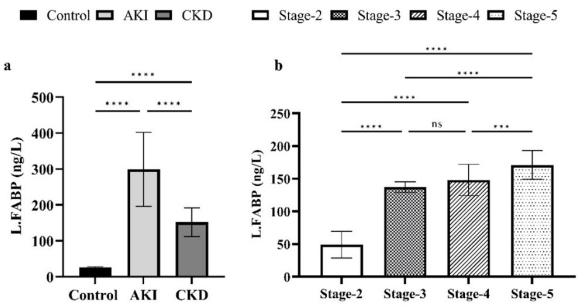


**Figure 1: Level of serum L. FABP among control, AKI, CKD (a) and among CKD stages (stage-2 to stage-5) (b).** Data were statistically analyzed by Tukey multiple comparison test and one-way ANOVA. "ns" indicates non-significant, asterisks (*) indicate statistical significance. ****= significance level (P<0.0001).

Serum DKK3 (Dickkopf-3) participates in Wnt signaling pathways and is associated with kidney development and disease. To better elucidate the relation, serum DKK3 was compared with CKD, AKI patients and control. Serum DKK3 was measured using the Human DKK3 ELISA Kit (E2065Hu). AKI patients exhibited the highest mean serum DKK3 level (21 ng/ml±9.8), followed by CKD patients (6.6 ng/ml±2.3), while controls had the lowest (0.6 ng/ml±0.3) (**Figure 2a**). Additionally, DKK3 levels varied across different CKD stages, with statistical significance (p≤0.001). Mean serum DKK3 values in CKD stages were 1.9 ng/ml±0.90 for stage-2, 3.90 ng/ml±0.6 for stage-3, 5.6 ng/ml±1.6 for stage-4, and 8.2 ng/ml±0.8 for stage-5 (**Figure 2b**). This indicates that higher DKK3 levels correlate with increased injury, making it a potential predictor for CKD progression, particularly in AKI and advanced CKD stages.

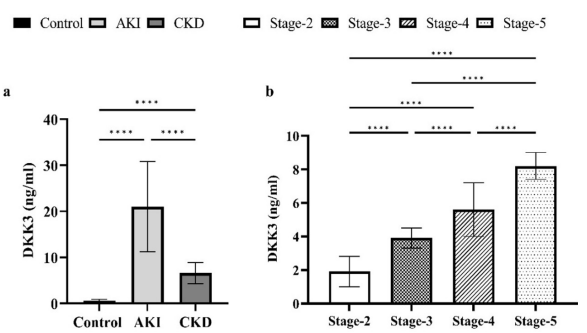


**Figure 2: Level of serum DKK3 among AKI, CKD, control (a) and among CKD stages (stage-2 to stage-5) (b).** Data were statistically analyzed by Tukey multiple comparison test and one-way ANOVA. ****= significance level (P<0.0001).

**Table 1: Serum L-FABP and DKK3 levels among normal, AKI, CKD, and CKD stages.**

| Category | L-FABP | | | DKK3 | | |
|---|---|---|---|---|---|---|
| | Mean (ng/L) | SD | N | Mean (ng/ml) | SD | N |
| Control | 26 | 2.3 | 70 | 0.6 | 0.3 | 70 |
| AKI | 299 | 103 | 17 | 21 | 9.8 | 17 |
| CKD | 152 | 40 | 113 | 6.6 | 2.3 | 113 |
| CKD stage-2 | 49 | 20.4 | 10 | 1.9 | 0.9 | 10 |
| CKD stage-3 | 137 | 8 | 14 | 3.9 | 0.6 | 14 |
| CKD stage-4 | 148 | 24 | 21 | 5.6 | 1.6 | 21 |
| CKD stage-5 | 171 | 22 | 68 | 8.2 | 0.8 | 68 |
| p value | p<0.0001 | | | p<0.0001 | | |

*Note: SD= Standard deviation, N= Total number of participants*

The comparison between the serum values of L-FABP and DKK3 with AKI and different stages of CKD is shown in figure 3. L-FABP, shown in blue in the figure, highest level in AKI and increases with a rise in the stage of the disease, while DKK3 shows as red line in figure, DKK3 level increase with increase in disease progression and highest in stage-5 (**Table 1**).

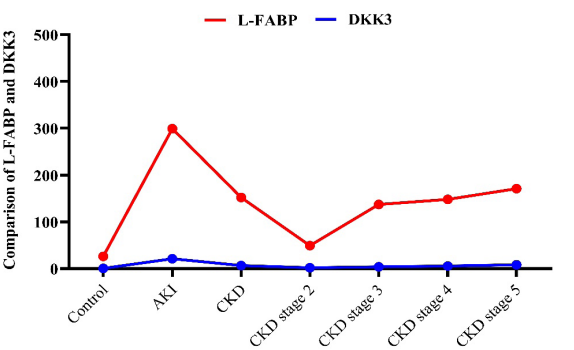


**Figure 3: Comparison of serum L-FABP and DKK3 among AKI and CKD stages.** Blue line shows the level of serum L-FABP and red line show the level of serum DKK3.

The diagnostic performance of the two investigated indicators for CKD was assessed using ROC analysis, which showed that the markers had an AUC: 0.9937, 0.9912 for L. FABP and DKK3, respectively described in Table 2. Therefore, ROC analysis showed excellent CKD detection in both biomarkers (AUC > 0.99). The correlations were measured using the Pearson correlation coefficient (r) and are accompanied by their corresponding p-values, providing a comprehensive understanding of the associations among the biomarkers. The correlation between two independent variables, i.e. L-FABP and DKK3, among cases and controls. The results of the current study found that L-FABP shows a strong positive correlation with DKK3 among cases. However, among controls,

**Table 2: Diagnostic performance of biomarkers for CKD detection.**

| Biomarkers | Category | AUC | Sensitivity | Specificity | Youden index | 95% CI | p value |
|---|---|---|---|---|---|---|---|
| FABP (ng/L) | CKD | 0.9937 | 0.936544 | 0.969251 | 0.91 | 0.9739 to 0.9940 | <0.0001 |
| | AKI | 0.7800 | 0.665445 | 0.860713 | 0.52 | 0.8700 to 0.8864 | <0.0001 |
| DKK3 (ng/ml) | CKD | 0.9912 | 0.955184 | 0.963305 | 0.91 | 0.9821 to 0.9900 | <0.0001 |
| | AKI | 0.8500 | 0.722228 | 0.805556 | 0.53 | 0.8960 to 0.9974 | <0.0001 |

*Note: AUC = Areas Under the Curves, 95% CI = 95% Confidence Interval*

L-FABP shows negative correlation with DKK3. Notably, several strong positive correlations emerge, indicating potential interconnectedness and shared behaviors among these biomarkers.

## DISCUSSION

The study focused on CKD biomarkers L-FABP and DKK3. AKI and CKD patients exhibited higher levels compared to controls, with AKI patients showing the highest concentrations. In the instance of CKD, an increase in L-FABP and DKK3 levels with an increase in stage demonstrated their role in CKD progression and prognosis.

Early detection of renal impairment is crucial for patients with CKD.[4] Clinical practice mainly relies on eGFR, renal histopathology, albuminuria, and clinical symptoms to diagnose CKD using serum creatinine rather than GFR. Factors such as illness and fever complicate the evaluation of proteinuria and eGFR, affecting CKD diagnosis and treatment timeliness.[4,13-15] Novel biomarkers like L-FABP and DKK3 are emerging non-invasive tools for early kidney injury detection, overcoming the limitations of traditional markers that indicate advanced damage.[9-11] This study involving 200 participants, L-FABP and DKK3 were utilized for CKD detection, with 130 diagnosed with kidney disease based on eGFR and creatinine levels, while 70 were healthy controls. Sun et al found that higher L-FABP levels were linked to an increased chance of developing AKI and seeing the disease worsen, demonstrating that human L-FABP as a renal tubular damage biomarker,[13] which is consistent with our study whereas, the level of L-FABP was significantly highest in AKI, compared to both CKD and control and the level of L-FABP was higher in CKD compared to control. Further, the highest level of serum L-FABP was seen in patients with CKD stage-5 and stage-4 as compared to stage-3 and stage-2 which shows the level of L-FABP increases with disease progression. Consistent with our study Ostovar et al reported that the level of L-FABP were significantly higher in CKD in comparison to control group.[9] This aligns with findings from multiple studies indicating L-FABP's association with CKD development[16] and progression,[17] underscoring its importance as a kidney injury and repair biomarker, especially in renal tubules.

The current study found significantly elevated serum DKK3 levels in patients with AKI and CKD, with the highest levels in AKI, followed by CKD, and the lowest in controls. CKD stage-4 and stage-5 patients showed the highest DKK3 levels compared to stage-2 and stage-3 patients. Kamal et al. also found a notable increase in DKK3 levels in stage-3 CKD patients treated with vitamin D3.[18] Arjune et al. study reported elevated urinary DKK3 levels in patients with autosomal dominant polycystic kidney disease versus controls,[10] suggesting a correlation between DKK3 and kidney disease. Renal tubular cells produce DKK3 under stress, and increased urinary DKK3 levels can identify patients at high risk for short-term CKD development. When we compare both serum levels, we found L-FABP was highest level in AKI and increases with a rise in the stage of the disease, while DKK3 level increases with an increase in disease progression and is highest in CKD stage-5. Further, our study found that L-FABP shows a strong positive correlation with DKK3 among cases. However, among controls, L-FABP shows a negative correlation with DKK3. González et al. found a positive correlation of urinary DKK-3, L-FABP with interstitial fibrosis in IgA Nephropathy, which shows its relation with the kidney.[19]

## CONCLUSION

It is summarized that DKK3 and L-FABP are effective non-invasive biomarkers for early CKD detection. Our studies are consistent with previous studies that demonstrate DKK3 and L-FABP as excellent indicators for AKI detection. Further our studies found these biomarkers indicate CKD presence and correlate with its various stages, showing significant potential and demonstrate excellent detection performance (AUC >0.99). Therefore, incorporating them into routine screening for high-risk populations could improve early diagnosis and management, ultimately leading to better clinical outcomes and potentially delaying or preventing the need for dialysis.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.


## AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

| | |
|---|---|
| Conception or Design: | BF, KY |
| Acquisition, Analysis or Interpretation of Data: | BF, KY, ZHQ, MMJK, KG |
| Manuscript Writing & Approval: | BF, KY, ZHQ, MMJK, MSL |

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.