The Egyptian Journal of Hospital Medicine (April 2023) Vol. 91, Page 4210-4214

Plasma Biomarker Dickkopf-3 and its Association with Chronic Kidney Disease:Review Article

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ABSTRACT

A secreted glycoprotein known as Dickkopf-related protein 3 (DKK3) has been linked to the pathophysiology of numerous illnesses. According to recent research, urine DKK3 may be used as a possible biomarker to track the onset of renal disease and assess the efficacy of treatments.

INTRODUCTION

A major public health issue on a global scale, significant morbidity, a shortened life expectancy, and an excessive use of medical resources are all symptoms of chronic kidney disease (CKD). Around 800 million people, or up to 15% of the world's population, have CKD, and the prevalence of the condition increases substantially with advancing age in the general population. [(1)].

Diabetic and/or hypertensive kidney damage are the main acute kidney injury (AKI), which occurs repeatedly, is one of the emerging causes of progressive chronic kidney disease (CKD) and kidney failure in the West. The progression of CKD is marked by a reduction in kidney function, and renal replacement therapy is necessary when end-stage kidney disease (ESKD) is reached [(2)].

The early stages of CKD are non-renal issues, together with a host of co-morbidities, contribute to the abnormally high rates of, for instance, cardiovascular mortality in CKD patients. This makes severe uremic complications caused by progressive CKD a "systemic" disease that has a significant effect on almost all organ systems. Therefore, accurate detection of individuals with continuing CKD progression has wide-ranging effects on their health as well as healthcare resource conservation [(3)].

Currently, the glomerular filtration rate (GFR) and albuminuria are acknowledged indicators of the long-term progression of CKD. Patients with CKD of diverse etiologies are categorised in the kidney disease based on their estimated GFR (e-GFR) and albuminuria: kidney disease progression is classified according to Improving Global Outcomes (KDIGO) standards as low, moderate, high, or extremely high [(4)].

Even within a given risk category, it can be challenging to predict an individual's course of CKD, especially when using disease-modifying pharmacological therapies. Thus, in order to improve the prognosis of kidney failure, risk equations have been created. These are heavily dependent on albuminuria and e-GFR, but they also take other clinical and biochemical factors into account. [(4)].

Although further expanding these equations and adding more progression indicators can boost their accuracy at the large population level to predict the 2- or 5-year likelihood for ESKD, the individual CKD course (i.e., dialysis or transplantation) is varied and difficult to predict by general equations. Indeed, research has demonstrated that both linear and non-linear GFR trajectories are associated with the advancement of CKD [(5)].

Additionally, some people's kidney function might hold steady for years without their renal disease progressing. For instance, in 1.7 million people from 35 cohorts with 12 344 ESKD incidences, a widely variable individual CKD development was seen, even within subjects classified in the same KDIGO risk category. Individuals with a baseline e-GFR of 35 mL/min/1.73 m^2 whose e-GFR remained stable within the first two years of the subsequent monitoring period had an 18% chance of having ESKD after ten years [(6)].

Patients with the same e-GFR at baseline (35 mL/min/1.73 m^2), however, who saw a mean e-GFR reduction of 57% in the first two years, had a 99% chance of developing ESKD in the ensuing ten years. Consequently, it is difficult to identify people who are at risk for rapid advancement, regardless of the etiology, but it is crucial for each patient [(7)].

Recent investigations conducted in the general population and in patients at high risk found that a considerable GFR loss could occur even in the absence of higher-grade albuminuria for developing CKD (termed non-proteinuric CKD pathway). Consequently, the requirement for biomarkers that enable the identification of patients with CKD progression is so important [(3)].

Received: 10/11/2022
Accepted: 12/01/2023

The Wnt pathway, tubular cell stress and progressive tubulointerstitial fibrosis:

In addition to being the main compartment of the kidney, the renal tubulointerstitial compartment is also susceptible to many ailments, including damage brought on by hypoxia and toxicity. Despite the long-held belief that tubular epithelia cells (TECs) are the primary target of such damage, recent experimental results revealed that TECs play a significant role in the development of CKD [(2)].

TECs can change in phenotype and function in response to injury, acting as pro-fibrotic and pro-inflammatory cells as a result. They generate a variety of bioactive chemicals that keep the harm going and ultimately result in the epithelial-mesenchymal transition and irreversible scarring of the kidneys. The latter is referred to as tubulointerstitial fibrosis, and it is the pathological hallmark of numerous CKD entities with various underlying causes that eventually result in organ failure [(4)].

However, there are currently no biomarkers for this particular renal pathologic course. This is crucial since the development of specialized treatments to arrest the course of CKD depends on a deeper comprehension of the mechanisms causing tubulointerstitial fibrosis. Numerous pro-fibrotic substances released by TECs have been found in experimental research, such as transforming growth factor beta and platelet-derived growth factor (TGF-b) [(3)]. Furthermore, it has been demonstrated that TECs can influence particular pathways, including the pathways involved in tubulointerstitial fibrosis, Wingless-Int1 (Wnt)/b-catenin and Notch [(8)].

Different cellular activities, including The Wnt/b-catenin signaling cascade affects a variety of processes, including proliferation, migration, polarity, and the production of cytokines that promote fibrogenesis. Numerous factors can either activate or inhibit Wnt signaling. In addition to the activity and/or concentration of the ligands and co-activators/co-inhibitors, important parameters in influencing the Wnt signaling include the time and duration of activity, as well as the interaction with other cytokine-triggered pathways [(4)].

The Wnt pathways in the kidney are a complicated system, and their effects may vary depending on whether TEC injury is acute or chronic. Either kidney damage advances, or repair processes predominate, depending on the intensity and length of activation. According to a theory, TECs have the ability to create Wnt ligands stimulate adjacent fibroblasts paracrinely to promote tubulointerstitial fibrosis, which consequently accelerates CKD [(8)].

Dickkopf-3 (DKK3) in kidney disease:

One of the DKK1-4 family members is Dickkopf-3 (DKK3) of glycoproteins, which control the Wnt signaling pathway. DKK genes are responsible for encoding them include the Dkk3-related gene Dkkl1 and a small gene family with four members that has undergone evolutionary conservation (Dkk1-4). The local inhibition of Wnt-regulated activities, including as limb growth and eye formation, by DKK proteins is crucial for the development of vertebrates [(2)].

In addition, numerous studies using experimental CKD models have demonstrated important impacts on Wnt/b-catenin signaling by DKK proteins. In a study using high-throughput single-nucleotide polymorphism (SNP) genotyping of 173 candidate genes in 794 white people from 227 families with autosomal dominant polycystic kidney disease, a genetic variation within the DKK3 locus was linked to a more severe disease development (ADPKD) [(9)].

Federico *et al.* [(10)] presented proof that DKK3 is expressed during kidney development, is inhibited in adulthood, and is reactivated during pathological circumstances, such as kidney tissue damage. After unilateral ureter ligation and in a mouse model of adenine nephropathy, when compared to wild-type mice, TEC damage and renal interstitial fibrosis were significantly reduced in Dkk3-deficient mice. Similar outcomes were attained using antibody-mediated DKK3 inhibition.

Parallel to this, the authors found that unilateral ureter ligation of Dkk3 mice resulted in an increase of T lymphocytes (mostly The kidneys contain Foxp3 regulatory T cells and Th1 cells that make interferon c. The scientists found that a genetic Dkk3 deficit within tubular epithelial cells was adequate to prevent kidney fibrosis and tubular injury after unilateral ureter ligation by using Dkk3fl/flPax8Cre in rats. This shows that TEC is essential for the development of renal fibrosis [(10)].

DKK3 appears to be a part of evolutionary conserved gene clusters that are expressed during disease (or "stress") states and re-expressed during developmental processes. Because of this, it is intriguing since prior in vitro studies showed that DKK3 can, depending on the tissue examined, either activate or repress canonical Wnt/b-catenin signaling [(4)].

Urinary DKK3; source and origin:

DKK3 was not found in the urine of healthy mice, however it has been shown that animals fed an adenine-rich diet have much increased urinary quantities of the protein. Luciferase and mCherry are expressed under the regulatory domains of the Dkk3 gene in reporter mice. Using bioluminescence imaging, no luciferase activity was found in the healthy control mice. In contrast,

luciferase activity has been found inside the wounded kidney two days following unilateral ureter ligation [10].

Cherry co-localized with aquaporin 1 and fluorescence (a marker for proximal TEC) and aquaporin 2 (a sign for distal TEC) to a lesser extent, but was undetectable in other kidney compartments. As a result, TEC seems to be the kidney's exclusive source of DKK3. Due to this, numerous authors discovered that CKD patients' urine DKK3 concentrations were much greater than those of apparently healthy individuals in the general population [3].

DKK3 can be detected in plasma since it is expressed in other organs as well. This suggests that, following glomerular damage, filtered plasma DKK3 may also be the source of urine DKK3. DKK3 has a 38 kDa calculated molecular weight. However, it has been shown that DKK3 may be extensively glycosylated, resulting in a rise in its molecular weight to 60–70 kDa [8].

Recent research by numerous authors revealed that participants without CKD did not exhibit a correlation between urine concentrations of DKK3 and albuminuria. According to the 0.258 correlation coefficient between urine DKK3 and albuminuria, albuminuria is responsible for 25.8% of the urine DKK3 variability [11].

Greater albuminuria (>300 mg/g creatinine) was related with greater urine DKK3 levels, which were measured as part of the CARE FOR HOME (Cardiovascular and Renal Outcome in CKD Patients) study, which involved 575 CKD patients with a variety of underlying conditions; as opposed to individuals whose urine albumin excretion was 30 mg/g. But in 48.6% of the subjects with higher grade albuminuria and in some cases even below the detection limit, urine DKK3 levels remained modest (i.e., 1000 pg/mg creatinine) [7].

Therefore, despite the fact that plasma DKK3's molecular mass may not considerably differ from, for example, albumin, the reason why it may not penetrate the glomerular barrier in these patients is still unknown. This may be due to high molecular weight complexes that inhibit plasma DKK3 from overcoming the glomerular barrier or interactions between plasma DKK3 and other elements of the blood plasma [2].

Future research is required to examine the precise pathway of plasma and urine DKK3 after renal damage. **Zewinger *et al.*** failed to find a significant correlation between DKK3 in plasma and urine. However, because DKK3 is only expressed in the TEC of the injured kidney, it may be possible to use urine DKK3 as a non-invasive diagnostic biomarker for TEC injury that is still occurring at the onset of CKD [11].

By using hybridoma cells to produce antibodies against DKK3, the German Cancer Research Center in Heidelberg also devised an enzyme-linked immunosorbent test (ELISA) with detection parameters particularly designed for detecting the DKK3 protein in human urine. In the EU, the ELISA has been authorized for use as a tool for patient diagnosis (ReFiNE, DiaRen UG, Homburg/Saar, Germany) [11].

According to studies, DKK3 is stable in urine samples kept at 4°C for up to 24 hours (10% breakdown). An alternative is to instantly freeze urine samples (0.5 mL) at -20°C (for longer periods at -80°C) until they are analyzed. The assay's lowest detection limit is 30 pg/mL. The repeatable urine sample readings' intra-assay test variabilities fall between 3.1% in the lower detection range (500 pg/mL) and 3.5% in the higher detection range (1500 pg/mL). 4.7% The values for inter-assay test variability are 5.1% in the higher detection range and 5.1% in the lower detection range. A cross-reactivity with additional DKK family proteins is not present [7].

DKK3: a biomarker of short-term CKD progression:

Prior to study inclusion, patients undergoing University Diagnostic Kidney Biopsies Hospital Innsbruck in Austria and participants in the Supportive Versus Immunosuppressive Therapy for the Treatment of Progressive IgA Nephropathy (STOP- IgAN) trial were included in a large study that examined the relationship between urinary DKK3 and tubulointerstitial fibrosis [12].

Intriguingly, **Zewinger *et al.*** discovered that in both individuals' biopsy samples were primarily interstitial diseases as well as patients with primarily glomerular diseases, greater urine DKK3 concentrations were strongly linked (P 0.001) with higher-grade tubulointerstitial fibrosis [11].

With a mean follow-up, the prospective CARE FOR HOME study of 5.1 years, which included patients of varied CKD etiologies, they additionally evaluated the association between urine DKK3 concentrations and annual variations of e-GFR in order to further investigate this issue in people. A total of 2035 person-years from annual patient visits were available for a 1-year block analysis for this reason [11].

In addition, **Rauen *et al.*** [12] evaluated urine DKK3 levels in participants in the STOP-IgAN experiment who had IgA nephropathy. After adjusting for a number of clinical factors, such as baseline e-GFR and albuminuria, the CARE FOR HOME study found that urine DKK3 concentrations were substantially and independently linked with a reduction in e-GFR in the next 12 months.

In addition, a mean e-GFR drop of 12.2% during the 6-month run-in phase was independently linked to urine DKK3 >1000 pg/mg creatinine. In STOP-IgAN, the addition of urinary DKK3 to a model that included age, sex, body mass index, systolic blood pressure, e-GFR, and albuminuria markedly improved integrated discrimination, net reclassification improvement (NRI), and c-statistics for the prediction of >0 and >5% decrease of e-GFR during the run-in phase [12].

In the study by **Zewinger *et al.*** [11] a rise in urine DKK3 concentration was linked to a significant e-GFR reduction within the subsequent first six months of the treatment period, whereas constant or declining urinary DKK3 indicated a more favorable path of renal function. This outcome was unrelated to how the treatment arms were randomly assigned. These results emphasize urinary DKK3 as a biomarker for short-term CKD advancement, which may be crucial for nephrologists in particular to monitor medications that stop or even stop progression.

In contrast to determining the long-term prognosis of CKD by considering specific renal endpoints like ESRD or a 40% GFR drop after several years, the evaluation of short-term loss of GFR takes a different approach. While urine DKK3 shows a patient's short-term loss of kidney function, the latter provides a risk assessment of how many patients will experience a renal endpoint. Future research must evaluate the relationship between urine DKK3 and the aforementioned predefined renal outcomes. [3].

Even after accounting for albuminuria, variations in urine DKK3 levels were independently linked with variations in e-GFR in both cohorts, the CARE FOR HOME study and the randomized STOP-IgAN trial. In addition, urine DKK3 in patients with normal albumin excretion rate similarly indicated the deterioration in renal function. This suggests that DKK3 production in the urine and albuminuria/proteinuria may not be connected by the same pathophysiological pathways. Therefore, more research into the mechanisms of renal disease progression in non-proteinuric CKD is necessary [8].

Schunk *et al.* [7] examined urinary DKK3 concentrations in these patients after particular treatment initiation to visualize the individual course of DKK3 in patients with primary kidney disorders (i.e., complement C3 glomerulonephritis, granulomatosis with polyangiitis, and microscopic polyangiitis). Notably, they discovered that urine DKK3 decreased within 30 days of the start of the prescribed treatment (cyclophosphamide or rituximab/corticosteroids).

DKK3 levels in the urine returned to normal before kidney function did. Larger studies have been started to see if DKK3 in urine could indicate the efficacy of treatment for various CKD types even before GFR increases or stabilizes, renal function [2].

DKK3: a biomarker of risk prediction of AKI and AKI–CKD transition:

It is generally known that AKI, especially recurrent AKI, is an increasing factor in the development of both progressive CKD and ESKD. For instance, AKI, which affects 26.0% to 28.5% of individuals following heart surgery, is the most common consequence. Due to a rising percentage of older, AKI is becoming more common, particularly in multi-morbid individuals after heart surgery [3].

Acute kidney disease, which is the name for prolonged renal impairment lasting up to 90 days, or (seemingly) complete restoration of kidney function can occur over the course of AKI, which can be extremely varied (AKD). Last but not least, in some individuals, kidney function continues to diminish or even becomes permanently reduced; this process is now known as the AKI-CKD transition [4].

People with AKI are 8.8 times more likely to develop CKD than people without AKI. As a result, it is possible to think of AKI, AKD, and CKD as a "kidney injury continuum" that ultimately results in ESKD. The severe health and socioeconomic effects of AKI, particularly its progression to CKD, make it imperative to identify those who are at higher risk in order to apply preventative strategies for the prevention of AKI and the consequent loss of kidney function [8].

Therefore, several publications have investigated the therapeutic applicability of urine DKK3 preoperatively measured as a marker for postoperative AKI and consequently the progression of patients from AKI to CKD undergoing elective heart surgery. In fact, they discovered that urinary DKK3 prior to surgery was independently linked to a noticeably greater risk of AKI following surgery. The risk of AKI was predicted by urinary DKK3 among patients whose e-GFR was normal before to surgery or whose renal function seemed to be normal [2].

Notably, those allocated to the trajectory group experienced substantial e-GFR loss when AKI and CKD progressed during an extended period of follow-up were substantially more likely to have greater baseline urine DKK3. Accordingly, subjects with higher baseline DKK3 had reduced e-GFR following long-term follow-up [3].

Prior to heart surgery, patients were randomly assigned to receive remote ischemia preconditioning (RIPC) or a sham operation in the prospective RenalRIP (Renal Effects of Remote ischemia Preconditioning in heart Surgery) investigation, corroborated these findings. [13].

Finally, Renal RIP was only linked to a decreased risk of postoperative AKI among patients with preoperatively increased urine DKK3, according to **Schunk *et al.*** [14] post hoc analysis of the data. To determine whether individuals with high urine DKK3 would benefit from particular therapeutic measures, additional prospective studies are required.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest: There are no conflicts of interest, according to the authors.

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