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NGAL , KIM-1, and MCP-1 as Biomarkers of Kidney Injury

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Abstract: Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein that was initially thought to be associated with human neutrophil gelatinase, which is secreted by activated neutrophils. Many clinical studies have followed these observations . Numerous reports have proved that NGAL is synthesized in kidney tissue following several mechanisms of kidney injury, such as ischemic, nephrotoxic like contrast material injection for radiological purposes, or septic. It is nowadays known that NGAL is a marker of renal tubular damage as it is released from the distal tube. It is filtered through the glomerular membrane and is reabsorbed in the proximal tubule of the kidney. Kidney injury molecule-1 (KIM-1) is a marker for proximal tubular injury, the hallmark of virtually all proteinuric, toxic and ischaemic renal diseases. The selective KIM-1 expression by injured proximal tubular cells provides a strong evidence for using KIM-1 as a biomarker of damage. When renal epithelial cells die, a soluble KIM-1 together with the fluid may be entrained to the interstitium and enter therefrom the bloodstream . Higher KIM-1 concentration in blood together with its increased contents in urine can also reflect injury of the kidney tubular apparatus. Findings of several studies have shown that urinary KIM-1 is an early marker of AKI induced by the administration of the contrast agent to the patients who undergo coronary or peripheral angiography. Monocyte chemoattractant protein-1 (MCP-1), also referred to as chemokine ligand 2 (CCL2), belongs to the CC subfamily of cytokines. Increased glomerular synthesis of CC chemokines, including MCP-1, has been shown to correlate with monocyte/macrophage infiltration in experimental models of antibody and immune complex–mediated glomerulonephritis

Keywords: *NGAL, KIM-1, MCP-1*

Introduction

Over the years, the increasing use of diagnostic and interventional procedures requiring the use of contrast media (CM) has progressively increased the population exposed to the risk of CI-AKI.

Iodinated contrast materials (ICM) are widely used in many diagnostics and surgical procedures. However, it is well established that CM exposure causes iatrogenic kidney impairment, which incidence is highly increasing, especially, in patients with preexisting cardiovascular, diabetes or renal disease. Therefore, CM-associated kidney dysfunction variates from slight serum creatinine (SCr) increase to severe Acute Kidney

Injury (AKI) AKI secondary to CM injection is called contrast-induced nephropathy (CIN) or contrast-induced AKI (CI-AKI) ***(1***)..

Worryingly, the enormous burden of CM used in contemporary clinical practice explains why CI-AKI is one of the top leading forms of hospital-acquired renal disease, being in fact, the third most common cause of AKI ***(2).***

CI-AKI is estimated as constituting the third cause of AKI in hospitalized patients, following prerenal kidney failure caused by reduced perfusion and that due to the administration of nephrotoxic medications, representing about 10% of all cases of AKI ***(2).***

The overall incidence of CI-AKI is is very variable , mainly due to the use of numerous definitions of CI-AKI, the heterogeneity of the populations investigated and the different procedures under examination ***(3).***

In recent decades, the identification of new biomarkers of AKI has been the subject of interest by scientists worldwide. However, the predictive, diagnostic and prognostic ability of biomarkers in the context of iodinated contrast administration, has been less studied. In recent years, many additional potential biomarkers have been newly described for the early detection of tubular dysfunction/lesion associated with CM administration, to reliably measure CI-AKI, and thus prevent patient outcomes.

Some of them are well characterized and categorized and could be divided into different groups in response to different physiological conditions: some involved in glomerular filtration e.g cyststin c, others related to the inflammatory response e.g. MCP-1,YKL-40,IL-18 and tubular cell injury e.g KIM-1,NGAL and LFABP or with a not well-defined relationship with the disease and new emergent biomarkers under study e.g CTGF and suPAR ***(4)***.

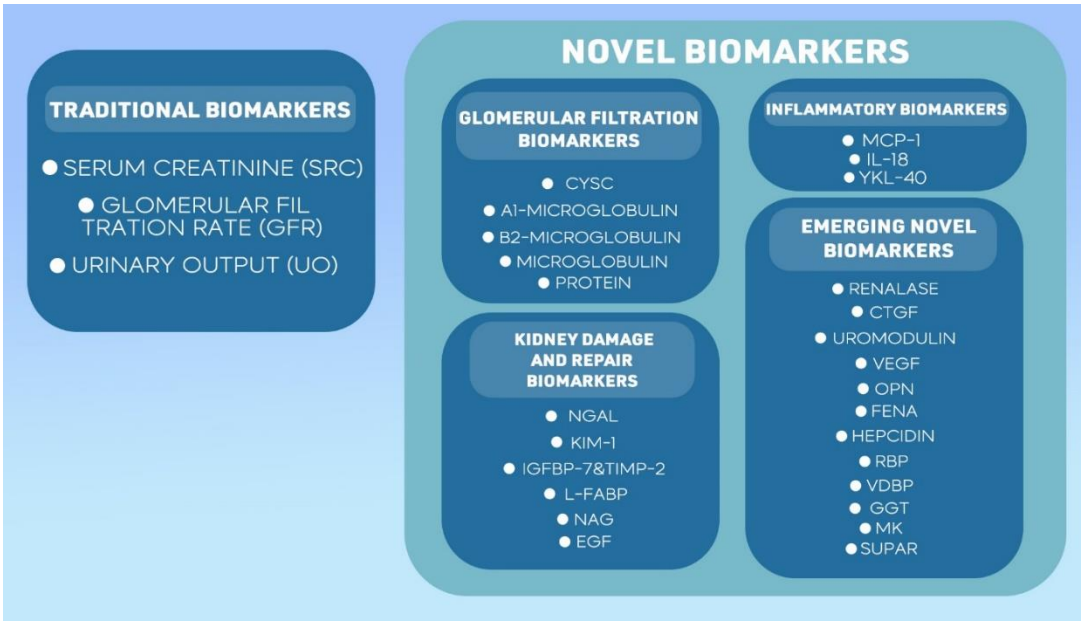

Figure (1) Traditional and novel biomarkers classification.

1-Neutrophil Gelatinase-Associated Lipocalin (NGAL):

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein that was initially thought to be associated with human neutrophil gelatinase, which is secreted by activated neutrophils . Although neutrophils are the main source of NGAL, their expression is also found in numerous human tissues, including tubular cells in the kidney, heart, lung, liver, stomach, colon, epithelial cells, macrophages, dendritic cells and adipocytes **(5*).***

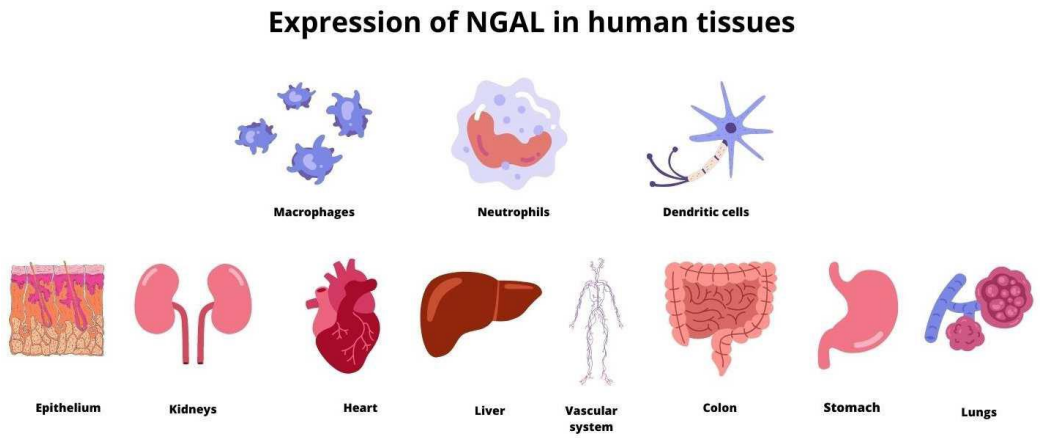

Figure (2): The Expression of NGAL in Numerous Human Tissue (6).

Its structure is made of 8 β-strands that form a β-barrel enclosing a calyx forming a three-dimensional barrel which contains a ligand-binding site called calyx which allows receptors to attach to the surface of membranes and create bigger molecules ***(7)***.

Neutrophil chemoattractants, specifically N-formylated tripeptides, with leukotriene B4 and the platelet-activating factor, are the major group of ligands that connect to the binding side of NGAL ***(7)***.

There are three forms of NGAL:

1- Monomeric NGAL, a 25-kDa glycoprotein mainly released from neutrophils and epithelial tissues, including renal tubular epithelial cells
2- Homodimeric NGAL, a 45-kDa protein produced by neutrophils
3- Heterodimeric NGAL, a 135-kDa heterodimer of NGAL with matrix metalloproteinase (MMP-9). The connection of NGAL to MMP-9 increases the activity of MMP-9 and protects against its degradation. This results in enhanced proteolysis and the dissolution of collagen and contributes to fibrosis . Renal tubular cells produce this heterodimer when subjected to stress ***(8).***

Two receptors for NGAL can be found:

a) Lipocalin-2 receptor (24p3R): The role of the lipocalin-2 receptor is multifactorial. It regulates intracellular iron concentrations. It is also expressed in cardiomyocytes with up-regulation during myocarditis and is known to be involved in smooth muscle cell proliferation, cardiac remodeling, and cardiac fibrosis ***(9)***.

b) Megalin receptor. The role of the megalin receptor is not yet well understood. Studies have shown that it binds NGAL with high affinity: much higher than other lipocalins. Its expression can be observed in cardiomyocytes, kidney and ileum epithelial cells, lung ependyma, epididymis, immune cells, and numerous types of cancer cells. It is involved in various cancer processes such as proliferation, migration, angiogenesis, immunotolerance, and multidrug resistance ***(10)***.

The Biological Role of NGAL:

NGAL is protein involved in iron activity. The molecule of NGAL-containing iron interacts with receptors on the cell surface. Then, it is transported into the cell and releases iron inside. NGAL that is not bound to iron also interacts with the cell surface receptors, which results in an intracellular iron transfer out of the cell ***(11)***.

1- NGAL in Infections:

NGAL plays a role as acute phase reactant that includes antibacterial immune response. Inflammatory cytokines induce NGAL expression in neutrophils, epithelial cells, or hepatocytes***.*** The injury of epithelial cells in the intestine, stomach, liver, or lungs during infections results in an increase in plasma NGAL concentrations . NGAL modulates iron transport as part of antibacterial immunity. During inflammatory processes, bacteria synthesize siderophores. The high affinity of siderophores for iron results in its dissociation from lactoferrin and transferrin and its transfer into the pathogen ***(12).***

The macrophage of Toll-like receptor (TLR) stimulation up-regulates the NGAL gene and enhances NGAL synthesis. NGAL sequestrates siderophores, prevents bacteria from obtaining iron, and thus decreases bacteria growth and multiplication, as the pathogen's ability to proliferate is often dependent on the bioavailability of iron. NGAL has been observed to prevent the production of siderophores by Escherichia coli, which could be involved in pneumonia. NGAL also protects the respiratory system from other types of infections, such as Stapylococcus aureus, Klebsiella pneumoniae, or Mycobacterium tuberculosis. Additionally, NGAL up-regulates bacterial clearance from the urinary system ***(13).***

It has been found that the function of neutrophils is impaired in the state of an NGAL deficit. This may result in the dysfunction of chemotaxis and the adhesion and migration of inflammatory cells. NGAL concentrations are increased in sepsis and correlate with inflammatory parameters such as interleukin-6 (IL-6), interleukin-10 (IL-10), vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), tumor necrosis factoralpha (TNF-alpha), the C-reactive protein (CRP) and leukocytes account [43–45]. Additionally, plasma NGAL levels are higher in patients with septic shock and sepsis-related organ failure compared to those with a milder course of sepsis. However, further studies are necessary to assess if the risk of mortality is also higher in individuals with elevated plasma NGAL concentrations [43,47,48]. NGAL is also involved in fungal and viral infections ***(14)***.

NGAL in Metabolic Complications:

NGAL concentrations rise in many pathological states. NGAL is expressed in numerous types of tissues, and its concentrations increase during injury. Inflammatory cytokines induce NGAL expression in neutrophils, epithelial cells, or hepatocytes [53–55]. Adipose tissue is also the source of NGAL, NGAL concentrations are higher in obesity, diabetes mellitus type 2, and nonalcoholic fatty liver disease**.** There are studies that have proved that the estimation of NGAL concentrations in serum could be useful in predicting the metabolic complications caused by obesity. NGAL is an independent risk factor for insulin resistance, systolic blood pressure, and lipid metabolism disorders ***(15).***

NGAL urinary excretion is a marker of diabetic nephropathy and is detected in urine early as a result of high serum glucose concentrations, even before kidney injury [64,65]. Urine NGAL concentrations correlate with the stage of renal damage in diabetic nephropathy ***(16).***

NGAL in Carcinogenesis:

NGAL is also known as a growth factor. It stimulates the proliferation and differentiation of epithelial cells . Due to this fact, the role of NGAL has been assessed in different types of cancer. Studies have demonstrated that NGAL promotes breast cancer progression through the induction of mesenchymal markers such as vimentin or fibronectin. Also it has a role in progression of thyroid and gastric cancers. Hopefully, NGAL could even be a biomarker of malignancy and the aim of anticancer therapies in the future, but further research is necessary ***(17).***

NGAL in Arrhythmias:

As NGAL participates in cardiac remodeling, it could also be hypothesized that it is also involved in the occurrence of atrial fibrillation (AF) episodes. The maintenance of sinus rhythm after an episode of atrial fibrillation and electrical cardioversion depends on various factors, e.g., the left atrium size, age, or the longevity of atrial fibrillation. The study of Mlodawska found that plasma concentrations of the NGAL/MMP complex could predict AF recurrence after successful electrical cardioversion in obese patients. The increased concentrations of serum NGAL/MMP levels were positively associated with the recurrence of AF ***(18)***.

NGAL in Kidney Diseases:

In 2003 Mishra proposed that NGAL could be an early biomarker of acute kidney injury (AKI), initially in experimental and then clinical studies. They found that in a mouse model of renal ischemia injury, NGAL was a very sensitive marker of ischemic AKI, and its urine concentrations were associated with the severity and duration of ischemia ***(19)***.

Many clinical studies have followed these observations . Numerous reports have proved that NGAL is synthesized in kidney tissue following several mechanisms of kidney injury, such as ischemic, nephrotoxic like contrast material injection for radiological purposes, or septic ***(14)***.

NGAL can be detected in plasma within two hours of AKI, with a concentration peak after 6 h. Increased serum NGAL levels were observed for approximately five days after AKI before they decreased. AKI also resulted in the elevation of urine NGAL levels. Elevated serum and urine NGAL, due to AKI, were observed 24 h earlier than the increase in creatinineThere was no superiority of plasma NGAL over urine NGAL and urine NGAL over plasma NGAL in AKI, and these can be used according to laboratory preference ***(20)***.

It is nowadays known that NGAL is a marker of renal tubular damage as it is released from the distal tube. It is filtered through the glomerular membrane and is reabsorbed in the proximal tubule of the kidney. The NGAL observed in urine is caused by proximal tubular damage or originates from its up-regulated synthesis in the distal part of the nephron, especially in the ascending limb and Henle's loop, and in the collecting duct ***(21)***.

The expression of NGAL in the regenerating tubular epithelial cells increases significantly after kidney injury. Thus, NGAL in urine during AKI often originates from an impaired reabsorption in the proximal tubule segments and also from its increased production and secretion in the distal parts of the nephron.

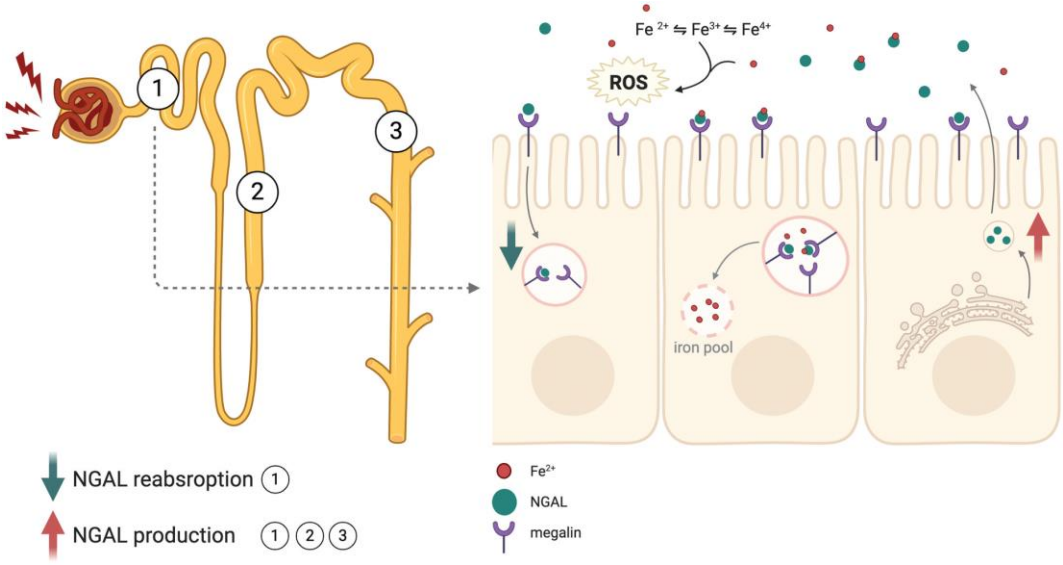

Figure (3): Elevation of Urine NGAL Levels During kidney Injury. In response to kidney injury, epithelial cells from the proximal tubule, thick ascending loop of Henle, and the collecting duct increase NGAL expression. The proximal tubule also reduces its uptake of filtrated NGAL. In the kidney, NGAL may a play role in iron chelation and transportation ***(22).***

Increased plasma NGAL levels during AKI are multifactorial. One of the sources of NGAL in the serum is the activation process of neutrophils and monocytes during the acute phase of the reaction ***(23)***.

Additionally, the synthesis of NGAL in the liver and lungs during AKI is significantly elevated ***(21)***.

Moreover, a decrease in renal function results in the accumulation of NGAL in plasma, and an increase in its serum concentrations could be observed ***(25)***.

NGAL in the prediction of AKI:

Preclinical transcriptome profiling studies identified NGAL to be one of the most upregulated genes in the kidney very early after acute injury in animal models. Downstream proteomic analyses also revealed NGAL to be one of the most highly induced proteins in the kidney after ischemic or nephrotoxic AKI in animal models ***(25)***.

The incidental finding that NGAL protein was easily detected in the urine soon after AKI in animal studies has initiated a number of translational studies to evaluate NGAL as a non-invasive biomarker in human AKI. In

a cross-sectional study of adults with established AKI (doubling of serum creatinine) from varying etiologies, a marked increase in urine and serum NGAL was documented by western blotting when compared with normal controls ***(26)***.

Urine and serum NGAL levels correlated with serum creatinine an d kidney biopsies in subjects with AKI who demonstrated intense accumulation of immunoreactive NGAL in cortical tubules, confirming NGAL as a sensitive index of established AKI in humans. A number of subsequent studies have now implicated NGAL as a promising, non-invasive, troponin-like, early diagnostic biomarker for AKI in various common clinical settings. NGAL appears to fulfill many characteristics of an ideal AKI biomarker.

Table 1. NGAL as an AKI biomarker.

NGAL properties
Non-invasive, rapid, inexpensive method in urine or blood
Sensitive for early diagnosis
Amenable to existing clinical assay platforms
High gradient to allow risk stratification
Specific to AKI (AKI versus CKD vs. Systemic Disease)*
Differential diagnosis between pre-renal azotemia and AKI
The increase is proportional to injury or loss of renal function
Associated with a clear pathophysiological mechanism
Results predict clinical outcomes and treatment efficacy

Note: Plasma NGAL may be detected in chronic kidney disease (CKD), chronic hypertension, systemic infections, inflammatory conditions and malignancies.6,7 Urine NGAL may be detected in CKD, lupus nephritis and urinary tract infections.6,7 In all these situations, NGAL values are generally substantially blunted compared to those typically measured in AKI ***(26)***.

NGAL in CI-AKI:

Several investigators have examined the role of plasma and urine NGAL as a predictive biomarker of CI-AKI in adults and children undergoing contrast administration and revealed good diagnostic and prognostic performance ***(27)***.

In a prospective study of 91 children with congenital heart disease undergoing elective cardiac catheterization and angiography with contrast administration, both urine and plasma NGAL measured at 2-h post-procedure predicted CI-AKI, defined as a 50% increase in SCr from baseline, with an area under the receiver-operating characteristic curve (AUC-ROC) of 0.91– 0.92. A study from China detected CI-AKI in 8.7% of adults with normal renal function undergoing coronary angiography and found that urinary NGAL measured by a research-based assay at 24-h postprocedure increased significantly in the CI-AKI group, but not in the non-CI-AKI group ***(28)***.

Finally, a meta-analysis revealed an overall AUC-ROC of 0.894 for CIN prediction, when plasma or urine NGAL was measured within 6 h after contrast administration for coronary procedures ***(27)***. In brief, current evidence suggests that NGAL could help in early AKI detection.

2-Kidney Injury Molecule Type 1 (KIM-1):

Kidney injury molecule-1 (KIM-1) is a marker for proximal tubular injury, the hallmark of virtually all proteinuric, toxic and ischaemic renal diseases.

Recently, much attention has been paid to its possible pathophysiological role in modulating tubular damage and repair. In this respect, it is interesting that the best biomarkers often turn out to be important in damage modulation and some even become therapeutic targets ***(29)***.

Structural and Biochemical Aspects of KIM-1:

KIM-1 is a type I transmembrane glycoprotein, It is a 104 kDa peptide, comprising a 14 kDa membrane-bound fragment and a 90 kDa soluble portion. The extracellular portion contains a six-cysteine

immunoglobulin-like domain and a Thr/Ser-Pro-rich domain, which is characteristic of mucin-like O-glycosylated proteins. The cytoplasmic portion is relatively short with two splice variants, KIM-1a and KIM-1b. The KIM-1a variant lacks the tyrosine kinase phosphorylation motif and is mainly expressed in the liver. The KIM-1 genes have high homology with the monkey gene for hepatitis A virus cell receptor 1 (HAVcr-1), which is expressed by hepatocytes and could promote cellular entry of the virus in certain conditions ***(30)***.

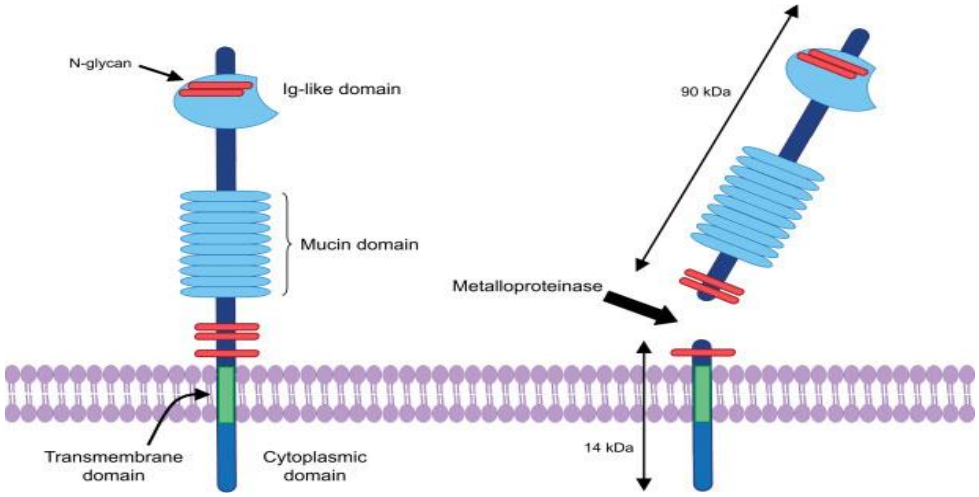

Figure (4): Structure of KIM-1.

The protein is a type-1 membrane protein with most of the protein made up of an extracellular domain that consists of a signal peptide, an Ig domain and a mucin domain. There is one transmembrane domain and a short intracellular domain with at least one important tyrosine phosphorylation domain. The protein can be cleaved by a metalloproteinase, after which the ectodomain (90 kDa) appears in the urine, leaving a 14 kDa membrane-bound fragment that is
tyrosine-phosphorylated (Tyr-P) ***(31)***.

KIM-1 is also known as T-cell immunoglobulin mucin domains- 1 (TIM-1) because of its expression at low levels by subpopulations of activated T cells. TIM-1 is a costimulatory molecule of T cells; it can enhance T-cell proliferation and cytokine production. The KIM-1b variant contains two conserved tyrosine residues and a tyrosine kinase hosphorylation motif; it is mainly expressed in the kidney. The cleavage of KIM-1 is related to metalloproteinase. Zhang et al.11 revealed that constitutive KIM-1 shedding was mediated by extracellular signal-regulated kinase activation and that the cleavage was accelerated by p38 mitogenactivated protein kinase activation ***(32)***.

KIM-1 expression:

Previous studies have showed that KIM-1 was mostly undetectable in healthy kidney tissue, while it was is abundantly expressed in post-ischaemic kidneys. Subsequently, KIM-1 was shown to be up-regulated in various human primary and secondary kidney diseases and in allograft nephropathy .The majority of KIM-1-positive tubules in various human renal diseases approximately 90%) are of proximal origin, as was identified by double labelling studies with aquaporin-1 (marker for proximal tubules). KIM-1 is localized in the apical membrane of dilated tubules in acute and chronic tubular injury. Localization of KIM- 1 expression appears to be related to the susceptibility of specific tubular segments to different types of injury ***(33)***.

Tubular KIM-1 expression is related to tubulointerstitial damage and inflammation. Many factors that initiate tubular epithelial cell injury result in KIM-1 protein expression. In acute renal damage, KIM-1 gene and

protein products are up-regulated 3 h after experimental renal ischaemia–reperfusion injury. Cisplatin, folic acid, *S*-(1,1,2,2-tertrafluoroethyl)-1-cysteine , cyclosporine , iodinated contrast agents and other nephrotoxicants that cause acute tubular cell damage all result in KIM-1 up-regulation ***(34)***.

KIM-1 may play a role in the regeneration process of tubule epithelial cells. After kidney injury, the proximal tubule epithelium would regenerate. This involves dedifferentiation and proliferation of viable cells bordering the damaged areas, to reconstitute an intact functional epithelial layer which is accompanied by dramatic upregulation of KIM-1***(34)***.

The vimentin colocalized with KIM-1 in most of the tubules in human renal disease. Vimentin is an intermediate filament involved in tubule dedifferentiation. The KIM-1-positive tubule cells had a dedifferentiated phenotype and correlated with tissue osteopontin and a-smooth muscle actin (a-SMA) levels. Osteopontin is a tubule-derived protein involved in chemotaxis and repair ***(33)***.

Also, KIM-1 is also expressed, at much lower levels, in lymphocytes and it has also been reported to be expressed in the cochlea in response to cisplatin-induced injury ***(35)***.

KIM-1 as a Marker and Predictor of Kidney Disease:

The selective KIM-1 expression by injured proximal tubular cells provides a strong evidence for using KIM-1 as a biomarker of damage. When renal epithelial cells die, a soluble KIM-1 together with the fluid may be entrained to the interstitium and enter therefrom the bloodstream . Higher KIM-1 concentration in blood together with its increased contents in urine can also reflect injury of the kidney tubular apparatus ***(36)***.

KIM-1 possesses the properties of ideal marker of renal proximal tubule epithelium injury : in the normal kidney, KIM-1 expression is determined in trace quantities; in ischemic or toxic kidney injury, activation of KIM-1 synthesis in the cells of the damaged tubules and its increased expression on the apical cell membrane is observed; shedding of KIM-1 from the cell surface results in considerable increase of its content in urine and/or in the circulating blood. According to the data of the experimental studies on animals, KIM-1 expression in the epithelial cells of the renal proximal tubules as well as its concentration in urine and blood plasma correlate with the severity of the pathological process in the kidneys as no other organs express KIM- 1 to a degree that would influence renal excretion of KIM-1 ***(37)***.

KIM-1 not only functions as a marker but also has predictive value for acute kidney injury. A one-unit increase in normalized urinary KIM-1 is associated with a 12-fold higher odds ratio for the presence of ischaemic acute tubular necrosis. Moreover, Liangos *et al* showed that urinary KIM-1 is predictive for adverse clinical outcomes in a cohort of 201 hospitalized patients with acute kidney injury. Patients within the highest KIM-1 quartile have a 3.2-fold higher odds ratio for dialysis or hospital death compared to patients within the lowest quartile ***(38)***.

Findings of several studies have shown that urinary KIM-1 is an early marker of AKI induced by the administration of the contrast agent to the patients who undergo coronary or peripheral angiography. Li *et al.* determined uKIM-1 in diabetic patients who underwent coronarography due to coronary artery disease and they confirmed that high KIM-1 level predicted CI-AKI ***(8).*** Another study of consecutive patients with normal SCr undergoing angiographic procedure demonstrated a significant elevation in uKIM-1 after 24 hours of coronary angiography (during which iodixanol or iopromide were used), and no changes were observed in estimated GFR up to 48 hours after the angiography ***(39)***.

3-Monocyte chemotactic peptide-1 (MCP-1)

Monocyte chemoattractant protein-1 (MCP-1), also referred to as chemokine ligand 2 (CCL2), belongs to the CC subfamily of cytokines. The main producers of MCP-1 are monocytes and macrophages, although it is also expressed by various other cell types such as endothelial cells, fibroblasts, epithelial cells, smooth muscle cells, mesangial cells, astrocytes, and microglia ***(40)***.

MCP-1 Structure and Fuctions:

Mature human MCP-1 is a small protein (13 kDa) comprising 76 amino acid residues ***(32)***.

The primary structure of biological activity has 2 important regions: amino acids 10 to 13 and amino acids 34 to 35. The secondary structure of MCP-1 is formed by a 4-stranded β-sheet, a short unstructured N-terminal

loop, and a C-terminal α-helix, which lies above the Greek bond formed by β-folding . The N-terminal plays an important role in receptor activation ***(41)***.

In general, MCP-1 exists as a monomer or a dimer. MCP-1 monomers combine with each other at the N-terminus to form dimers, whereas the 2 C-terminal α-helices enclose the chamber. It has been speculated that there is a receptor-binding functional area in this domain that can bind to receptors. The N-terminus is a chemotactic functional region that can activate receptors to mediate downstream signaling. Most researchers believe that both the monomeric and dimeric forms of MCP-1 are biologically active. Upon binding to its receptor, MCP-1 can induce homing, migration, activation, differentiation,and development of lymphocytes and NK cells; facilitate infiltration of monocytes and macrophages; promote inflammation occurrence; stimulate angiogenesis; as well as exert fibrotic effects ***(42)***.

Origin And Regulation of Renal MCP-1*:*

MCP-1 is synthesized by a range of cell types in the kidney, including intrinsic kidney cells and infiltrating leucocyte. Glomerular mesangial cells release MCP-1 in vitro following stimulation with a variety of stimuli, including inflammatory cytokines [interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-a)], immune complexes, metabolic factors (glucose, glycation end products) and danger-associated molecular patterns (DAMPs), such as extracellular adenosine triphosphate ***(43)***.

Podocytes release MCP-1 following stimulation with glycation end products, TNF-a and transforming growth factor beta (TGF-b) in vitro. Stimulation of podocytes [which express CC chemokine receptor type 2 (CCR2)] by MCP-1 downregulates nephrin, an alteration that could contribute to development of proteinuria in diabetic nephropathy and focal segmental glomerulosclerosis (FSGS) . Renal tubular cells also produce MCP-1 in response to IL-1 and TNF-a , thrombin and albumin. The wide variety of cells and stimuli that are known to release and respond to MCP-1 highlight its importance but also raise questions as to whether it could ever be useful as a specific disease biomarker or selective therapeutic target ***(44)***.

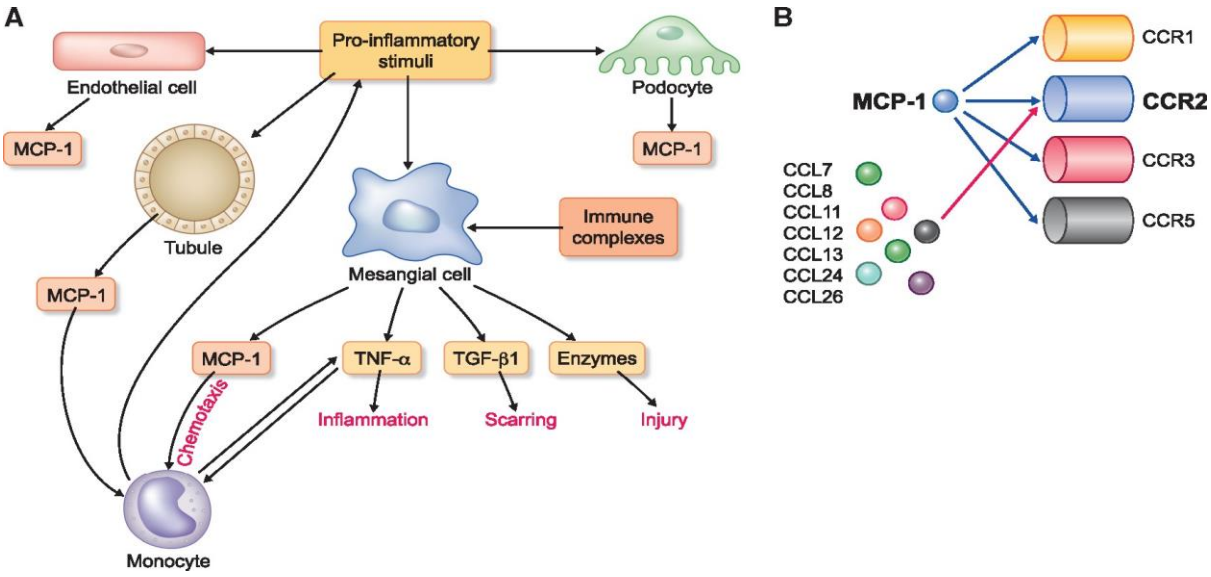

Figure (5): The renal MCP-1–CCR2 axis. (A) Cytokines, inflammatory cells and the pathogenesis of kidney disease. MCP-1 can be synthesized by a range of cell types including macrophages, podocytes, mesangial cells, endothelial cells and tubular cells. (B) The interaction between MCP-1 and CCR2 is not exclusive, as MCP-1 can also bind to CCR1, CCR3 and CCR5. Conversely, CCR2 has been shown to bind multiple chemokines such as CCL7, CCL8, CCL11, CCL12, CL13, CCL24 and CCL26 ***(45)***.

Expression of MCP-1 in Kidney Diseases:

Increased glomerular synthesis of CC chemokines, including MCP-1, has been shown to correlate with monocyte/macrophage infiltration in experimental models of antibody and immune complex–mediated glomerulonephritis. In clinical diseases, increased MCP-1 expression in human renal biopsies and excretion in urine has been detected in a number of proliferative glomerulonephritis associated with monocyte/ macrophage infiltration, such as immunoglobulin A (IgA) nephropathy, lupus nephritis, renal vasculitis and Goodpasture's syndrome ***(46)***.

The role of urinary MCP-1 as a disease marker has also been investigated in ANCA-associated renal vasculitis. In a seminal study, urinary MCP-1 was found to be elevated in patients with active renal vasculitis but not in patients with systemic vasculitis sparing the kidney. Most patients had a decrease in urinary MCP-1 following successful broad immunosuppressive treatment ***(46)***.

Diabetic nephropathy:

Although diabetic nephropathy is now the most common cause of kidney failure, the contribution of monocytes/macrophages in its pathogenesis has not been studied until recently.

In renal biopsies of patients with diabetic nephropathy, increased expression of MCP-1 and macrophage infiltration in the glomeruli and tubulointerstitium has been reported. Similarly, increased numbers of glomerular macrophages have been detected in rodent models of diabetic nephropathy ***(46)***.

Of significance, proteinuria and glomerular macrophages were significantly reduced in Mcp1 knockout mice following the experimental induction of diabetes. Increased levels of uMCP-1 as well as other cytokines and profibrotic growth factors [TGF-b, connective tissue growth factor (CTGF)] have been detected in patients with diabetic nephropathy. In a longitudinal study over 6 years, the uMCP-1:Cr ratio was found to be predictive of the rate of estimated glomerular filtration rate (eGFR) decline ***(46)***.

Regarding plasma MCP-1, it was showed that MCP-1 besides stimulated and recruited monocytes/ macrophages are related to damage of the kidneys in diabetic nephropathy The increase in MCP-1 could aggravate the release of oxidoreductase and apoptosis of membrane podocytes, leading to urinary protein leakage ***(47)***.

MCP-1 not only affects the activation of inflammatory signaling pathways but also promotes urinary protein leakage.

In a case-cohort study, 894 patients with DN were observed for approximately 8.7 years, showing that higher levels of plasma MCP-1 were related to diabetic nephropathy progression ***(47)***.

Acute Kidney Injury:

The baseline estimated glomerular filtration rate (eGFR) of 2,351 participants in the SPRINT study was below 60 ml/min/ 1.73m2. Exploratory factor analysis (EFA) was employed to capture distinct tubular pathophysiological processes, while a linear mixedeffect model was utilized to evaluate the association between each factor and longitudinal changes in eGFR. Cox proportional hazard regression analysis was conducted to assess the relationship between tubular factor scores and acute kidney injury (AKI). Among the 10 biomarkers examined, EFA revealed a reflection of tubular injury/fibrosis through KIM-1 and MCP-1, which were independently associated with an increased risk of AKI ***(48)***.

Additionally, recent studies have shown that MCP-1 in combination with other markers (e.g., epidermal growth factor –EGF-, KIM-1, neutrophil gelatinase-associated lipocalin –NGAL-) appears to represent a potent diagnostic and prognostic biomarker of tubulointerstitial injury and repair. Therefore, MCP-1 alone or in combination with other molecules could be considered a useful biomarker in AKI, which could also be extrapolated to CI-AKI. In fact, in an experimental CI-AKI model, 5 days after CM injection MCP-1 protein levels were found to be elevated in the renal tubules of CI-AKI rats, accompanied by increased concentrations of IL-6 and TNF-α in the kidneys and the serum, ROS production, cell death, renal dysfunction and an increased excretion of other urinary AKI biomarkers.

Although MCP-1 appears to be a promising biomarker for CI-AKI, data are currently only available in experimental models, not in human trials, and only once CI-AKI has already occurred. Therefore, despite

existing data on the correlation between MCP-1 levels and the diagnosis and prognosis of tubulointerstitial damage and repair, little is known about its efficacy in the diagnosis and prognosis of AKI and/or in humans ***(4)***.

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