Chapter 2
Insights from Proteomics in Kidney Disease Diagnosis and Various *In Vitro* and *In Vivo* Experimental Models

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Abstract   Kidney disease is emerging as a significant cause of mortality and public health burden worldwide. Diabetes, hypertension, obesity, and drug toxicity are the leading causes of kidney damage. The biomarkers and experimental models of kidney disease play an important role in diagnosis, prognosis, and follow-up treatment. Therefore, detailed knowledge about molecular markers and experimental models could be helpful to understand kidney diseases. Proteomic technology contributes to clinical research, particularly biomarker discovery and prognosis of kidney disease by estimating protein expression. Here, we review various kidney biomarkers which are efficient and associated with early detection of chronic kidney disease, diabetic nephropathy and acute kidney injury compared to surrogate markers. Also, we highlight the recent progress of proteomic quantification techniques like gel-based and gel-free techniques primarily used for biomarker discovery. Various *in vitro* cell line models used for screening and molecular studies were explained. Further, the different rodent and alternative models like zebrafish and drosophila used in kidney research are also discussed in detail. In conclusion, this chapter aims to review molecular biomarkers, the role of proteomics in kidney diseases, and different *in vitro* and *in vivo* models for understanding disease pathogenesis and therapeutics development.

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Abbreviations

ADMA	Asymmetric dimethylarginine
DOCA	Deoxycorticosterone acetate
ELISA	Enzyme-linked immunosorbent assays
eNOS	endothelial nitric oxide synthase
GeLC	Gel electrophoresis Liquid Chromatography-Mass Spectrometry
GFR	Glomerular Filtration Rate
IL-18	Interleukin 18
IL-1β	Interleukin-1β
KIM-1	Kidney Injury Molecule 1
LC-MS	Liquid Chromatography-Mass Spectrometry
LDH	Lactate dehydrogenase
L-FABP	Liver-fatty acid-binding protein
MCP-1	Monocyte chemoattractant protein-1
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
RBP4	Retinol-binding protein 4
$\alpha_1 M$	α_1-microglobulin
α-GST/π-GST	α/π-Glutathione s-transferase
$\beta_2 M$	β-microglobulin

2.1 Introduction

Kidney disease is an important contributor to health burden and mortality globally. More than 2.5 million people receive kidney replacement therapy, and the number is projected to double by 2030. The disease screening programme in countries like USA, Australia, and Norway have shown kidney diseasemarkers in more than 10% of the adult population (Bikbov et al. 2020). Three million people in UK alone are diagnosed with kidney ailments; however, more than one million people are undiagnosed (Outtandy et al. 2019). Chronic kidney disease is the 12th most common cause of death worldwide. In India, areas like Goa and Odisha were reported to have high chronic kidney disease incidence of unknown etiology (Jayasekara et al. 2015). According to the global burden of disease, approximately 1.5 million people died from kidney disease in 2015 alone, which was almost 32% higher from 2005 (Wang et al. 2016).

The term "Kidney disease" is used for any abnormality or changes that occur in kidney function. The abrupt change in glomerular filtration rate, increased serum creatinine, and decreased urine output over 6 to 24 h is termed as acute kidney

injury (Peerapornratana et al. 2019). It is mainly characterized by azotemia and oliguria. Acute kidney injury was defined by Acute Dialysis Quality Initiative (ADQI) as risk, injury, failure, loss of kidney function, end stage kidney disease (RIFLE), and later adopted by the acute kidney injury network (Lopes and Jorge 2013). Sepsis, ischemia, and drug toxicity are the leading causes of acute kidney injury. Recent studies suggest that acute kidney injury may lead to the development of chronic kidney disease, which requires kidney replacement therapy (Peerapornratana et al. 2019). Chronic kidney disease is characterized by kidney impairment that leads to decreased glomerular filtration rate (less than 60 ml/min/1.73m^2), elevated albuminuria, and serum creatinine. In addition, kidney injury causes deterioration in kidney function, resulting impaired urine output and higher connective tissue deposition in the kidney to cause fibrotic disorders (Levin et al. 2013). End-stage kidney disease is the last stage when the kidney cannot work and identified by a glomerular filtration rate of less than 15 ml/min (Sgambat et al. 2019). In the last few decades, an increasing prevalence of diseases such as obesity, diabetes and hypertension predisposed individuals to chronic kidney disease. Chronic kidney disease has been recognized as a significant risk factor for the higher rate of cardiovascular disease and mortality. Therefore, its early detection and prevention is a health-care priority in both developed and developing countries (Matovinović 2009).

The prognosis of kidney disease is greatly facilitated by the detection of biomarkers. Biomarkers are protein/enzymes released by damaged kidney tissue, and their presence directly reflects pathological condition. Further, the identification of proteins, enzymes and transcription factors in pathological conditions will increase our understanding about the molecular regulation of disease. The use of proteomics for disease diagnosis has recently been gathered pace (Wu and Fenton 2018). Proteomic approaches are applied for qualitative and quantitative estimation of biomarkers in different samples like plasma, urine, or tissue that will be useful for early diagnosis and treatment of kidney disease. Experimental models have a critical role in studying the pathology and pharmacology of kidney diseases. The *in vitro* and *in vivo* models provide the best insights into the mechanism of kidney disease and identify potential therapeutic targets, which further help clinical science for understanding disease and develop medications. Cell lines, zebrafish, and drosophila provides great alternatives to rodent and primates in screening the compound and studying the molecular pathways in renal diseases (Bao et al. 2018). This chapter provides an overview of various biomarkers for early diagnosis of kidney disease, proteomic technologies for their identification and validation, and different non-transgenic experimental models to study kidney diseases based on published research articles.

2.2   Pathophysiology of Kidney Disease

To understand the pathophysiology of kidney disease, the renal structure, physiology, and various mechanism of kidney injury must be taken into consideration. The kidney is a bean-shaped organ located in the upper abdominal area. Broadly kidneys are divided into two regions, *viz.* cortex and medulla. The cortex is mainly composed of the glomeruli, proximal and distal tubules, whereas collecting ducts and loop of Henle from the medullary part (Wang et al. 2017a, b). The nephron is the kidney's functional unit and its development in human occur during the 36th week of gestation period. The number of nephrons varies in an individual with an average of 0.2 to more than 2.5 million. Although when body mass increases the nephron hypertrophy occurs like in the case of obesity, pregnancy, and kidney transplantation (Bertram et al. 2011). The primary functions of nephron include glomerular filtration, tubular reabsorption, and secretion. Glomerulus consists of bundles of capillaries, mesangial cells, and podocytes enclosed by bowman's capsule, forming kidney corpuscles. It filters the blood plasma and allows waste products to pass through tubules (Fig. 2.1) (Wang et al. 2017a, b). The direct damage to glomerular (mesangial, podocytes, and endothelial cells), and tubular cells lead to impaired kidney function. The change in kidney blood flow due to cytokines and chemokines, alteration in glomerular filtration rate (due to disturbance in electrostatic barrier), drugs, and genetic factors are the primary reasons for the kidney injury (Matovinović 2009). The kidney injury can be acute or chronic, depending upon the duration of the disease process. Acute kidney injury causes sudden kidney perfusion changes, resulting in reduced glomerular filtration rate and blood flow (Basile et al. 2011). The significant pathological changes include inflammation, cytokines activation, tubular, epithelial, and endothelial cell injury (Bellomo et al. 2017). Ischemia is the leading causes of acute kidney injury that results in tubular cell damage due to ATP depletion and redistribution of Na^+/K^+ ATPase pump from basolateral to apical membrane of proximal tubular cells. This further results

in cytoskeleton structure disturbance, loss of cell-cell interaction, brush border damage, and cell swelling (Shaw et al. 2018; Chawla and Kimmel 2012). The kidney blood flow reduction due to kidney injury induces conversion of prorenin to renin and angiotensinogen activation. Angiotensinogen converting enzyme convert angiotensinogen into angiotensin I and activates angiotensin II, leading to vasoconstriction and high blood flow. Angiotensin II causes the secretion of aldosterone, leading to impairment of glomerular barrio function by reducing the expression of podocytes protein responsible for maintaining glomerular barrier function (Fig. 2.2) (Benigni et al. 2004; Lim 2014). The altered glomerular filtration rate and pressure lead to glomerular hypertension and nephron hypertrophy. This further increases the shear stress of podocytes resulting in podocyte detachment, glomerulosclerosis, and kidney injury (Romagnani et al. 2017). Ischemic kidney injury also causes some epigenetic changes like DNA methylation, acetylation and mRNA expression contributing to kidney impairment (Rodriguez-Romo et al. 2015).
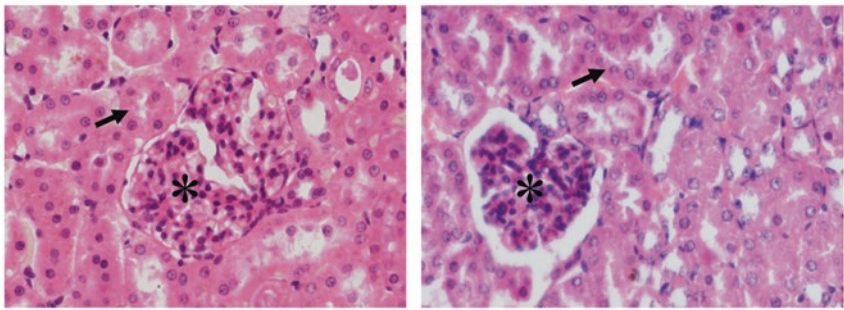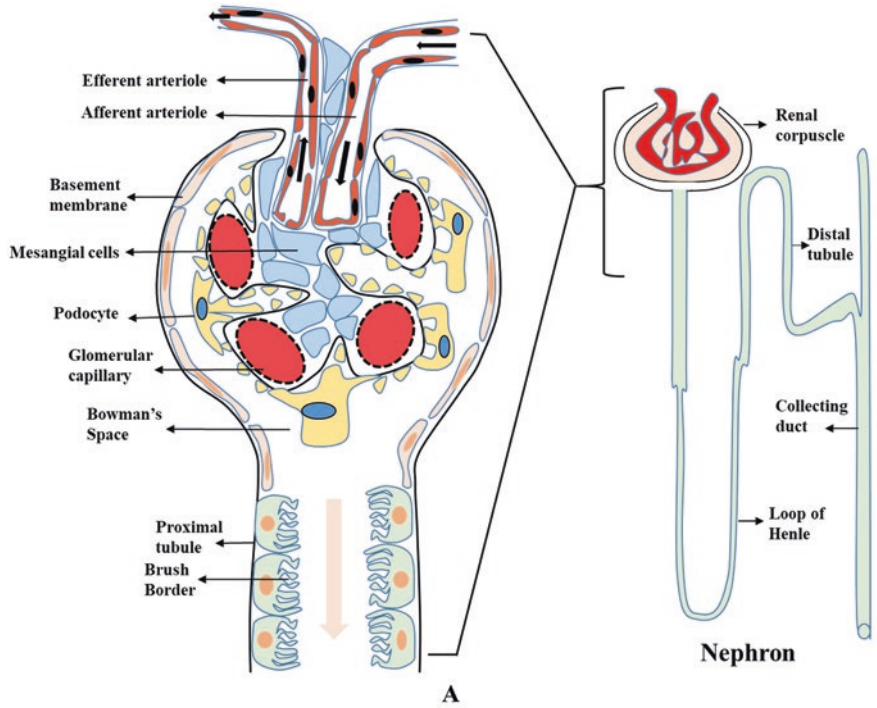
Fig. 2.1 (**a**) Showing different parts of the nephron and glomerulus. The blood enters the glomerulus through the afferent arteriole and leaves through the efferent. The glomerular capillaries, podocytes and mesangial cell together form a filtration bed in the glomerulus. The glomerulus is followed by proximal convoluted tubules with brush border epithelial cells, the loop of Henle, distal convoluted tubules and collecting ducts; (**b**) Histology of rat kidney showing normal glomerulus (asterisk) and proximal convoluted tubule with brush border epithelium (arrow); (**c**) Mouse kidney showing normal glomeruli (asterisks) and proximal convoluted tubules (arrow)

If the kidney function abnormalities remain for more than 3 months, the condition is known as chronic kidney disease. Chronic kidney disease is identified by albuminuria, nephron loss, impaired globular filtration rate, hypertrophy of nephrons, and kidney fibrosis (Romagnani et al. 2017). Its development is associated with three factors: first is cause-specific, second is related to fibrosis and dysfunction, and third is related to progressive damage to nephrons (Zhong et al. 2017). The tubules and interstitium form approximately 90% of the kidney volume. In proteinuria and tubulointerstitial damage, excessive protein reabsorption in proximal tubules is increased via lysosomal processing that causes lysosomal rupture resulting in tubular toxicity. Kidney injury activates the pro-inflammatory, and profibrotic factors contributing to kidney fibrosis development. Chemokine secretion in proteinuria may induce secretion of TGF-β, and intercellular adhesion molecule. The degree of tubulointerstitial fibrosis is directly linked to the level of expression of adhesion molecule (Fig. 2.2) (Nogueira et al. 2017).

Hypertension is one of the primary reasons for kidney damage. Increased blood pressure level for the long term can cause epithelial damage resulting in reduced
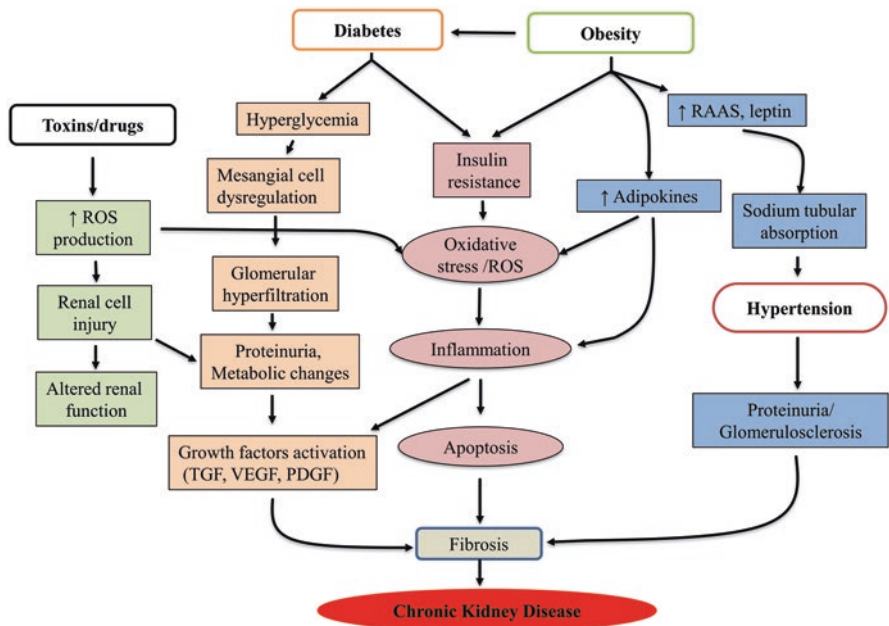

Fig. 2.2 Schematic representation of the pathogenesis of kidney diseases. The hyperglycemia and insulin resistance in diabetes leads to glomerular function changes, oxidative stress, inflammation, and activation of growth factors leading to fibrotic changes. Obesity increases adipokine levels and oxidative stress in the renal tissue. The activation of RAAS increases tubular absorption of sodium, leading to hypertension and pathological changes in the kidneys. Toxin/drugs cause direct injury to the renal cells and cause impairment of renal function. PDGF: Platelet-derived growth factor; RAAS: Renin-angiotensin-aldosterone system; ROS: Reactive oxygen species; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor

globular filtration rate arterial stenosis, arterial stiffening, and kidney ischemia (Fenoglio et al. 2019). Nephron loss increases the glomerular workload leading to glomerular hypertension, which further activates chronic kidney disease pathways. Similarly, the diabetic condition causes microvascular complications in the kidney leading to albuminuria, proteinuria, and decreased glomerular filtration rate (Orasanu and Plutzky 2009; Ioannou 2017). In diabetic nephropathy (DN), proximal tubules are unable to reduce the glucose transport rate due to hyperglycemia leading to hypertrophy, hyperfiltration, elevated reactive oxygen species generation, and angiotensinogen production (Fig. 2.2) (Vallon 2011). Most glomerular diseases are caused by a defective immune system, metabolism, and stress-related injuries. Proliferative glomerular disease is characterized by immunoglobulins accumulation, leading to glomerular injury and inflammation. The non-proliferative glomerular disease occurs without any glomerular inflammation, and immunoglobulin accumulation as in case diabetic, metabolic, and toxins related damage (Fine and Norman 2008). Some inherited diseases like nail-patella syndrome, thin membrane, and partial lipodystrophy also affect the glomerular function.

2.3 Molecular Biomarkers of Kidney Diseases

Biomarkers are defined as 'characteristic' or 'measurable/quantifiable biological parameters' that differentiate between normal biological processes to pathogenic and other clinical conditions (Provenzano et al. 2020). Molecular biomarkers represent disease-associated proteins for various pathophysiology of kidney, including acute kidney failure, chronic kidney disease, autoimmune kidney disease, and kidney cancer. Biomarker's most important feature is the ease of availability, sensitivity, specificity, reproducibility, and non-invasive and easy-to-perform in the clinical laboratory. The application of molecular biomarkers in clinical practice is vital for the early diagnosis of disease, leading to a better prognosis with improved clinical outcome. Besides, biomarkers were also used to differentiate disease subtype, etiology, predicting the disease severity, and drug development. Although no clear guidelines are available for molecular biomarkers identification; however, the two most common approaches; deductive and inductive were used for kidney biomarkers identification. Deductive approaches include protein study of specific diseases like inflammation, while inductive methods include studying a tissue at a particular condition using different genomic and proteomic techniques followed by bioinformatics analysis. In the recent past, numerous studies have been reported for a better understanding of biomarkers and their role in the early diagnosis of kidney disease (Vaidya et al. 2008; Nguyen and Devarajan 2008; Lopez-Giacoman and Madero 2015; Dobrek and Thor 2016; Alicic et al. 2017; Malhotra and Siew 2017). Here, we discuss prominent biomarkers associated with kidney disease (Table 2.1).

Measurement of glomerular filtration rate is considered to be a direct assessment of kidney functions. In clinical practices, estimation of glomerular filtration rate is usually estimated through measuring alternate markers like serum creatinine (a

Table 2.1 Various molecular markers of kidney disease

Biomarkers	Tissue	Renal disease	Biological function	References
NGAL	Serum, urine	AKI,CKD,DN	Indicate renal injury	Mitsnefes et al. (2007), Nguyen and Devarajan (2008), Bolignano et al. (2009), Kohl et al. (2020)
Cys-C	Serum, urine	CKD, DN, AKI	Glomerular and tubular dysfunction	Vaidya et al. (2008), Kim et al. (2013), Lopez-Giacoman and Madero (2015)
KIM-1	Urine	CKD, DN, AKI	Tubular dysfunction	Vaidya et al. (2005) Uwaezuoke (2017)
NAG	Plasma, urine	CKD, DN, AKI	Tubular injury	Vaidya et al. (2008)
L-FABP	Plasma, urine	CKD, DN, AKI	Renal injury	Vaidya et al. (2008), Bonventre et al. (2010), Lopez-Giacoman and Madero (2015)
β_2M	Plasma and urine	CKD,DN,AKI	Tubular injury	Lopez-Giacoman and Madero (2015), Vaidya et al. (2008)
α_1M	Urine	AKI	Tubular injury	Hart (2005)
RBP4	Urine	CKD, AKI	Tubular dysfunction	Lopez-Giacoman and Madero (2015)
IL-18	Urine	CKD, AKI, DN	Tubular damage	Miyauchi et al. (2009)
Clustrin	Urine	CKD, AKI, DN	Tubular damage	Vaidya et al. (2008)
ADMA	Plasma, urine	CKD,DN	Kidney disease	Raptis et al. (2013)
MCP-1	Urine	CKD, DN	Renal injury	Wada et al. 2000 and Lopez-Giacoman and Madero (2015)
α-GST/π-GST	Urine	AKI	Renal injury	Harrison et al. (1989)
NHE3	Urine	AKI	Tubular injury	du Cheyron et al. (2003)
Fetuin A	Urine	AKI	Tubular damage	Zhou et al. (2006)
TIMP-2 and IGFBP7	Urine	AKI	G_1 cell-cycle arrest	Kashani et al. (2013)
Osteopontin	Urine	AKI	Kidney disease	Vaidya et al. (2008)
Clustrin	Urine	AKI	Tubular injury	Vaidya et al. (2008)
FGF-23	Urine	CKD		Isakova et al. (2011)

Abbreviations: *AKI* Acute Kidney Injury, *CKD* Chronic Kidney Disease, *DN* Diabetic Nephropathy, *NGAL* Neutrophil gelatinase-associated lipocalin, *Cys-C* cysteine protease inhibitor, *KIM-1* Kidney Injury Molecule 1, *NAG* N-acetyl-β-D-glucosaminidase, *L-FABP* Liver-fatty acid-binding protein 1, *β_2M* β-microglobulin, *α_1M* α₁-microglobulin, *RBP4* Retinol-binding protein 4, *IL-18* Interleukin 18, *ADMA* Asymmetric dimethylarginine, *MCP-1* Monocyte chemoattractant protein-1, *α-GST/π-GST* α/π-Glutathione s-transferase, *NHE3* Sodium–hydrogen exchanger isoform, *TIMP-2* Tissue Inhibitor of Metalloproteinases-2, *IGFBP7* Insulin-Like Growth Factor-Binding Protein-7, *FGF-23* fibroblast growth factor-23

113 kDa metabolic waste product of muscle-derived from creatine degradation), albuminuria (excess albumin excretion in urine), blood urea nitrogen, and urine output. Prediction of kidney function through increased serum creatinine has some limitations as its concentration may be influenced by patient muscle mass, physical activity, diet, time log between kidney injury that results in low predictive value (Uchino 2010). In kidney disease, albuminuria and glomerular filtration rate are inversely proportional, i.e., higher albuminuria lowers the glomerular filtration rate. Increased albuminuria results due to diabetes, hypertensive nephrosclerosis, and glomerular disease. Excess albuminuria directly affects kidney glomeruli and tubules or induces inflammation that results in kidney damage. However, in contrast to previous report MacIsaac et al. (2014) found that microalbumin concentration is moderately associated with kidney disease progress. Besides, many diabetic kidney disease cases are associated with non-proteinuria (Tsalamandris et al. 1994). Blood urea nitrogen usually increases as glomerular filtration rate decreases; however, urea production may be related to a protein diet and tissue breakdown. Due to insensitivity, non-specificity, and delayed response, these surrogate markers are not sufficient and reliable for early diagnosis of kidney disease. Therefore, identifying novel, sensitive, and specific biomarkers for prior and accurate diagnosis of kidney disease is essential, particularly for reflecting intrinsic organ injury.

Identifying kidney biomarkers in the urine mainly depends on the change in glomerular permeability or tubular resorption, and increased synthesis/release of a biomarker in response to tissue damage. Alteration in glomerular permeability and tubular damage is the leading cause of kidney disease. Tubular protein like neutrophil gelatinase-associated lipocalin (NGAL) is associated with damaged glomerular and tubular epithelial cells of the kidney. An increased concentration of neutrophil gelatinase-associated lipocalin in blood/urine is used to detect acute kidney injury, kidney transplant, and kidney disease associated with diabetes, hypertension, and atherosclerosis (Mitsnefes et al. 2007; Nguyen and Devarajan 2008; Bolignano et al. 2009; Kohl et al. 2020). Cystatin C (13.4-kDa) an indicator of glomerular and tubular damage. An increased concentration of cystatin C in urine has been used to predict chronic kidney disease and diabetic nephropathy (Lopez-Giacoman and Madero 2015; Kim et al. 2013). However, in patients with kidney transplants and cardiac surgery, an increase of 50% urinary cystatin C level was reported that represents a potential biomarker for acute kidney injury (Vaidya et al. 2008).

Kidney injury molecule 1 (KIM-1) is a transmembrane protein usually detected in urine after tubulointerstitial impairment. An increased level of KIM-1 in urine has been reported in chronic kidney disease and diabetic nephropathy (Uwaezuoke 2017). Likewise, its levels significantly increases in acute kidney injury patients with ischemic and cardiac surgery (Vaidya et al. 2005). A lysosomal enzyme in the proximal tubule, N-acetyl-β-D-glucosaminidase (NAG) has been associated with altered urine levels in tubular damage in acute kidney injury, chronic kidney disease and diabetic nephropathy (Vaidya et al. 2008). However, the non-specificity of NAG for acute kidney injury limits its use as biomarkers. Liver-fatty acid-binding protein 1 (L-FABP; intracellular binding protein of fatty acid, highly expressed in tubular damage) has been used as next-generation biomarkers to diagnose kidney disease

(Vaidya et al. 2008; Bonventre et al. 2010; Lopez-Giacoman and Madero 2015). β-microglobulin (β₂M), a 11.8 kDa light chain of major histocompatibility class 1 protein is normally filtered through the glomerulus and reabsorbed by proximal tubular cells. Its increased concentration in urine indicates tubular dysfunctions and act as an early marker for kidney disease (Lopez-Giacoman and Madero 2015). However, rapid degradation in urine with a pH less than 6.0 at room temperature limits its use as biomarkers for acute kidney injury. Similarly, increased urinary α₁-microglobulin (α₁M) level has been used as a sensitive biomarker for proximal tubular damage in acute kidney injury (Hart 2005).

Other protein/enzymes associated with tubular damage like retinol-binding protein 4 (RBP4; a 21kD protein involves in vitamin A transport from liver to other tissue), Interleukin 18 (IL-18; an inflammatory cytokine), and Clusterin; 75 kD-disulfide-linked heterodimeric glycoprotein have been used for detection of chronic kidney disease, acute kidney injury and diabetic nephropathy (Vaidya et al. 2008; Miyauchi et al. 2009; Lopez-Giacoman and Madero 2015). Further, C reactive protein belongs to a protein family known as pentraxin, is associated with endothelial injury and impaired vasodilation in the kidney leading to glomerular damage and progressive kidney function impairment. Another protein monocyte chemoattractant protein-1 (MCP-1; a member of chemotactic cytokines affects kidney structure and function), asymmetric dimethylarginine (ADMA) and fibroblast growth factor-23 also has been used to diagnosse chronic kidney disease and diabetic nephropathy (Wada et al. 2000; Bonventre et al. 2010; Isakova et al. 2011; Raptis et al. 2013; Lopez-Giacoman and Madero 2015). Various other glomerular proteins like transferrin (serum iron-binding protein), immunoglobulin G, laminin (components of glomerular basement membrane), type IV collagen (components of the glomerular basement membrane and mesangial matrix), fibronectin1 (intrinsic component of the glomerular extracellular matrix), resistin (Serum marker for reduced glomerular filtration), prostaglandin D2 synthase (Increased serum level indicates impaired kidney function), ceruloplasmin, glycosaminoglycans, lipocalintype, podocalyxin, vascular endothelial growth factor, human cartilage glycoprotein-40, and nephrin has been used for early diagnosis of chronic kidney disease (Dobrek and Thor 2016). Furthermore, proteins associated with cell cycle regulation (G₁ cell-cycle arrest) including insulin-like growth factor-binding protein-7, tissue inhibitor of metalloproteinases-2, α/π-glutathione s-transferase (α-GST/π-GST), sodium–hydrogen exchanger isoform, fetuin-A, osteopontin, clusterin have been identified as early acute kidney diseasebiomarkers (Harrison et al. 1989; du Cheyron et al. 2003; Zhou et al. 2006; Vaidya et al. 2008; Kashani et al. 2013).

One of the most crucial reasons for the progression of any disease is oxidative stress. Oxidative stress leads to generation of reactive oxygen species, and at higher concentrations, they produce unwanted modification of protein, lipid, and DNA. Of a particular interest, protein oxidative products and protein carbonyls were used as molecular markers as they significantly increase in chronic kidney disease and end stage renal disease patients (Himmelfarb et al. 2000; Mitrogianni et al. 2009; Machowska et al. 2016). Mitrogianni et al. (2009) reported that the carbonylation of albumin gradually increases with chronic kidney disease progression. Further, lipid

peroxidation products; malondialdehyde, 4-hydroxynonenal, thiobarbituric acid reactive substances, and isoprostanes such as 8-iso-prostaglandin F2α were used as biomarkers for chronic kidney disease (Sugiura and Wada 2009). Moreover, urinary 8-oxo-7,8-dihydro-2-deoxyguanosine; an oxidative modification of DNA is a typical biomarker used for diabetic nephropathy. Recently, Michele et al. reviewed in detail about molecular biomarkers involved in chronic kidney disease. They discussed the serum level of oxidative stress-induced biomarkers involved in tissue remodelling, and metabolism; myeloperoxidase, matrix metalloproteinase (matrix metalloproteinase-2, −8 and − 9), endopeptidases, tissue inhibitor of metalloproteinases-1, C-reactive protein and fibroblast growth factor-23 were increased significantly. Using these biomarkers in clinical practice, diagnosis and prognosis of kidney diseases at an early stage becomes more accurate than surrogate markers. In addition, these biomarkers will also increase our molecular understanding about pathophysiology of kidney damage that could be utilised for developing novel therapy to treat kidney diseases.

2.4   Proteomics in Kidney Disease

Proteome; analogue of genome, may be defined as "total protein complement of a cell, tissue or of an organism at a particular time, condition or environment" (Pennington et al. 1997). The emergence of mass spectrometry with better specificity and sensitivity, enrichment of less abundant proteins and depletion of abundant proteins with different pre-fractionation steps, computer tools for data processing, peptide sequencing, and protein identification has paved the way for analysis of protein and peptides at large scale in high-throughput mode (Matthiesen et al. 2004). Proteomic techniques have been used for the identification and quantification of protein that will be essential to inference the biological functions. Mostly two complementary approaches are common in proteomics; top-down or bottom-up. In top-down approach, isolated proteins were first fractionated, and intact protein was trypsin digested, followed by data acquisition and analysis by mass spectrometry (Han et al. 2006). This approach allows identification of protein, post-translational modifications, and protein isoforms. In contrast, the bottom-up approach includes characterization of protein through peptides generated from protein digestion (Yates 2004). The bottom-up approach is generally called shotgun proteomics when it is performed on a mixture of proteins (Yates 1998). In general, shotgun proteomic experiment includes trypsin digestion of protein mixture to generate peptides, resulting peptides mixture was fractionated and identified by mass spectrometry.

In the past few decades, significant progress has been made to explore the mechanism of kidney disease progression and biomarker discovery for disease diagnosis, prognosis, and their follow-up treatment through proteomic approaches (Mischak et al. 2007; Chen et al. 2018). In this context, body fluids (blood and urine) are seem to be ideal for the identification of new biomarkers as they contained secreted/ leaked proteins associated with a disease or altered state of tissue or organ (Lescuyer

et al. 2007). Among different body fluids (blood/plasma/serum), proteome is highly complex in which peptide concentration can vary ten order of magnitude. However, urine proteome is comparatively less complex, and the pathophysiological state of kidney is indicated by change in urinary proteome. Considering urine for proteomic study has several advantages as it collected noninvasively in large volume, stored at 37 °C for several hours without any proteolytic degradation and it devoid highly abundant plasma proteins that create hindrance in the extraction process. In this section, we discussed proteomic approaches involved in biomarkers discovery for kidney disease and also highlighting the advantage and its limitation.

A large number of proteomic techniques like capillary electrophoresis, two-dimensional gel electrophoresis, surface-enhanced laser desorption/ionization, liquid chromatography-mass spectrometry (LC-MS), and among others have been used for the identification of kidney biomarkers (Mischak et al. 2015; L'Imperio et al. 2016; Magalhães et al. 2016; Van et al. 2017). Capillary electrophoresis coupled with mass spectrometry is one of the important proteomic technique for fractionation of protein with good resolution in the presence of electric field using capillary in a single step (Maier and Schmitt-Kopplin 2016). It has been used for the identification of biomarker peptides where sample complexity is the limiting factor. In 2010, a human urinary peptidome panel developed CKD273 classifier; composed of 273 specific peptide markers for diabetic kidney disease (Good et al. 2010). Most of the peptide biomarker of CKD273 classifier is derived from collagens, serum albumin, uromodulin, membrane-associated progesterone receptor component 1, sodium/potassium-transporting ATPase γ chain, haemoglobin α chain, α1-antitrypsin, and fibrinogen α chain proteins. CKD273 classifier has been used to diagnose diabetic nephropathy (Zürbig et al. 2012), hypertensive nephropathy (Ovrehus et al. 2015), and progression to end-stage renal disease (Pontillo et al. 2017). In addition, it also predicts the progress of chronic kidney disease from non-albuminuria to macroalbuminuria (Roscioni et al. 2013).

Two dimensional gel electrophoresis coupled with mass spectrometry is another powerful tool to fractionate and visualize thousands of proteins from a complex biological sample. The protein fractionation takes place using two fundamental properties of protein; isoelectric point and molecular weight. After protein fractionation gels were stained with Coomassie brilliant blue/silver stain), and analysed through different software e.g. PDQuest. Further differential protein spots were excised from the gel, trypsin digested, and identified by mass spectrometry. This technique has several advantages like protein quantification, detection of post-translational modification, and protein isoforms and wide application in clinical pathology for biomarker discovery (Park et al. 2006). Ferlizza et al. (2015) reported a reference map of urinary proteome of 23 healthy cats and 17 chronic kidney disease associated cats and found an increased abundance of retinol-binding protein, cystatin M and apolipoprotein-H while a decreased abundance of uromodulin and cauxin that could be a candidate biomarker for chronic disease disease. Recently performed urinary proteome of 22 healthy dogs and 28 dogs with chronic kidney disease using gel electrophoresis revealed uromodulin could be a putative biomarker for chronic kidney disease (Ferlizza et al. 2020). However, two dimensional gel

electrophoresis approach is limited by poor reproducibility, less coverage, and unable to detect low abundant proteins. These limitations were overcome by the use of differential gel electrophoresis in which proteins isolated from different treatments were labelled with a specific fluorophore, pooled and run in a single gel. The main advantage of differential gel electrophoresis is less number of replicate gel that increase the reproducibility of the experiment. Fluorophore has four-fold higher linear response than Coomassie brilliant blue/silver stain that results in detection of low abundant proteins (Tannu and Hemby 2006; Sui et al. 2010). Recently, Romanova et al. (2020) examined serum sample of 26 adult chronic kidney disease patients and 10 healthy adults using differential gel electrophoresis and identify 46 differentially expressed proteins, among which 21 proteins were further quantified by multiple reaction monitoring and enzyme-linked immunosorbent assays (ELISA) suggesting these 21 proteins could be an early biomarker for chronic kidney disease progression (Romanova et al. 2020).

In order to identify low abundant protein, another approach has been developed known as multidimensional protein identification technology that allows identification proteins in complex mixtures without prior separation of protein. In this approach, protein lysate was first digested with trypsin followed by separation of peptides using strong cation exchange chromatography and later on by C18 reversed-phase. The resulting peptides were analyzed by mass spectrometry for protein identification. Another proteomic technique known as GeLC has been developed for complex samples in which proteins were first fractionated by one dimensional gel electrophoresis, fractionated protein bands were chopped into slices, digested, extracted and analyzed by reversed phase nanoLC-MS that gives better protein identification. However, above described shotgun approaches perform quantification at peptide level and lack information on the intact protein. In addition, these techniques have poor reproducibility in liquid chromatography separation, variable ion signal, and strong ion suppression effect that makes it challenging. However, these limitations were overcome by chemical tagging or by metabolic labelling of protein during growth of biological material. Zhang et al. performed proteomic profiling of serum samples with acute kidney allograft rejection using isobaric tag for relative and absolute quantification and identified 109 differential proteins that will be helpful for the identification of allograft rejection (Zhang et al. 2020). Further, a label-free proteomics analysis has been developed, which involves the comparison of normalized relative peptide peak intensities between multiple LC-MS datasets using different software that results identification and quantification of peptides and proteins. This technique reduces time for spectra search and simultaneously increases the throughput of biomarker discovery. Recently studied urinary proteome of acute kidney injury patients using gel-free proteomic approach, identified 1810 proteins in which 168 were differential proteins. Further validation through ELISA showed that annexin A5, neutrophil gelatinase-associated lipocalin, and protein S100-P are promising biomarkers for acute kidney injure (Jung et al. 2020).

Once the biomarkers were identified from proteomic techniques quantification/ validation was performed through affinity-based assay like ELISA and western

blotting. ELISA is a highly sensitive and specific method for absolute quantification of proteins; however, prior to ELISA molecular structure of protein must be known for purification of protein and generation of antibody to validate its specificity. In addition, it has low dynamic range that needs optimum dilution of antibody for cross-reactivity of antigen for the standard curve (Vaidya et al. 2008). Further, mass spectrometry based absolute quantification of protein with high sensitivity has been developed for validation of biomarker. The two methods are multiple reaction monitoring mode or selected reaction monitoring, which are not discussed in this chapter (Aebersold et al. 2013; Picotti and Aebersold 2012; Nilsson et al. 2010). Therefore, recent development in proteomic techniques offers great promise towards the identification of kidney biomarkers for early disease diagnosis; however, there is a need to develop proteomic techniques used for validation of biomarkers specificity.

2.5 Experimental Model for Studying Kidney Diseases

2.5.1 In Vitro *Models*

The cell lines are one of the reliable methods to study kidney diseases as they can closely mimic the cells' physiological condition in the body. Cell lines also provide an advantage over animal models for cost and ethical constraints. In the primary cell culture, cells are directly separated and propagated from the tissue. It is still used as a useful tool in drug screening and toxicity studies; however, they have limitation of their growth capacity. The secondary cell line or immortalized cells overcome this gap and divide indefinitely (Faria et al. 2019). Various types of glomerular and tubular cell lines are used to study kidney disease as *in vitro* models.

2.5.1.1 Podocytes and Mesangial Cell Lines

Podocytes are the glomerular cells having a role in the filtration and maintenance of the slit diaphragm. These cells are used as *in vitro* models to study the disease condition and can be isolated from rat, mouse and human (Ni et al. 2012). The altered fatty acid metabolism in diabetes and obesity affects the podocytes. Palmitic acid treatment of the cultured podocytes was reported to alter the gene expression profile of stearoyl-CoA desaturase-1, stearoyl-CoA desaturase-2, and carnitine palmitoyltransferase and induced cell apoptosis, which mimics the altered fatty acid metabolism in the diabetic nephropathy (Sieber et al. 2013). Conditionally immortalized human podocyte's treatment with glucose (5 nM and 25 nM), angiotensin II and methylglyoxal (an advanced glycated end-product precursor) mimic the conditions of diabetic nephropathy. Methylglyoxal (1.5 mM) treatment increased the oxidative stress, decreased the expression of nephrin-like protein 1 and Tjp1/ZO-1, which are tight junction proteins and maintains the podocyte integrity and slit diaphragm (He

et al. 2020). Mesangial cells are perivascular pericytes in glomerulus involved in mesangial matrix synthesis, maintenance of glomerular hemodynamics, and filtration process. IP15 is a human stable immortalized mesangial cell line with the ability to contract with angiotensin II (L'Azou et al. 2007). Spontaneously transformed rat mesangial cell lines treatment with 25 mM D-glucose resulted in enhanced caspase-3 activity, decreased Bax: Bcl-2 ratio, cell apoptosis, inhibition of nuclear factor kappa B, and transforming growth factor beta activation. High glucose treatment of mesangial cells induces their proliferation via toll-like receptor 4 dependent inhibition of hydrogen sulfide synthesis (Khera et al. 2006). Both podocytes and mesangial cells are the important cells of glomerulus, and their cell lines are very useful in studying the glomerular function in renal disease.

2.5.1.2 Proximal Tubular Epithelial Cell Lines

Proximal tubular epithelial cells perform the primary function of reabsorption of numerous solute present in the glomerular filtrate. These cells are highly prone to injury in kidney toxicity or disease conditions and thus are used as a potential *in vitro* model system to study kidney diseases. Human kidney cells immortalized by transduction with human papillomavirus 16 E6/E7 genes are the most commonly used tubular cell line. Human kidney 2 cells treated with Tenofovir for 72 h showed cell cytotoxicity, oxidative stress, mitochondrial dysfunction, upregulation of tumor necrosis factor-alpha and caspases (3 and 9). These cells mimic the various conditions related to Tenofovir induced nephrotoxicity (Murphy et al. 2017). The human renal proximal tubule epithelial cells immortalized by the human telomerase reverse transcriptase form the RPTEC/TERT1 cell line. These are extensively used to study human kidney disease conditions, including kidney cancer (Simon-Friedt et al. 2015). Human embryonic kidney 293 is another human embryonic kidney cell derived line used for *in vitro* studies. The cell's treatment with nephrotoxic molecule ochratoxin A (25 μM) showed the impairment of miRNA biogenesis correlated to cancer and signal transduction pathways (Zhao et al. 2016).

Normal rat kidney-52E epithelial cells are used to study kidney disease and for toxicological drug screening. The treatment of epithelial cells with transforming growth factor beta-1 activates the various pathway of kidney fibrosis. It induces cells' transition into spindle-like cells and activates Smad2/3 signalling pathway with up-regulation of alpha smooth muscle actin, fibronectin, and integrin-linked kinase expressions (Wei et al. 2015). Similarly, treatment of these cells with high glucose concentration (30 mM) mimics the physiological conditions of diabetic nephropathy. Glucose treatment induces oxidative stress and activates the pro-inflammatory cytokines (TNF-α and IL-1β) and fibrogenic factors (TGF-β1) in the kidney epithelial cells (Sharma et al. 2019). Caki-1 is a human clear cell renal cell carcinoma (ccRCC) line with the morphology of epithelial cells, and used to study renal cancer. Other cells are porcine kidney cells (LLC-PK1) and Madin-Darby canine kidney cells (MDCK) (Barnett et al. 2018). Together, the previous reports suggest that *in vitro* kidney disease models are vital for renal molecular studies,

drug screening, and safety assessment. The range of *in vitro* renal models allows researchers to select the most suitable model to answer their scientific questions.

2.5.2 In Vivo *Rodent Models of Kidney Disease (Non-transgenic)*

2.5.2.1 Acute Kidney Injury Models

Animal models are widely used for understanding the human kidney disease as they mimic the human condition to a more considerable extent. Various type of non-transgenic *in vivo* models are enlisted in Table 2.2.

Ischaemia-Reperfusion (I/R) Model

Ischaemia-reperfusion is one of the primary reasons of acute kidney injury. It triggers robust oxidative damage and inflammatory response in the tubular cells, leading to lipid peroxidation, mitochondrial damage, and cell death. Ischemia-reperfusion induced acute kidney injury can be developed in both rat and mouse. This model involves a surgical procedure in which midline dorsal incision is used to locate the kidneys and bilateral clamping of kidney pedicles for 30 minutes. The kidneys are observed for color change before removing the clamps. The renal ischaemia-reperfusion results in a sharp increase in the serum creatinine and blood urea nitrogen. Kidney histopathology showed cast formation, severe tubular dilation, loss of proximal tubular brush border, cell apoptosis, and interstitial inflammation (LiGong et al. 2020; Zheng et al. 2019). This model is a robust model to study the pathophysiology of I/R induced acute kidney damage.

Acetaminophen

Acetaminophen is a widely used antipyretic and analgesic drug. It is a safe drug at therapeutic doses; however, overdosing of the drug causes hepatotoxicity and nephrotoxicity. At higher doses, harmful metabolites like N-acetyl-p-benzoquinone imine are produced from acetaminophen leading to severe oxidative damage and kidney toxicity. A single oral dose of paracetamol (500 mg/kg BW) administered to rats resulted in elevated kidney injury markers (serum urea and creatinine), lipid peroxidation, glutathione depletion, and reduced activities of antioxidant enzymes (super oxide dismutase, catalase, and glutathione peroxidase) (Kandemir et al. 2017). Mice treated with acetaminophen for 24 h at a dose of 300 mg/kg (intraperitoneal) increased the creatinine, blood urea nitrogen, and cystatin C levels. Acetaminophen treatment also elevated the markers of tubular injury *viz*. kidney injury molecule−1 and neutrophil gelatinase-associated lipocalin by 5 and 347-fold

Table 2.2 Various non-transgenic rodent models of kidney diseases

Agent	Model/strain	Method	Characteristics	References
Acute kidney injury				
Acetaminophen	Sprague-Dawley (SD) rats	500 mg/kg BW and 2 g/kg, single oral dose	↑ Serum urea and creatinine, lipid peroxidation, oxidative stress and tubular damage	Kandemir et al. (2017)
	BALB/c mice	300 mg/kg BW intraperitoneal, single dose	↑ Creatinine, BUN, cystatin C, NGAL and KIM-1	Hua et al. (2018)
	SD rat	2 g/kg BW single dose	↑ Urea, creatinine, MDA and TNF-α	Haidara et al. (2020)
Adriamycin	SD rat	2 mg/kg BW intravenous for 20 days	Lipid peroxidation and oxidative stress	Okuda et al. (1986)
	BALB/c	10 mg/kg BW intravenous, single dose	Proteinuria, glomerulosclerosis, tubular atrophy and fibrosis	Tan et al. 2013
Cisplatin	SD rat	7 mg/kg BW intraperitoneal, and 6 mg/kg BW, single dose	↑ BUN, creatinine and TNF-α Oxidative stress, tubular degeneration and altered urine output	El Amir et al. (2019)
Cyclophosphamide	Albino rats	75 mg/kg BW, intraperitoneal (six doses)	Altered urine output, serum Na+, serum kidney injury markers and tubular damage	El-Shabrawy et al. (2020)
	Swiss albino mice	CP (200 mg/kg BW intraperitoneal) two doses	↑ BUN, creatinine, LDH and Tubular degeneration, inflammation and apoptosis	Sharma et al. (2017)
Cyclosporine A	SD rat	7.5 mg/kg,15 mg/kg and 20 mg/kg BW subcutaneous	↓ GFR rate, ↑ creatinine levels, endothelial dysfunction and tubular atrophy	Bing et al. (2006) Huang et al. 2018

(continued)

Table 2.2 (continued)

Agent	Model/strain	Method	Characteristics	References
Ischaemia-reperfusion model	SD rat	Bilateral clamping of renal pedicles for 30 minutes	↑ BUN and creatinine, tubular injury, cast formation, loss of brush border and apoptosis	Gong et al. (2020)
	C57bl/6 mice		Oxidative stress, tubular degeneration, cell apoptosis, inflammation and fibrosis	Zheng et al. (2019)
Mercuric chloride	SD rat	1 mg/kg and 3.5 mg/kg BW (intraperitoneal)	Proximal tubular damage, mitochondrial swelling, vacuolization and apoptosis	Stacchiotti et al. (2003)
		2 mg/kg BW subcutaneous	↑ BUN, creatinine, ↓ GSH and GPx, glomerular and tubular degeneration, inflammation	Gao et al. (2016)
Renal fibrosis				
Carbon tetrachloride	Wistar rat	1.5 mL/kg BW of 30% CCl_4 in olive oil along with 1 gm of BSA as a 3 mL solution in NS	Connective tissue proliferation, protein cast and lymphocytic infiltrations in peritubular area	Limbu et al. (2017)
	Swiss albino mice	0.5 ml/ kg BW /1 ml olive oil for 4 weeks	↑ Collagen deposition and α-SMA upregulation, oxidative stress, ↑ TNF-α and IL-6	Ma et al. (2020)
		1.5 ml/kg BW (1:1 v/v with olive oil) intraperitoneal twice a week for 15 days	Lipid peroxidation, oxidative stress, ↑cytokines and apoptosis	Safhi (2018)

(continued)

Table 2.2 (continued)

Agent	Model/strain	Method	Characteristics	References
Vanadate	Wistar rat	0.125 mg/mL for 12 weeks	↓fluid intake, urine output, body weight, and urinary creatinine ↑ MDA, cystatin C and KIM-1	Ścibior et al. (2014)
		0.9 mg/kg BW subcutaneous for 16 days	↑ collagen deposition and inflammation	Al-Bayati et al. (1989)
Folic acid	CD1 mice	240 mg/kg BW intraperitoneal	Folic acid crystals deposition, tubular obstruction, necrosis and interstitial fibrosis	Yuan et al. (2003)
	C57BL/6 mice	250 mg/kg BW, intraperitoneal, single dose	Impairment of renal function, lipid peroxidation, oxidative stress, inflammation and fibrosis	Li et al. (2020) and Hsu et al. (2020)
Nephrectomy	129/Sv and C57BL/6JRj	5/6 nephrectomy of left kidney through left laparotomy	↑ Serum creatinine and urinary albumin/creatinine ratio glomerulosclerosis, tubular damage and fibrosis	Hamzaoui et al. (2020)
	C57BL/6 mice	Upper and lower poles of left kidney were ligated	↑ BUN, creatinine and proteinuria ↑ α-SMA and severe renal fibrosis	Tan et al. (2019)
Unilateral ureteral obstruction	C57BL/6 mice	Ligation of left ureter	↑ BUN and creatinine Pyelonephrosis, renal papillary necrosis, tubular degeneration and fibrosis	Gu et al. (2019, 2020)

Table 2.2 (continued)

Agent	Model/strain	Method	Characteristics	References
Chronic kidney disease				
Adenine	Wistar rat	0.25% adenine in diet for 16 weeks	↑ Body weight BUN, creatinine, LDH activity, and potassium concentration glomerulosclerosis, and tubular atrophy	Diwan et al. (2013)
	SD rats	Dietary adenine (0.75%) for 3 weeks and unilateral nephrectomy	Hypocalcemia, hyperphosphatemia, anemia and ↓ GFR Tubular degeneration, inflammation and interstitial fibrosis	Abellán et al. (2019)
High calorie diet	Wistar rat	Special diet (carbohydrate 56%, 30% fat, and 14% protein) for 28 weeks	Renal vasorelaxation, ↓ catalase activity and catalase mRNA transcripts IL-1β and endothelial dysfunction	Rangel Silvares et al. (2019)
	Wistar rat	High fructose diet (35%) for 16 weeks	↑ BUN, creatinine, lipid peroxidation, amyloid deposition, atherosclerosis and vacuolar degeneration of tubular epithelial cells	Bratoeva et al. (2017)
	Rcc:Han WIST rats	Cafeteria diet for 14 weeks	↓ Plasma antioxidant capacity, cytoplasmic antioxidant enzymes and induction of apoptosis	La Russa et al. (2019)
	C57BL/6 mice	HFD (60% calorie from fat) for 16 weeks	↑ Weight gain, blood glucose, BUN and creatinine, albuminuria, oxidative stress Glomerular and tubular degeneration and apoptosis	Sun et al. (2020)

Table 2.2 (continued)

Agent	Model/strain	Method	Characteristics	References
DOCA	SD rat	Uninephrectomy and DOCA (50 mg pellet) and 0.9% NaCl and 0.2% KCl in drinking water for 4 to 16 weeks	↑ Albumin/creatinine ratio, urinary KIM-1 marker excretion, ↓ GFR, accumulation of glomerular extracellular matrix, collagen and mesangial proliferation	Wang et al. (2017a, b)
	SD rat	DOCA (3.3 mg/day BW subcutaneous) along with 1% NaCl +0.2% KCl in the drinking water	Higher blood pressure with proteinuria and glomerulosclerosis	Polichnowski et al. (2017)
Streptozotocin	SD rats	50 mg/kg BW intraperitoneal, single dose	↑ BUN and creatinine, lipid peroxidation and ↓ antioxidant parameters (SOD, CAT and GPx), mesangial proliferation, thickening of basement membrane, glomerular hypertrophy and degeneration	Qi et al. (2020)
	SD rats	60 mg/kg BW intraperitoneal, single dose	↑ Blood glucose, urinary volume, urinary protein excretion, lipid peroxidation and eNOS immunoreactivity	Nakhoul et al. (2006)

Table 2.2 (continued)

Agent	Model/strain	Method	Characteristics	References
Lupus nephritis	(NZB X NZW)F1 (B/W) lupus-prone mice MRL/lpr lupus mice and BXSB mice strain	Spontaneous models	Deposition of immune complexes in subendothelium or mesangium, mesangial proliferation, glomerulonephritis, and tubular degeneration	McGaha and Madaio (2014)
	BALB/c mice	0.5 ml Pristane (2,6,10,14 tetramethylpentadecane) intraperitoneal	Moderate proteinuria and proliferative glomerulonephritis	Satoh et al. (1995)
Thy-1 nephritis model	Brown Norway/ RijHsd rat	Single intravenous injection of mouse or rabbit anti-thymocyte monoclonal antibody (OX-7 1 mg/kg bw)	Proteinuria, hematuria, mesangiolysis, mononuclear infiltration and endothelial damage	Westerweel et al. (2012)

Abbreviations: *α-SMA* Alpha smooth muscle actin, *BW* Body weight, *BUN* Blood Urea nitrogen, *CAT* Catalase, *DOCA* Deoxycorticosterone acetate, *eNOS* endothelial nitric oxide synthase, *GFR* Glomerular Filtration Rate, *GPx* Glutathione Peroxidase, *GSH* Glutathione, *IL-6* Interleukin-6, *IL-1β* Interleukin--1β, *KIM-1* Kidney Injury Molecule 1, *LDH* lactate dehydrogenase, *MDA* Malondialdehyde, *NGAL* Neutrophil gelatinase-associated lipocalin, *SOD* Superoxide dismutase, *TNF- α* Tumor necrosis factor-alpha

at mRNA levels, respectively (Hua et al. 2018). In another study, a single dose of acetaminophen (2 g/kg) to rats resulted in elevated kidney injury markers in serum, alterations in the ultrastructure of the proximal convoluted tubule, lipid peroxidation and high tumor necrosis factor-alpha (Haidara et al. 2020). Acetaminophen induced renal injury model is used for long time and one of the preferred models to study acute kidney injury till date.

Adriamycin

It is an anti-neoplastic and antibiotic drug used to study chronic glomerular disease. Rats treated with adriamycin for short term manifest glomerular lesions, fibrosis, and kidney damage. A dose of 2 mg/kg by intravenous route in Sprague Dawley rats was given for twenty days to induce kidney disease (Okuda et al. 1986). Adriamycin causes oxidative stress and lipid peroxidation in the glomerular epithelial cells. A 10 mg/kg dose of adriamycin to C57/balbc mice *via* tail vein resulted in proteinuria, podocytes injury, glomerular damage, tubular atrophy, and fibrosis. The adriamycin-induced kidney damage model mimics the human disease of focal segmental glomerulosclerosis (Tan et al. 2013). The adriamycin toxicity is mainly attributed to the

oxidative damage caused to cellular components including the plasma membrane, lipids, and mitochondria.

Cisplatin

Cisplatin is a common anticancer agent used for the treatment of solid tumors. Kidney damage is one of the side effects of this drug; therefore, this is used to develop kidney injury models in biomedical research. Cisplatin-induced acute kidney injury involves the induction of oxidative stress, kidney inflammation, vascular and tubular injury. Cisplatin was reported to impair kidney functions, induce tubular damage, kidney cast formation, and increased neutrophil gelatinase-associated lipocalin expression in kidneys of mice (Wu et al. 2020). Cisplatin (7 mg/kg BW intraperitoneal, single dose) reported elevating the kidney injury markers (blood urea nitrogen and creatinine) and tumor necrosis factor-alpha in the serum of SD rats. The kidney tubule showed vacuolar degeneration and necrosis on microscopic evaluation (El Amir et al. 2019). In another study, cisplatin treatment (6 mg/kg BW, single dose) increased the serum urea, creatinine, and urine glucose levels in rats. The drug also altered the urine output and osmolality excretion rate (Hosseinian et al. 2016). Overall, cisplatin triggers complex molecular pathways involving tubular cell injury, inflammatory response, and cell death.

Cyclophosphamide

Cyclophosphamide is a widely used agent for a range of neoplastic conditions, autoimmune diseases, and organ transplantation. Nephrotoxicity is one of the primary side effects of this drug. Cyclophosphamide gets metabolized in the liver into highly reactive compound acrolein, which is responsible for oxidative damage and cell toxicity (Mahmoud 2014). Cyclophosphamide (200 mg/kg intraperitoneal) administration for two consecutive days resulted in elevated creatinine and blood urea nitrogen in mice. The drug resulted in proximal tubular swelling, glomerular degeneration, and elevated pro-inflammatory cytokines (Interleukin-1β and tumor necrosis factor-alpha). Cyclophosphamide also induced cell apoptosis by altering caspase 3/9 activity and Bax/Bcl-2 ratio (Sharma et al. 2017). In a recent study, nephrotoxicity was induced by administering cyclophosphamide (75 mg/kg bw, intraperitoneal) on days 3, 4, 5, 19, 20, and 21 of the trial. The agent altered the urine volume, serum Na^+ and serum kidney injury markers. The kidney tissue showed pathological changes with lipid peroxidation, elevated pro-inflammatory cytokines and apoptotic changes (El-Shabrawy et al. 2020). The induction of oxidative stress, inflammation, cell injury, and apoptosis in the kidney tissue by cyclophosphamide makes it a suitable model to study drug-induced renal damage.

Cyclosporine A

Cyclosporine A is an immunosuppressant agent used to increase the efficacy of organ transplantation. It is a calcineurin inhibitor, and its mechanism of action is linked to the side effect of nephrotoxicity. Cyclosporine A interacts with the renal tubular cell membrane, and long-term use leads to tubulointerstitial nephropathy and kidney fibrosis. The drug is used to study interstitial fibrosis in rodent models; however, there are limitations of cost, hepatic dysfunction, and higher doses in animal studies than in clinical trials (Zhao et al. 2015; Nogueira et al. 2017). Cyclosporine A is given by subcutaneous route (7.5 mg/kg and 15 mg/kg) in rats for 28 days resulted in decreased glomerular filtration rate with an elevated creatinine level. Morphological investigation showed tubular atrophy with endothelial dysfunction (Bing et al. 2006). In another study on rats, cyclosporine A (30 mg/kg) treatment elevated serum blood urea nitrogen and creatinine levels and reduced the endogenous creatinine clearance. Cyclosporine A also induced kidney cell injury by causing vacuolar degeneration in glomerular cells (Huang et al. 2018). The short-term administration of cyclosporine A produces acute renal cell damage, however, long term use may lead to the development of chronic tubule-interstitial nephropathy.

Mercuric Chloride (Hgcl$_2$)

Mercuric chloride is used to induce kidney damage by triggering oxidative stress in the organ. The toxicity mechanism involves disturbing membrane potential, precipitation of proteins, alteration in protein synthesis, and intracellular calcium homeostasis (Zalups 2000; Dhanapriya et al. 2016). HgCl$_2$ at 1 mg/kg and 3.5 mg/kg BW (intraperitoneal) doses induced nuclear and cytoplasmic changes in the proximal tubular cells. The ultrastructural pathology showed nucleolar segregation, mitochondrial swelling, vacuolization, loss of brush border, necrosis and apoptosis of tubular epithelial cells (Stacchiotti et al. 2003). Similarly, rats treated with a single dose of HgCl2 (2 mg/kg) subcutaneously showed elevated kidney serum injury markers (Blood urea nitrogen and creatinine), depletion of glutathione level, and decreased glutathione peroxidase activity. The kidneys appeared enlarged and whitish in color. Microscopically, glomerular degeneration, swelling of proximal tubule epithelial cell, granular degeneration, infiltration of inflammatory cells, and extensive necrosis were reported (Gao et al. 2016). The HgCl$_2$ induced kidney damage is a classical model to study acute kidney injury. Overall, various analgesic, anticancer, immunosuppressive drugs, and chemicals induced acute kidney injury model offers a great opportunity to explore the pathophysiology and molecular mechanisms of acute kidney injury.

2.5.2.2 Kidney Fibrosis Models

Carbon Tetrachloride

Carbon tetrachloride is a potent toxin to induce oxidative injury in various physiological conditions. The metabolism of CCl_4 in the liver leads to the generation of trichloromethyl radical ($CCl_3\bullet$) and trichloromethyl peroxyl radical ($CCl_3O_2\bullet$) which causes oxidative tissue injury by binding with the macromolecules (lipids, proteins, and DNA) (Chhimwal et al. 2020). The administration of carbon tetrachloride (0.5 ml/kg BW/1 ml olive oil) to mice for 4 weeks induced kidney fibrosis by deposition of collagen and upregulation of α-smooth muscle actin. It also promoted oxidative stress and inflammatory response by activation of proinflammatory cytokines (Interleukin-6 and tumor necrosis factor-alpha) (Ma et al. 2020). A combination of carbon tetrachloride and bovine serum albumin (1.5 mL/kg of 30% carbon tetrachloride in olive oil and 1 g of bovine serum albumin as a 3 mL solution in NS) was reported to induce kidney fibrosis in female rats. The combination was found to develop the early stages of kidney fibrosis (Limbu et al. 2017). In another study on Swiss albino mice, carbon tetrachloride treatment at 1.5 ml/kg (1:1 v/v with olive oil) intraperitoneally twice a week for 15 days resulted in lipid peroxidation and disruption of antioxidant defense in the kidney tissue. It also increased the cytokines' levels with the induction of apoptosis in the kidney cells (Safhi 2018). Free radical generation, lipid peroxidation, inflammation, and increased fibrous tissue deposition are the main steps involved in carbon tetrachloride-induced renal fibrosis.

Vanadate and Folic Acid

Vanadate is pro-oxidant, can alter the oxidative stress levels in the cells, and activates oxidative damage mechanisms. An exposure of sodium metavanadate (0.125 mg V/mL) to rats for 12 weeks decreased the fluid intake, urine output, body weight, and urinary creatinine excretion rate. The relative kidney weight and the malondialdehyde levels were also found elevated. The study suggested that cystatin C and KIM-1 might be the most appropriate makers to evaluate sodium metavanadate-induced alterations in kidney function (Ścibior et al. 2014). Vanadate-induced kidney fibrosis is basically dose-dependent. A dose of 0.9 mg/kg for 16 days *via* the subcutaneous route to rats resulted in kidney fibrosis and inflammation. Pathological and other biochemical parameters confirmed the kidney injury. After 12 days of administration, cellular proliferation was observed in the cortex and medulla, and fibrotic changes appeared in the glomerular tuft and interstitium. The collagen deposition in the kidney was reported highest after 25th day of the trial (Al-Bayati et al. 1989).

Folic acid, also known as vitamin B, is water-soluble and helps the body in the production of new cells. The administration of folic acid to (240 mg/kg by intraperitoneal route) mice reported depositing folic acid crystals and obstruction in the tubules within 2 weeks leading to acute tubular necrosis and nephrotoxicity. The

animals were reported to develop interstitial fibrosis by the end of fourth week (Yuan et al. 2003). In another study, the folic acid treatment in mice (250 mg/kg BW, i.p., single dose) resulted in kidney function impairment, lipid peroxidation, and reduction in the kidney antioxidant parameters. Besides, severe glomerular inflammation, tubular swelling, kidney fibrosis, and apoptosis were also noticed. (Li et al. 2020; Hsu et al. 2020). Deposition of folic acid crystal is the main triggering point for folic acid-induced renal fibrosis.

Nephrectomy and Unilateral Ureteral Obstruction Models

These methods are commonly used in the study of kidney disease; however, there is disadvantage of performing the surgery, loss of kidney tissue and mortality. Gender and strain (Sprague Dawley are resistant then Wistar rats) also influence kidney disease progression in the experimental model. Hamzaoui et al. (2020) developed a kidney disease model in 129/Sv and C57BL/6JRj mice by performing 5/6 nephrectomy of the left kidney through left laparotomy. A significant increase was observed in the creatinine and urinary albumin/creatinine ratio after 12 weeks. Histopathological evaluation showed glomerulosclerosis, tubular damage, perivascular, and interstitial fibrosis, vascular thickening and inflammation in the kidney tissue. Recently a highly efficient method was developed in C57BL/6 mice to reduce the mortality and infection in the 5/6 nephrectomy model of kidney fibrosis. In this method, the right kidney was removed surgically, and after 1 week the upper and lower poles of the left kidney were ligated directly to cause necrosis of ligated poles. The method results in high levels of serum blood urea nitrogen, creatinine, and proteinuria after 12 weeks. The tissue also showed increased expression of smooth muscle alpha-actin and severe kidney fibrosis (Tan et al. 2019).

Unilateral ureteral obstruction is a model of urinary path obstruction resulting in damage of kidney structures. It leads to kidney enlargement and hydronephrosis. Unilateral ureteral obstruction model is the most frequently used model to study non-immunological tubulointerstitial fibrosis (Grande et al. 2010). In this model, the left kidney of mouse is exposed surgically, and ligation of left ureter is performed. Unilateral ureteral obstruction results in an elevation in the serum kidney injury markers (blood urea nitrogen and creatinine). The kidney tissue shows typical pathology of obstructive nephropathy with pyelonephrosis, renal papillary necrosis, severe tubular dilatation and atrophy, interstitial inflammation, and fibrosis (Gu et al. 2019). Similarly, Gu et al. (2020) showed that Unilateral ureteral obstruction in mice resulted in proinflammatory activation cytokines and kidney fibrosis along with impairment of kidney function. This is a frequently used model; however, the drawback is that a complete obstruction rarely occurs in humans.

2.5.2.3   Chronic Kidney Disease Models

Adenine

Adenine administration in the diet (0.25% to 0.75%) was used as a potential model to study chronic kidney disease in rodents. Adenine is converted to 2,8-dihydroxyadenine, which deposit as crystals in the proximal tubular cells, leading to tubular degeneration and kidney damage. Administration of 0.25% adenine in the diet for 16 weeks in rats mimics the slow progression of chronic kidney disease in humans. The rats showed weight gain with increased blood urea nitrogen, creatinine, lactate dehydrogenase (LDH) activity, and potassium concentration. Microscopically, the kidney showed glomerulosclerosis, loss of proximal tubular brush border epithelium and tubular atrophy (Diwan et al. 2013). In another method, dietary adenine (0.75%) for 3 weeks combined with unilateral nephrectomy in rats was used to develop chronic kidney disease. The rats develop hypocalcemia, hyperphosphatemia, and anemia along with altered glomerular filtration rate. Histopathology of kidneys showed tubular degeneration and dilatation, adenine crystal deposits, mononuclear cell infiltration and interstitial fibrosis (Abellán et al. 2019). The adenine-induced model is also preferred to study the chronic kidney disease associated secondary hyperparathyroidism and hyperphosphatemia related to mineral bone disease.

Streptozotocin

Streptozotocin is a N-acetyl glucosamine analogue transported to pancreatic beta cells by GLUT-2 receptors, causing toxicity and used to develop diabetic nephropathy in rodents. Diabetic nephropathy is the highest cause of chronic kidney disease and end-stage kidney disease. At high doses, streptozotocin can also lead to nonspecific kidney toxicity; therefore, single or multiple low doses are used to induce diabetic nephropathy in mice. Streptozotocin at a dose of 60 mg/kg BW i.p. was reported to induce diabetic nephropathy in Sprague Dawley rats. The rats showed elevated blood glucose, urinary volume, and urinary protein excretion. The kidney tissue also showed increased lipid peroxidation and endothelial nitric oxide synthase immunoreactivity (Nakhoul et al. 2006). Similarly, streptozotocin (50 mg/kg BW i.p.) administration to male Sprague Dawley rats elevated the kidney injury markers (blood urea nitrogen and creatinine), lipid peroxidation and antioxidant parameters (superoxide dismutase, catalase and glutathione peroxidase). Histopathologically, kidney tissue showed glomerular hypertrophy, mesangial proliferation, thickening of the basement membrane, glomerular epithelial cell swelling, and infiltration of inflammatory cells (Qi et al. 2020). Streptozotocin is very effective in inducing the diabetic condition in rodent; therefore, this model is highly preferred to study diabetic nephropathy in experimental animals.

Deoxycorticosterone Acetate Salt

Deoxycorticosterone acetate (DOCA), a synthetic mineralocorticoid derivative, and high salt intake are used to induce hypertension and kidney damage in rodents. This model mimics the human disease condition to a greater extent. The uninephrecto-mized rats receiving deoxycorticosterone acetate (DOCA; 50 mg pellet) and 0.9% NaCl plus 0.2% KCl in drinking water develop increased systolic blood pressure and kidney and heart weights after 4 to 16 weeks. The DOCA salt treatment decreased GFR and increased the urinary albumin/creatinine ratio and urinary kidney injury molecule-1 marker excretion. Microscopically, kidney tissue showed accumulation of extracellular matrix in glomeruli, mesangial proliferation, and collagen deposition in the kidney tissue (Wang et al. 2017a, b). Similarly, Sprague Dawley rats administered with DOCA (3.3 mg/day s.c.) and 1% NaCl plus 0.2% KCl in the drinking water developed higher blood pressure with proteinuria after 6 weeks. The kidney pathology showed the development of glomerulosclerosis in rats (Polichnowski et al. 2017). Hypertension is one of the primary reasons for chronic kidney disease, as high blood pressure causes deleterious effects on kidney vasculature. DOCA salt-induced model provides an opportunity to study the pathophysiology of hypertension-induced chronic kidney disease in experimental animals.

Diet-Induced Chronic Kidney Disease Models

A metabolic syndrome is a group of complications that includes obesity, diabetes, hypertension, and dyslipidemia. Patients with metabolic syndrome are having more risk of kidney injury (Nashar and Egan 2014). An obese person is more prone to chronic kidney injury, although the exact mechanism is not clear. A study reported that a few weeks of weight gain leads to glomerular cell proliferation and structural changes in the kidney (Laurentius et al. 2019). Lifestyle and high-calorie diet are significant factors for the development of metabolic syndrome and related complications. There are many diets induced rodent model which are used to study the kidney damage.

A high-fat diet contains a high-fat percentage, increasing the calorie intake and leading to obesity and related complications. Feeding of high-fat diet (60% calorie from fat) to C57BL/6 mice for 16 weeks resulted in excessive weight gain, elevated blood glucose levels, and kidney function impairment. The mice showed increased accumulation of blood urea nitrogen, creatinine and albuminuria. The kidneys showed marked degeneration in the glomeruli and tubules, leading to damaged glomerular filtration barrier and apoptosis of tubular epithelial cells. The intake of high-fat diet triggers lipogenic pathways in the kidney tissue and increases kidney triglyceride and cholesterol contents. It also increases oxidative stress and induction of pro-apoptotic pathways in the kidney tissue (Sun et al. 2020). A daily intake of high-fat diet (carbohydrate 56%, 30% fat, and 14% protein) for 28 weeks in Wistar rats altered kidney vasorelaxation, decreased catalase activity, and catalase mRNA

transcripts. The high-fat diet fed rats showed upregulation of interleukin-1β and endothelial dysfunction in the kidneys. The study emphasized that vascular changes as a result of high caloric intake play an important role in kidney dysfunction (Rangel Silvares et al. 2019). Similarly, a high fructose diet is also used to induce kidney damage. High fructose ingestion affects blood pressure, adenosine triphosphate depletion, and uric acid generation, leading to glomerular hypertension, tubulointerstitial damage, and inflammation (Johnson et al. 2010). Male Wistar rats exposed to a high fructose diet (35%) for 16 weeks resulted in obesity, diabetes, and kidney impairments. The fructose-rich diet elevated the serum kidney injury markers (blood urea nitrogen and creatinine) and malondialdehyde levels in the kidney tissue. Microscopically, kidney tissue showed amyloid deposition, atherosclerosis, vacuolar degeneration of tubular epithelial cells, and eosinophilia in the distal tubules (Bratoeva et al. 2017). Recently**,** La Russa et al. (2019) showed that ad libitum feeding of cafeteria diet (cookies, snakes, chocolates, potato chips) for 14 weeks used to develop obesity and associated kidney disease in rats. This induced severe prooxidant effects by reducing the plasma antioxidant capacity, cytoplasmic antioxidant enzymes, and induction of apoptosis in the kidney cells.

Lupus and Thy-1 Nephritis Models

Kidney damage resulting from the autoimmune reaction is one of the comorbidities related to Systemic Lupus Erythematosus. The disease is characterized by deposition of immune complexes in sub-endothelium or mesangium, mesangial proliferation, glomerulonephritis, deposition of casts in the tubules and tubular degeneration (McGaha and Madaio 2014). There are mice strains that show spontaneous development of the lupus nephritis *viz.* (NZB X NZW) F1 (B/W) lupus-prone mice, deoxyribonuclease 1 (Dnase1)-deficient mice, MRL/lpr lupus mice, and BXSB mice strain. However, intraperitoneal administration of pristane (2,6,10,14 tetramethyl-pentadecane) to mice is one of the potential inducible lupus nephritis models. BALB/c mice injected with pristine were reported to develop moderate proteinuria and proliferative glomerulonephritis after six months (Satoh et al. 1995). The Thy-1 antigen is mainly present on thymocytes, however, also expressed on the glomerular mesangial cells. Thy-1 nephritis is an experimental mesangio-proliferative glomerulonephritis model, induced by the single intravenous injection of mouse or rabbit anti-thymocyte monoclonal antibody (Yang et al. 2010). The disease condition is characterized by marked proteinuria, hematuria, mesangiolysis, mononuclear infiltration, and endothelial damage (Westerweel et al. 2012). An autoimmune reaction is one of the major cause of chronic kidney disease, and existing animal models are very useful to explore the molecular aspect of such predisposition.

Immunoglobulin A Nephropathy

Immunoglobulin A (IgA) nephropathy is one of the leading cause of chronic kidney disease and kidney damage worldwide. The condition is characterized by the deposition of IgA1-immune complexes in the mesangium with co-deposition of variable immunoglobulin G/M and complement C3 (Suzuki and Suzuki 2018). Immunoglobulin A nephropathy can be induced in rats by oral and intravenous administration of 0.1% bovine serum albumin for 12 weeks. The rats immunized with bovine serum albumin showed severe proteinuria and a higher urine protein/creatinine ratio. Microscopically, the rat kidneys showed mesangial proliferation and expansion and diffuse deposition of Immunoglobulin A in glomeruli. The kidney tissue showed upregulation of proinflammatory cytokines and activation of kidney NLR family pyrin domain containing-3 inflammasome (He et al. 2015). Similarly, another study used a combination of bovine serum albumin (400 mg/kg, orally), carbon tetrachloride (0.1 ml dissolved in 0.5 ml castor oil, s.c.), and lipopolysaccharide (0.05 mg, i.v.) to induce IgA nephropathy in rats (Wei et al. 2017). Zhang et al. (2010) used a fusion protein MBP-20 consist of maltose-binding protein and a peptide containing 20-amino-acid from *Staphylococcus aureus* to induce IgA nephropathy in Balb/c mice. Mice were immunized with MBP-20 (0.5 mg) for 21 weeks. The mice showed hematuria and increased protein/creatinine ratio. Kidney tissue showed mesangial matrix proliferation and expansion in light microscopy and electron-dense deposits in the mesangium and subendothelium, ultrastructurally. The model was closely found to mimic the clinical and pathological features of IgA nephropathy.

2.6 Alternative *In Vivo* Models for Kidney Injury

2.6.1 *Zebrafish Model*

There is an increasing interest in Zebrafish (*Danio rerio*) as a model to study kidney diseases. A less maturation time, the large number of offspring, easy handling, low rearing cost, and less ethical constraints make them favourites for the high throughput screening of molecules for various disorders (Katoch and Patial 2020). The transparency of embryos provides an added advantage of studying organs *in situ.* The genetic models of kidney diseases can be easily created in zebrafish. Exposure to nephrotoxic drugs like cisplatin and gentamicin are used to induce acute kidney injury in zebrafish (Hentschel and Bonventre 2005; Sharma et al. 2014). These compounds' toxicity leads to cellular vacuolization, loss of brush border, degeneration of proximal convoluted tubules necrosis, and inflammation. Gentamicin administration to zebrafish at (0–100 mg) different concentrations produced pathological kidney tissue changes. A dose-dependent kidney damage was observed in adult zebrafish, and high doses lead to the degeneration of glomerulus and proximal

tubules (Kato et al. 2020). Adriamycin treatment to 3–4 dpf zebrafish (1,2,4 mg/ml) for 24 and 48 h affected the fish survival and caused acute kidney injury. Similarly, puromycin was used to study the podocyte injury in zebrafish. Both the models are used to determine the podocyte injury and glomerular damage in zebrafish larvae and side effects to cardiovascular injury (Rider et al. 2018). Many other agents like acetaminophen, benzo(a)pyrene, sodium benzoate, and mycotoxins are used to induce acute kidney injury in zebrafish (Sharma et al. 2014). Overall, zebrafish has emerged as a promising model to study acute kidney injury, chronic kidney disease, and kidney related drug developmental studies.

2.6.2 Drosophila Model

Drosophila is an excellent model for understanding the development of the human kidney system. It is quick, inexpensive, and has extensive functional similarities to mammalian kidney function (Dow et al. 1995; Millet-Boureima et al. 2019). The compact genome of drosophila is wholly sequenced, and around 70% of genes have human homologs. Drosophila has a small body size and fastest filtration rate. They have a separate section for their kidney function, the Malpighian tubules (similar to kidney tubules) and two clusters of nephrocytes within the body cavity (identical to podocytes in the glomerular kidney) (Millet-Boureima et al. 2019). The fly's kidney system is aglomerular, and active transport is responsible for urine formation rather than selective reabsorption (Millet-Boureima et al. 2018; Dow and Romero 2010).

Drosophila nephrocytes were used to study the glomerular filtration barrier disruption and podocyte function in kidney disease. Garland and pericardial are the two types of nephrocytes present in the drosophila. The fenestrated endothelium is absent in nephrocytes, and two barriers include the basement membrane and nephrocyte slit diaphragm. The nephrocytes can be dissected out quickly and used for filtration and uptake studies. Chemical induced kidney injury can be created in the flies by feeding the compound (Marelja and Simons 2019). Malpighian tubules are also used to study kidney physiology and diseases due to their simple anatomy and functional similarity. They contain high cytochrome P450 and glutathione transferase levels and perform the function of detoxification as by mammalian kidney tubules. Malpighian tubules have been used as models to study human calcium oxalate nephrolithiasis and polycystic kidney disease (Millet-Boureima et al. 2018). Research on drosophila resulted in the discovery of sub lethal human V-ATPase mutations leading to kidney tubular acidosis (Dow and Romero 2010). Therefore, drosophila provides a rapid model system to study kidney development, physiology, and disease pathology.

2.7 Chronic Kidney Disease in Animals

There is limited information available on chronic kidney disease in large animals. However, chronic kidney disease was well reported in cats and dogs with more prevalence in older animals. A study reported more than 30% of cats and 10% of dogs over 15 years of age with chronic kidney disease at the University of Minnesota Veterinary Medical Centre. The clinical manifestation of chronic kidney disease in small animals is generally seen as loss or retention of compounds. Moreover, kidneys' inability to regulate water balance results in polydipsia and polyuria (Polzin 2011; Bartges 2012). Potassium, sodium, calcium, and phosphorus are the major minerals affected by chronic kidney disease. However, phosphorus retention is the major effect that occurs during chronic kidney disease, which is exhibited as increased blood phosphorus concentration known as hyperphosphatemia. Alteration in calcium level is the second change that occurs in the case of chronic kidney disease. The calcium level in the body may either decrease or increase, while phosphorus generally increases (Elliott 2006). Animals with chronic kidney disease have increased urine volume, which enhances the excretion of water soluble vitamins. Further, loss of these vitamins results in anorexia which is directly associated with several renal complications. Similarly, humans with renal failure have less ability to excrete certain vitamins. Acid-base abnormalities are most commonly seen in cats and dogs as the kidney's function is to retain bicarbonate ions and excrete hydrogen ions. However, kidney failure results in more retention of hydrogen ions and less reabsorption of bicarbonate ions resulting in metabolic acidosis (Elliott et al. 2003). Proteinuria is also associated with chronic kidney disease in both cats and dogs. An initial urine protein: creatinine ratio greater than 1.0 in dogs is directly associated with a greater risk of developing uremic crisis and death (Vaden and Elliott 2016).

Renal hyperparathyroidism is the secondary consequence of chronic kidney disease. Generally, in human, the bone quality is decreased, and the risk of fracture is increased during this condition. Similar effects are also observed in companion animals, especially in cats and dogs (Segev et al. 2016). Systemic and glomerular hypertension has also been correlated with the development and the progression of kidney diseases in both rodent models and humans. Similarly, Hypoxia has also been reported to contribute in the progression of chronic kidney disease. However, in animals, there relatively less information is available which directly demonstrates the relation between hypoxia and progression of chronic kidney disease (King et al. 2007). Excessive consumption of phosphorous in daily diet leads to phosphorous nephritis in different species like humans, dogs, cats and rats. Feeding of high phosphorous to cats resulted in microalbuminuria and glucosuria, indicating renal damage (Dobenecker et al. 2018). Therefore, diet modification is considered as one of the important interventions in preventing the development of chronic kidney disease in small animals.

2.8   Conclusion and Future Perspective

Kidney diseases are one of the major heath burden on the global economy. Researchers are continuously trying to understand the underlying disease mechanism and to develop highly effective tools for the early detection of disease. Early diagnosis is essential for the management and better prognosis of the disease. In the past few decades, significant progress has been achieved in the identification of clinically relevant kidney markers from body fluids, particularly from plasma/serum and urine. For this, proteomic techniques like electrophoresis, chromatography, protein array, chemical tagging/metabolic labelling coupled with mass spectrometry offer not only for biomarker identification but also increase our understanding of the molecular process underlying kidney disease. In proteomic study, major limitation is the false-positive error, which was rule out using probability based auto validation of peptides by database search. In addition, to achieve high throughput data in the animal experiment, we should consider large sample size, standardized method for sample collection and preparation, advanced proteomic techniques, robust statistical models, proper normalization of data, and extensive validation that often requires strong collaboration with research laboratory across multidiscipline. Furthermore, the selection of body fluids and abundance of biomarkers will be important towards biomarker discovery and diagnosis of kidney disease. The experimental models play a crucial role in the understanding of various disease conditions. This chapter mainly focused on non-transgenic kidney disease models; however, different transgenic models are equally important. None of the experimental models mimics the human disease entirely; however, careful selection of experimental models can provide valuable understandings of disease pathogenesis.

Declaration of Interest   Authors declare no conflict of interest.

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