Experimental Models of Chronic Kidney Disease – A Review

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Abstract. Study on chronic kidney disease (CKD) requires reliable animal models because it is a major worldwide health concern. It has a substantial global health burden, a variety of etiologies, and complicated progression pathways. The many in vivo models used in CKD research are thoroughly analysed in this review. The models are grouped according to the etiology of CKD, which includes drug-induced nephrotoxicity, glomerulonephritis, diabetic nephropathy, and hypertension-induced renal damage. Each model's benefits and drawbacks are discussed with an emphasis on how relevant each is to a particular CKD pathology. New developments like genetically modified models and sophisticated imaging methods are examined. It is emphasized how crucial translational research is to verifying results from studies conducted on animals that are applied to humans. To sum up, this review highlights how important in vivo models are to understanding the pathophysiology of CKD and to developing new treatments. Including a variety of models that capture the complexity of CKD facilitates understanding.

Keywords: CKD, *In vivo*, Mouse model & Rat model.

Introduction

The progressive loss of kidney function over time is a sign of chronic kidney disease. Renal failure and the development of end-stage kidney disease are the two main outcomes of chronic kidney disease, a complicated and diverse illness. (ESKD) as well as heart conditions. Disease-related complications increase the likelihood of cardiovascular-related morbidities and quicken the course of CKD.[1] Changes in the kidney's structure and function are the main symptoms of chronic kidney disease, a chronic condition with a broad range of etiologies.[2] It is most frequently caused by diabetes and hypertension. is a separate risk factor for hospitalization, cognitive decline, cardiovascular disease, and all-cause death. Age, proteinuria, and kidney function level all influence the risk of cardiovascular disease and all-cause mortality in older individuals with chronic kidney disease, which is frequently higher than the risk of ESRD progression.[3] The general term which includes a variety of illnesses affecting the structure and function of the kidney is chronic renal disease. The differences in how diseases occur is influenced by the pathology, severity, and rate of progression in part.[4] The clinical and financial impact of its related problems, together with its great prevalence, have not improved the disease's overall level of awareness. Globally, only 10% of people at high risk and 6% of the general population are aware that they have chronic kidney disease.[5]

FPCS 2024, November 13-14, Bengaluru, India

DOI 10.4108/eai.13-11-2024.2355682

Between 8% and 16% of people worldwide suffer from chronic kidney disease, which is typically neglected by patients and physicians. As stated by Aglo it is more common in low- and middle-income nations than in high-income ones.[6] The rate at which plasma passes through the functional nephrons in the kidneys, or glomerular filtration rate, or GFR, is the most accurate indicator of overall kidney function.[7]

Glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m2, albuminuria of at least 30 mg per 24 hours, or signs of kidney destruction (e.g., hematuria or structural abnormalities such as poly cystic kidney disease plastic kidneys) persisting for more than three months. Six Worldwide, diabetes and/or hypertension are the most prevalent causes of chronic kidney disease. [8] However, glomerulonephritis, infections, and environmental exposures (including air pollution, air pollution, herbal medicines, and pesticides) are also common in Asia, sub-Saharan Africa, and many developing nations. A fourth risk factor for CHD is genetic risk.[9]

Udhanam is a green area with a diverse range of flora and wildlife, comprising numerous villages situated in different mandals within the Srikakulam district of Andhra Pradesh, India. According to Ganguli agronomy producers are severely affected with chronic kidney disease. [10] Animal models of chronic kidney disease offer the chance to study the molecular pathophysiology of the disease, examine disease-specific processes, and evaluate potential new treatments. We present a variety of animal models in this review that replicate different aspects of human chronic kidney disease.[11]

Stages of CKD

According to the estimated glomerular filtration rate (eGFR), which estimates how well the kidneys are removing waste from the blood, the phases of chronic kidney disease are normally categorized. Five stages of chronic kidney disease are defined by the National Kidney Foundation (NKF) as follows: [11]

Stage 1: Damage to the kidneys with a GFR of Normal or High (≥90 mL/min/1.73 m2)

At this point, the GFR is either normal or only slightly decreased, despite signs of renal injury (such as protein in the urine). At this stage, renal function is still as normal.

Stage 2: Moderately Reduced GFR (60-89 mL/min/1.73 m2)

There may be few symptoms despite a moderate reduction in kidney function. Similar to Stage1, renal damage may be evident.

Stage 3: Moderate GFR Decrease

To prevent progress, management and changes in lifestyle become more crucial.

Kidney function is moderately reduced, and symptoms may become more noticeable.

Stage 4: Severe Decrease in GFR

Significantly reduced kidney function increases the risk of problems.

Stage 5: End-Stage kidney Disease (ESKD)

The kidneys can no long support the body's requirements with their current level of function.[12]

Mechanism of Action

1. Initiation Factors: • Diabetes Mellitus • Hypertension • Glomerulonephritis • Polycystic Kidney Disease • Other causes of kidney damage

2. Renal Injury: • Damage to nephrons (functional units of the kidney) • Inflammation • Oxidative stress

3. Progressive Damage: • Compensation by hypertrophy of remaining nephrons • Slow decline in kidney function over time

4. Fibrosis and Scarring: • Accumulation of fibrous tissue in the kidneys • Scarring of renal tissue

5. Reduced Filtration Capacity:• Decline in the number and function of nephrons • Reduced ability to filter waste products and excess fluids
6. Complications: • Anaemia • Bone disease • Cardiovascular problems • Electrolyte imbalances • Other systemic issues
7. Advanced CKD: • Consideration of renal replacement therapies • Dialysis • Kidney Transplantation
8. Management: • Address underlying causes (e.g., control blood pressure, manage diabetes) • Symptomatic management • Regular monitoring and follow-up [13]

Pathophysiology of Chronic Kidney Disease

An array of complex elements interacts to cause the pathophysiology of chronic kidney disease, which usually progresses through multiple phases.

The following are the main mechanisms involved in the pathophysiology of CKD: [14,15,16]

1. Hemodynamic Modifications: Renal hypoperfusion, or reduced blood flow to the kidneys, can be caused by diseases like atherosclerosis or hypertension. Renal blood vessel injury may result from prolonged decreased blood flow.
2. Inflammatory response: The onset and course of chronic kidney disease are significantly influenced by chronic inflammation. Numerous things, such as infections, autoimmune disorders, or other shocks to the kidneys, might start inflammatory processes.
3. Oxidative Stress: Oxidative stress can be caused by a decrease in antioxidant defences and an increase in the generation of reactive oxygen species (ROS). In addition to causing damage to renal cells, oxidative stress causes fibrosis and inflammation.
4. Glomerular Hyperfiltration: The kidneys may initially increase the filtration rate in the remaining healthy nephrons to make up for the damage. this might result in glomerular hyperfiltration, which increases stress on the nephrons and accelerates their eventual deterioration.
5. Hypertension: One of the main causes of chronic high blood pressure disease chronic kidney disease is damage to the kidneys' narrow blood capillaries. renal disease causes hypertension, which in turn complicates renal damage.
6. Metabolic Factors: One of the biggest risk factors for chronic kidney disease is diabetes mellitus. Diabetic nephropathy is a condition where kidney damage from high blood glucose levels occurs gradually. Diabeticemia is one of the other metabolic variables that might cause kidney injury
7. Genetic Factors: Some persons may be genetically inclined to acquire specific types of kidney illnesses. Polycystic kidney disease, for example, is a hereditary condition that can lead to CKD.
8. Immune System Disorders: CKD can occur as a result of illnesses like lupus nephritis, in which the immune system targets the kidneys.
9. Fibrosis: Renal tissue fibrosis and progressive scarring compromise the kidneys' ability to function normally. Oxidative stress, long-term inflammation.

Risk Factors

Risk factors for chronic kidney disease include diabetes, hypertension, aging, a family history of kidney disease, smoking, obesity, cardiovascular disease, autoimmune diseases, urinary tract infections, prior episodes of acute kidney injury, and certain ethnicities (e.g., African, American). [17] Reducing the chance of chronic kidney disease requires keeping an eye on kidney health, taking care of underlying illnesses, and leading a healthy lifestyle. The advancement of an illness can be slowed or stopped with early discovery and treatment. [18,19]

***In vitro* models**

In vitro models of chronic kidney disease are essential for understanding disease mechanisms, drug discovery, and prospective therapeutic approaches. These models imitate the structural and functional characteristics of kidney tissue impacted by chronic kidney disease, allowing researchers to better understand disease development in a controlled setting. Here are the primary *in vitro* CKD models:

1. Cell Culture Models 2. Kidney Organoids 3. 3D Bioprinting and Scaffolds
4. Kidney-on-a-Chip Models 5. Genetically Engineered Models

1) Cell Culture Models [20,21]

Primary Renal Cells: These are cells that come directly from renal tissues and comprise proximal tubular cells, podocytes, mesangial cells, and endothelial cells. Primary cells retain some physiological properties of kidney cells and are useful for short-term investigations.

Immortalized Cell Lines: These are genetically modified cells that may divide indefinitely, making them suitable for repeat research. HK-2 (human proximal tubular cells) and HEK293 cells are common cell lines utilized in chronic kidney disease models.

Co-Culture Systems: Different renal cell types can be cultivated alongside one another to better imitate kidney microenvironments. Combining tubular and endothelial cells, for example, allows researchers to investigate intercellular connections, inflammation, and fibrosis in chronic kidney disease.

2. Kidney Organoids [22,23]

Stem Cell-Derived Organoids: Human induced pluripotent stem cells (iPSCs) and embryonic stem cells can be differentiated into kidney organoids. These three-dimensional constructs contain a variety of kidney cell types and more closely resemble the architecture and function of renal tissue than 2D cultures.

Organoid models: Can be produced with substances such as TGF-β to induce fibrosis, hypoxia to simulate ischemia, or high glucose to represent diabetic kidney disease.

3. 3d Bioprinting and Scaffolds[24,25]

Bioprinting Kidney Tissue Models: Advanced 3D bioprinting techniques enable the production of kidney tissue with exact spatial organization of various cell types and extracellular matrix components. These models can more accurately reproduce kidney structure and are especially valuable for long-term studies of chronic kidney disease development.

Hydrogels and Extracellular Matrix (ECM) Scaffolds: 3D hydrogels can help kidney cells by increasing cellular connections and simulating CKD microenvironments. ECM scaffolds are made from decellularized kidney tissues and utilized to culture renal cells, allowing for a more natural interaction in kidney tissue-like environments.

4. Kidney-on-a-Chip Models[26]

These models use microfluidic devices to simulate kidney activities such as filtration and reabsorption. They enable the use of shear stress, flow, and other physiological parameters to imitate kidney settings, making them valuable for studying how chronic stress or toxins affect kidney function.

5. Genetically Engineered Models[27]

CRISPR/Cas9 Gene Editing: Genetic modification of kidney cell lines (such as podocytes or proximal tubular cells) can result in CKD-associated mutations. This enables the modelling of CKD variants such as autosomal dominant polycystic kidney disease (ADPKD) or Alport syndrome, allowing for disease-specific research.

Advantages: • Controlled Environment • High-Throughput Screening • Mechanistic Insights • Reduced Animal Use

Limitations: • Lack of Complexity • Reduced Vascularization • Short Lifespan • Limited Predictability

Applications: • Utilized to investigate disease pathways such as fibrosis, inflammation, and tubular damage. • They are also useful for evaluating medication candidates, conducting toxicity studies, and testing therapy targeted at slowing or reversing CKD progression.

***In vivo* models**

In vivo models of chronic kidney disease are critical for researching disease development and systemic consequences in living organisms. These models, mainly based on rodents, replicate chronic kidney disease symptoms such as fibrosis, inflammation, and proteinuria, allowing for in-depth research into disease causes, drug testing, and prospective therapy methods. The following are a few popular *in vivo* techniques for researching chronic kidney disease in animals. [28]

1. Animal model: a) Rat Models:(i) 5/6 Nephrectomy Model (ii) Unilateral ureteral obstruction
   b) Mouse Models: (i) Adenine – induced nephropathy
   (ii) Stz induced diabetic nephropathy
2. Genetically Modified Models : Genetically hypertensive rats
3. Induced Renal Injury Model: Renal – ischemia reperfusion model
4. Diet Induced Models: High fat diet method

1. Animal Model:

a) Rat Models:

(i) 5/6 Nephrectomy Model[29,30,31]

The 5/6 nephrectomy model is a prominent rat surgical method for simulating chronic kidney disease. By eliminating approximately 5/6 of the renal mass, it efficiently simulates nephron function loss, allowing researchers to analyze CKD development, underlying causes, and prospective therapeutic approaches.

Materials and Reagents: • Anaesthesia agent (ketamine, isoflurane, etc.) • Analgesics (buprenorphine) for the treatment of pain • Antibiotics (enrofloxacin) to prevent infections • Sterile saline irrigation and hydration solution • Solution containing benzodine or chlorhexidine for pre-operative skin preparation.

Procedure:

• The 5/6 nephrectomy model mimic chronic kidney disease in rat by performing many surgical procedures • After anesthetic is administered, a midline abdominal incision is performed to provide access to the kidneys • One kidney is completely removed (1/2 nephrectomy), followed by the excision of one-third of the remaining kidney (1/3 nephrectomy), with some nephron mass preserved • After surgery, the abdomen is closed with clips, and the mouse is cared for with warmth, water, and drugs. Weight, health, and renal function are monitored as part of the follow-up, with periodic necropsies performed to assess disease progression • Ethical standards and institutional authorizations must be thoroughly observed throughout the procedure.

Advantages: •Controlled Environment •Therapeutic Testing •Mimics Chronic Kidney disease

Limitations: • Species Differences • Surgical Risks • Variable outcomes

(ii) Unilateral Ureteral Obstruction[32,33,34,35]

One of the most important tools for research on chronic kidney disease is the unilateral ureteral obstruction (UUO) model. UUO causes kidney damage similar to human chronic kidney disease, including inflammation, fibrosis, and functional deterioration, by blocking one ureter. This model facilitates controlled research on the pathophysiology of chronic kidney disease and treatment approaches. Although compensatory renal modifications are a drawback, UUO's capacity to replicate characteristics of the disease makes it a priceless tool for preclinical research. researchers obtain insights into disease pathways and test possible therapeutics.

Materials and Reagents: • Aesthetic (e.g., xylazine/ketamine, isoflurane) • Using betadine or another antiseptic • Sterile saline irrigation solution • Analgesic medication (such as buprenorphine) • Antibiotic cream or solution for the treatment of wounds

Procedure:

The method begins by anesthetizing the animal and testing reflexes to ensure unconsciousness. After shaving, the surgical site is disinfected with antiseptic. A tiny incision is made in the flank or abdomen to gain access to the kidney and ureter, which are gently exposed. The incision is closed with surgical clips or sutures, and analgesics such as buprenorphine are provided to relieve pain. Postoperative care includes monitoring hydration and body temperature, administering antibiotic ointment to avoid infection, and adhering to hospital standards. Urine and blood samples are collected on a continuous basis to evaluate the animal's health and renal function, as well as histological analysis. Ethical norms must be followed throughout the procedure.

Advantages: • Simplicity • Reproducibility • Pathophysiology Insight

Limitations: • Acute Phase Response • Species Specificity • Variable Responses

b) Mouse Models:

(i) Adenine – Induced Nephropathy[36,37,38,39]

An extensively utilized experimental model for kidney damage in the study of renal disorders in animals, especially rodents, is adenine-induced nephropathy. Adenine-induced nephropathy in rodents can be caused using the following wide summary of materials, reagents, and process.

Materials and Reagents: • Adenine • Animals • Standard rodent chow • Cages and bedding • Drinking water • laboratory equipment • Chemicals and Reagents

Procedure:

The adenine-induced nephropathy model begins with a solution made by dissolving adenine powder in drinking water, typically at concentrations ranging from 0.5% to 0.15%. Animals should be exposed to the laboratory environment for several days. They are subsequently provided access to adenine-containing water for two to four weeks, whereas control groups are given ordinary water. Monitoring entails evaluating changes in behaviour, body weight, and urine production, as well as conducting biochemical analyses on blood and urine samples to evaluate kidney function. After the trial is completed, the animals are euthanized for histological analysis of kidney tissues, which allows for the evaluation of adenine-induced damage and nephropathy research.

Advantages: • Controlled Environment • Easy to administer and requires less surgical intervention • Versatility

Limitations: • Potential Complications • Acute vs. Chronic Response • Potential Complications • Results may not be fully applicable to human renal disease.

(ii) Stz Induced Diabetic Nephropathy[40,41,42,43]

Diabetic nephropathy is a primary cause of end-stage renal disease and one of the most prevalent microvascular consequences of diabetes mellitus. The pathophysiology of diabetic kidney disease in humans is commonly replicated in animals by the use of STZ-induced diabetic nephropathy. The naturally occurring chemical molecule streptozotocin selectively kills pancreatic β-cells, resulting in hyper glycemia and insulin insufficiency, which subsequently exacerbates the development of nephropathy.

Materials and Reagents: • Streptozotocin • Animals • Standard rodent chow • Cages and bedding • Sterile saline • Diabetes monitoring equipment • Laboratory equipment • Chemicals and reagents

Procedure:

The streptozotocin (STZ) model for generating diabetes consists of several important phases. First, STZ is dissolved in sterile saline at a dosage of 50-100 mg/kg for rats and 150-200 mg/kg for mice. Before receiving an intraperitoneal injection of STZ, animals should adapt to the lab environment. Blood glucose levels are subsequently monitored on a regular basis to confirm hyperglycemia, which usually occurs within a few days. Monitoring involves body weight, food and water intake, and biochemical tests of blood and urine samples to determine kidney function. At regular intervals, animals may be sacrificed for histological evaluation of kidney tissues. The data analysis focuses on determining the extent of diabetic kidney damage and comparing outcomes between diabetic and control groups.

Advantages: • Diabetes Induction • Rapid Onset • Well-Characterized • Reproducibility

2. Genetically Modified Models:

Genetically Hypertensive Rats[45,46,47,48]

The genetically hypertensive rat model is used to study chronic kidney disease (CKD), which mimics hypertension-induced renal damage. These rats have sustained elevated blood pressure, which causes glomerular damage, fibrosis, and progressive kidney impairment. This model sheds light on the pathways that link hypertension and CKD, allowing for more effective therapeutic research.

Materials and Reagents: • Devices that measure blood pressure (such as telemetry and tail-cuff systems). • Surgical instruments (for performing procedures including surgery). apparatus for anaesthesia. • Tools for tissue analysis and sampling (such as a microscope and centrifuge). • anaesthetics (such as ketamine/xylazine, isoflurane, etc.). • Sterile saline injection solution. • Anticoagulants (like EDTA) are used in blood sample. • Molecular biology tools for genetic examination. • Physiological solutions (such as phosphate-buffered saline) for tissue preparation or perfusion.

Staining agents for histology, such as haematoxylin and eosin.

Procedure:

The genetically hypertensive rat model consists of multiple procedural steps. Rats are housed in conditions of controlled humidity, temperature, and lighting, with frequent health checks as per institutional requirements. Blood pressure is measured using appropriate techniques, such as telemetry or the tail-cuff method, and recorded at predetermined intervals. Experimental interventions, such as drugs or dietary changes, are applied in accordance with research objectives, and the effects on physiological parameters are monitored. Surgical methods may

be used to collect tissue samples for molecular analysis or histological inspection. Finally, data are evaluated using proper statistical methods to determine differences in blood pressure and other physiological outcomes across experimental groups**.**

Advantages: • Relevance • Consistency • Longitudinal Studies

Limitation • Genetic Background • Cost and Time

3. Induced Renal Injury Model

Renal – Ischemia Reperfusion Model [49,50,51,52]

Acute kidney damage (AKI) is the result of renal ischemia-reperfusion injury, a common consequence in a variety of clinical conditions. The process involves a brief interruption of blood flow followed by its restoration. This cascade of injury includes pathways leading to oxidative stress, inflammation, cell death, and ATP depletion. Its mechanisms need to be investigated in order to create new treatments. This disease is replicated in rodent models, which facilitate mechanistic comprehension, treatment evaluation, and identification of biomarkers. Our research seeks to clarify these processes, assess the effectiveness of interventions, and identify biomarkers. Using a model of rodents, we performed thorough assessments. Our research could help shape focused approaches for managing and preventing AKI, which could lead to better clinical results.

Materials and reagents: • Anaesthetics (such as xylazine and ketamine) • Phosphate-buffered saline (PBS), often known as sterile saline solution • Antibiotics (such as cefazolin) to prevent infections (optional) • For skin preparation, use a solution of benzodine • lubricating eye cream to protect the eyes during surgery • Analgesic medication, such as buprenorphine, to relieve pain following surgery Vascular clips or microvascular clamps

Procedure:

The surgical process for causing renal ischemia begins with preoperative preparation, which includes anesthetizing the animal and keeping its body temperature stable with a heat lamp. the surgical region is sterilized before exposing the kidneys through a midline incision. ischemia is caused by clamping the renal artery and monitoring with kidney blanching, which normally lasts 20-45 minutes. the clamps are then loosened to restore blood flow (reperfusion), and the wound is closed with stitches. analgesics are administered after surgery, and pain is monitored. renal function is measured using blood and urine samples, while histological and molecular investigations of kidney tissues are used to explore injury and healing mechanisms.

Advantages: • Mimics Clinical Conditions • Research Versatility • Rapid Assessment

Limitations: • Surgical Risks • Acute Phase Focus • Variability in Response

4. Diet Induced Models

High Fat Diet Method [53,54,55,56]

The rat high-fat diet (HFD) model is essential for simulating the human metabolic syndrome and diet-induced obesity. This model causes metabolic changes similar to those seen in obese people by feeding mice meals high in fat content compared to normal chow. The pathogenesis of diseases like insulin resistance, dyslipidaemia, inflammation, and cardiovascular disorders has been identified by HFD research. Additionally, it gives researchers a platform to evaluate medications, dietary supplements, and lifestyle changes in the treatment of metabolic disorders brought on by a diet high in fat. Developing effective solutions to address obesity and its related health consequences requires a thorough understanding of the mechanisms behind

HFD-induced metabolic dysfunction. Thus, the HFD model is an essential of preclinical research, providing information that is essential for treating the epidemic of obesity around the world.

Materials and Reagents: • High-fat rat diet: Get a customized rodent feed that has a high fat content (45–60% kcal from fat, on average) • The standard rodent diet: which is used as a control, usually has a lower fat content • Cages for animals: Give rats suitable habitat, making sure they have enough room and ventilation • Rats' food is delivered to them via feeding hoppers • Make sure there is always access to water by using water bottles or automated watering devices • Animal scale: Required to track changes in body weight during the duration of the study • Sterile water • PBS: For use in handling and cleaning

Procedure:

To prepare for a high-fat diet (HFD) study, procure a high-fat rat food and store it properly. If the diet is powdered, mix it with sterile water. Allow rats to adjust in their new surroundings for at least one week. Assign groups, with the experimental group having constant access to HFD and the controls receiving standard chow. Regularly examine your body weight, dietary intake, and overall condition. The HFD intervention can last from weeks to months, with optional assessments such lipid profile, body composition, metabolic evaluations, glucose tolerance testing, and tissue histology performed as needed.

Advantages: • Relevance to Human Conditions • Allows for the investigation of the chronic impacts of obesity and metabolic syndrome over long durations.

Limitation: • Nutritional Factors • Species Differences

Conclusion

In vitro and in vivo chronic kidney disease (CKD) models are essential for improving our understanding of disease mechanisms, drug development, and possible therapeutics. In vitro models, such as cell culture systems and renal organoids, provide controlled conditions for investigating CKD processes and medication effects, although they are limited in complexity and predictability. In vivo models, primarily based on rats, accurately imitate CKD symptoms and systemic effects, enabling extensive investigation. They encounter hurdles, such as species variations and surgical risks. Together, these models provide important insights into CKD pathogenesis and therapeutic choices.

Acknowledgements.
The authors would like to express their heartfelt gratitude to the administration and staff of Acharya & BM Reddy College of Pharmacy for their continuous support and encouragement throughout the process.

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