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UNLOCKING THE POTENTIAL OF ZEBRAFISH: A COMPREHENSIVE GUIDE TO ACUTE KIDNEY DISEASE RESEARCH

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Abstract: Zebrafish serves as a powerful tool to understand Acute Kidney Disease (AKD). Zebrafish models have been used to mimic clinical manifestations of renal failure, but they lack in specific structures for understanding the overall development. The development of the renal system, the genes related to the progression of AKI and its recovery in zebrafish have been mentioned. HNF-1 also known as hepatocyte nuclear factor and ATF3 or Activating Transcription Factor has also been considered a potential therapeutic target. Zebrafish have a pair of ambiguous genes namely, wt1a and wt1b for regeneration of nephrons. Various drugs such as nonsteroidal anti-inflammatory medicines, angiotensin converting enzyme inhibitors and aminoglycoside antibiotics have been discussed upon as models for AKD in zebrafish. Various biomarkers of kidney disease such as Neutrophil gelatinase-associated lipocalin (NGAL) have been briefly mentioned. A review based on developmental stages of AKD followed by genes responsible for regeneration of kidney in zebrafish has been developed with special focus on common zebrafish models and its biomarkers. Zebrafish is a potential experimental model which can be extensively used to study human inherited diseases due to its fecundity, rapid development, optical transparency, and accessibility to genetic manipulation tools.

Index terms: **zebrafish, acute kidney injury, regeneration, HNF1, AFT3, NGAL**

1. Introduction

The prime role of the renal system is to filter body waste, accompanied with regulation of body fluid and maintenance of normal blood pressure.[1](Kamei 2015) Acute kidney injury (AKI) formerly known as acute renal failure is marked by elevated levels of creatinine along with fall in urine volume leading to unanticipated yet reversible waning of kidney function.(Goyal 2019) Histological changes characterised by cessation, programmed cell death or necroptosis are prominent indicators for early stages of AKI.(Zhang 2019) Standardized measures have been introduced afresh to enable consistent evaluation of the severity of AKI and its effects on early necrosis in survivors.(Ferenbach 2016) Uncomplicated visual display of embryos in the first five days post fertilization in zebrafish can show mutations in genes needed for development of important organ systems such as the nervous system, cardiovascular system, circulatory system, renal system.(Amsterdam 1999) Even though there is a universal rise in AKI and Chronic kidney disease (CKD) which is correlated with fatality, clinical diagnosis and therapeutic interpositions are underdeveloped. (Bao 2018) The scientific community is still concerned about the translational gap between the practical implementation of experimental therapeutics for AKI in the clinical context. Experienced teams of researchers in a variety of model systems must work closely with clinical researchers to build trustworthy preclinical evidence to support more targeted therapies in patients with AKI. (Hukriede 2022)

Understanding the pathophysiology underlying the onset of acute renal failure has greatly benefited from the creation of many animal models such as rodents. They mimic the clinical manifestations of several aetiologies related to renal failure. (Singh 2012) Zebrafish (*Danio rerio*) has proved itself as a tiny, multifaceted vertebrate organism. It's genetic accessibility allows modelling of human genetic illnesses and the analysis of underlying cellular networks and physiological systems.(Gehrig 2018) Adult fish can develop new nephrons throughout their lives, unlike mammals, to promote their growth and in response to injury. The zebrafish pronephros has also served as a valuable model for differentiation of nephrogenic mesoderm and kidney cell types, along with patterning of nephrons, illnesses affecting size of tubular lumen and related factors. (Drummond 2010)

Despite extensive research there is absence of specific models for understanding the progress of acute kidney disease. The present review throws light upon the progression of acute kidney disease in zebrafish by correlating it with existing models related to induction of kidney disease in zebrafish. This review article also looks upon some of the prevalent gene expressions that lead to the pathogenesis of AKI, methods of setting up models for AKI, as well as the regeneration of the renal system in zebrafish.

2. Aetiology of Acute Kidney Disease in human

2.1. Introduction:

Filtration of nitrogenous waste and its excretion from blood are the crucial tasks performed by the kidney. Intrinsic renal disorders like acute tubular necrosis and interstitial nephritis affects the glomerulus. This in turn releases vasoconstrictors from the afferent

pathways of the renal system, prolonged renal ischemia, sepsis, and nephrotoxins being some of them. Prolonged exposure of these may induce cellular damage and prerenal injury that can further develop into renal injury.(Thodani 1996) Injuries to the renal tubule such as ischemic shock, toxic aggregation, or obstruction of tubulointerstitial processes with inflammation and edema, or a primary decrease in the glomerular filtering ability can all lead to acute renal failure.

2.2. Prerenal Failure:

Prerenal failure or prerenal azotaemia, is a condition in which renal glomerular function stays unaffected but filtration is constrained due to factors affecting renal perfusion. Additionally, volume responsive and volume nonresponsive prerenal azotaemia have been distinguished. The former is simple to understand, while the latter is more complicated. Additionally intravenous volume has insignificant effect on restoring kidney perfusion and function in volume-unresponsive types.(Molitoris 2022) Postrenal failure, also known as postrenal azotaemia, is the medical term for renal insufficiency caused by obstruction of the urine outflow system. Effects of prerenal failure and intrinsic renal failure caused by ischemia and nephrotoxins mostly leads to episodes of acute renal failure.

2.3. Elevated parameters of kidney function:

Reduced ability to remove waste products from the body and impaired renal function are certain indications of elevated blood urea nitrogen (BUN) and creatinine levels.(Basile 2012) According to recent studies, the deterioration of kidney function can be reasonably anticipated by baseline glomerular filtration rate (GFR), albuminuria, and blood biochemical indicators. (Gluck 2019) The decline in GFR is caused by several processes. In Fig.1, the possible changes related to reduction in GFR are represented. Inflammation due to ischemia could possibly produce an extreme vasoconstriction, caused by endothelial damage and tubule-glomerular feedback indirectly reduces glomerular filtration directly. (Khalil 2008)

[Fig 1 belongs here]

2.4. Renal obstruction:

Most post-renal causes are a result of obstructive reasons such as filtration system congestion or a change in the filtration driving forces. The most frequent ones include blood clots, tumours of the kidney or ureter, and any obstruction of the urethra. A solitary obstruction may not certainly manifest as AKI, particularly if the barrier develops gradually. For example, in the case of a tumour, a normally functioning kidney can take over the function of the damaged kidney. Therefore, bladder outlet inhibition is the most frequent cause of post-renal AKI.(Goyal)

3. Structure of kidney in zebrafish

Zebrafish serves as an outstanding model for developmental genetics in vertebrates with similarities in nephron organization and physiology with mammals. The embryo of zebrafish develops a simple pronephros with two nephrons, serving as a platform upon which a mesonephros with hundreds of nephrons are built throughout the larval stages.(Gerlach 2013) A secured system of blood filtration, tubular absorption, and fluid discharge is formed by the pronephric nephrons of zebrafish. The kidney in zebrafish (*Danio rerio*) is a compressed organ, situated along the dorsal inner body wall. Zebrafish nephrons present in the head, trunk and tail are physically distinct from one another.(Bates 2019) All kidney types follow comparable developmental processes. (Ref. Table 1)

[Table 1 belongs here]

3.1. Embryonic progression:

During first two days of embryonic growth, a cascade of changes occurs leading to pronephric kidney development, followed by successive inclusion of three predominant components namely, the pronephric ducts, tubules and the glomerulus. The overall development of the pronephric ducts occurs within 24 hours post fertilization (hpf). (Kimmel 1990) From the second day after fertilization, the pronephros begins to regulate the embryo's fluid and solute homeostasis. Plenty of molecular pathways involved in kidney maturation and homeostasis along with renal vascular morphogenesis have been found to be highly conserved. (Simic 2013)

In Fig.2, a brief comparison between human and zebrafish nephron has been illustrated. The zebrafish pronephros is an elongated version of the mammalian nephron, comprising of a glomerulus equipped with podocytes, two proximal and distal tubules. The expression of recognized signalling molecules and transcription factors in the developing pronephros is controlled by entry points of pathways that essentially regulate early pronephric development. (Kim 1998) Major nephron component and cell types are retained in zebrafish at the cellular level. The structure of the podocytes in the glomerulus of the zebrafish and mammals are very comparable. The regular perfusion for the pronephric glomerulus provides evidence that podocytes take measure to organize vessel ingrowth.(Carmeleit 1996)

[Fig 2 belongs here]

3.2. Cilio genesis:

Motile cilia are present in the pronephric duct and tubule of zebrafish, in contrast to the cilia observed on the epithelial cells of kidney in mammals. Although the precise function of the gene in vertebrate embryos is still unknown, research related to studies of polycstin1 and polycystin2, state them to be responsible for autosomal dominant polycystic kidney disease (PKD).(Nauli 2003) Observations in research specify cilia in the zebrafish pronephros to be motile with ‘‘9+2’’ microtubule doublet arrangement i.e., a typical characteristic of motile cilia and flagella. A disruption in cilia structure or motility resulted in the formation of pronephric cysts with left-right asymmetry defects.(Hildebrandt 2007) Similarly malformed or immobile cilia can be linked with its inability to transport fluid or particles in the lumen of the pronephric ducts hence, accounting for fluid aggregation in the

anterior segments of the pronephros. One of the major differences between the zebrafish and mammalian nephron is the absence of the Henle loop in zebrafish, which serves as a counter current multiplier to create the medullary osmotic gradient for water conservation in mammals (Ref. Table 2). This is because zebrafish are freshwater fishes, making this segment useless for them.(Fatma 2021)

[Table 2 belongs here]

4. Development of Disease

Renal ischemia reperfusion (IR), sepsis and nephrotoxins are some of the general causes of AKI. Sublethal and lethal injury of renal tubular epithelial cells is promoted by AKI pathologically. Prior CKD serves as a crucial risk factor for AKI progression; patients with pre-existing CKD recover partially but often encounter aggravated subsequent renal deterioration; and patients with de novo AKI are more prone to be affected by proteinuria, elevated risks towards cardiovascular diseases, and progressive CKD. (Shu 2019)

Renal biopsy studies are often used to understand the acute phase of the disease. Endothelial injury occurs initially accompanied with vasoconstriction which results in decreased oxygen supply. Platelet aggregation is promoted through ligand expression, inflammatory factors such as monocytes and neutrophils deposit complement through alternative pathways. Tubular dysfunction, oliguria and decreased filtration rate of glomerulus via tubulo-glomerular feedback is a result of tubular injury and necrosis. These are the other significant changes following altered oxygen availability.(Ferenbach 2016)

The term CAKUT (congenital anomalies of the kidney and urinary tract) is referred to an extended range of structural anomalies related to the kidney such as obstruction of the ureteropelvic junction, vesicoureteral reflux kidney agenesis and dysplasia. The most recurring genetic causes of CAKUT at present time are mutations in pax2 and hnf-1. Insights have been gained from studying pax2 and hnf1 mutant zebrafish, two genes that encode impersonation factors necessary for the regional determination of nephrons. (Jerman 2017)

4.1. Expression of genes

4.1.1. Pax gene:

Embryonic spinal cord and the midbrain-hindbrain barrier of rodents exhibit spatial and temporal overlaps in the Pax2/5/8 (Paired box) gene expression. As in mammals, two Pax genes are expressed during kidney morphogenesis of zebrafish. Pax2 and Pax8 expression outside of the central nervous system overlaps in the developing excretory system partially. Pax2a expression is constrained to the neck area and anterior pronephric tubule of zebrafish, Na+/K+ ATPase signal being absent in the former regions. The expression of wt1a expands from the glomerular region to the neck region, guiding to a patterning issue. (Pfeffer 1998)

4.1.2. HNF-1 gene:

HNF1b controls a number of genes affecting the aetiology of renal cystic disease along with those involved in nephrogenesis itself.(Verhave 2016) Results based on the unavailability of overt diabetes suggest that alterations in HNF-1 gene may be discovered in a patient in due course, especially in children suffering from cystic kidney disease and chronic renal failure with pelvicalyceal anomalies. (Mache 2002) The Hippo signalling pathway on the other hand has been linked to the formation of zebrafish renal cysts. However, earlier research related to renal cyst formation was patchy and chiefly depended on morpholino-mediated gene knockdown. More research based on gene knockout is necessary to obtain promising evidence.(Ren 2021) The pro-viral insertion in a gene proves to be resembling the vHnf1 gene in humans, which is the cause of the zebrafish mutants hi548, hi1843, and hi2169, resembles a single complementation group. The transactivation domain, which distinguishes Hnf1 from vHnf1, is included in the sequence that is homologous to both the fish and the human genes. Additionally, zebrafish genes have similar geographic and temporal pattern of expression like vHnf1 gene, proving it to be a homolog of the vHnf1 gene. (Sun 2001)

4.1.3. AFT-3 gene:

In the cell line HK-2 of human renal proximal tubule, doxorubicin-induced cytotoxicity was mediated by ATF3. Higher levels of ATF3 is regarded as a preliminary diagnostic indicator for AKI. (Kato 2020) However, since many non-neuronal cells and tissues expresses ATF3 constitutively and plays numerous roles in the body, it should be considered as a potential therapeutic target. (Katz 2022) A potential treatment for kidney disease involves preventing ferroptosis-related fibrosis by inhibiting the Smad3/ATF3/SLC7A11 pathway. (Zhu 2020) Another study further explained ATF3 induction and its beneficial effects on promoting axonal regrowth as a feasible method for increasing neural regeneration in the nervous system of vertebrates. In the zebrafish model, the up regulation of ATF3 may also contribute to the survival of motor neurons and then enhance spinal cord regrowth. In contrast, it was proved that ATF3 knockdown prevented axon regeneration after zebrafish spinal cord injury.(Wang 2017)

4.1.4. c-Fos/AP-1:

Since treatment of a particular c-Fos/AP-1 inhibitor reduced renal inflammation in an AKI mouse model by endotoxin-induction, it is thought that c-Fos is linked to kidney injury. The zebrafish kidneys these genomic biomarker candidates presented characteristic increase in expression along with histological abnormalities, and vice versa in the absence of nephrotoxicity throughout the body. By direct attachment to AP-1 motifs present in the promoters of these genes, c-Fos/AP-1 regulates the inflammatory cytokines expressions such as TNF by adhering to these genes.(Ishida 2015) In an investigation, specific c-Fos/AP-

1 inhibitor, T-5224 exhibited dose-dependently inhibition of LPS-induced TNF-production. Inhibition of TNF is regarded as a powerful therapy for septic AKI. (Miyazaki 2012)

4.2. Neo-nephrogenesis

Regeneration of tubular epithelium and neo-nephrogenesis are a set of procedures that occur and imbricate throughout the regeneration time course, according to research related to regeneration and repair in zebrafish following AKI. (McCampbell 2014) Neo-nephrogenesis is the process by which adult zebrafish with AKI simultaneously produce new functioning nephrons, making them different from humans and other mammals in this regard is representative of several other vertebrate fishes such as goldfish, skate, and medaka. In neo-nephrogenesis, basophilic interstitial cell aggregates, develop, multiply, and transform into functional nephrons between 2-3 weeks following damage,based on activity wt1b and lhx1a promoters following AKI.(Poureetezadi 2016)

4.2.1. Wt1a/b:

The teleostean model has been used to research genetic abnormalities of the kidney and adrenals because of the discovery of wt1 and sf-1 zebrafish counterparts. Zebrafish consists of two related wt1 genes namely, wt1a and wt1b, which are both necessary for kidney development. While early pronephric glomerular formation is influenced by wt1a, the final kidney's nephrogenesis is influenced by wt1b. (Chou 2017) Wt1a and wt1b serve distinct roles in the formation of the zebrafish pronephros, based on evidences that knockdown of both wt1 genes led to abnormalities that were severe compared to either case alone.(Perner 2016) In fact, it was discovered that a wt1 homolog exclusive to zebrafish, wt1b, exhibits a distinctive pattern in the development and regeneration of nephrons, serving as a useful indicator to monitor maturation of mesonephric patterns and regeneration in vivo. It also helps to segregate and define the specification and regulation of the presumed renal progenitor population inside the mesonephros region of the zebrafish. (Zhou 2010)

4.3. Renal ciliopathy

Recent research says that primary cilia abnormalities may also contribute to the development of renal cystic kidneys. The Hedgehog (Hh), Wnt, platelet-derived growth factor receptor (PDGFR), and mechano-signalling associated with the PKD1/2 complex are only a few of the intracellular signalling pathways that are regulated by primary cilia. (Ko 2013) Pkd1 and Pkd2 mutation-based PKD models in zebrafish have been developed resulting in cystic kidney disease. Col2a1 collagen knockdown partially reversed this phenotype. Zebrafish not only serves as a useful model for discovering novel genes responsible for the emergence of cystic kidney disease, but has also resulted in the discovery of ideal genes involved in cilio-genesis. (Corkins 2021)

4.4. Hypoxia inducible factors in kidney injury

In a variety of organisms, HIF plays a pivotal role in maintaining oxygen homeostasis and regulating responses to hypoxia. (Jaakkola 2001) HIF is a protein heterodimer made up of the constitutively produced subunit HIF out of the various available inducible subunits (HIF-1, HIF-2, or HIF-3). Specific proteins with prolyl hydroxylase domains hydroxylate two essential prolyl residues of HIF during hypoxia (PHDs). The abundance of mutations in the PHD-VHL-HIF pathway highlights its critical function in erythropoiesis and other physiological systems intended to keep oxygen homeostasis. (Semenza 2011)

5. Drugs involved in Acute Kidney Disease

5.1. NSAIDS induced injury

Acute renal failure is a condition that some patients develop after taking nonsteroidal anti-inflammatory medicines. (Davidman 1991) In a study it was hypothesized that the incidence of major renal issues associated with the use of these medications was significantly higher than is generally thought. (Shankel 1992) In a meta-analysis, traditional NSAID users had an enhanced AKI risk that was statistically significant. The pooled risk ratios, which ranged from 1.58 to 2.11, were rather stable among all classical NSAIDs. (Ungprasert 2015) Another study offered some evidence that the risk of AKI varies among the various NSAIDs. NSAIDs that are more selective may have a higher safety profile in this area. Most patients suffering from CKD, diabetes, exposure to contrast media, or those on nephrotoxic medications (ACEi /ARBs) or diuretics should always take NSAIDs with caution. (Lafrance 2009)

5.2. ACE inhibitor induced injury

Angiotensin-converting enzyme inhibitors serves as the primary cause of acute renal failure in a small population of patients, which can be altered after stopping the medication, in situations where glomerular filtration is supremely dependent on efferent vascular tone conciliated by angiotensin II.(Navis 1996)The main reason of this underutilization of ACE inhibitors in patients with heart failure is due to the pre-existing renal insufficiency or the elevated blood creatinine level following the initiation of ACE inhibitor therapy . (Ahmed 2002) Additionally, this effect is more evident in people who already have renal insufficiency. (Bakris 2000)

5.3. Aminoglycoside antibiotics induced injury

The most effective treatments for serious infections are aminoglycosides. Even in older patients, these medications might be prescribed since, the urgency of treating a severe illness overrides any concern about kidney damage. A significant side effect of aminoglycoside antibiotics is nephrotoxicity. Typically, five to seven days into treatment, the clinical picture is one of non-oliguric acute renal failure with a minor to moderate decline in creatinine clearance.(Moore 1984) Inflicting gentamicin into zebrafish produces a potent, synchronous response of regeneration, resulting in production of many new nephrons and subsequent progression through stages of development, augmentation, and differentiation of the kidney (Ref. Fig.3). The natural history, host, and pharmacological variables that influence the onset of gentamicin and amikacin induced nephrotoxicity are better understood because of a study related to nephrotoxicity associated with aminoglycoside therapy. (Smith 1978)

[Fig 3 belongs here]

6. Kidney Regeneration

Tubular epithelium proliferates and undergoes dedifferentiation following kidney damage in mammals. It has been shown that certain fish species, including medaka and zebrafish, have an amazing ability for regeneration after suffering an acute kidney injury. Adult fish kidneys keep and repair their functions in a different way than kidney regeneration in mammals. Zebrafish as a model contributes towards understanding mammalian renal regeneration and how it could be therapeutically triggered since they constantly add nephrons and regenerate them from scratch following injury. (Diep 2011)

6.1. Wnt signalling

An investigation reported use of genetic and pharmacological processes for testing of signalling route induced by kidney injury. Wnt signalling also proved to play significant role in multiple functions in zebrafish precursor mediated regeneration of kidney. The development of novel nephron cell clusters with high authorised Wnt reporter activity, characterised by fzd9b-, lef1-, and lhx1a positivity, was preceded by Wnt9b expression present in injury induced kidney distal tubules. As a vital step in the creation of new tubules, standard Wnt signalling was considered necessary for cell proliferation.(Kamei 2019)

6.2. Omi/HtrA2 protease inhibitors

The apoptosis-related protease acts as a selective inhibitor of Omi/HtrA2, has been indicated to be effective in treating nephrotoxicity induced by gentamicin, cisplatin and AKI in larval zebrafish.(Sanz 2013) Renal failure was averted, and survival was improved by a selective inhibitor of Omi/HtrA2, a serine protease involved in apoptosis induced by cisplatin. This shielding effect was shown in a gentamicin induced nephrotoxic mouse model. As a result, zebrafish offers a distinctive model system that can be used for drug testing and genetic alteration.(Hentschel 2005)

7. Biomarkers for Kidney Disease

7.1. NGAL

Novel early AKI markers are required to enhance AKI avoidance, identification, treatment, and prognosis prediction. Recent data show that NGAL and AKI are closely related. A plenty of experiments and clinical investigations have shown that AKI greatly enhances the expression of urine and serum NGAL.(Shang 2017) Early progenitor cells in the metanephric mesenchyme responded to pure NGAL by proliferating. This was followed by epithelial differentiation of these cells and the formation of nephron like structures that expressed glomerular, proximal, and distal tubular surface cellular markers. (Soni 2010)

7.2. KIM-1

As a distinguishable marker of proximal tubular injury, with characteristic proteinuria and ischemic renal disorders, KIM-1 has been developed. In several animal models used in kidney illness, including models of injury caused by ischemia and different nephrotoxins, KIM-1 serves as a very sensitive and specific marker of kidney injury. After AKI, KIM-1 is the protein responsible for extensive expression in the proximal tubules. It is a phosphatidylserine receptor that helps renal PTCs phagocytose apoptotic cells and oxidised lipids.(Yang 2015)

8. Benefits of Zebrafish model

The zebrafish (*Danio rerio*) can be utilised as a versatile, minute vertebrate organism widely used to study diseases in humans. (Gehrig 2018) In order to unravel the secrets of kidney development and diseases in human, zebrafish has turned out to be a powerful experimental model of utmost amenability.(Naylor 2017) For example, *ex-vivo* development of zebrafish and *in-vivo* study of anatomical, cellular, and molecular events due to its transparency.(Pickart 2014) The comparison of the zebrafish reference genome to that of human reveals over 70% of human genes to have at least a single clear zebrafish orthologue. The excellent quality of the genome assembly offers a greater knowledge of important genomic characteristics like distinctive palindromic content, a lack of pseudogenes, a plethora of genes specific to zebrafish on chromosome 4 and chromosomal areas that affect sex identification.(CF 2013) The larval model gains significant advantages such as the zebrafish's fecundity, rapid development, optical transparency, and accessibility to a wide range of genetic manipulation instruments. It also demands less time and effort than traditional models.(Wen 2018) Even on the vast scale needed for genetic screening, whole-mount immunohistochemistry and RNA in situ hybridization are practical due to the tiny size of the zebrafish embryo and early larvae. Many aspects related to vertebrate embryogenesis, such as the neural crest or the notochord and organogenesis lack in any obvious counterparts in invertebrates, and a comprehensive molecular understanding of vertebrate development require an amalgamation of genetic approaches, molecular characterization, and experimental analysis in vertebrate model systems such as the zebrafish. (Driever 1996)

The transparent embryo of the zebrafish, along with the rapid development of adult kidney (by 4 days after conception), make it extremely convenient to investigate. (Sander 2014) Large kidney cysts can be seen under a dissecting scope without the need for staining since they are transparent. Due of the genetic closeness of humans to zebrafish, we may examine the effects of mutations in certain genes that serves a role in renal physiology and better understand how the same gene affects humans. (Outtandy 2019) Using genetic screening with zebrafish, many genes that were initially thought to be unrelated to kidney conditions have now been correlated. (Morales 2017)

9. Conclusion

A complete in depth understanding of the process of origin of Acute kidney injury is yet unknown. Zebra fish is presently gaining significance in studies related to human diseases. This review tried to highlight the effectiveness of zebrafish model to evaluate acute kidney disease. We mainly tried to focus upon various genes related to zebrafish involved in acute kidney disease and their

effect on the expression in the kidney. Progress of kidney injury in adult zebrafish has been developed at various stages of kidney development. Various methods responsible for the induction of AKI were also discussed along with possible biomarkers for kidney disease.

The above-mentioned fields of investigation must be further looked upon with the purpose of setting up zebrafish as a more prominent model in kidney related diseases in humans. The genetic validations used must be established along with better methods of estimation. Available routes of drug administration must also be looked upon. Moreover, the rapid absorption of drugs through skin and gills based on surface area can affect the drug absorption. However other routes such as intraperitoneal injection can avoid these variations. Comparative studies of zebrafish with other animal models and humans can help in proper study related to the pharmacokinetic and pharmacodynamics of drugs necessary for the therapy and cure of acute kidney injury. Based on the review it can be concluded that in spite of certain drawbacks, zebrafish can be used widely in understanding the buildout and advancement of acute kidney disease.

Authorship:

Ishani Chowdhury: Data curation, Writing- Original draft preparation, Visualization. **Surendra Vada:** Conceptualization, Supervision. **Febisha A:** Visualization, Writing-Reviewing & Editing. **Sushree Swaraj:** Writing- Reviewing & Editing. **Manjunatha PM:** Supervision

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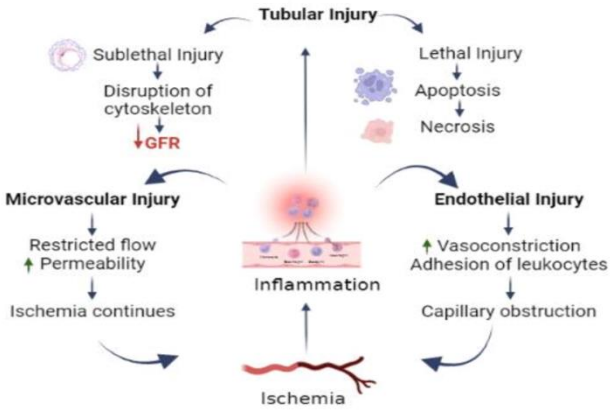

Figure 1: Sustained decline in GFR (Glomerular Filtration Rate) due to tubular and vascular injury in extended AKI (Acute Kidney Injury)

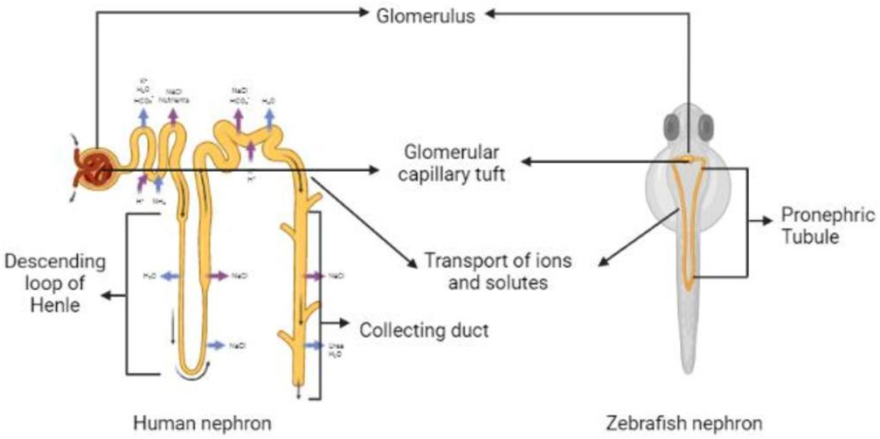

Figure 2: The differential functional characteristics between the vertebrate nephron and the zebrafish pronephric nephron

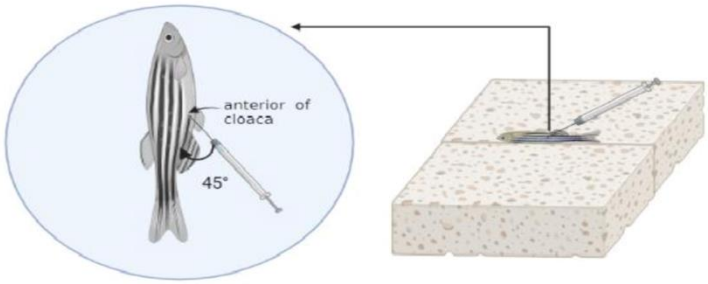

Figure 3: Intraperitoneal administration of Gentamicin injection in adult zebrafish

Table 1: Developmental process of the kidney in zebrafish

Stages of Development of kidney	
A	intermediate mesoderm spurring to initiate renal primordium
B	resurfacing of epithelium and development of nephric duct
C	ornamentation of nephron into unique segments
D	vascularization of nephron for the purpose of filtration of blood

Table 2: The kidney types in humans and zebrafish

Forms of kidney	Human	Zebrafish
Pronephros	6 to 10 pair of tubules develop on the 22nd day of gestation and end by the 4th week.	A solitary pair of tubules whose development is complete and functional by 48 hpf*.
Mesonephros	30–40 mesonephric tubules formation starts from the 25th day, and continues till the 8th week of pregnancy, and degenerate gradually after that.	300 tubules begin to develop at 10 days post-fertilization, become functional at 14 days post-fertilization, and continue to mature throughout the rest of development.
Metanephros	Nephrogenesis, which produces between 300,000 and 1 million nephrons, develop from the fifth week of pregnancy and is complete by the 36th week.	No formation

*hpf: hours post fertilisation

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