University of Kerbala

College of Applied Medical Sciences

Department of Clinical Laboratories

Evaluation of the Levels of Neutrophil Gelatinase-Associated Lipocalin NGAL, Kidney Injury Molecule-1, and Cystatin-C as Early Predicting Complications of Acute Kidney Injury (AKI) in Male Pediatric in Kerbala Province

A thesis

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Dedication

Thanks to Almighty **"Allah"** always and forever for his gifts.

To **" my Mom"** For her love and support, To my protective angel **"my Dad"**

To a pure soul, friend, and supporter in difficult times, for his help and encouragement **"My husband".**To my little prince **"my son Redha"**

To my **" sisters, brother"** who waited for my graduation with passion.

To my supportive **"friends "**who surround me in difficult times.

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I'm doing this humble work

"Halah R. Al-Sabah"

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Summary

Acute kidney injury (AKI) is defined by a rapid increase in serum creatinine, a decrease in urine output, and a rapid decline in glomerular filtration rate (GFR).

The present study included (120) individuals of pediatric males with a mean of age (5.94±4.9) years patients collected from Karbala Children's Teaching Hospital during the period from September 2023 to February 2024 divided into two groups (30) intact males used as control and (90) male patients with AKI then categories to three subdivision according to the stages of AKI disease as to Risk, Injury, Failure, Loss End-stage (RIFLE) criteria as follow: stage (1),(2), and (3) include (32), (27), and (31) respactively. Then estimation of the serum concentration of these biochemical markers. The present result found a significant difference at P≤0.001 in increased KIM-1, NGAL, and Cystatin C (1533.36±404.21), (5.69±1.23), (12.50±2.88) respectively as compared to control (716.64±50.72), (2.30±0.28), and (5.32±1.71) respectively in all stages used in the study. The result found a significant difference at P≤0.001 in the age of patients—the younger age found in stage (3) (4.31±4.03) years. Also, BMI showed a high decrease in all patients (14.45±1.9) compared to control. Creatinine, Urea, and Blood Urea Nitrogen (BUN) revealed a significant increase at P≤0.001 (1.61±1.81),(104.38±65.27), and (16.24±10.15) respectively than control (0.42±0.13),(20.61±5.13), and (3.21±0.80) respectively, while significant decrease at P≤0.001 in urine output for all stages compared to control, the higher decline found in stage(3) (0.14±0.09) as compared to control (91.56±0.46), a negative correlation found between KIM-1 and Hemoglobin (Hb), Albumin, and urine output (-0.295),(-0.458),

and (-0.629) respectively and positive correlation between KIM-1 with C Reactive Protien (CRP), urea, creatinine, and BUN (0.279),(0.366),(0.236), and (0.366) respectively, a negative correlation found between NGAL and Hb, Red Blood Cells (RBC), Albumin, and urine output (-0.315),(-0.166),(-0.508), and (-0.727) respectively and positive correlation between NGAL with CRP, urea, creatinine, and BUN (0.312),(0.456),(0.231), and (0.455) respectively, a negative correlation found between Cystatin C and Hb, Albumin, and urine output (-0.299),(-0.453), and (-0.675) respectively and positive correlation between Cystatin C with CRP , urea, creatinine, and BUN (0.278),(0.366),(0.214), and (0.366) respectively.

The study finding eGFR by sCr, GFR by sCr & Cyc C, and GFR by Cys C revealed a significant decrease at $P \leq 0.001$ (49.60±27.64), (20.68±7.85), and (7.32±2.84) respectively in all patients as compared to control (109.71±13.06), (50.30±7.73), and (16.30±5.07) respectively, and the highest decline for GFR found in Cystatin C for all stages. In conclusion, the present study showed biomarkers containing KIM-1, NGAL, and Cystatin C had the best predictive assurance for AKI. The study revealed that Cystatin C-based GFR reflects a decline in GFR with worsening AKI better than creatinine-based GFR which means a better marker of renal function in early-stage AKI.

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List of Abbreviations

Abbreviation	Fill name
ACE	**Angiotensin-Converting Enzyme**
ADQI	**Acute Dialysis Quality Initiative**
AKD	**Acute Kidney Disease**
AKI	**Acute Kidney Injury**
AKIN	**The Acute Kidney Injury Network**
ARS	**Artificial Renal Support**
AT2	**Angiotincin II Type 2**
BCG	**Bromo Cresol Green**
BMI	**Body Mass Index**
BUN	**Blood Urea Nitrogen**

CBC	**Complete Blood Count**
CDC	**Centers for Disease Control and Prevention**
CKD	**Chronic Kidney Disease**
Cl	**Chloride**
CR\KRT	**Continuous Renal\Kidney Replacement Therapy**
CRP	**C Reactive Protein**
Cys C	**Cystatin C**
EDTA tube	**Ethylene Diamine Tetraacetic Acid tube**
eGFR	**estimated Glomerular Filturation Rate**
ELISA	**The Enzyme-Linked Immunosorbent Assay**
ESRD	**End Stage Renal Disease**
GFR	**Glomerular Filturation Rate**
GLDH	**Glutamate Dehydrogenase**
HAVCR1	**Hepatitis A Virus Cellular Receptor 1**
Hb	**Hemoglobin**
HCT	**Hematocrit**
HD	**Hemodialysis**
HEPES buffer	**4-Hydroxyethylpiperazineethanesulfonic Acid**
ICU	**Intensive Care Units**
K	**Potassium**
KD	**Kidney Disease**
KDIGO	**The Kidney Disease Global Outcomes**
KIM-1	**Kidney Injury Molecule -1**
KRT\RRT	**Kidney\Renal Replacement Therapy**
MMP-9	**Matrix Metalloproteinase-9**

Na	**Sodium**
NADH	**Nicotinamide Adenine Dinucleotide+Hydrogen**
NGAL	**Neutrophil Gelatinase-Associated Lipocalin**
NICU	**Neonatal Intensive Care Units**
NSAID	**Non-Steroidal Anti-Inflammatory Drugs**
OD	**Optical Density**
PD	**Peritoneal Dialysis**
PICU	**Pediatric Intensive Care Units**
PLT	**Platelet**
RAAS	**Renin -Angiotensin-Aldosterone System**
RBC	**Red Blood Cells**
RFT	**Renal Function Tests**
RIFLE	**Risk, Injury, Failure, Loss, End-stage Kidney Disease**
ROS	**Reactive Oxygen Species**
sCr	**Serum Creatinine**
TIM-1	**T-cell Immunoglobulin and Mucin domain-1**
TRIS buffer	**Tris hydroxymethyl Aminomethane**
TWBC	**Total White Blood Cells**
Ungal	**Urinary Neutrophil Gelatinase-Associated Lipocalin**
WBC	**White Blood Cells**

Chapter One:

Introduction

Kidney injury (KD) can be divided into two types acute kidney injury (AKI) (cases that develop rapidly) and chronic kidney disease (CKD) (those that are long-term) (Salim *et al*.,2022).

Acute kidney injury (AKI) is characterized by a sudden decline in the kidney's ability to filter waste products, leading to an increase in waste substances such as urea and creatinine in the bloodstream with or without changes in urine output , it has been referred to as Acute Kidney Failure (AKF) in the past (Kondabolu *et al*., 2023).

(AKI) remains a common and significant sign problem in the last decade, between 5% and 20% of critically ill patients in the intensive care unit (ICU) have an episode of AKI, with acute tubular necrosis (ATN) accounting for about 75% of cases (Khairul *et al*., 2023).

The most common causes of AKI are septic shock, ischemia, and nephrotoxins. AKI has been conceptually destitute as a rapid decline in glomerular glomerular rate (GFR) that occurs over hours and days. It propels to a clinical syndrome characterized by a rapid decrease in renal excretory function, with the accumulation of products of nitrogen metabolism such as creatinine and urea clinically unmeasured waste products. The AKI time framework is 7 days (Kishore *et al*., 2023).

Early detection of AKI biomarkers can significantly reduce renal damage, prevent the progression of Chronic Kidney Disease (CKD), as well as to reduce medical costs and time (Luft, 2021). The current AKI definitions are based on two basic criteria: changes in serum creatinine and how much urine is made, according to that AKI includes three subdivision:

prerenal, renal, and postrenal events. Serum creatinine and blood urea used in conjunction with urine output have been the traditional means of diagnosing and managing AKI and are measured by glomerular filtration rate (GFR) (Freiz *et al*., 2020).

Kidney Injury Molecule-1 (KIM-1) is a type 1 transmembrane glycoprotein. It has two extracellular domains and is most expressed on T-cell surfaces. Its expression is minimal in healthy kidneys but markedly increases succeeding kidney injury in proximal tubular cells (Jana *et al*.,2023). The reason is that the ectodomain of KIM-1 undergoes cleavage and can be detected in blood and urine (Anandkumar *et al*., 2023).

Human Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a glycoprotein mainly secreted from epithelial cells, kidney tubular cells, hepatocytes, activated neutrophils, and pathological conditions like inflammation (Yousef *et al.,*2023), both blood and urinary NGAL respond to the damage that occurs in the renal tubular, it's filtered through the membrane of glomerular renal corpuscles mainly by endocytosis in the proximal tubule, the role of NGAL in renal damage response is believed to be protective (Huang *et al*., 2022).

Cystatin C is a protein from the group of cysteine protease inhibitors. It is produced in all nucleated cells at a constant rate and has a low molecular weight (Jančič *et al*., 2022), it is an effective biomarker of glomerular filtration due to its production and renal elimination by filtration (Ma *et al*., 2023), contrary to creatinine, which is dependent on height, weight, age,

sex, nutritional status, and inflammatory processes, and its concentrations are increased in acute and chronic kidney disease (Bhosale & Kulkarni, 2020).

Aims of the Study:-

Early detection of AKI biomarkers can significantly reduce renal damage, improve the results in the long term, and prevent the progression of chronic kidney disease (CKD), also help identify patients who can benefit from early intervention before the AKI becomes clinically detectable.

Early diagnosis of renal disease complication or regeneration is essential for the prognosis of patients as well as to reduce medical costs and time and this is done by measurement:

1. The level of some biomarkers such as (Cystatin C, Kidney Injury Molecule-1 (KIM-1), and NGAL) that are related to early predicting renal injury in different stages of AKI then compared to the control.
2. The traditional renal test (urea, creatinine, albumin, BUN, electrolytes) in different stages of AKI then found correlated with biomarkers
3. Different hematological parameters (RBC, WBC, Hb).
4. The correlation between these markers with proinflammatory markers (CRP).
5. The GFR with different markers: GFR by Cr, GFR by Cys, and GFR by (Cr+Cys), and estimated the best marker to determine decline in renal function.

Chapter two:

Literature Review

2. Kidney Disease (KD)

The kidneys' organ primary role is to remove toxins from the blood and produce urine from waste (Taylor, 2023). Numerous homeostatic functions are related to this, including as blood pressure and volume regulation, pH regulation, and electrolyte composition regulation. Because of this, any malfunction in the kidney's ability to function could potentially have detrimental effects on the urinary system as well as other organ systems (Levey *et al*., 2020).

The kidney carries out the following tasks: electrolyte regulation volume, and control of nitrogenous excretion of waste. Many drugs are eliminated by getting rid of exogenous substances. Several hormones, including erythropoietin, are synthesized. For instance, low molecular weight proteins metabolize insulin (Fishman & Singer, 2023).

Renal disease results from kidney damage, leading to the loss of the normal function of filtering waste in the body. Immunological responses, medication exposure, and genetic abnormalities can all result in renal damage. Modifications to lifestyle affect behavioral risk factor modification, as well as the development and progression of kidney disease (Anandkumar *et al*., 2023).

Kidney dysfunction is broadly categorized as follows:

Table (2-1) lists those recommended as a result of Kidney Disease: Improving Global Outcomes (KDIGO’s) consensus conferences. Some of the more prominent criteria are summarized below.

Table (2-1): Defining criteria for kidney dysfunction (Levey, *et al*.,2020).

Category	Key defining criteria
Acute kidney diseases and disorders	For ≤3 months, one of the following: ● GFR: <60ml per minute per 1.73m2 ● GFR: ≥35% reduction ● SCr: >50% increase ● Presence of indicators of kidney damage
Acute kidney injury	One of the following: ● SCr: >0.3mg/dl increase in two days ● SCr: >50% increase in one week ● Urine output: oliguria (<0.5ml per kg per hour) for >6 hours
Chronic kidney disease	For >3 months, one of the following: ● GFR: <60ml per minute per 1.73m2 ● Presence of indicators of kidney damage

2.1 Chronic Kidney Disease (CKD)

The CKD is defined as abnormalities in the structure or function of the kidney, occurring within three months after the loss of kidney function in kidney failure levels , which have health implications (Dwi *et al*., 2020).

Chronic kidney disease (CKD) is defined as a persistent abnormality in kidney structure or function (e.g., glomerular filtration rate (GFR) < 60ml/min/1.73m) for more than 3 months, CKD affects 8% to 16% of the population worldwide (Chen *et al*., 2021).

The CKD is slowly progressive and leads to irreversible loss of nephrons, end-stage renal disease, and/or early death. Therefore, CKD represents a

worldwide major concern and its prevalence continues to rise, also it is one of the most common diseases (Ruiz-Ortegn *et al*., 2020).

One of the major complications of CKD is hemodialysis catheter use which causes bloodstream infection, which is associated with an increased risk of systemic infection complication, hospitalization, and death (Salim *et al*., 2022).

(CKD) It is a growing issue in adults but also significant in children, albeit less frequent. Research estimates the prevalence to be 74.7 cases per million children. Children with CKD have risk factors for increased morbidity, mortality, and diminished quality of life. In a significant number of cases, kidney failure develops before the age of 20 (Jančič *et al*., 2022) .

Traditional clinical endpoints in clinical trials in patients with CKD are kidney failure and doubling of serum creatinine. However, these endpoints are late events in the progression of CKD and take a long time to manifest (Waijer *et al*., 2022).

2.2 Acute Kidney Injury (AKI)

Acute kidney injury (AKI) is a rapid loss of kidney function following: failure to maintain fluid, acid balance, and electrolyte homeostasis (Gupta, B. *et al*.,2023).

The AKI is defined by a rapid increase in serum creatinine, a decrease in urine output, or both, this clinical syndrome is linked to a sudden decline in kidney function that can happen within hours to days and is frequently accompanied by oliguria (Luft, 2021), AKI is a serious problem in children

and adolescents and can rapidly progress to chronic kidney disease and result in the need for dialysis if not diagnosed promptly (Abbasi *et al*., 2020).

Acute Kidney Injury is linked to higher morbidity, mortality, and hospital expense rates in about 15.3% of hospitalized patients suffering from acute kidney injury (AKI). Additionally, there is a chance that chronic kidney disease (CKD) will later develop (Kishore *et al*., 2023).

The following are the mechanisms related to kidney injury markers:

1. Inadequate filtering barriers

2. decreasing tubular reabsorption

3. Increased tubular protein release as a result of cell damage

4. Inflammatory cells are activated and release activation products in response to damage (Freiz *et al*., 2020).

2.2.1 Epidemiology of Acute Kidney Injury (AKI)

An abrupt deterioration of kidney function characterizes Acute Kidney Injury (AKI) and is common in critically ill children and adults. It occurs in approximately 30% of pediatric intensive care units (PICU). Pediatric AKI has been linked to increased morbidity and mortality even after accounting for other risk factors. It also poses a long-term risk for hypertension and CKD (Cho, *et al* .,2020).

Recently, many study have been conducted in the field of pediatric AKI following adult studies, which have sparked new interest . AKI occurs in

13–78% of critically ill patients (Chávez-Íñiguez *et al*.,2023), AKI causes approximately 2 million deaths worldwide each year according to (Jana *et al*., 2022) .

According to (Hoste EAJ, *et al*.,2018) AKI reported 22.3% in North America,31% in South America, also 19.3%, 20.8%, 25.2%, and 23.8% in each of North, west, south, and East Europe respectively, while 0.7%,1.7%,23.5%, and 13.4% in North, west-central, and East Africa respectively. In Central Asia 9%, in East Asia 19.4%, south Asia 7.5%, and in Australia 16.9%.

In Iraqi study on 210 AKI patients, the incidence of AKI was 39% in which stage 1 (8.2%), stage 2(16.7%), and stage 3(19.2%), among patients with AKI 43.7% of them referred to the nephrologist, and 12.2% received Renal Replacement Therapy (RRT), complete renal recovery, partial recovery, and death were observed in 47.6%,12.2%, and 40.2%, respectively (Jalal Al-Mukhtar & Amin, 2024).

Acute kidney injury is one of the most common and important problems in the intensive care unit (ICU). AKI has been reported to occur in as few as 20% to >50% of patients in ICUs around the world. A study of AKI incidence were conducted in the resource-limited settings of lower- and middle-income countries (Srisawat, 2020).

Acute kidney injury is a frequent and fatal complication in trauma patients and carries high morbidity and mortality, if the diagnosis is delayed, in the ICU population, AKI is common with an incidence of 1% to 25%, depending on the criteria used for definition, and is associated with 50% to 70%

mortality. The presence of even mild AKI is associated with an almost two-fold increase in ICU mortality as well as greater duration of mechanical ventilation and length of stay in the ICU. The etiology of AKI in critically ill patients is multifactorial. However, this often leads to delays in diagnosis and failure to intervene at an earlier stage (Gupta *et al*.,2023).

A meta-analysis reported a worldwide incidence of pediatric AKI (33.7%) and a high AKI-associated mortality risk (13.8%), however, this meta-analysis included studies with variable AKI definitions and was mostly limited to high-income countries; hence, it was not accurately representative of the overall incidence of AKI across the world in the pediatric population (Meena *et al*.,2023).

2.2.2 Etiology of Acute Kidney Injury (AKI)

AKI is frequently brought on by sepsis, nephrotoxin exposure, or acute tubular necrosis, Volume depletion, urinary blockage, quickly progressing glomerulonephritis, and acute interstitial nephritis are additional causes of AKI, in this study at Madrid 13 tertiary hospital facilities, 748 individuals with AKI were assessed as part of Acute tubular necrosis (45%) and renal disease (21%) were found to be the most common causes of AKI (Huang *et al*.,2021).

(25–66) % of newborn infants with respiratory distress syndrome (RDS) develop AKI One of the most significant causes of acute kidney injury (AKI) has been revealed to be inadequate lung maturation and the development of RDS in premature newborns According to reports (Ider *et al*., 2023).

Several studies have found an association between AKI and cardiovascular outcomes (Schytz *et al*., 2022).

Recently published Indian data reveals the spectrum of AKI as post-sepsis (22.5%), post-surgical (21%), post-trauma (28%), post-cardiac events (10.7%), pregnancy-related AKI (15%), AKI in the pediatric age group (25.1%), thus, there is an urgent need to develop health strategies to reduce the enormous growing burden of AKI and mitigate its outcomes, there was inadequate assessment of patients at risk of AKI and approximately 60% of post-admission AKI was predictable with 21% of AKI being avoidable (Kishore *et al*., 2023).

The AKI is often observed in neonates, and about 8%-24% were observed among those in the neonatal intensive care units (NICU) (Mehrkesh, *et al*., 2022).

2.2.3 Types of acute kidney injury (AKI):

2.2.3.1 Prerenal causes

A type of acute kidney injury caused by decreased blood flow to the kidney. This is a type of systemic hypoperfusion caused by hypotension or hypovolemia or by selective kidney hypoperfusion produced by kidney aortic stenosis and dissection (Kadhim *et al*., 2022).

Reversible kidney hypoperfusion associated with hypotension, volume depletion (due to hemorrhage, diarrhea, dehydration, severe burns, or diuretics), Organ failure(such as pancreatitis and liver disease, which causes a shift of fluid in the abdomen), heart failure or heart attack, Severe allergic

reactions, or overuse of medications (such as nonsteroidal anti-inflammatory drugs (NSAIDs, such as ibuprofen, and naproxen) and angiotensin-converting enzyme (ACE) inhibitors) (Nehomar,2020).

2.2.3.2 Intra-renal causes

Direct injury to the kidney or renal parenchyma due to factors, such as cancer, systemic Infection, sepsis, interstitial nephritis, allergic reaction to medications, scleroderma or other connective tissue disorder, or damage to the renal tubules (such as glomerulonephritis, vasculitis, or thrombotic microangiography) (Ng *et al*., 2020).

The internal type occurs in sepsis-induced acute tubular necrosis and renal ischemia, in general, it can be helpful to think of damage to the four major structures of the kidney when considering etiologies of intrinsic renal failure. These four structures are (Kadhim *et al*., 2022) :

1-Tubular damage 2-Glomerular damage 3-Interstitial damage

4- Vascular damage

2.2.3.3 Postrenal causes

That is obstruction of the urinary tract leads to increased pressure in the Bowman's capsule and reduced glomerular filtration rate (GFR) caused by: renal stones, tumors, prostatic enlargement, and congenital problems (Kadhim *et al*., 2022).

Acute kidney injury impacts tubular function in both the proximal and distal tubules (Sun *et al*.,2023), a severe obstruction to urine flow that

defines them. A blocked urinary tract elevates intratubular pressure, which lowers GFR. Acute urinary tract obstruction can also result in reduced renal blood flow and inflammatory processes, both of which reduce GFR (Ng *et al*.,2020).

2.2.4 Classification of Acute Kidney Injury (AKI)

The Kidney Disease Improving Global Outcomes (KDIGO) criteria is a classification system, which divides AKI into three stages for pediatrics. These stages help clinicians assess the severity of AKI and guide appropriate management and treatment decisions. The KDIGO Clinical Practice Guideline for Acute Kidney Injury, published by provides recommendations for the diagnosis, evaluation, and management of AKI (Cho, 2020).

The guideline includes a classification system for AKI based on the severity of the condition, determined by changes in serum creatinine levels and urine output, the International Society of Nephrology's initiative for AKI focuses on the goal of zero deaths from AKI by 2025, this initiative emphasizes the importance of early detection and prevention of AKI to reduce mortality rates, the KDIGO guideline for AKI specifically addresses its application in the United States, the commentary discusses the implications of the guideline for clinical practice and highlights areas where further research is needed (Meena *et al*.,2023).

2.2.5 Distribution of AKI in Gender

Because gender affects the incidence, prevalence, and progression of kidney disease, men are more progressive than women in systemic diseases

that cause kidney failure, this example involves multiple factors: diet, kidney, and glomerular size, hemodynamic variations, and the direct impacts of sex hormones, where estrogen will protect kidney tissue and reduce the rate of progression by blocking apoptosis and inflammatory processes (Neugarten & Golestaneh,2022).

2.2.6 Diagnosis of Acute Kidney Injury (AKI)

The first standardized definition of AKI was published in 2004, called (Risk, Injury, Failure, Loss, End-stage) RIFLE. Three years afterward, these criteria were adapted to the pediatric population, the Acute Kidney Injury Network (AKIN) criteria were established in 2007 and are based on the RIFLE (risk, injury, failure, loss, end-stage, kidney disease) criteria which are viewed in **Table (2-2)**. The most recent one is the KDIGO rating system published in 2012. The pediatric RIFLE (p RIFLE) acronym since then, this criterion has undergone two other modifications. AKI diagnosis is debatable because there lack of any established diagnostic criteria, while certain standards, such as the RIFLE, AKIN, and KDIGO criteria, have been published (Salvador, 2020).

Kidney Disease: Improving Global Outcomes (KDIGO) published the KDIGO standard for the assessment and management of AKI in 2012. It is based in part on the AKIN and RIFLE criteria. RIFLE criteria include parameters present during the whole course of the condition, ranging from kidney injury to end-stage renal failure which is viewed in **Table (2-2)** (Kellum *et al*., 2021).

The criteria divide AKI into three levels, namely, risk, injury, and failure, according to changes in SCr, GFR, and urine volume. The prognosis of AKI is classified into two levels, namely, (loss of renal function and end-stage renal disease (ESRD)) and two levels (loss, end-stage) represent that development into CKD, based on the time of complete loss of renal function (Meena, 2023).

The time timeframe for AKI is seven days. The two primary criteria used to define AKI recently are changes in blood creatinine levels and the amount of urine produced (Luft, 2021).

Table (2-2): Pediatric modified RIFLE (p RIFLE) criteria for Diagnosis and Classification of AKI (Salvador, 2020; Gupta *et al*., 2023).

Class	**Serum creatinine**	**Urine output**
Risk (stage 1)	Elevated SCr ≥0.3 mg\dl of 105% – 200 %× baseline	Or Urine output <0.5 mg/kg/h for > 6 h
Injury (stage 2)	Elevated SCr to ≥200%-300% ×baseline	Or Urine output <0.5 mg/kg/h for >12 h
Failure (stage 3)	Elevated SCr to >300%× baseline; or an elevated of SCr ≥4 mg/dL with acute elevated or at least ≥0.5 mg/dL or RRT	Or Urine output <0.3 mg/kg/h for >24 h or anuria for >12 h
Loss	Need for RRT for >4 weeks	
End-stage	Need for RRT for >3 months	

2.2.6.1 Biomarkers for Acute Kidney Injury (AKI)

2.2.6.1.1 Kidney Injury Molecule-1 (KIM-1)

Kidney Injury Molecule (KIM1) (also known as TIM-1 and HAVCR) (Hepatitis A Virus Cellular Receptor 1 (HAVCR1) in hepatocytes, and T-cell immunoglobulin and mucin domain 1). KIM-1 is a type 1 transmembrane glycoprotein (104Da), It is primarily expressed on the surface of T-cells and has two extracellular kidney injuries in proximal tubular cells (Brilland *et al*., 2023).

The reason is that the ectodomain of KIM-1 undergoes cleavage and can be detected in blood and urine (Uma *et al*. 2022), a study showed that both urine and serum KIM-1 were noticed to be increased after 12 hours while serum creatinine increased after 48 hours in AKI (Mohammed El-Esawy *et al*., 2020).

It is expressed in low levels in the kidney and other organs, but its expression is accentuated in pre-renal kidney injury and after reperfusion (Nehomar, 2020).

According to (Zakiyanov *et al*.,2021) study KIM-1 is involved in the degradation of cell surface components and regulation of multiple cellular processes such as cell-to-cell interaction, cell proliferation , and cell signaling pathways and to this KIM-1 has anti-inflammatory and protective properties, more recently, elevated circulating levels of KIM-1 in blood were associated with acute and chronic kidney damage (Brilland *et al*.,2023), according to (Anandkumar *et al*.,2023) measuring KIM-1 expression might aid in the early diagnosis of AKI, increases in KIM-1 concentration can be

regarded as a sign of an ischemic kidney (Mehrkesh *et al*., 2022), related to is present in the proximal tubular epithelium, which is susceptible to injury due to ischemia (Dase *et al*.,2022), elevated levels of KIM-1 can be detected in the blood and can be used as a biomarker of kidney injury (Aljorani *et al.*, 2023).

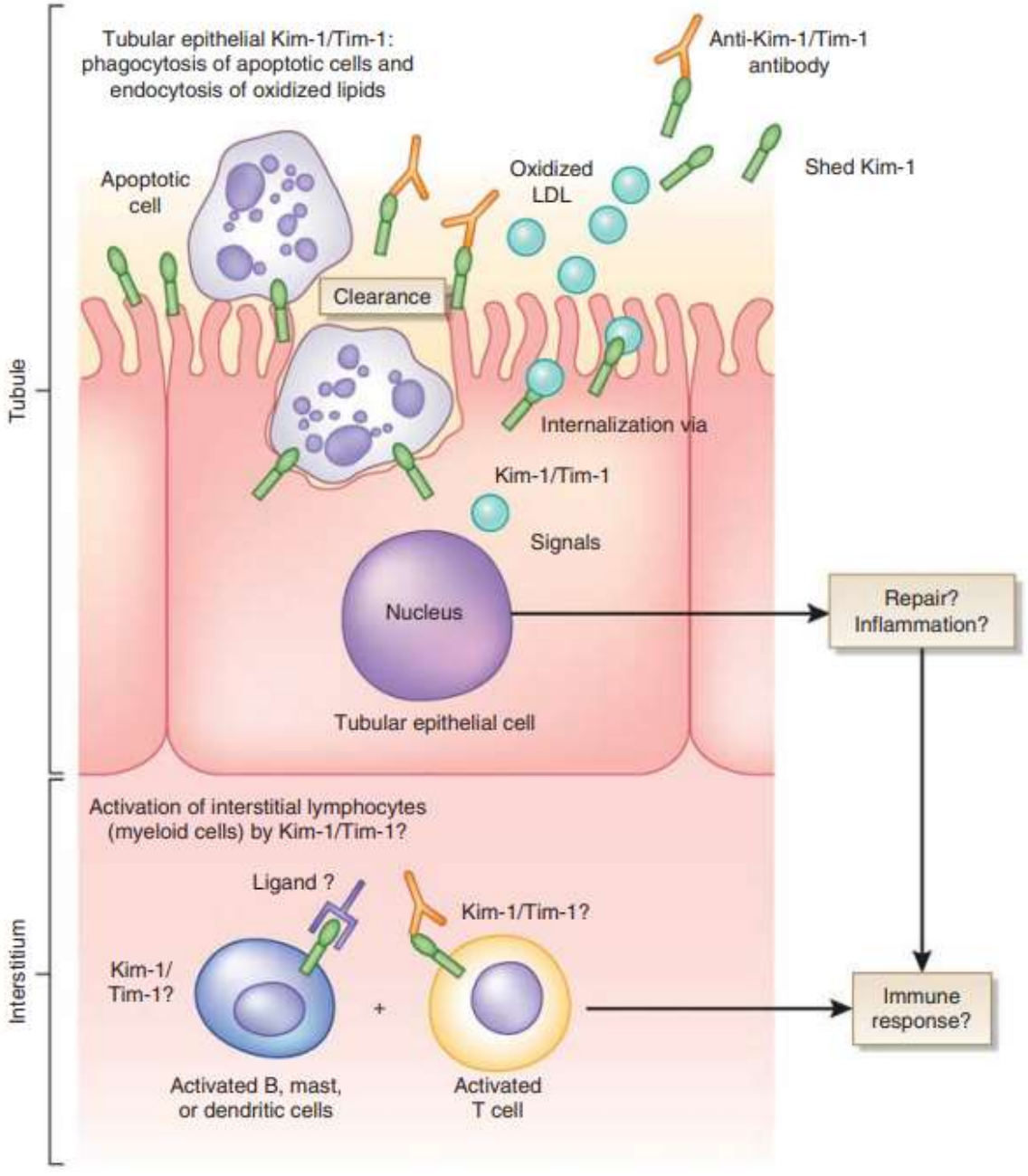

Figure (2-1): Shows the expression of KIM-1\TIM-1(Ichimura *et al*., 2012).

TIM-1: T-cell immunoglobulin and mucin domain 1),

KIM-1: Kidney Injury Molecule

2.2.6.1.2 Neutrophil gelatinase-associated lipocalin (NGAL)

NGAL is a member of the lipocalin superfamily and one of the most reliable markers of renal epithelial injury. NGAL is a secretory protein of activated neutrophils with a single polypeptide and a molecular weight of 25 kDa. Initially, NGAL was localized inside the granules of the neutrophils during the maturation process that occurs in the bone marrow (Marakala, 2022), and has emerged as an early predictive biomarker for acute kidney injury (AKI), its presence in tissue is identified in the breast, colon, and kidney and is increased in epithelial injury and neoplastic conditions. Serum and urinary levels are found to be significantly raised in conditions affecting the kidney (Naqvi *et al*., 2023).

Under physiological conditions, serum NGAL is filtered through the glomerulus and nearly completely reabsorbed by the proximal tubule through the protic transporter megalin, NGAL is present in 3 forms; a 25 kDa monomer released predominantly by the renal tubules; a 45 kDa dimer predominantly secreted by neutrophils as part of a systemic inflammatory response not specific to renal injury; and a 135 kDa NGAL/matrix metalloproteinase-9 (MMP-9) complex (Whitehead, *et al*.,2022), and (Romejko *et al*.,2023) believed that NGAL's role as protective against renal damage.

Neutrophil gelatinase-associated lipocalin (NGAL) acts as a growth and differentiation factor in multiple cell types, including developing and mature renal epithelia, and some of this activity is enhanced in the presence of siderophore-iron complexes. The molecule is responsible for iron traffic within renal epithelia (Luft, 2021).

NGAL has been reported to be superior to sCr for predicting the prognosis and severity of AKI according to (Whitehead *et al*.,2022).

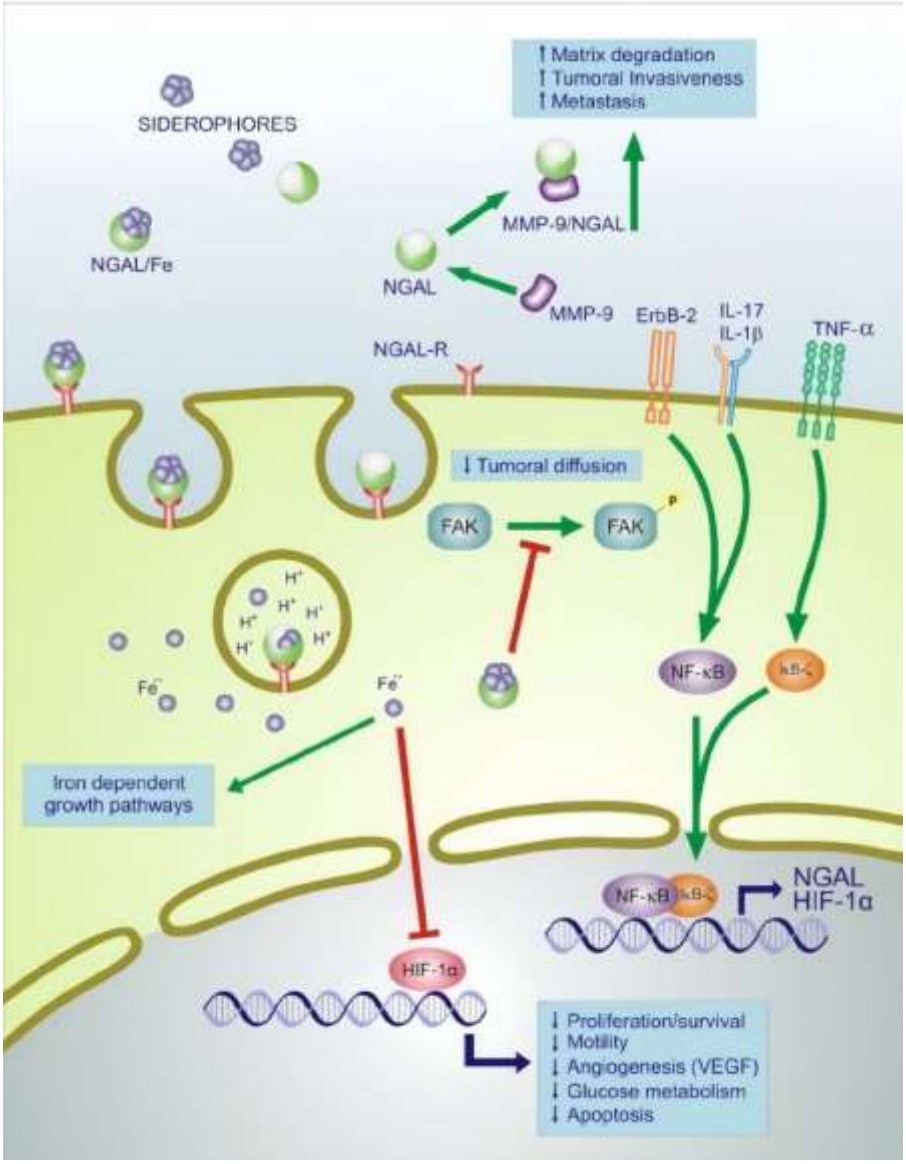

Figure (2-2): Showed the role of NGAL on survival, motility, angiogenic, apoptotic, and glucose metabolism. Green lines with arrows indicate activation of the pathway, red lines with blocked ends indicate inhibition of the pathway, and blue lines indicate transcriptional activation and additional events (Candido *et al*., 2014).

NGAL was initially discovered in the granules of neutrophils. It is almost completely reabsorbed in the proximal tubule, and elevated levels can indicate proximal tubular damage (Srisawat , 2020).

This pro-inflammatory mediator is produced in response to tissue damage, making it a biomarker for early detection of kidney injury. Among them are inflammatory cells, such as neutrophils called macrophages, which penetrate the kidneys during the inflammatory stage and release NGAL according to (Nehomar, 2020).

The NGAL is a small circulating protein that is highly modulated in a wide variety of pathological situations, making it a useful biomarker of various disease states. It is one of the best markers of acute kidney injury, as it is rapidly released after tubular damage. However, a growing body of evidence highlights an important role for NGAL beyond that of a biomarker of renal dysfunction. Indeed, a study have demonstrated a role for NGAL in both cardiovascular and renal diseases (Abdulameer, *et al*.,2022).

2.2.6.1.3 Cystatin C

Cystatin C is a low molecular weight protein produced in the nucleated cells and is present in relatively high amounts in a variety of physiological fluids, most notably seminal fluid, cerebral fluid, and synovial fluid.

Recently, serum cystatin C was proposed as a new endogenous marker of GFR. Cystatin C is a reliable biochemical marker with very promising results worldwide (Kadhim *et al*., 2022), Cystatin C is released into the bloodstream with a half-life of 2 hours and is detected in almost all body fluids. It has a molecular mass of 13 kDa that make it almost freely filtered through the normal glomerular basement membrane and almost completely reabsorbed and degraded by the normal proximal tubular cells. It is not secreted in the tubules and also not reabsorbed back into the serum, therefore Cystatin C was extensively investigated as a marker of renal function to assess the glomerular filtration rate (Tahir *et al*.,2022).

Serum cystatin C has been proposed as an ideal marker for assessing GFR, a study have shown that Serum Cys C is a more sensitive indicator of an early and mild reduction in renal functions than sCr (Luft, 2021).

Serum Cystatin C can be an important biomarker in predicting acute kidney injury (AKI); its urinary excretion reflects tubular damage, and it has moderate diagnostic utility. Its concentrations are elevated in both acute and chronic kidney disease. Unlike creatinine, Cystatin C levels are not influenced by factors such as height, weight, age, sex, nutritional status, and inflammatory processes. In the absence of renal disease, the serum concentration of cystatin C is increased in patients with liver illness, thyroid disease, and during glucocorticoid treatment (Jančič *et al*., 2022).

Cys C level rises earlier (12–24 h after insult) than that of serum creatinine in AKI patients, Studies have evaluated the use of CysC as an endogenous marker of kidney function, generally in populations at risk of or with CKD, showing CysC per- forms comparably or superior to the diagnostic accuracy

of SCr for the discrimination of normal from impaired kidney function (Cho, 2020).

Cys C is being investigated as a new endogenous serum biomarker that is sensitive for the early evaluation of changes in eGFR as a filtration marker. Most studies demonstrate that serum CysC levels are more closely connected to eGFR than SCr. Serum CysC concentrations have also been proven to be unaffected by some inflammatory diseases or metabolic abnormalities (Yahya, *et al*.,2023).

2.2.6.2 Renal Function Tests (RFT)

2.2.6.2.1 Serum Creatinine

Serum creatinine is an amino acid compound derived from creatine metabolism in skeletal muscle and dietary meat intake. Creatinine has a molecular weight of 113 Da, is released into the plasma at a relatively constant rate, is freely filtered by the glomerulus, and is not reabsorbed or metabolized by the kidney. Accordingly, the clearance of SCr is the most widely used means for estimating glomerular filtration rate (GFR) and SCr levels generally have an inverse relationship to GFR (Nehomar, 2020).

Serum Cr is used as a gold standard diagnostic criterion for AKI, but its use is limited due to its inability to indicate the initial stage of renal injury (Uma *et al*. 2022).

At birth, serum creatinine is elevated due to its placental passage during pregnancy and does not accurately reflect GFR. however, its level might be useful in observing its dynamics an additional rise or failure to fall

appropriately might indicate an acute kidney injury, to which neonates are especially prone. On the contrary, cystatin C does not cross the placenta and seems to correspond to GFR better (Jančič *et al*., 2022).

Measurement of serum creatinine (SCr) has long been used as a key indicator of kidney function and is a core element in the staging of AKI. Despite its long-standing and continued use (Hwang *et al*., 2022).

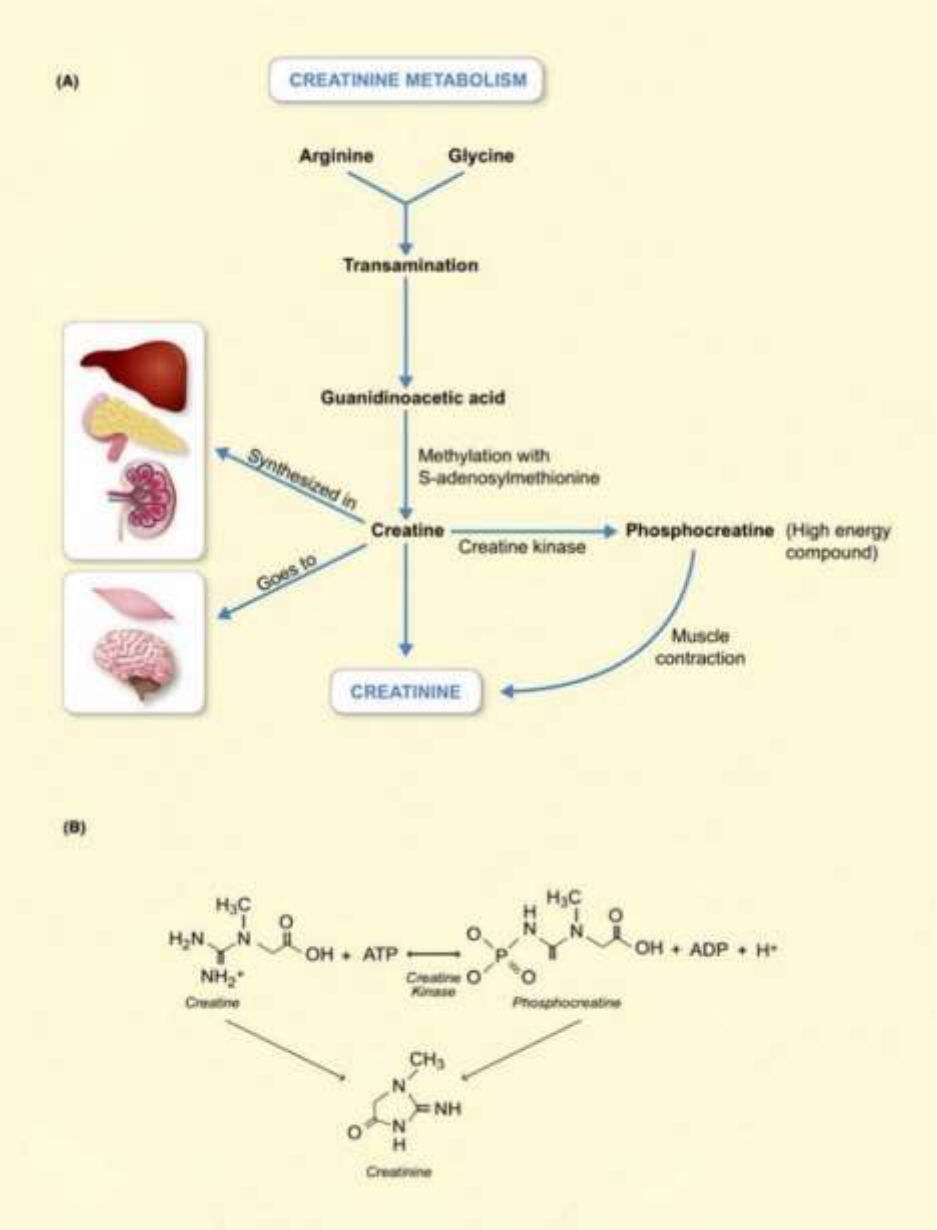

Figure (2-3): Creatinine formation. The daily-produced product is freely filtered, nonprotein bound, and appears in the urine (Luft, 2021).

2.2.6.2.2 Serum Urea

Urea has a small molecular weight of 60Da, it's the major nitrogen-containing product of protein catabolism. Urea originates from the breakdown of proteins and amino acids in the urea cycle in the liver, to detoxify/neutralize ammonia (Duff & Baruteau ,2022). Most of the blood urea (90%) is excreted in the urine. Urea is filtered freely in the glomeruli and is partially reabsorbed by the tubules, which is correlated to the reabsorption of water. Plasma urea is also dependent on the protein load in the body increases in high protein diet, catabolism, steroid treatment, and infections and gastrointestinal bleeding (Salvador ,2020).

According to (Luft, 2021), urea is one of the renal function parameters and has an observational increase in Acute Kidney Injury.

2.2.6.2.3 Blood Urea Nitrogen (BUN)

Blood urea nitrogen is one of the medical tests used to diagnose kidney disease. BUN also increases in the following state: ingestion of food with a high nitrogen content, hypovolemic shock, heart failure, gastrointestinal hemorrhage, fever, and increased catabolism, BUN is more sensitive for patients who have renal failure, it has been prognostic in other many conditions like acute pancreatitis, acute pneumonia, and acute intracerebral hemorrhage, blood urea nitrogen in the prediction of in-hospital mortality of patients with acute aortic dissection (Kadhim *et al*., 2022).

A study documented that BUN levels are associated with adverse dependently with of renal function. Although the exact pathophysiological mechanism underlying this association is unclear, biologically plausible hypotheses have been postulated and BUN has been labeled as a sensitive index of neurohormonal activation over and above any decline in renal filtration functions (Harazim *et al*., 2023)

2.2.6.2.4 Urine Output

Acute Kidney Injury (AKI) is associated with a sudden decline in kidney function within hours to days. Serum creatinine elevation and decreased urine output alone are insufficient for diagnosing AKI, as their sensitivity and specificity are too low to confirm a diagnosis. Many studies suggest biomarkers such as Cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and others are being explored to detect AKI earlier and predict prognosis more accurately than urine output and serum creatinine levels (Cho, 2020).

To measure the patient's urine output:

1. Note the time at which the patient's catheter bag/chamber was last emptied.

2. Inspect the catheter bag/chamber at eye level and note the volume of urine present (typically in milliliters).

3. Record the volume of urine on the relevant urine output chart and the time of the measurement.

4. If the catheter bag is full, this should be emptied after your recording (Kocadal, *et al*.,2023)

2.2.6.2.5 Glomerular Filtration Rate (GFR)

The kidney maintains body homeostasis via its complex 3-dimensional (3D) nephron structure. When this structural organization is altered, renal pathophysiology ensues, kidney diseases are characterized by the development of renal lesions, whether this reflects the fact that specific nephron segments can be differently damaged, or alternatively, that some nephrons have different susceptibilities to be injured (Blanc *et al.,* 2021).

The nephron is the microscopic structural and functional unit of the kidney. It is composed of a renal corpuscle and a renal tubule. The renal corpuscle consists of a tuft of capillaries called a glomerulus and a cup-shaped structure called Bowman's capsule. The renal tubule extends from the capsule. The capsule and tubule are connected and are composed of epithelial cells with a lumen. A healthy adult has 1 to 1.5 million nephrons in each kidney (Lote *et al*., 1994).

GFR is currently the best indicator of renal function (Freiz *et al*., 2020). Since it is known that a considerable decrease in glomerular filtration rate will result in a rise in serum creatinine (SCr), this method has been used to diagnose acute kidney injury (AKI) for the past 50 years. The basis of glomerular filtration is eliminating waste products from the body. In normal conditions, renal blood flow corresponds to 5-6 mls/g/min with a pressure of 60-100 mmHg, this is required to keep the kidneys functioning normally (Nehomar, 2020).

Monitoring the progress of the kidney disease can therefore be difficult as in the initial stages of a decline in kidney function, there are no clinical signs. Due to children's growth and development, changes in muscle mass and growth impair GFR estimation based solely on serum creatinine values. Though approaches involving ionizing agents, big-volume blood samples, or timed voiding have limited use in children, more invasive ways of measuring GFR are more reliable (Jančič *et al*., 2022), because direct measurement of GFR is exhausting and expensive to undertake, estimated GFR (eGFR) equations were developed from easily measurable markers including serum creatinine, to provide rapid, repeatable, and inexpensive estimations of kidney function. Serum creatinine is converted into a clinically meaningful value by mathematical transformation, and scores of eGFR formulae have now been published (Nankivell *et al*., 2020).

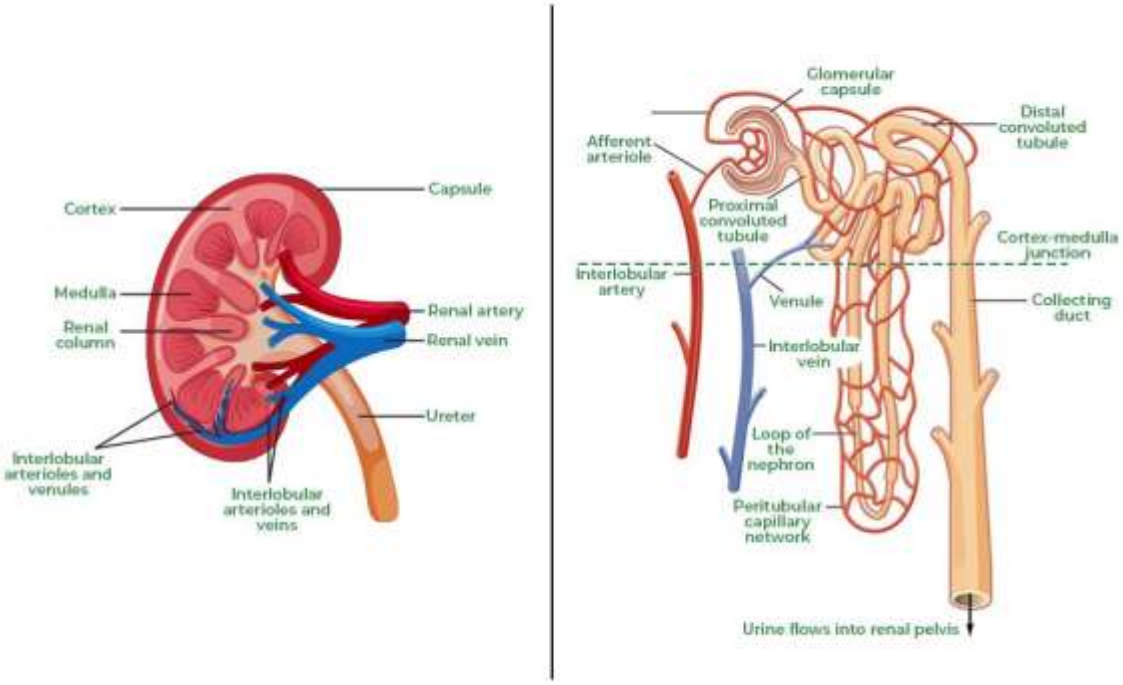

Figure (2-4): Structure of nephron (Blanc *et al.,* 2021).

2.2.6.2.6 Electrolyte {Sodium (Na), Potassium (K), Chloride (Cl)}

2.2.6.2.6.1 Sodium (Na)

Sodium excretion, rather than intake, primarily regulates sodium homeostasis. The glomerular filtration rate (GFR) is the main contributor to the regulation of sodium homeostasis. Changes in GFR affect the amount of sodium that is filtered. Other factors such as intrarenal blood flow, renal prostaglandins, and natriuretic peptides also play a role, albeit more limited, in sodium regulation. The Na+-K+-ATPase pump maintains cellular membrane potential. Under normal physiologic circumstances, the body's sodium intake matches sodium losses (Nogueira *et al*., 2022; Shahrin *et al*.,2020)

2.2.6.2.6.2 Potassium (K)

Hyperkalemia, defined as an increase in potassium levels above 5 mmol/L, is particularly harmful in patients with AKI and should be treated urgently to avoid potential life-threatening arrhythmias (Nehomar, 2020).

Severe hyperkalemia is a life-threatening medical emergency, which is unresponsive in medical management and is one of the most common indications for peritoneal and hemodialysis in newborns (Nogueira *et al*., 2022).

Particularly hyperkalemia puts patients at higher risk of death. Thus, electrolyte disorders are potentially useful for the prediction of the outcome of AKI if needed for RRT or in-hospital death, besides their diagnostic value (Erfurt *et al*., 2023)

2.2.6.3.6.3 Chloride (Cl)

The chloride ion has major physiological significance, which includes the regulation of extracellular and intracellular volume and acid-base homeostasis. Chloride is the most abundant extracellular anion and accounts for about one-third of extracellular fluid tonicity. Intracellular chloride ranges from 20 to 40% of total body chloride and is most concentrated within erythrocytes (70–80 mEq/l) (Rein & Coca, 2019), according to (Chen *et al*.,2021) electrolyte disorders including hyperchloremia useful in AKI prediction.

Treatment of electrolyte disorders in AKI typically involves correcting the underlying cause and restoring electrolyte balance through intravenous fluids and electrolyte replacement therapy and by attention to electrolyte intake during the initial course of AKI with frequent evaluation and specific therapies (Moss ,2022).

2.2.6.3 Hematological Parameters

2.2.6.3.1 Blood Hemoglobin (Hb) & Red blood cells (RBC)

The study describes that a toxic uremic environment accounts for the decreased RBC life span. Reductions of up to 70% in total RBC survival have been reported in uremic patients. Because both anemia and erythrocyte

transfusion are separately linked to cardiac surgery-associated acute kidney injury CS-AKI, the ideal threshold for intraoperative erythrocyte transfusion is crucial, also iron deficiency is also a reason for anemia in these patients. (Peng *et al*., 2022).

The major cause for a decreased count of RBC is a reduction in the production of erythropoietin from the kidney, that related to erythropoiesis suppression, these factors are considered toxic substances normally metabolized or eliminated by kidneys such as guanidine and derivatives which adversely affect the survival of erythrocytes (Kadhim *et al*., 2020).

Hemoglobin levels decrease in AKI patients due to the decline of RBC and it varies depending on the age of the child (Lee *et al*., 2020).

2.2.6.3.2 White Blood cells (WBC)

Acute renal damage has been linked to elevated total white blood cell (TWBC) counts. Lymphopenia and neutrophil leukocytosis are frequently found in AKI patients (Jang *et al*., 2020).

WBCs play a crucial role in the pathogenesis of AKI, during AKI, white blood cells are recruited to the site of injury in response to inflammatory signals. These cells release cytokines and chemokines that further amplify the inflammatory response, leading to tissue damage and renal dysfunction. Neutrophils are the most abundant type of white blood cell involved in AKI. They are attracted to the site of injury by chemotactic signals and release reactive oxygen species (ROS) and proteases that contribute to tissue damage. Additionally, neutrophils can adhere to the endothelial cells of the renal vasculature, leading to impaired blood flow and ischemia (Liu *et al*.,

2022), white blood cells, contribute to the development of AKI , and play a critical role in the pathogenesis of AKI by amplifying the inflammatory response and contributing to tissue damage according to (Deng *et al*.,2022).

2.2.6.4 Biochemical Parameters

2.2.6.4.1 Serum Albumin

Decreased levels of albumin in patients can be related to low intake of protein, insulin resistance, anabolic effects /other hormone growth factors, metabolic acidosis, and the release of inflammatory cytokines, which promote the degradation of proteins Diabetes-related nephropathy and blood pressure increases can exacerbate proteinuria, which lowers blood albumin levels and activation of inflammatory cytokines that stimulate protein breakdown rise in blood pressure and nephropathy in diabetes can increase proteinuria which also results in lower levels of albumin in the blood (Asih Imro *et al*., 2018).

Patients who undergo AKI loss of appetite are at increased risk of death and malnutrition. Protein-energy or protein-calorie malnutrition is a pathological condition with muscle and fat tissue loss. Among the biomarkers of malnutrition that allow for quick identification and correspond to the profile of our population. hypoalbuminemia has been associated with lower survival (Castillo Velarde *et al*., 2020).

The relationship between serum albumin levels and acute kidney injury (AKI) in critically ill patients. The researchers found that lower serum albumin levels were associated with a higher risk of AKI development and increased mortality rates. They suggest that monitoring serum albumin

levels may be a useful tool in predicting and preventing AKI in critically ill patients (Simsek *et al*., 2022).

2.2.6.4.2 C Reactive Protein (CRP)

The liver produces CRP as part of the acute phase reaction in response to a variety of pro-inflammatory cytokines. CRP has wide acceptance as a reliable indicator of systematic inflammation and tissue damage (Kaddam & Kaddam, 2020). Hepatocytes produce CRP, the main indicator of systemic inflammation including acute renal injury and disease severity, and can be useful in the diagnosis and monitoring of disease conditions (Abed *et al*., 2022).

CRP is an acute-phase protein that is produced by the liver in response to inflammation. Elevated levels of CRP have been found in patients with AKI, but it is unclear whether CRP plays a direct role in the pathogenesis of AKI or if it is simply a marker of inflammation (Simsek *et al*., 2022).

2.2.7 Complications of Acute Kidney Injury (AKI)

Patients with acute kidney injury (AKI) may present with symptoms specific to their underlying conditions, such as heart failure, sepsis, cirrhosis, and systemic vasculitis. If there is true involvement of renal function, the dynamic process is as follows: Stage 1, Stage 2, and Stage 3. Numerous complications can occur if the AKI is left untreated or persists; these include hyperkalemia, uremia, volume overload, metabolic acidosis, and progression to chronic kidney disease (CKD) (Nehomar, 2020).

AKI leads to higher death rates and more risks of the development of chronic kidney illness, which can range from discrete changes in biochemical markers to kidney failure requiring artificial renal support (ARS), the most widely available studies deal primarily with AKI requiring ARS, reporting mortality rates between 11% and 63% in pediatric patients (Mehrkesh *et al.* 2022).

Long-established complications of AKI include electrolyte abnormalities, volume overload, and uremia. These "traditional" complications can be managed with dialysis. AKI has been shown in a study to increase susceptibility to infection, double the rate of respiratory failure, and directly and indirectly impair cardiac function. While the mechanisms of these systemic effects remain to be fully explained, given that the mortality of AKI remains high despite RRT, it appears that it is not the loss of renal clearance but rather the association with multiorgan dysfunction that makes AKI so deadly (Griffin *et al*., 2020).

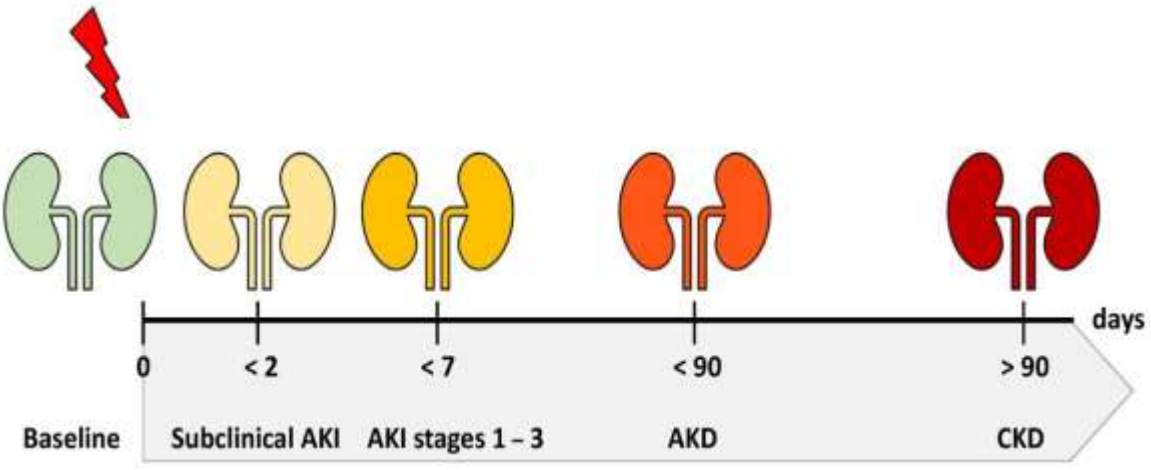

Figure (2-5): The spectrum of disease: subclinical AKI (acute kidney injury), AKD (acute kidney disease), and CKD (chronic kidney disease) (Massoth & Zarbock,2021).

2.2.8 Treatment of AKI and Prevention of Development of CKD

The prevalence of acute kidney injury indicates that approximately 10% of children admitted to intensive care units develop AKI, and the effect of this failure on mortality is significant (Abbasi *et al*., 2020).

The prognosis of AKI depends on factors including:

- The underlying cause;
- The stage of progression;
- Whether any previous kidney injury has been sustained;
- The presence of associated conditions (Goyal *et al*., 2022).

Although new biomarkers appear to detect AKI earlier and predict prognosis more accurately than traditional markers, they are not frequently used in clinical settings. There is no validated pharmacological intervention for AKI, so prevention and early detection are the mainstays of treatment. Preventing AKI and increasing prognosis has been shown to require optimization of blood pressure and volume status, avoidance of nephrotoxins, and adequate nutritional assistance for individuals at high risk or in the early stages of the disease. However, when conservative measures fail, renal replacement therapy such as hemodialysis, peritoneal dialysis, continuous hemofiltration, and hemodialysis are required (Cho, 2020).

Prevention of AKI can be achieved through:

1- early detection, and

2- treatment (Kishore *et al*. 2023).

Kidney (renal) replacement therapy (KRT, also known as RRT), is considered early when conservative measures fail. (Fishman & Singer, 2023). KRT modalities include peritoneal dialysis (PD), hemodialysis (HD), and continuous renal replacement therapy (CKRT) (Parolin *et al*., 2023). Hemodialysis offers the advantage of rapidly correcting fluid or electrolyte imbalances but requires patients to tolerate a large extracorporeal volume. (Starr *et al*., 2021; Parolin *et al*., 2023), general preventative measures include restoring intravascular volume, avoiding hypotension and renal ischemia, and carefully adjusting nephrotoxic medications by close monitoring of drug levels and kidney function. Several pharmacological agents, including mannitol, loop diuretics, low-dose dopamine, fenoldopam, and N-acetylcysteine, have been studied in pediatric AKI with no convincing evidence of benefit but potential adverse side effects. None of these agents is routinely recommended to prevent AKI or its progression. Quality improvement initiatives like the Nephrotoxic Injury Negated by Just-in-time Action (NINJA) have demonstrated successful and sustained reductions of AKI by 24–62% in hospitalized at-risk children. This was achieved through the implementation of daily serum creatinine monitoring and reduced exposure to nephrotoxins (Goldstein *et al*., 2020).

Chapter three:

Materials & Methods

3.1 Instruments and Laboratory Equipment utilized in the present study:

The important instruments and equipment found in Table (3-1)

Table (3-1):-List of the instruments with their company and origin.

NO.	Instrument Name	Company	Origin
1	Automated hematology analyzer	Swe lab Alfa PLUS	Sweden
2	Centrifuge	Universal 16 A	Germany
3	Cobas Integra 400 plus	Roche COBAS	Germany
4	Cotton	Dunya cotton	Iraq
5	EDTA tube	Thomas Scientific	USA
6	Enzyme-linked immune sorbent assay (ELISA) system	Human	Germany
7	Eppendorf tubes	Fisher Scientific	USA
8	EX-DS Electrolyte analyzer	JOKOH	Japan
9	Gel &clot activator tube	Biozek	United Kingdom
10	Gloves	T G S	Malaysia
11	Human Reader HS	Human	Germany
12	Incubator	Jrad	China
13	Micropipettes	Huma pette	Germany
14	Multichannel Micropipettes, fixed with different sizes	Slamed	Germany
15	Refrigerator	Concord	Lebanon
16	Spectrophotometer	Human	Germany

3.2 Kits used in the present study

The important kits found in Table (3-2) in the present study:

Table (3-2):- List the kits with their companies and origins in the present study.

NO.	Kit	Company	Origin
1	Albumin kit	LINEAR CHEMICALS S.L	Spain
2	CBC kit	Swe lab Alfa PLUS	Sweden
3	Chloride (Cl) kit	JOKOH	Japan
4	Creatinine kit	Roche COBAS	Germany
5	CRP kit	Roche COBAS	Germany
6	Cystatin C Kit	Cloud clone	USA
7	KIM-1 kit	Cloud clone	USA
8	NGAL kit	Cloud clone	USA
9	Potassium (K) kit	JOKOH	Japan
10	Sodium (Na) kit	JOKOH	Japan
11	Urea kit	Roche COBAS	Germany

3.3 Study Design

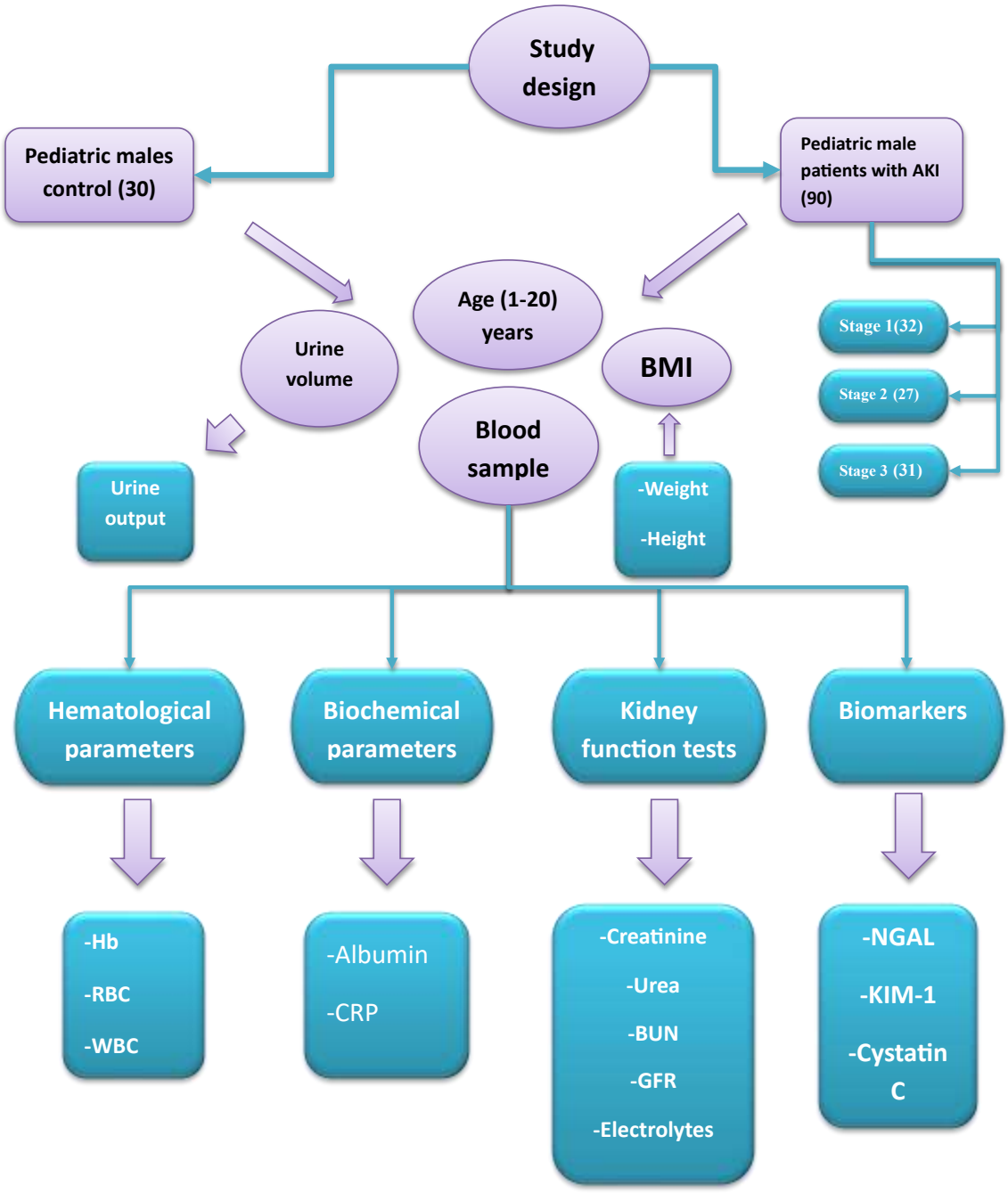

Figure (3-1): Diagram showing the study design plan.

3.4 Collection of Data

This study was a case-control done on the Kerbala population. Blood samples were collected from a total of in a period from September 2023 to April 2024. The number of volunteers in the current study was divided into two groups as the following:

Group 1. Acute Kidney Injury (AKI) includes (90) patients divided into the following subdivision:

A-Stage 1 (N=32).

B-Stage 2 (N=27).

C-Stage 3 (N=31).

In this study, a total of 90 blood samples were collected from pediatric male patients with a range of ages between (1 year to 20 years) according to the 2015 classification of pediatric patients by the National Institute of Child Health and Human Development in Table (3-3) on mean (5.94 ±4.93) years in Karbala Children's Teaching Hospital. Patients who had signs and symptoms were diagnosed with AKI by a specialist physician who was managing the patients and sent to the laboratory for investigations. Patient groups were classified according to characteristic symptoms, laboratory investigations, and depending on RIFEL, published by provides recommendations for the diagnosis, evaluation, and management of AKI shown in Table (3-4). The questionnaire shown in Appendix 1 contains descriptive information about each patient including: Name, age, Laboratory test, treatment, and whether patients have any other diseases or not.

Table (3-3): the 2015 classification of pediatric patients by the National Institute of Child Health and Human Development (Schellack, *et al*.,2017).

Classification	**Monthes\ years**
Neonates	0–27 days
Infants	Birth to12 months
Toddlers	13 months to 2 years
Early childhood	2–5 years
Middle Childhood	6–11 years
Early adolescence	12–18 years
Late adolescence	19–21 years

Table (3-4): Pediatric modified RIFLE (p RIFLE) criteria for Diagnosis and Classification of AKI (Salvador, C. L.,2020; Gupta *et al*., 2023).

Class	**Serum creatinine**	**Urine output**
Risk (stage 1)	Elevated SCr ≥0.3 mg\dl of 105% – 200 %× baseline	Or Urine output <0.5 mg/kg/h for > 6 h
Injury (stage 2)	Elevated SCr to ≥200%-300% ×baseline	Or Urine output <0.5 mg/kg/h for >12 h
Failure (stage 3)	Elevated SCr to >300%× baseline; or an elevated of SCr ≥4 mg/dL with acute elevated or at least ≥0.5 mg/dL or RRT	Or Urine output <0.3 mg/kg/h for >24 h or anuria for >12 h
Loss	Need for RRT for >4 weeks	
End-stage	Need for RRT for >3 months	

SCr = serum creatinine,RRT= Renal Replacement Therapy

<u>Group 2</u>. Healthy control (HC) (No.30).

This group includes (30) intact pediatric male individuals with a range of ages (1 year to 20 years) on mean (7.70±5.02) years in Karbala Teaching Hospital for Children , who were volunteers without symptoms or chronic disease at the time of collection samples. They were age and sex matching to the patient's group.

3.5 Inclusion Criteria:

- Age from toddler period (13 months) to late adolescence (21 years).
- Any case with an acute increase in renal indices and decreased urine output.
- Only male patients.

3.6 Exclusion Criteria:

- Congenital anomaly of the renal system.
- Small size kidney (Atrophy).
- Any association with chronic diseases such as diabetes mellitus type 1, or congenital heart disease.
- Chronic Kidney Disease (CKD)
- Female patients.
- Adult patients.
- Thyroid disease.
- Liver disease.

- Patients that are less than 1 year old (Neonates and infants).

3.7 Collection of Blood Samples

Five milliliters (5mL) of venous blood were collected from each patient and control subjects via vein puncture with a sterile syringe, and all samples were identified by their specific numbers (ID) generated by the laboratory information system. Blood was put in the EDTA tube to prevent clotting of blood for complete blood count (CBC) to measure hemoglobin, white blood cells, and red blood cells and the jell plain tube and let clot (to get the serum) then, centrifuged at (4000 rpm) for five minutes. The serum was separated from the blood contents and was conserved in the sterile Eppendorf tubes, labeled with the sample name, and kept in deep freezing ($^{-}$20 C) used for Cystatin C, NGAL, and KIM-1. Subsequently, the remainder serum was used for creatinine, urea, electrolyte, albumin, and CRP.

3.8 Ethics Statement

This study protocol was accepted after it was reviewed by a medical ethics committee at the University of Karbala College of Applied Medical. Also, the study achieves the permission of research ethics in Karbala Children's Teaching Hospital. The study objectives were described to all participants and verbal approvals were obtained from them. Sampling processing and laboratory biochemistry assay investigations for studied parameters were carried out in the laboratories of the previously mentioned hospitals.

3.9 Estimation of Biomarkers

3.9.1 Estimation of BMI

Weight by Kg, height by Meter and calculate body mass index (BMI):

BMI = Weight (kg)/height (m2) (Blackburn *et al*., 2014).

classified as:

Table (3-5): The Centers for Disease Control and Prevention (CDC) recommends BMI categorization for pediatric (Rashid *et al*., 2023). .

Classification	BMI
Severe Thinness	< 16
Moderate Thinness	16 - 17
Mild Thinness	17 - 18.5
Normal	18.5 - 25
Overweight	25 - 30
Obese Class I	30 - 35
Obese Class II	35 - 40
Obese Class III	> 40

3.9.2 Estimation of Biochemical Markers by ELISA Kit

3.9.2.1 Detection of KIM-1 (Cloud clone\USA)

The principle of KIM-1 identification in serum was measured by using ELISA technique in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the(appendix V) .

3.9.2.2 Detection of NGAL (Cloud clone\USA)

The principle of NGAL identification in serum was measured by using ELISA technique in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the(appendix V) .

3.9.2.3 Detection of Cystatin C (Cloud clone\USA)

The principle of Cystatin C identification in serum was measured by using ELISA technique in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the(appendix V) .

3.9.3 Estimation of Renal Function Tests

3.9.3.1 Determination of Creatinine

The principle of creatinine identification in serum was measured by using Cobas Integra 400 plus (Roche COPAS\Germany) in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix VI) (Jaffe *et al*.,1886 &Fabiny DL, *et al*.,1971).

3.9.3.2 Determination of Urea

The principle of urea identification in serum was measured by using Cobas Integra 400 plus (Roche COPAS\Germany) in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix VI) (Richterich R, *et al* .,1978 & Talke H, *et al*.,1965 & Tiffany TO, *et al*.,1972 & Sampson EJ, *et al*.,1980).

3.9.3.3 Determination of BUN

BUN (mg\dl) = urea (mmol\l) × 2.8 (Wang, Y., *et al*.,2023)**.**

3.9.3.4 Determination of Urine Output

Urine Output = Volume of Urine\ number of hours (Kocadal, K. *et al*.,2023).

3.9.3.5 Determination of GFR

The GFR identification was measured by using three formulas that are:

Formulas Used:

1-Estimated GFR for pediatrics using Schwartz formula "Bedside Schwartz":

$$\textbf{eGFR = 0.413 x (height in cm) ÷ serum Cr}$$

2- Estimated GFR for pediatric using Cystatin C "Cystatin C-based equation"

$$\textbf{eGFR= 70.69 × (Cyc C)}^{\textbf{-0.931}}$$

3- Estimated GFR for pediatric using creatinine and cystatin C together "Creatinine-Cystatin C -Based CKID Equation"

$$\textbf{eGFR = 39.8 x [ht/Scr]}^{\textbf{0.456}}\textbf{ x [1.8/cysC]}^{\textbf{0.418}}\textbf{ x [30/BUN]}^{\textbf{0.079}}\textbf{ x [1.076}^{\textbf{male}}\textbf{] x [ht/1.4]}^{\textbf{0.179}}$$

(Schwartz GJ, *et al*.,2012; Grubb A, *et al*.,2010).

Estimated GFR was calculated by simple software programs in IOS software, using the CKD-EPI Creatinine equation (2021), Cystatin C-based equation (2012), and Creatinine-cystatin C-based CKiD equation (2012).

3.9.3.6 Determination of Electrolyte Sodium (Na), Potassium (K), and Chloride (Cl)

The principle of electrolytes identification in serum was measured by using EX-DS Electrolyte Analyzer (JOKOH\Japan) in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix VI)

3.9.4 Estimation of Hematological Parameters;

Determination of CBC to calculate hemoglobin (Hb), white blood cells (WBC), and red blood cells (RBC)**;** Determination of Complete Blood Count (CBC) by Automated hematology analyzer (Swe lab Alfa PLUS)\ Sweden in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix VII) (Whitepaper: Hematology analyzers: 3-part or 5-part, that is the question. Boule Diagnostics, WP31183, Edition 4 (2021).

3.9.5 Estimation of Biochemical Parameters

3.9.5.1 Determination of Albumin

The principle of albumin identification in serum was measured by using (Colorimetric Method) by (LINEAR CHEMICAL S.L\Spain) method in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix VIII) (Doumas, *et al*., 1971;Tietz. N.W.,1987).

3.9.5.2 Determination of CRP

The principle of CRP identification in serum was measured by using Cobas Integra 400 plus (Roche COPAS\Germany) in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix VIII) (Senju O, *et al*.,1986 &Price CP, *et al*., 1987 & Eda S, *et al*.,1998).

3.10 Statistical Analysis

Statistical Package of Social Science (SPSS) Version 24.0 was used to analyze the data. The comparison, among groups, was made by using analysis of variance (ANOVA table), A T-test was employed to assess the significance of arithmetic means the correlation between KIM-1, NGAL, and Cystatin C level and other variables was assessed using Spearman correlation. The statistically significant threshold was set at ($P \leq 0.01$).

Chapter Four:

Results & Discussions

4.1 Distribution of studied groups according to age

The present result in Table (4-1) demonstrated a high significant decrease at (P≤0.01) in age between stage 3 of AKI (4.31±4.03) and control (7.70±5.02) and stage 1 (7.73±5.13), while no significant decrease between stage 1(7.73±5.13), stage 2 (5.66±5.00) with control and stage 1, stage 3 with stage 2, on the other hand a non-significant decrease in P≥0.09 between all patients (5.94±4.93) and the control group (7.70±5.02).

Table (4-1): The age of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Age (year)	Control (a)	30	7.70 a	5.02	0.01**	2.48
	stage 1 (b)	32	7.73 b	5.13		
	stage 2 (c)	27	5.66 c	5.00		
	stage 3 (d)	31	4.31 d a b	4.03		
	All patients	90	5.94	4.93	0.09	

N=number of sample. S.D= standard deviation. LSD =least significant difference.**=high significant decrease(p≤0.01)

4.2 Distribution of studied groups according to weight

The present result in Table (4-2) demonstrated a very high significant decrease at (P≤0.001) in weight between stage 2 (18.65±12.65) and stage 3 (15.33±10.10) with control (27.85±16.80) and between stage 1 (25.33±15.30) and stage 3. while no significant decrease between stage 1 with control, and between stage 2 with stage 1 and stage 3, also, a high significant difference in P≤0.01 between all patients (19.88±13.45) and the control group (27.85±16.80).

Table (4-2): The weight of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Weight (Kg)	Control (a)	30	27.85 a	16.80	0.001***	6.96
	stage 1 (b)	32	25.33 b	15.30		
	stage 2 (c)	27	18.65 c a	12.65		
	stage 3 (d)	31	15.33 d a b	10.10		
	All patient	90	19.88	13.45	0.01**	

N=number of sample. S.D= standard deviation. LSD least significant difference.**=high significant decrease($p \leq 0.01$) ,***= very high significant decrease ($p \leq 0.001$).

4. 3 Distribution of studied groups according to height

The present result in Table (4-3) demonstrated a high significant decrease at ($P \leq 0.01$) in height between stage 3 (0.98±0.29) and control (1.22±0.32) and among stage 1 (1.24±0.35) with stage 2 (1.06±0.38) and stage 3. There is no significant decrease between stage 1, stage 2 with control and stage 2 with stage 3, also, a non-significant decrease in $P \geq 0.09$ between all patients (1.10±0.36) and the control group (1.22±0.32).

Table (4-3): The height of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Height (m)	Control (a)	30	1.22 a	0.32	0.01 **	0.17
	stage 1 (b)	32	1.24 b	0.35		
	stage 2 (c)	27	1.06 c b	0.38		
	stage 3 (d)	31	0.98 d a b	0.29		
	All patient	90	1.10	0.36	0.09	

N=number of sample. S.D= standard deviation. LSD least significant difference.

**=high significant decrease($p \leq 0.01$) .

4.4 Distribution of studied groups according to BMI

The present result in Table (4-4) demonstrated a very high significant decrease at (P≤0.001) in BMI among stage 1 (14.75±1.83), stage 2 (13.84±1.93), stage 3 (14.67±1.87) stages with control (16.92±2.54). while non-significant decrease between Stage 1, stage 2, and Stage 3 with each other.

Also, a very high significant decrease in P≤0.001 between all patients (14.45±1.90) and the control group (16.92±2.54).

Table (4-4): The BMI of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
BMI (kg\m2)	Control (a)	30	16.92 a	2.54	0.001***	1.03
	stage 1 (b)	32	14.75 b a	1.83		
	stage 2 (c)	27	13.84 c a	1.93		
	stage 3 (d)	31	14.67 d a	1.87		
	All patient	90	14.45	1.90	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant decrease (p≤0.001).

4.5 Distribution of study population according to the age group classification for each parameter

Table (4-5) shows a very high significant differences (P≤0.001) in the age among all age groups (1-4), (5-9), and (10 & more),(1.85±0.88),(6.80±1.62), and (13.42±1.98) respectively in stage 1. Also, stage 2,3 found very high significant differences (P≤0.001) in the age among all age groups.

Table (4-5): Classification of the stages of AKI pediatric male patients according to different age groups

variable	stage	Ages	N	Mean	S. D	p-value	LSD
Age(year)	Stage 1	1--4	10	1.85	0.88	0.001***	1.40
		5--9	10	6.80	1.62		
		10 & more	12	13.42	1.98		
	Stage 2	1--4	15	1.84	1.04	0.001***	1.85
		5--9	4	6.55	1.86		
		10 & more	8	12.38	2.45		
	Stage 3	1--4	20	1.78	1.01	0.001***	1.22
		5--9	7	7.00	1.41		
		10 & more	4	12.25	2.87		

N=number of sample. S.D= standard deviation. LSD =least significant difference.

***= very high significant decrease ($p \leq 0.001$).

Table (4-6) shows very high significant differences ($P \leq 0.001$) in the weight among all age groups (1-4), (5-9), and (10 & more),(9.15±2.25),(20.50±5.50), and (42.83±6.12) respectively in stage 1. Also, stage 2,3 found very high significant differences ($P \leq 0.001$) in the weight among all age groups.

Table (4-6): compare the weight of pediatric male patients of AKI stages according to age group in different stages

variable	stage	Ages	N	Mean	S. D	p-value	LSD
Weight (kg)	Stage 1	1--4	10	9.15	2.25	0.001***	4.40
		5--9	10	20.50	5.50		
		10 & more	12	42.83	6.12		
	Stage 2	1--4	15	9.77	2.85	0.001***	2.01
		5--9	4	18.00	5.35		
		10 & more	8	35.63	8.19		
	Stage 3	1--4	20	9.47	2.64	0.001***	5.01
		5--9	7	20.86	6.18		
		10 & more	4	35.00	9.13		

N=number of sample. S.D= standard deviation. LSD =least significant difference.

***= very high significant decrease ($p \leq 0.001$).

Table (4-7) shows very high significant differences ($P \leq 0.001$) in the height among all age groups (1-4), (5-9), and (10 & more),(0.81±0.08),(1.22±0.16), and (1.61±0.08) respectively in stage 1. Also, stage 2,3 found very high significant differences ($P \leq 0.001$) in the weight among all age groups.

Table (4-8) shows very high significant differences ($P \leq 0.001$) in the BMI among all age groups (1-4), (5-9), and (10 & more),(13.88±1.34),(13.61±0.85), and (16.43±1.58) respectively in stage 1, while there is a non-significant difference in BMI among age groups in stage 2 and 3.

Table (4-7): compare the height of pediatric male patients of AKI stages according to age group in different stages

variable	stage	Ages	N	Mean	S. D	p-value	LSD
Height (m)	Stage 1	1--4	10	0.81	0.08	0.001***	0.10
		5--9	10	1.22	0.16		
		10 & more	12	1.61	0.08		
	Stage 2	1--4	15	0.78	0.22	0.001***	6.34
		5--9	4	1.20	0.10		
		10 & more	8	1.53	0.09		
	Stage 3	1--4	20	0.80	0.12	0.001***	0.13
		5--9	7	1.19	0.13		
		10 & more	4	1.53	0.10		

N=number of sample. S.D= standard deviation. LSD =least significant difference.

***= very high significant decrease ($p \leq 0.001$).

Table (4-8): Compare the BMI for AKI stages according to age group classification Compare the BMI for AKI stages according to age group classification

variable	stage	Ages	N	Mean	S. D	p-value	LSD
BMI ($Kg\backslash m^2$)	Stage 1	1--4	10	13.88	1.34	0.001***	1.15
		5--9	10	13.61	0.85		
		10 & more	12	16.43	1.58		
	Stage 2	1--4	15	13.73	1.56	N.S	
		5--9	4	12.25	1.42		
		10 & more	8	14.85	2.35		
	Stage 3	1--4	20	14.70	1.98	N.S	
		5--9	7	14.50	1.72		
		10 & more	4	14.80	2.08		

N=number of sample. S.D= standard deviation. LSD =least significant difference.N.S=Non-significant**.** ***= very high significant decrease ($p \leq 0.001$).

In the current study, nutritional status measurements of growth parameters of children enrolled nonsignificant differences between patient and control

groups in age and height but there is significant variation in weight, and BMI showed between patient and control groups and because of the proportion in this study will indicate that the majority of pediatrics with AKI suffer malnutrition and inactivity with a nutritional deficiency in comparison with pediatrics with normal or moderate dietary deficiency and this agreement with (Rashid *et al*.,2023), BMI was the most effective factor in predicting varying degrees of malnutrition (Ghorbani *et al*., 2020) and disagreement with (Li *et al*., 2020) that demonstrated high BMI increases the risk of AKI.

4.6 Renal Biomarker

4.6.1 Serum level of KIM-1

The present result in Table (4-9) demonstrated a very high significant increase ($P \leq 0.001$) in KIM-1 among stage 1 (1501.64±417.02), stage 2 (1638.91±354.85), stage 3 (1474.16±425.63) with the control group (716.64±50.72). while no significant increase between each stage with another stage.

Also, a very high significant increase in $P \leq 0.001$ between all patients (1533.36±404.21) and the control group (716.64±50.72).

This study found that KIM-1 has increased concentrations in AKI patients compared to the control related to KIM-1 is expressed in low levels in the kidney and other organs in normal kidneys, but its expression is accentuated in kidney injury because it is a protein released by tubular cell damage (Fazel *et al*., 2020).

Table (4-9): The KIM-1 of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
KIM-1 (ng\ml)	Control (a)	30	716.64 a	50.72	0.001***	181.75
	stage 1 (b)	32	1501.64 b a	417.02		
	stage 2 (c)	27	1638.91 c a	354.85		
	stage 3 (d)	31	1474.16 d a	425.63		
	All patient	90	1533.36	404.21	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase ($p \leq 0.001$).

KIM-1 is released into the circulation following kidney proximal tubule damage. Tubular cell polarity is lost after damage, and KIM-1 may be discharged directly into the interstitium. Furthermore, increased trans-epithelial permeability after tubular injury causes tubular contents to seep back into the circulation. In addition, increased microvascular permeability contributes significantly to the pathogenesis of kidney damage, in renal microvascular endothelial cells, the actin cytoskeleton architecture is disrupted, with loss of cell-cell and cell-matrix adhesion junctions, and endothelial cells are separated from the basement membrane, allowing KIM-1 to enter the circulation, the study found that elevated levels of KIM-1 can be detected in the blood and can be used as a biomarker of kidney injury (Aljorani *et al*., 2023) .

KIM-1 is secreted by inflammatory cells as macrophages that enter the kidneys during the inflammatory phase and are markedly up-regulated in the proximal tubule in the post-ischemic kidney. After all, this pro-inflammatory mediator is produced because of tissue damage, which serves as a biomarker for kidney injury early detection (Luft, 2021), and

disagreement with (Capelli *et al*., 2020) who demonstrated a decrease in KIM-1 in AKI in kidney injury in their study.

4.6.2 Serum level of NGAL

The present result in Table (4-10) demonstrated a very high significant increase ($P \leq 0.001$) in NGAL among stage 1 (5.51±1.23), stage 2 (5.84±1.08), stage 3 (5.75±1.36) with the control group (2.30±0.28) and between stage 2 and stage 3. while no significant increase between stage 1 and stage 2 and between stage 1 and stage 3.

Also, a very high significant increase in $P \leq 0.001$ between all patients (5.69±1.23) and the control group (2.30±0.28).

Table (4-10): The NGAL of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
NGAL (ng\ml)	Control (a)	30	2.30 a	0.28	0.001***	0.56
	stage 1 (b)	32	5.51 b a	1.23		
	stage 2 (c)	27	5.84 c a	1.08		
	stage 3 (d)	31	5.75 d a	1.36		
	All patient	90	5.69	1.23	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase ($p \leq 0.001$) ,ns= non-significant.

This study proved that NGAL concentrations increased in AKI patients. NGAL, initially discovered in the granules of neutrophils, is reabsorbed in the proximal tubule. Increased levels can indicate proximal tubular damage (Srisawat , 2020) because this pro-inflammatory mediator is produced due

to tissue damage, which serves as a biomarker for early kidney injury detection

NGAL, which is secreted by epithelial and neutrophil, was found to appear early in acute renal tubular injury (Quang *et al*., 2020).

The production of NGAL can increase up to 1000 times in Henle's loop and the distal tubule when AKI is present. This finding is consistent with (Menez *et al*., 2019), but contradicts (Capelli *et al*., 2020), who demonstrated only a slight increase in NGAL levels in their study of kidney injury.

NGAL in human neutrophils was reported as kidney tubular injury early marker in various patient populations and hence was nicknamed “kidney troponin.” It is released by renal tubular cells in cases of acute tubular damage and may be detected within a few hours of the tubular insult and even in the absence of functional AKI. NGAL released by neutrophils also plays a role in rapid inflammatory modulations. Both rapid inflammatory reactions and early renal dysfunction are important prognostic markers in acute coronary syndromes (Zahler *et al*., 2022)

4.6.3 Serum Level of Cystatin C

The present result in Table (4-11) demonstrated a very high significant increase ($P \leq 0.001$) in Cystatin C among AKI patients’ stage 1 (12.52±3.02), stage 2 (13.23±2.42), stage 3 (11.85±3.04) with the control group (5.32±1.71) and between stage 2 and stage 3. while no significant increase between stage 1 and stage 2 and between stage 1 and stage 3.

Also, a very high significant increase in P≤0.001 between all patients (12.50±2.88) and the control group (5.32±1.71).

This study proved that cystatin C was found to increase concentrations AKI patients in compared to normal. That is because Cystatin C is produced at a constant rate in all nucleated cells investigated, freely filtered by the glomeruli, and almost completely reabsorbed in the proximal tubule. in normal kidneys, it is expressed in low levels in the kidney and other organs, but its expression is elevated in kidney injury because of decreased glomerular filtration rate it can be eliminated almost exclusively from the bloodstream by glomerular filtration in the kidney, if kidney function and glomerular filtration rate decline, the blood levels of Cystatin C increase (Nehomar, 2020; Luft, 2021).

Table (4-11): The Cys C of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Cystatin C (ng\ml)	Control (a)	30	5.32 a	1.71	0.001***	1.36
	stage 1 (b)	32	12.52 b a	3.02		
	stage 2 (c)	27	13.23 c a	2.42		
	stage 3 (d)	31	11.85 d a c	3.04		
	All patient	90	12.50	2.88	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase (p≤0.001).

Cys C is believed to be neither actively secreted into the tubular lumen nor reabsorbed into the plasma. After filtration, Cys C is normally completely reabsorbed by proximal renal tubular epithelial cells, through

megalin receptor-induced endocytosis, and catabolized. There is virtually no detection of Cys C in the urine; however, it can be measured. Indeed, elevated urine Cys C may indicate tubular epithelial damage, and has been proposed as an additional urine biomarker for AKI (Naqvi *et al*., 2023b) Cystatin C is an endogenous biomarker of renal function produced by all nucleated cells at a near-constant rate, independent of muscle mass, and is cleared from circulation through glomerular filtration without reabsorption or secretion. Cys C performs equally as Scr as a marker of renal function in most AKI cases and outperforms sCr in some cases (Gharaibeh *et al*., 2018), and this study disagreement with (Capelli *et al*., 2020) demonstrated a decrease of cystatin C in kidney injury in their study.

4.7 Renal Function Tests

4.7.1 Creatinine

The present result in Table (4-12) demonstrated a very high significant increase ($P \leq 0.001$) in creatinine between stage 3 (3.18±2.36) and control (0.42±0.13), stage 3 and stage 1 (0.62±0.18), and stage 3 and stage 2 (0.97±0.36). while nonsignificant increase between stage 1, stage 2 with control, and stage 1 with stage 2.

Also, a very high significant increase in $P \leq 0.001$ between all patients (1.61±1.81) and the control group (0.42±0.13).

This study demonstrates that developed serum creatinine in AKI patients is defined as an increase in serum creatinine, and this is because Scr is the product of creatine metabolism with a small molecular weight, most of which passes through glomerular filtration, and almost all of the Scr formed

in the body can be excreted by urine. Reduced cardiac output and decreased blood volume due to diuretic use, resulting in renal insufficiency, decreased glomerular filtration rate, and increased sCr (Wang *et al*., 2023).

Table (4-12): The creatinine of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Creatinine (mg\dl)	Control(a)	30	0.42 a	0.13	0.001***	0.63
	stage 1 (b)	32	0.62 b	0.18		
	stage 2 (c)	27	0.97 c	0.36		
	stage 3 (d)	31	3.18 d a b c	2.36		
	All patient	90	1.61	1.81	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase (p≤0.001).

Creatinine elimination by the kidney and when the kidney damage causes an increase in serum creatinine. Even so, the diagnosis and stratification of acute kidney injury are made by obtaining serum creatinine levels and this agreement with (Nehomar, 2020) and disagreement with (Capelli *et al*., 2020) which demonstrated that No changes in sCr were observed in their study of kidney injury.

4.7.2 Blood Urea

The present result in Table (4-13) demonstrated a very high significant increase (P≤0.001) in urea between stage 1 (61.10±17.99), stage 2 (74.85±24.42), and stage 3 (174.79±62.30) with control (20.61±5.13),

between stage 1 and stage 3, and between stage 2 and stage 3. while the nonsignificant increase between stage 1 and stage 2.

Also, a very high significant increase in P≤0.001 between all patients (104.38±65.27) and the control group (20.61±5.13).

Table (4-13): The urea of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Urea (mg\dl)	Control (a)	30	20.61 a	5.13	0.001***	17.47
	stage 1 (b)	32	61.10 b a	17.99		
	stage 2 (c)	27	74.85 c a	24.42		
	stage 3 (d)	31	174.79 d a b c	62.30		
	All patient	90	104.38	65.27	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase (p≤0.001)

The study shows an increase in blood urea related to urea is it product of proteins and nitrogen metabolism, urea is the most abundant substance in the blood of uremic people (Noman Salman *et al*., 2022), and due to the decline of GFR as a result of early cellular damage, which also lowers total renal function. This causes a decrease in urine output as well as elevated serum urea levels (Cook, *et al*.,2022). This phase begins with the initial insult or injury to the kidneys. It is characterized by a decrease in renal blood flow and/or direct damage to the kidney tissue. Kidney function may decline, but it is still potentially reversible with appropriate interventions (Beck *et al*., 2023).

Urea is not age-related in the same way as creatinine but reflects fluid and protein intake as well as renal function, volume depletion increases renal

tubular uptake, causing increased serum urea, in pediatrics, urine volume depletion is most often caused by less intake or gastrointestinal losses, and this agreement (Salvador, 2019).

4.7.3 BUN

The present result in Table (4-14) demonstrated a very high significant increase (P≤0.001) in BUN among all stages with control and stage 1 (9.50±2.80), stage 2 (27.19±9.69) with stage 3 (11.64±3.80). while nonsignificant increase between stage 1 and stage 2.

Also, a very high significant increase in P≤0.001 between all patients (16.24±10.15) and the control group (3.21±0.80).

This study demonstrated an increase of BUN related to its reabsorption by renal tubular filtration, A serum urea level can be represented as a molar concentration or even as a mass concentration the levels of serum mass concentration can be defined for the entire urea molecule or nitrogen equivalents [blood urea nitrogen (BUN)] in a 60/28 ratio (O=C=(NH2)=Urea=molecular weight =60 there are 2 molecules of Nitrogen(N)=28 gram) (Noman Salman *et al.,* 2022), BUN can change quickly enough during injury because individuals with normal renal function have a functional reserve that compensates for nephron injury (Mohamed Ali El-Esawy *et al*., 2020) and this agreement with (Bjornstad *et al*.,2023).

Table (4-14): The BUN of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
	Control (a)	30	3.21 a	0.80	0.001***	2.84

BUN (mg\dl)	stage 1 (b)	32	9.50 b a	2.80		
	stage 2 (c)	27	11.64 c a	3.80		
	stage 3 (d)	31	27.19 d a b c	9.69		
	All patient	90	16.24	10.15	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase (p≤0.001)

The normal of increased urea nitrogen is ingestion of food with a high nitrogen content, glomerular filtration rate, hypovolemic shock, heart failure, gastrointestinal hemorrhage, fever, and increased catabolism. Urea-derived nitrogen in the bloodstream (a substance formed by the breakdown of protein in the liver). BUN is more sensitive for patients who have renal failure (Kadhim *et al*., 2022).

4.7.4 Urine output

The present result in Table (4-15) demonstrated a very high significant decrease (P≤0.001) in urine output among stage 1 (0.39±0.06), stage 2 (0.38±0.05), stage 3 (0.14±0.09) with the control group (1.56±0.46) and between stage 1, stage 2 with stage 3. while no significant decrease between stage 1 and stage 2.

Also, a very high significant decrease in P≤0.001 between all patients (0.30±0.13) and the control group (1.56±0.46).

Table (4-15): The urine output of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
urine output (ml\kg\hr)	Control (a)	30	1.56 a	0.46	0.001***	0.12
	stage 1 (b)	32	0.39 b a	0.06		
	stage 2 (c)	27	0.38 c a	0.05		

	stage 3 (d)	31	0.14 d a b c	0.09		
	All patient	90	0.30	0.13	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant decrease ($p \leq 0.001$).

In a recent study, it was shown that urine output decreases are related to the pathophysiology of Acute Kidney Injury (AKI), which varies depending on the various conditions associated with its development. About 20% of cardiac output is required for renal perfusion, which constitutes a significant portion of the total intravascular volume (Nehomar, 2020). Once in the kidney, it is distributed in a specific manner through the renal arteries, which branch into glomerular arterioles and eventually form a network of capillaries that facilitate glomerular filtration.

This process involves filtering waste substances and other molecules (e.g., proteins). In normal cases, renal blood flow corresponds to 5-6 mL/g/min with a pressure of 60-100 mmHg, which is important to maintain normal kidney function. Renal blood flow is primarily regulated by multiple factors that involve extrarenal processes, such as vascular tone, neurohormonal processes, and vasodilator/vasoconstrictor substances, among others. Hence, failure in any of these mechanisms will cause hypoxia, decrease GFR, and impair the normal production of urine, leading to reduced urine output (Luft, 2021).

To diagnose and stratify AKI, serum creatinine levels and urine output are assessed. A decrease in urine output alone is insufficient for AKI diagnosis, as its sensitivity and specificity are too low to confirm a diagnosis. It is crucial to consider that a healthy renal functional reserve can mitigate

the increase in serum creatinine. This is supported by (Capelli *et al*.,2020), who showed that AKI is characterized by an elevation in serum creatinine (sCr) or changes in urine output.

4.7.5 GFR

The result of GFR by sCr was detected as a decrease among AKI patients in each stage of AKI was (81.40±4.08), (48.25±8.10), and (17.94±8.80) in stage 1, stage 2, and stage 3 respectively than in control groups (109.71±13.06) as shown in Table (4-16). The analytical study demonstrated very high significant decrease at ($P \leq 0.001$) between all stages with control and between stages 2 and 3 with stage 1 and between stage 2 and stage 3, also, a very high significant decrease in $P \leq 0.001$ between all patients (49.60±27.64) and the control group (109.71±13.06).

Table (4-16): The GFR by sCr of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S. D.	P-value	LSD
GFR by sCr (ml\min\1.73m2)	Control (a)	30	109.71 a	13.06	0.001***	4.70
	stage 1(b)	32	81.40 b a	4.08		
	stage 2 (c)	27	48.25 c a b	8.10		
	stage 3(d)	31	17.94 d a b c	8.80		
	All patient	90	49.60	27.64	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant decrease ($p \leq 0.001$).

The result of GFR by sCr & Cys C was detected as a decrease among AKI patients in each stage of AKI was (28.56±4.71), (20.74±2.99), and (12.48±4.07) in stage 1, stage 2, and stage 3 respectively than in control groups (50.30±7.73) as shown in Table (4-17). The analytical study

demonstrated very high significant decrease at ($P \leq 0.001$) among all stages with control and between stage 2 and stage 3 with stage 1 and between stage 2 and stage 3 and between stage 1 and stage 2.

Also, a very high significant decrease in $P \leq 0.001$ between all patients (20.68±7.85) and the control group (50.30±7.73).

Table (4-17): The GFR by sCr and Cys C of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
GFR by sCr and Cystatin C (ml\min\1.73m^2)	Control (a)	30	50.30 a	7.73	0.001***	2.71
	stage 1 (b)	32	28.56 b a	4.71		
	stage 2 (c)	27	20.74 c a b	2.99		
	stage 3 (d)	31	12.48 d a b c	4.07		
	All patient	90	20.68	7.85	0.001***	

N=number of sample. S.D= standard deviation. LSD =least significant difference.

***= very high significant decrease ($p \leq 0.001$).

The result of GFR by Cys C formula was detected as a decrease among AKI patients in each stage of AKI was (7.41±2.84), (6.70±2.16), and (7.77±3.32) in stage 1, stage 2, and stage 3 respectively than in control groups (16.30±5.07) as shown in figure (4-18). The analytical study demonstrated very high significant decrease at ($P \leq 0.001$) between all stages with control. while there is no significant difference between each stage.

Also, a very high significant decrease in $P \leq 0.001$ between all patients (7.32±2.84) and the control group 16.30±5.07).

Table (4-18): The GFR by Cys C of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
GFR by Cystatin C ((ml\min\1.73m2)	Control (a)	30	16.30 a	5.07	0.001***	1.84
	stage 1 (b)	32	7.41 b a	2.84		
	stage 2 (c)	27	6.70 c a	2.16		
	stage 3 (d)	31	7.77 d a	3.32		
	All patient	90	7.32	2.84	0.001***	

N=number of sample. S.D= standard deviation. LSD =least significant difference.

***= very high significant decrease ($p \leq 0.001$)

This study demonstrated a significant reduction in GFR in AKI patient groups compared to the control groups in each of the formulas related to Pre-renal, renal, and post-renal AKI causes are summarized by the pathophysiology of AKI, which varies based on the various factors linked to its development a network of capillaries that perform glomerular filtration—the process of filtering waste products and other molecules like proteins. The removal of bodily wastes is the foundation of glomerular filtration (Nehomar, 2020).

Renal blood flow is primarily regulated by multiple factors that involve extrarenal processes such as vascular tone, neurohormonal processes, and vasodilator/vasoconstrictor substances, among others. Hence, failure in any of these mechanisms will lead to hypoxia and then decrease blood flow causing a decrease in glomerular filtration to produce the normal quantity of urine Acute kidney injury is not a single disease, but instead consists of a loose syndrome collection of conditions including sepsis, cardiovascular causes, nephrotoxicity, urinary tract obstruction and in short—anything that

can cause glomerular filtration rate (GFR) to be reduced this agreement with (Delgado *et al*., 2022) quickly.

As shown in Figure (4-1), The result of GFR depends on the three markers used was detected as a decrease among AKI patients in each marker: GFR by sCr and Cystatin C, GFR by Cystatin C, and GFR (ml\min\1.73m2) by sCr was (20.68±7.85), (7.32±2.84), and (49.60±27.64) respectively, the analytical study demonstrated very high significant decrease for GFR in cystatin C parameter at (P≤0.001) between patient groups.

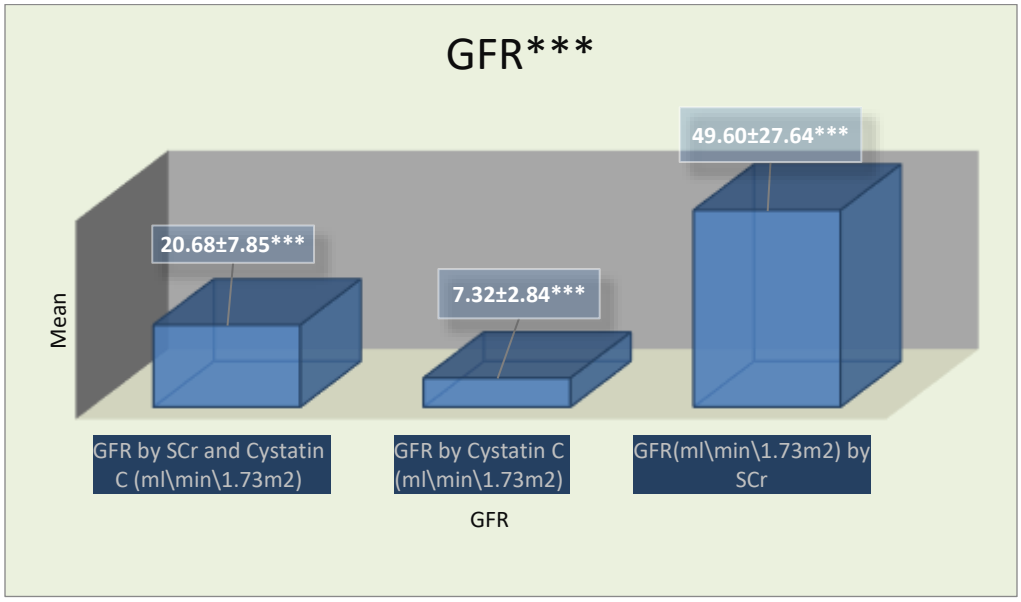

Figure (4-1): The level of GFR in pediatric male patients with AKI stages compared depends on three equations.

LSD=4.87 .***=very high Significant difference at P≥0.01

Figure (4-1) demonstrates three formulas to estimate GFR in patient groups and control groups and compares these three formulas. The first is performed by using sCr which is filtered by the glomerular and almost completely reabsorbed in the proximal tubule and the second by using Cystatin C another element that is filtered by the glomerular and in the

proximal tubule completely reabsorbed that constant production and renal elimination make it an excellent biomarker of glomerular filtration and this agrees with (Nehomar, 2020), and the third formula by using sCr and Cystatin C formula (Rizk DV, *et al*., 2018).

The comparison among these formulas shown in Figure (4-1) demonstrated that the Cystatin C formula more accurate in estimating GFR related to creatinine is inaccurate at detecting mild renal impairment, and creatinine levels can vary with muscle mass but little with protein intake whenever cystatin C might provide more accurate information compared to serum creatinine because it is unaffected by sex, age, and this agreement (Luft, 2021).

4.7.6 Electrolytes

4.7.6.1 Sodium (Na)

The present result in Table (4-19) demonstrated non-significant differences (P≥0.05) in the sodium among AKI patients' stage 1 (140.80±3.61), stage 2 (138.24±11.85), stage 3 (135.26±11.14), and the control group (137.17±3.61). Also, non-significant differences (P>0.01) among all stages with control and between each stage.

Also, a nonsignificant difference in (P>0.63) between all patients (138.12±10.60) and the control group (137.17±3.61).

Table (4-19): The sodium of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD

Na (mmol\l)	**Control (a)**	30	137.17 a	3.61	0.12	n. s
	stage 1 (b)	32	140.80 b	8.32		
	stage 2 (c)	27	138.24 c	11.85		
	stage 3 (d)	31	135.26 d	11.14		
	All patient	90	138.12	10.60	0.63	

N=number of sample. S.D= standard deviation. LSD least significant difference.

ns= non-significant.

This result is in agreement with (Shahrin, L., *et al*.,2020) who found a non-significant increase in Na electrolyte in AKI patients related to GFR changes, the excessive supply of sodium to the renal tubules disrupts the balance between oxygen nutrient production and demand, resulting in damage to tubular epithelial cells and leading to oxidative stress (Woitok, *et al*., 2020; Nehomar, 2020), and disagreement with (Nogueira *et al*., 2022) that demonstrated that electrolyte disorders are associated with worse outcomes, with increased hospitalization length and mortality, and (Zhi D *et al*., 2021) demonstrated hypernatremia is a frequent condition of life-threatening potential and found to occur in 9% of ICU patients. Hypernatremia can cause peripheral insulin resistance, hepatic gluconeogenesis impairment, neuropsychiatric impairment, and cardiac contractility dysfunction, while Hyponatremia occurs due to a combination of syndrome of inappropriate secretion of antidiuretic hormone and gastrointestinal fluid loss from vomiting and diarrhea (Nogueira *et al*., 2022).

4.7.6.2 Potassium (K)

The present result in Table (4-20) demonstrated a very high significant difference ($P \leq 0.001$) in potassium between stage 2 (4.96±0.99) and stage 3 (5.16±1.61) with control (4.25±0.45) and between stage 1(4.47±0.92) and stage 3, while no significant increase between stage 1 with control and between stage 1 and stage 2 and between stage 2 and stage 3.

Also, a high significant increase in $P \leq 0.01$ between all patients (4.85±1.25) and the control group (4.25±0.45).

Table (4-20): The potassium of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
K (mmol\l)	Control (a)	30	4.25 a	0.45	0.001***	0.54
	stage 1 (b)	32	4.47 b	0.92		
	stage 2 (c)	27	4.96 c a	0.99		
	stage 3 (d)	31	5.16 d a b	1.61		
	All patient	90	4.85	1.25	0.01**	

N=number of sample. S.D= standard deviation. LSD least significant difference.

=high significant increase($p \leq 0.01$) ,*= very high significant increase ($p \leq 0.001$).

This study showed the increase of potassium (K) related to many complications that can result from AKI, including metabolic acidosis, and elevated blood potassium levels with decreased excretion (Fazel *et al*., 2020), and disagreement with (Nogueira *et al*., 2022) that showed hypokalemia and demonstrated that; electrolyte disorders are associated with worse outcomes, with increased hospitalization length and mortality. Hyperkalemia is a well-known complication in AKI, it should be recognized instantly to prevent respective patients from severe cardiac arrhythmias. The data on AKI risk and outcomes of initial dyskalemia are quite limited in

contrast. (Erfurt *et al*., 2023) .Hyperkalemia and potassium variability are most likely AKI predictive. It must be prepended that both, serum potassium levels change in response to the intake of diuretics and/or angiotensin-converting enzyme (ACE)/angiotensin II type 2 (AT2) inhibitors. This aspect always needs to be considered if electrolyte disturbances are diagnosed before AKI. A distinct electrolyte disorder may reflect drug-induced effects (e.g., potassium and volume depletion), ultimately responsible for AKI. The same applies to AKI-associated alterations in the renin-angiotensin axis, which modify electrolyte serum levels also (Erfurt *et al*., 2023).

4.7.6.3 Chloride (Cl)

The present result in Table (4-21) demonstrated a significant increase ($P \leq 0.05$) in chloride between stage 1 (111.11±9.80) and control (103.26±2.81). while non-significant increase between stage 2 (107.31±13.65) or stage 3 (106.32±14.41) with control and between stage 2 and stage 3 and between each stage.

Also, a significant increase in $P \leq 0.03$ between all patients (4.85±1.25) and the control group (103.26±2.81).

Table (4-21): The chloride of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Cl (mmol\l)	Control (a)	30	103.26 a	2.81	0.05*	5.77
	stage 1 (b)	32	111.11 b a	9.80		

	stage 2 (c)	27	107.31 c	13.65		
	stage 3 (d)	31	106.32 d	14.41		
	All patient	90	108.32	12.74	0.03*	

N=number of sample. S.D= standard deviation. LSD least significant difference.

*=significant increase ($p \leq 0.05$)

The study showed an increase of chloride (Cl) related to the compromised ability to concentrate or dilute urine, In AKI, chloride levels may be elevated or decreased, depending on the underlying cause and severity of the injury. Elevated chloride levels may be associated with dehydration, while decreased levels may be due to fluid overload or impaired renal function, and this agreement (Rein, J. L., & Coca, S. G. 2019).

4.8 Hematological parameters

4.8.1 Hemoglobin (Hb)

The present result in Table (4-22) demonstrated a very high significant decrease ($P \leq 0.001$) in hemoglobin stage 1 (11.17±2.10), stage 2 (9.97±2.49), stage 3 (10.50±2.47) with the control group (12.54±0.85) and between stage 1 and stage 2. while no significant decrease between stage 2 and stage 3 and between stage 1 and stage 3.

Also, a very high significant decrease in $P \leq 0.001$ between all patients (10.58±2.38) and the control group (12.54±0.85).

Table (4-22): The hemoglobin of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Hb (g\dl)	**Control (a)**	30	12.54 a	0.85	0.001***	1.08
	stage 1 (b)	32	11.17 b a	2.10		

	stage 2 (c)	27	9.97 c a b	2.49		
	stage 3 (d)	31	10.50 d a	2.47		
	All patient	90	10.58	2.38	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant decrease ($p \leq 0.001$).

This study shows a decrease of Hemoglobin (Hb) related to anemia that considered various common causes of AKI and this agreement with (Radwan *et al*., 2023), and in epithelial cells in renal tubular, unable to secretion of erythropoietin hormone and leads to decreased hematopoiesis then decrease RBC and Hb this agreement with (Salim *et al*., 2022).

4.8.2 Red Blood Cell (RBC) count

The present result in Table (4-23) demonstrated high significant decrease ($P \leq 0.01$) in RBC between stage 2 (4.00±1.13) and stage 3 (4.00±0.96) with control (4.65±0.42) and also between stage 2 and stage 3 with stage 1 (4.43±0.85). while non-significant decrease between stage 1 and control and between stage 2 and stage 3.

Also, a high significant decrease in $P \leq 0.01$ between all patients (4.15±0.99) and the control group (4.65±0.42).

Table (4-23): The RBC count of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
RBC ($\times 10^6$\µl)	**Control (a)**	30	4.65 a	0.42	0.01**	0.40
	stage 1 (b)	32	4.43 b	0.85		
	stage 2 (c)	27	4.00 c a b	1.13		
	stage 3 (d)	31	4.00 d a b	0.96		

	All patient	90	4.15	0.99	0.01**	

N=number of sample. S.D= standard deviation. LSD least significant difference.

**=high significant decrease($p \leq 0.01$)

This study showed a decrease in RBC related to one or more causes one of these is a decrease in the erythropoietin production from the kidney, causing erythropoiesis suppression, the other cause, RBC with a short life span leading to a decreased count of RBC, that related to increase in the phosphatidylserine expression in the external surface of RBC, this will cause an increase in the damage of RBC by macrophage, therefore, decrease in cells survive. Other studies showed the causes of RBC decrease related to hemolytic factors therefore affecting the survival of RBC, these factors considered toxic substances normally metabolized or eliminated by kidneys such as guanidine and derivatives which is adversely affect the survival of erythrocytes (Salim *et al*., 2022), and this agreement with (Asaduzzaman *et al*., 2018).

4.8.3 White Blood Cells (WBC)

The present result in Table (4-24) demonstrated a very high significant increase ($P \leq 0.001$) in WBC between stage 2 (13.22±7.70) and stage 3 (14.86±9.40) with control (8.71±2.64) and between stage 1(11.02±6.00) and stage 3. while no significant increase between stage 1 and control and between stage 2 and stage 3 and between stage 1 and stage 2.

Also, a very high significant increase in $P \leq 0.001$ between all patients (13.00±7.89) and the control group (8.71±2.64).

This study shows an increase in WBC related to the total white Blood cell count had been reported to be high in cases of acute renal injury; neutrophil leukocytosis and lymphopenia are common. The cause of lymphopenia in uremia is obscure, as is also neutrophil leukocytosis in the absence of infection. It may be that substances retained in the body because of renal failure depress lymphopoiesis and stimulate granulopoiesis. However, the identity of such substances is unknown(Asaduzzaman *et al*., 2018) and this result is similar to (Pei *et al*., 2022).

Table (4-24): The WBC of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
WBC ($\times 10^3$ \µl)	Control (a)	30	8.71 a	2.64	0.001***	3.58
	stage 1 (b)	32	11.02 b	6.00		
	stage 2 (c)	27	13.22 c a	7.70		
	stage 3 (d)	31	14.86 d a b	9.40		
	All patient	90	13.00	7.89	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant increase ($p \leq 0.001$).

Leukocyte differential counts have long been used to determine the cause of infection. When an infection occurs, neutrophils and monocytes move to the peripheral site, which is essential for defending against pathogens. As the infection progresses, the number of neutrophils and monocytes increases rapidly, and the cells migrate to the inflamed tissue. Several studies have revealed a link between monocytes, infections, and immune mechanisms this agreement with (Lee *et al*., 2020).

4.9 Biochemical parameters

4.9.1 Albumin

The present result in Table (4-25) demonstrated a very high significant decrease (P≤0.001) in albumin among stage 1 (2.64±0.98), stage 2 (2.57±0.89), stage 3 (2.91±0.91) with the control group (4.25±0.56). while no significant decrease between each stage with another stage.

Also, a high significant decrease in P≤0.001 between all patients (2.71±0.93) and the control group (4.25±0.56).

Table (4-25): The albumin of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
Albumin (g\dl)	Control (a)	30	4.25 a	0.56	0.001***	0.43
	stage 1 (b)	32	2.64 b a	0.98		
	stage 2 (c)	27	2.57 c a	0.89		
	stage 3 (d)	31	2.91 d a	0.91		
	All patient	90	2.71	0.93	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.

***= very high significant decrease (p≤0.001).

The study results show a decrease in albumin related to malnutrition in patients with AKI that causes hypoalbuminemia and is associated with the effect of such other factors as proteinuria, a common problem in patients with renal failure. Several studies found that nephrinuria was associated with higher urine albumin concentrations and a decrease in serum ,nephrinuria may provide an early indicator of renal damage (Aljorani *et al.*, 2023). The specificity of albumin level as a nutritional marker decreases in case of

inflammation and fluid overload and acidemia also affects serum albumin levels that agreement with (Ghorbani *et al*., 2020; Li *et al*., 2020).

4.9.2 C Reactive Protein (CRP)

The present result in Table (4-26) Demonstrated a very high significant increase (P≤0.001) in CRP among stage 1 (33.93±64.75), stage 2 (35.81±29.45), stage 3 (54.29±64.41) with the control group (2.60±0.84). while no significant increase between each stage with another stage.

Also, a very high significant increase in P≤0.001 between all patients (41.51±56.56) and the control group (2.60±0.84).

Table (4-26): The CRP of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
CRP (mg\dl)	Control (a)	30	2.60 a	0.84	0.001***	24.34
	stage 1 (b)	32	33.93 b a	64.75		
	stage 2 (c)	27	35.81 c a	29.45		
	stage 3 (d)	31	54.29 d a	64.41		
	All patient	90	41.51	56.56	0.001***	

N=number of sample. S.D= standard deviation. LSD least significant difference.***= very high significant increase (p≤0.001).

The study demonstrated an increase in CRP related to AKI as the spread of inflammatory processes from the kidney to other organ systems also further increases WBC count this may be due to upregulation and the presence of interleukin-6 (IL-6) and cytokines such as tumor necrosis factor –α (TNF-α) in blood participate to inflammation in the uremic state (Kadhim, *et al*., 2020), presence of pro-inflammatory cytokines in dictate an inflammatory response. is also affected by metabolic changes and undernutrition and a direct and significant relationship between malnutrition

and inflammation with CRP level (Ghorbani *et al*., 2020), AKI as the spread of inflammatory processes from the kidney to other organ systems is also, affected by metabolic changes and undernutrition. Therefore, undernourished patients are more vulnerable to AKI. Therefore, malnutrition patients are more exhibition to AKI, and this agreement with (Li *et al*., 2020), which identified that nutritional markers (a low energetic intake, higher C-reactive protein level, etc.) are significantly associated with a risk of death in AKI patients. A StudY have demonstrated that malnutrition is related to poor prognosis in AKI, and CRP has a direct and significant relationship with malnutrition (Ghorbani *et al*., 2020). However, the elevation of traditional markers of inflammation such as CRP and WBC levels is also associated with an increased risk of heart failure and mortality after acute myocardial infarction one of the prerenal causes of acute kidney injury (Zahler *et al*., 2022).

4.10 Correlation Among Studied Groups

The result of the correlation coefficient of biomarkers in the pediatric male:

Table (4-27): The correlation between KIM-1 biomarkers with different hematological parameters, CRP, and electrolytes in the patient group.

Parameters	KIM-1(pg\ml)	
	r	P-Value
Na (mmol\l)	-0.065	P>0.05
k (mmol\l)	0.138	P>0.05
Cl (mmol\l)	0.103	P>0.05
CRP (mg\l)	0.279**	**P≤0.01**
Hb (g\dl)	-0.295**	**P≤0.01**

RBC ($\times 10^6$\µL)	-0.124	P>0.05
WBC ($\times 10^3$\µL)	0.097	P>0.05

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

According to Table (4-27) the findings showed a negative correlation (r= -0.295) existed between KIM-1 and Hb that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.279) existed between KIM-1 and CRP that was statistically significant at (P ≤0.01).

That demonstrated the positive correlation between KIM-1 and CRP by which AKI is one of the most common organ injuries. Some research showed that AKI is accompanied by inflammation in the kidney and each KIM-1 and CRP are pro-inflammatory proteins is that be a direct correlation between them (Wang *et al*., 2022).

On the other hand, that is a negative correlation between Hb and KIM-1 and related to anemia that is related to a reduction in the production of erythropoietin from the kidney, causing erythropoiesis suppression, also red blood cells with a short life span leading to lower in the count of RBC, considered common causes of AKI (Salim *et al*., 2022; Radwan *et al*., 2023) in addition to KIM-1 is present in the proximal tubular epithelium, and the proximal tubular epithelium is susceptible to injury due to ischemia (Dase, J.*et al*.,2022).

According to Table (4-28) the findings showed a direct association (r=0.366**) existed between KIM-1 and urea that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.236) existed

between KIM-1 and creatinine that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.458) existed between KIM-1 and albumin that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.366) existed between KIM-1 and BUN that was statistically significant at (P ≤0.01), and showed a negative correlation (r= -0.629) existed between KIM-1 and urine output that was statistically significant at (P ≤0.01).

That demonstrated the positive correlation between KIM-1 and urea, creatinine, and BUN related to each of the increase in the condition of kidney injury KIM-1 is an inflammatory biomarker, and elevated levels of KIM-1 can be detected in the blood and can be used as a biomarker of kidney injury (Aljorani *et al*., 2023), and each of urea (Salvador ,2020), creatinine (Nehomar, 2020), and BUN (S. H. Kadhim *et al.*, 2022) elevated related to excreted from the body depended on kidney filtration and due to kidney defect increase in blood.

In contrast, the negative correlation between KIM-1, albumin, and urine output related to the increase of KIM-1 in AKI condition related to the presence of inflammation, and the decrease of albumin related to activation of inflammatory cytokines that stimulate protein breakdown increase in blood pressure can increase proteinuria which also results in lower levels of albumin in the blood (Asih Imro *et al*., 2018), while the decrease in urine output related to Oliguria is defined as the production of inadequate volumes of urine in AKI (Kocadal, *et al*.,2023).

Table (4-28): The correlation between KIM-1 biomarkers with different renal function test parameters and other parameters in the patient group.

Parameters	KIM-1(pg\ml)	
	r	P-Value
Urea (mg\dl)	0.366**	**P ≤0.01**
Creatinine (mg\dl)	0.236**	**P ≤0.01**
Albumin (g\dl)	-0.458**	**P ≤0.01**
BUN (mg\dl)	0.366**	**P ≤0.01**
urine output(ml\kg\hr.)	-0.629**	**P ≤0.01**
Age(year)	-0.119	P>0.05
BMI (kg\m2)	-0.403**	**P ≤0.01**
Weight(kg)	-0.185*	P ≤0.05
Height (m)	-0.107	P>0.05
GFR by s Cr (ml\min\1.73m^2)	-0.503**	**P ≤0.01**
GFR by s Cr & cys C (ml\min\1.73m^2)	-0.690**	**P ≤0.01**
GFR by s Cr (ml\min\1.73m^2)	-0.811**	**P ≤0.01**

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

According to Table (4-28) the findings showed a negative correlation (r=-0.185) existed between KIM-1 and weight that was statistically significant at (P <0.05) and showed a negative correlation (r= -0.403) existed between KIM-1 and BMI that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.503) existed between KIM-1 and GFR by SCr that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.960) existed between KIM-1 and GFR by SCr & Cys C that was statistically significant at (P ≤0.01) and showed a negative

correlation (r= -0.811) existed between KIM-1 and GFR by Cys C that was statistically significant at (P ≤0.01).

That demonstrated a negative correlation between KIM-1, weight, BMI, and GFR related to the increase of KIM-1 in kidney injury compared to a decrease each of weight, BMI, and GFR related to malnutrition presence in kidney injury patients that cause low weight and BMI (Rashid *et al*.,2023), while a decrease in GFR one of the important markers of kidney injury (Freiz *et al*., 2020), on the other hand, an increase of KIM-1 is released into the circulation following kidney proximal tubule damage (Aljorani *et al.*, 2023).

According to Table (4-29) the findings showed a negative correlation (r= -0.315) existed between NGAL and Hb that was statistically significant at (P ≤0.01), and showed a positive correlation (r=0.312) existed between NGAL and CRP that was statistically significant at (P ≤0.01). The other correlation is mentioned in the previous paragraph.

That demonstrated the negative correlation between NGAL and Hb related to that NGAL is a pro-inflammatory mediator produced due to damage of tissue, which serves as a biomarker for kidney injury early detection (Nehomar, 2020) and Hb related to anemia that is related to AKI that can be correlated with a reduction in the production of erythropoietin from the kidney, causing erythropoiesis suppression, also red blood cells with a short life span leading to a lower in the count of RBC (Salim *et al*., 2022).

Table (4-29): The correlation between NGAL biomarkers with different hematological parameters,CRP ,and electrolytes in the patient group.

parameters	NGAL (ng\ml)	
	r	P-value
Na (mmol\l)	0.030	**P>0.05**
K (mmol\l)	0.296**	**P ≤0.01**
Cl (mmol\l)	0.189*	**P ≤0.05**
CRP (mg\l)	0.312**	**P≤0.01**
Hb (g\dl)	-0.315**	**P≤0.01**
RBC ($\times 10^6$\µL)	-0.166	P>0.05
WBC ($\times 10^3$\µL)	0.141	P>0.05

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

In contrast, there is a positive correlation between NGAL and CRP related to that each of them related to inflammation (Simsek, B.*et al*., 2022; Whitehead, S. J *et al*.,2022).

According to Table (4-29) the findings showed a positive correlation (r=0.296) existed between NGAL and K that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.189) existed between NGAL and Cl that was statistically significant at (P <0.05), that demonstrated the positive correlation between NGAL, Na, and K that related to each increase in kidney injury. An increase in sodium (Na) levels is associated with changes in glomerular filtration rate (GFR) and sodium delivery to the renal tubules. This imbalance between oxygen and nutrient supply and demand damages tubular epithelial cells, leading to oxidative stress (Shahrin, L., *et al*.,2020), increasing K related to many complications that can result from AKI, including metabolic acidosis, and elevated blood potassium levels with decreased excretion (Fazel *et al*., 2020), and An

increase in NGAL was initially discovered in the granules of neutrophils. It is almost completely reabsorbed in the proximal tubule, and elevated levels can indicate damage to the proximal tubules (Srisawat , 2020).

According to Table (4-30) the findings showed a positive correlation (r=0.456) existed between NGAL and urea that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.231) existed between NGAL and creatinine that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.508) existed between NGAL and albumin that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.455) existed between NGAL and BUN that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.727) existed between NGAL and urine output that was statistically significant at (P ≤0.01). The other correlation is mentioned in the previous paragraph.

That demonstrated the positive correlation between NGAL, creatinine, urea, and BUN related to the increase each of them related to kidney injury, NGAL can be secreted by impaired tubular cells and transferred into the serum which is a mechanism for how NGAL is used as a kidney injury biomarker Therefore, NGAL is not a direct biomarker of kidney function, not like BUN and Scr. The study still selected BUN and Scr as references to compare the diagnosis capability with NGAL(W. Wang *et al*., 2020).

Table (4-30): The correlation between NGAL biomarkers with differences in the patient group.

parameters	**NGAL (ng\ml)**	
	r	**P-value**

Cystatin C	0.850**	**P ≤0.01**
Urea (mg\dl)	0.456**	**P ≤0.01**
creatinine (mg\dl)	0.231*	P ≤ 0.05
Albumin (g\dl)	-0.508**	**P ≤0.01**
BUN (mg\dl)	0.455**	**P ≤0.01**
urine output (ml\kg\hr.)	-0.727**	**P ≤0.01**
Age(year)	-0.119	P>0.05
Weight (Kg)	-0.190*	P ≤ 0.05
Height (m)	-0.119	P >0.05
BMI (kg\m2)	-0.391**	**P ≤ 0.01**
GFR by sCr (ml\min\1.73m2)	-0.608**	**P ≤ 0.01**
GFR by sCr &cys C (ml\min\1.73m2)	-0.771**	**P ≤0.01**

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

According to Table (4-30) showed a negative correlation (r=-0.190) existed between NGAL and weight that was statistically significant at (P <0.05) and showed a negative correlation (r= -0.391) existed between NGAL and BMI that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.608) existed between NGAL and GFR by SCr that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.771) existed between NGAL and GFR by SCr & Cys C that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.850**) existed between NGAL and Cys C that was statistically significant at (P ≤0.01). The other correlation is mentioned in the previous paragraph.

That demonstrated a positive correlation between NGAL and Cys C related to the increase of related to kidney injury (Pei *et al*., 2022), while the negative correlation between NGAL, BMI, weight, and GFR is related to increased NGAL and decreased BMI, weight, and GFR.

Table (4-31): The correlation between Cys C biomarker with different hematological parameters in the patient group.

Parameters	Cystatin C (ng\ml)	
	r	P-Value
Na (mmol\l)	-0.002	P>0.05
k (mmol\l)	0.160	P>0.05
Cl (mmol\l)	0.111	P>0.05
CRP (mg\l)	0.278**	**P≤0.01**
Hb (g\dl)	-0.299**	**P≤0.01**
RBC ($\times 10^6$\µL)	-0.134	P>0.05
WBC ($\times 10^3$\µL)	0.149	P>0.05

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

According to Table (4-31) showed a negative correlation (r= -0.299) existed between Cys C and Hb that was statistically significant at (P ≤0.01), and showed a positive correlation (r=0.278) existed between Cys C and CRP that was statistically significant at (P ≤0.01). The other correlation is mentioned in the previous paragraph.

That demonstrated the negative correlation between Cys C and Hb related to anemia considered a common cause of AKI (Radwan *et al*., 2023), but Cys C is related to a defect in glomerular filtration that causes rises of Cys C (Luft, 2021).

On the other hand, there is a positive correlation between Cys C and CRP related to each of them associated with inflammation. Recently, it was reported that serum cystatin C concentration is well correlated with CRP levels (Wang *et al*., 2022).

According to Table (4-32) showed a positive correlation (r=0.366) existed between Cys C and urea that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.214) existed between Cys C and creatinine that was statistically significant at (P<0.05) and showed a negative correlation (r= -0.453) existed between Cys C and albumin that was statistically significant at (P ≤0.01) and showed a positive correlation (r=0.366) existed between Cys C and BUN that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.675) existed between Cys C and urine output that was statistically significant at (P ≤0.01). The other correlation is mentioned in the previous paragraph.

Table (4-32): The correlation between Cys C biomarker with different renal function test parameters in the patient group.

Parameters	**Cystatin C (ng\ml)**	
	r	**P-Value**
Urea (mg\dl)	0.366**	**P ≤0.01**
Creatinine (mg\dl)	0.214*	**P ≤0.05**
Albumin (g\dl)	-0.453**	**P ≤0.01**
BUN (mg\dl)	0.366**	**P ≤0.01**
urine output(ml\kg\hr.)	-0.675**	**P ≤0.01**
Age(year)	-0.039	P >0.05

BMI (kg\m2)	-0.452**	**P ≤0.01**
Weight (kg)	-0.127	P>0.05
Height (m)	-0.011	P>0.05
GFR by s Cr (ml\min\1.73m^2)	-0.522**	**P ≤0.01**
GFR by s Cr &cys C(ml\min\1.73m^2)	-0.761**	**P ≤0.01**
GFR by Cys C (ml\min\1.73m^2)	-0.922**	**P ≤0.01**

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

That demonstrated the positive correlation between Cys C and creatinine, urea, and BUN related to that each of them Cys C is a glomerular filtration biomarker Cystatin C levels increase earlier than urea and creatinine when renal is injured (Wang et al., 2022), while the indirect correlation between albumin and urine output is related to a decrease in each of them in kidney injury.

According to Table (4-32) showed a negative correlation (r= -0.452) existed between Cys C and BMI that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.522) existed between Cys C and GFR by SCr that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.761) existed between Cys C and GFR by SCr & Cys C that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.922) existed between Cys C and GFR by Cys C that was statistically significant at (P ≤0.01). The other correlation is mentioned in the previous paragraph.

That demonstrated a negative correlation between Cys C, BMI, and GFR related to an increase in Cys C, which is considered an early marker for the

diagnosis of AKI (Wang *et al*., 2022), while a decrease in each BMI due to malnutrition accompanied by kidney injury and a decrease in GFR.

The findings a positive correlation (r=0.785) existed between GFR by SCr & Cys C and GFR by Cys C that was statistically significant at ($P \leq 0.01$).

Demonstrated by a recent study by (Inker, *et al*.,2021) found that eGFR calculations that incorporate both creatinine and cystatin C outperform those that assess either creatinine or cystatin C alone. Serum creatinine (SCr) levels can be significantly influenced by factors such as overall muscle mass, recent muscle injury, glomerular filtration rate (GFR), and protein intake (Bellomo and See, 2021). Cystatin C-based eGFR is less influenced by age or ethnicity, but other factors such as obesity, inflammation, and smoking as well as intake of glucocorticoids may affect serum values (Rothenbacher, *et al*., 2020).

Chapter five:

Conclusions & Recommendations

Conclusion and Recommendation

5.1 Conclusions

The data obtained in this study enable us to conclude the following:

1- Increased concentration of cystatin C, KIM-1, and NAGL biomarkers in all stages of AKI patients, Early detection and intervention are considered crucial to decrease the damage caused, and reduce the chance of complications and mortality.

2-Serum cystatin C adds more value than other kidney function biomarkers in the early stages of AKI patients because is more accurate for measurement of GFR than creatinine and is less affected by age.

3-Disturbance in some hematological parameters in all stages of AKI patients.

4-Significant Positive correlation between KIM-1, NGAL, and Cystatin C with some renal tests (urea, creatinine, BUN, and CRP), while Significant negative correlation with (albumin, GFR, and urine output).

5-Most effected of growth factors such as BMI, Weight, and Height in patients with AKI, and this is related to many symptoms associated with AKI loss of activity.

5.2 Recommendations

The following points are recommended by future further studies:

1-For early treatment and to prevent complications, recommended to use serum cystatin C, which is considered the gold standard for GFR because it is more accurate and useful and not affected by gender, age, and other factors.

2-Increase future studies to validate the sensitivity and specificity of urine and plasma NGAL, KIM-1, and cystatin C as diagnostic biomarkers and as associated with the severity in different kinds of clinical renal injury could well improve knowledge.

3-Recommended to use the same biomarkers in adult or pediatric female patients with acute kidney injury (AKI) or chronic kidney diseases (CKD).

4-Future studies on other novel biomarkers such as (Urinary dickkopf-3 (DKK3), and Chemokine (C-C motif) ligand 2 (CCL2) in adults or pediatric males and females with AKI, or chronic kidney diseases (CKD).

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Appendices

Appendices I

No. ( )
Patient name:

Address: **Age of patient:**

Phone number: **Date:**

Patient	♂			
Congenital anomaly of the renal system		**Chronic kidney disease**		
diabetes mellitus type 1		**Congenital heart disease**		
Stage of AKI				
Clinical signs				
Puffiness of the face	**Yes**		**No**	
Puffiness of the feet	**Yes**		**No**	
Abdominal distention	**Yes**		**No**	
Fever	**Yes**		**No**	
Nausea, vomiting	**Yes**		**No**	
Feeding	**Good feeding**		**Poor feeding**	
Activity	**Good**		**Poor**	
Nephrotoxic drugs	**Yes**		**No**	
dehydration (bleeding, Anemia)	**Yes**		**No**	
Kidney stone, Obstruction	**Yes**		**No**	
Renal disease	**Yes**		**No**	
Other disease	**Yes**		**No**	

Weight (kg)		
Height (m)		
BMI (kg/m^2)		

	Biochemical parameters
1	**Serum creatinine**
2	**Blood urea**
3	**Electrolytes (Na, K, CL)**
4	**Albumin**
5	**GFR**
6	**Urine output**
7	**BUN**
8	**Hemoglobin**
9	**RBC**
10	**White Blood Cells (WBC)**
11	**C Reactive Protein (CRP)**
12	**Neutrophil gelatinase-associated lipocalin (NGAL)**
13	**Kidney injury molecule-1 (KIM-1)**
14	**Cystatin C**

Appendix II

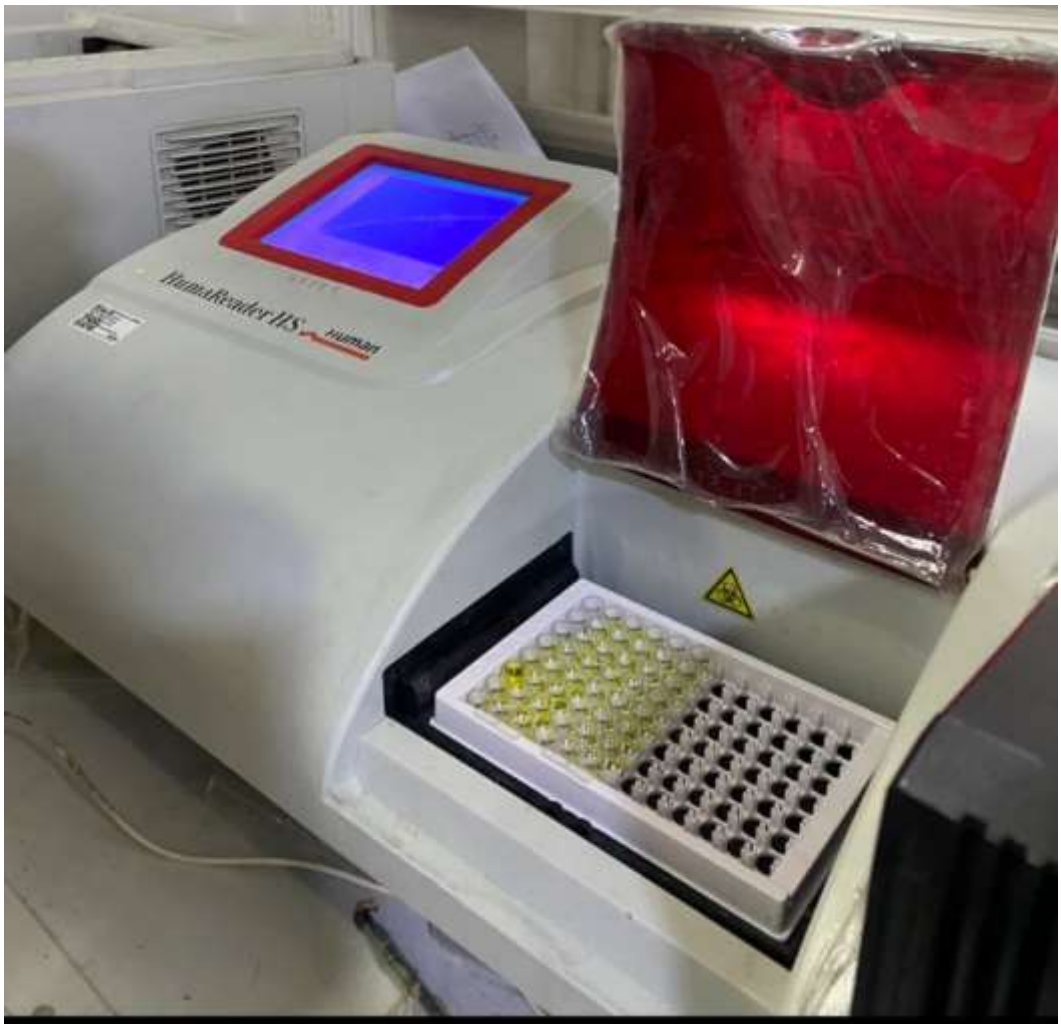
Cobas Integra 400 plus\Germany

Appendix III

EX-DS Electrolyte Analyzer\Japan

Appendix IV

Human Reader HS\Human



Appendix V

Estimation of Biochemical Markers by ELISA Kit

Detection of KIM-1 (Cloud clone\USA)

The KIM-1 test: Is a specific test for diagnosing patients with kidney disease. This test kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Kim-1 in human tissue homogenates, urine, cell culture supernates, and other biological fluids.

Principle

The test utilizes antibodies including a recombinant protein on the cellulose membrane with colloidal gold marked KIM-1 antigen as a tracer. The reagent is used to detect the antibody in serum according to the principle of double antibody sandwich and gold immunochromatography assay. The color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Kim-1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Procedure

1. Prepared all reagents, samples, and standards;

2. Added 100µL standard or sample to each well. Incubated for 1 hour at 37^{o}C;

3. Aspirated and added 100µL prepared **Detection Reagent A**. Incubate 1 hour at 37^{o}C;

4. Aspirated and washed 3 times;

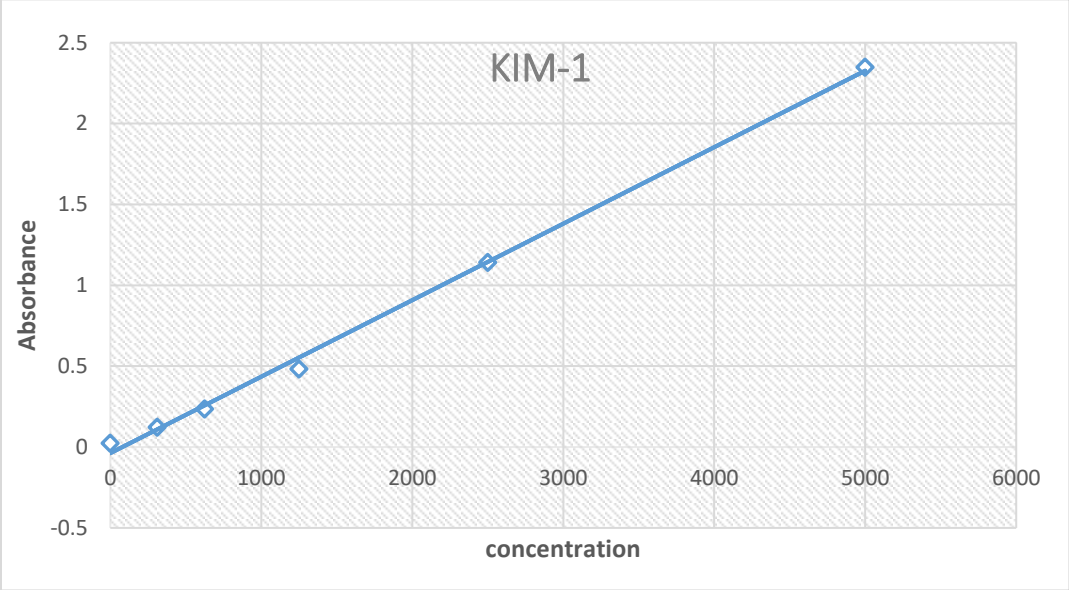
5. Added 100µL prepared **Detection Reagent B**. Incubated 30 minutes at 37^{o}C;

6. Aspirated and washed 5 times;

7. Added 90µL **Substrate Solution**. Incubated for 10-20 minutes at 37°C;

8. Added 50µL **Stop Solution**. Read at 450nm immediately.

Standard curve for KIM-1



Standard Curve for KIM-1.

Detection of NGAL (Cloud clone\USA)

The NGAL test: This is a specific test for diagnosing patients with Kidney disease. The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of NGAL in human serum, plasma, urine, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids.

Principle

The test utilizes antibodies including a recombinant protein on the cellulose membrane with colloidal gold marked NGAL antigen as a tracer. The reagent is used to detect the antibody in serum according to the principle of double antibody sandwich and gold immunochromatography assay. The color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of NGAL in the samples

is then determined by comparing the O.D. of the samples to the standard curve.

Procedure

1. Prepared all reagents, samples, and standards;

2. Added 100µL standard or sample to each well. Incubated for 1 hour at 37ºC;

3. Aspirated and added 100µL prepared **Detection Reagent A**. Incubated 1 hour at 37ºC;

4. Aspirated and washed 3 times;

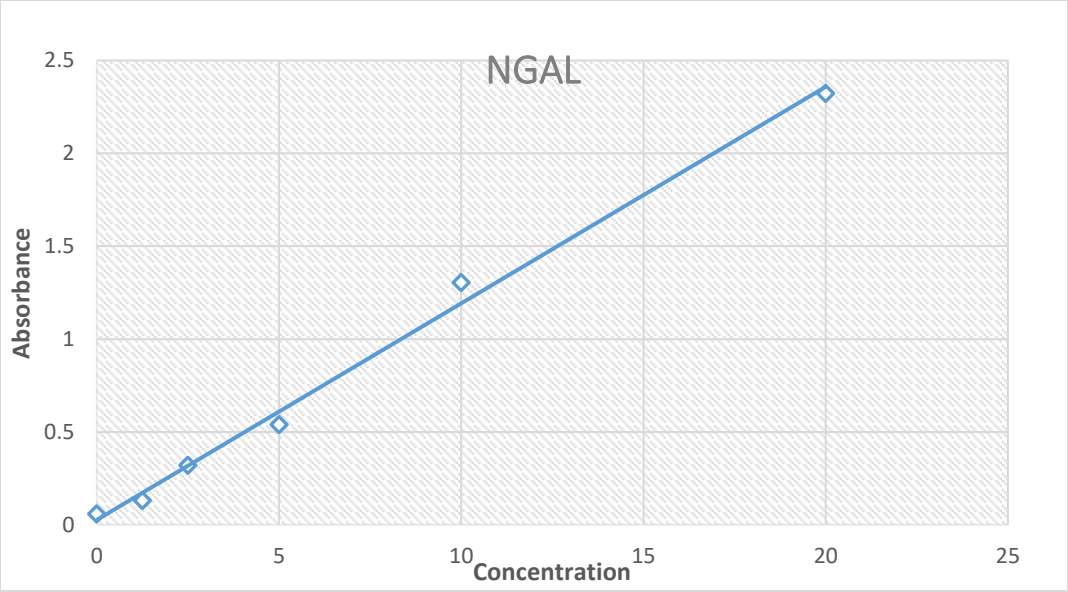
5. Added 100µL prepared **Detection Reagent B**. Incubated 30 minutes at 37ºC;

6. Aspirated and washed 5 times;

7. Added 90µL **Substrate Solution**. Incubated for 10-20 minutes at 37ºC;

8. Added 50µL **Stop Solution**. Read at 450nm immediately.

Standard curve for NGAL



Standard Curve for NGAL.

Detection of Cystatin C (Cloud clone\USA)

The Cystatin C test: Is a specific test for diagnosing patients with Kidney disease. The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Cystatin-C in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids.

Principle

The test utilizes antibodies including a recombinant protein on the cellulose membrane with colloidal gold marked Cys C antigen as a tracer. The reagent is used to detect the antibody in serum according to the principle of double antibody sandwich and gold immunochromatography assay. The color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Cys-C in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Procedure

1. Prepared all reagents, samples, and standards;

2. Added 100µL standard or sample to each well. Incubated for 1 hour at 37ºC;

3. Aspirated and added 100µL prepared **Detection Reagent A**. Incubated 1 hour at 37ºC;

4. Aspirated and washed 3 times;

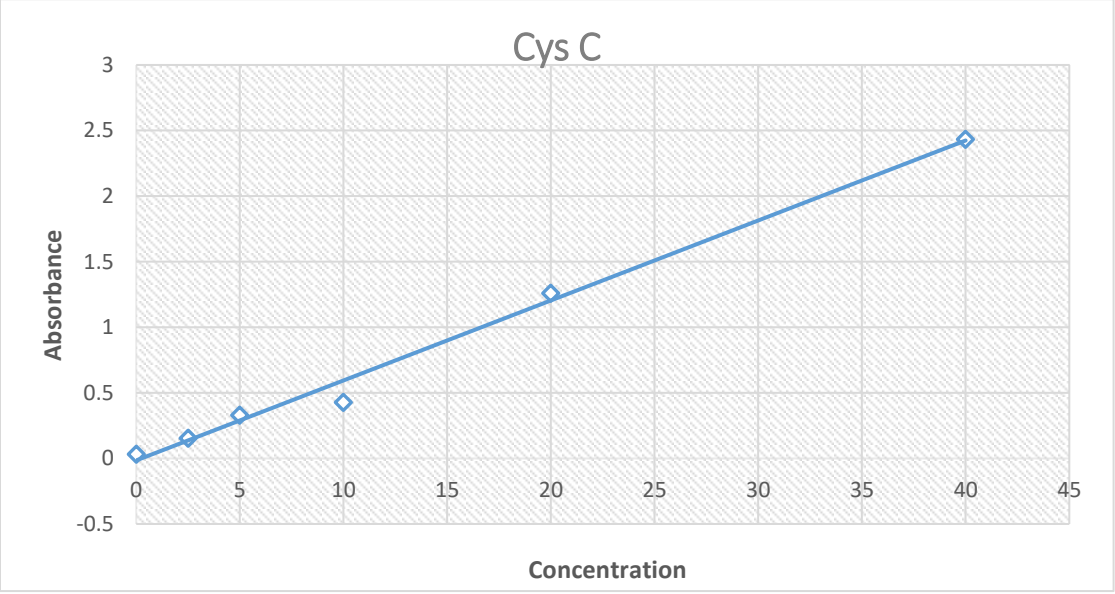
5. Added 100µL prepared **Detection Reagent B**. Incubated 30 minutes at 37ºC;

6. Aspirated and washed 5 times;

7. Added 90µL **Substrate Solution**. Incubated for 10-20 minutes at 37ºC;

8. Added 50µL **Stop Solution**. Read at 450nm immediately.

Standard Curve for Cystatin C



Standard Curve for Cystatin C

Appendix VI

Estimation of Renal Function Tests

Determination of Creatinine by Cobas Integra 400 plus (Roche COPAS\Germany)

Principle

This kinetic colorimetric assay is based on the Jaffé method. In an alkaline solution, creatinine forms a yellow-red complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. To correct for non-specific reactions caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -18 µmol/L (-0.2 mg/dL) (Jaffe *et al*.,1886 &Fabiny DL, *et al*.,1971).

Alkaline pH

Creatinine + picric acid ---------- > yellow-red complex

Reagents

R1 = Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH ≥ 13.5

SR = Picric acid: 38 mmol/L; pH 6.5; non-reactive buffer

R1 is in position B and SR is in position C

Calculation

Roche\Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Determination of Urea by Cobas Integra 400 plus (Roche COPAS\Germany)

Principle

Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction, 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce-glutamate. In this reaction, two moles of NADH are oxidized to NAD+ for each mole of urea hydrolyzed.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{-2}$$

$$NH_4^+ + \text{2-oxoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{L- glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and measurement photometrically (Richterich R, *et al* .,1978 & Talke H, *et al*.,1965 & Tiffany TO, *et al*.,1972 & Sampson EJ, *et al*.,1980).

Reagents

R1 = NaCl 9%

R2 = TRIS buffer: 220 mmol\L;2-oxoglutarate: 73 mmol\L; NADH: 2.5 mmol\L; ADP:6.5 mmol\L; urease (jack bean): ≥ 300 µkat\L; GLDH (bovine): ≥ 80 µkat\L; stabilizers; Ph 8.6.

Calculation

Roche\Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Determination of Electrolyte Sodium (Na), Potassium (K), and Chloride (Cl) by EX-DS Electrolyte Analyzer (JOKOH\Japan)

Principle

The principle of electrolyte identification in serum was measured by using an auto-chemistry analyzer following the criteria mentioned in the Kits instructions by the manufacturer.

Procedure

Fully Automated Procedure

1. Set the slide (Dry Slide Reagents).
2. Set the sample.
3. Press START.

Appendix VII

Estimation of Hematological Parameters;

Determination of CBC to calculate hemoglobin (Hb), white blood cells (WBC), and red blood cells (RBC)**;** Determination of Complete Blood Count (CBC) by Automated hematology analyzer (Swe lab Alfa PLUS)\ Sweden

Principle

The Swe lab Alfa Plus system is a multi-parameter quantitative automated hematology analyzer for in vitro diagnostic use under laboratory conditions. The Swe lab Alfa Plus is used for the enumeration of white blood cells (WBC); red blood cells (RBC); hemoglobin (Hb); and another blood component in EDTA anti-coagulated whole blood samples.

Reagent Setup

The Swe lab Alfa Plus system is interlocked with specified Boule reagents, Alfa Diluent and Alfa Lyse (hereafter referred to as Diluent and Lyse), for optimal performance. The reagent containers must be identified by the analyzer before the analysis of samples can begin.

Procedure

The device counts and sizes RBC and Hemoglobin using hydrodynamic impedance counting (sheath flow DC method). At the same time.

Sets of WBC, RBC, and Hb rules determine how positive parameter flags are handled by the instrument and/or operator and which samples are auto-validated by the DI. Manual differentials and RBC/Platelet morphology are entered via the DI. Pending Orders are checked via the Outstanding List in

EPIC (Whitepaper: Hematology analyzers: 3-part or 5-part, that is the question. Boule Diagnostics, WP31183, Edition 4 (2021).

Appendix VIII

Estimation of Biochemical Parameters

Determination of Albumin (Colorimetric Method) by (LINEAR CHEMICAL S.L\Spain)

Principle

The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample (Doumas, *et al*., 1971).

BCG + Albumin ----------→ BCG-albumin complex

Reagents

R1 Bromocresol reagent. Succinate buffer 75 mmol/L pH 4.2, BCG 0.12 mmol/L, Tensioactive 2 g/L (w/v).

CAL Albumin standard. Bovine serum albumin 5 g/dL (50 g/L)

Procedure

1. Bring reagents and samples to room temperature.

2. Pipette into labelled tubes:

3. Mixed and let the tubes stand for 1 minute at room temperature.

4. Read the absorbance (A) of the samples and the standard at 630 nm against the reagent blank.

The color is stable for 30 minutes protected from light.

Calculation

A Sample

———— x C Standard = g/dL albumin

A Standard

Samples with concentrations higher than 6 g/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

If results are to be expressed as SI units apply: g/dL x 10 = g/L (Tietz. N.W.,1987).

Reference values

Serum, plasma

The range of values for hospitalized individuals varies between 1.4

and 4.8 g/dL (Tietz.*et al*., 1976).

Determination of CRP by Cobas Integra 400 plus (Roche COPAS\Germany)

Principle

The latex particles coated with anti-CRP are agglutinated when they react with samples that contain C-reactive protein (CRP). The latex particle agglutination is proportional to the concentration of the CRP in the sample and can be measured by turbidimetry at 525 nm and 625 nm (Senju O, *et al*.,1986 &Price CP, *et al*., 1987 & Eda S, *et al*.,1998).

Reagents

HEPES buffer:1.79 mg

Anti-human CRP antibody (goat) Latex-conjugate:41.84 µg

Procedure

1- Insert the disc into the cobas b 101 instrument. Close the lid.

2- The measurement starts automatically

Calculation

Roche\Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

الخلاصه:

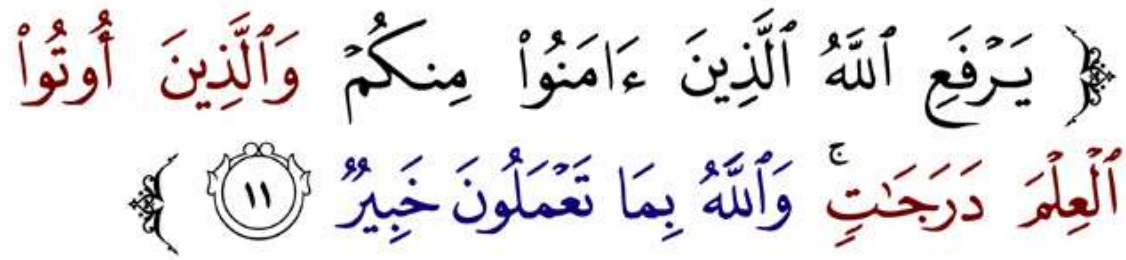
تعرَّف الإصابة الكلوية الحادة بأنها زيادة سريعة في نسبة الكرياتينين في المصل، انخفاض في إنتاج البول، وانخفاض سريع في معدل الترشيح الكبيبي.

وقد تضمنت الدراسة الحالية (120) فردًا من الذكور الأطفال بمتوسط عمر (5.94 ± 4.9) سنة تم جمعهم من مستشفى كربلاء التعليمي للأطفال خلال الفترة من سبتمبر 2023 إلى فبراير 2024 مقسمين إلى مجموعتين (30) من الذكور السليمين استخدموا كمجموعة سيطره و (90) من المرضى الذكور المصابين بالإصابة الكلوية الحادة ثم تم تصنيفهم إلى ثلاثة أقسام فرعية وفقًا (RIFLE)على النحو التالي : تشمل المرحله 1 و المرحله 2 و المرحله 3 (32) و (27) و (31) ثم تم تقدير تركيز هذه العلامات الكيميائيه الحيويه الليبوكالين المرتبط بالجيلاتيناز و جزئ أصابه الكلى الحاد -1 والسيستاتين- سي وجدت النتيجة الحالية فرقًا كبيرًا و (5.69 ± 1.23) و (12.50 ± 2.88) (1533.36 ± 404.21) في زيادة $P \leq 0.001$ عند على التوالي مقارنةً بمجموعه السيطره (716.64 ± 50.72) و (2.30 ± 0.28) و (5.32 ± 1.71 $P \leq$) على التوالي في جميع المراحل المستخدمة في الدراسة. وجدت النتيجة فرقًا كبيرًا عند في عمر المرضى - العمر الأصغر الموجود في المرحلة (3) (4.31 ± 4.03) سنة. كما 0.001 أظهر مؤشر كتلة الجسم انخفاضًا كبيرًا لدى جميع المرضى (14.45 ± 1.9) مقارنةً بمجموعه (1.81 ± 1.61) $P \leq 0.001$ زيادة كبيرة عند BUN السيطره. أظهر الكرياتينين واليوريا و ال و(104.38 ± 65.27) و(16.24 ± 10.15) على التوالي مقارنه بمجموعه السيطره (0.42 ± 0.13 P) و(20.61 ± 5.13) و(3.21 ± 0.80) على التوالي، بينما كان هناك انخفاض كبير عند في ناتج البول لجميع المراحل مقارنة بمجموعه السيطره، وكان الانخفاض الأعلى في $0.001 \leq$ المرحلة (3) (0.14 ± 0.09) مقارنه بمجموعه السيطره (91.56 ± 0.46)، كما وجد ارتباط والهيموغلوبين والألبومين ومخرجات البول (-0.295) و(-0.458) و(- KIM-1 سلبي بين والبروتين التفاعلي سي واليوريا والكرياتينين KIM-1 0.629) على التوالي وارتباط طردي بين واليوريا البولية (0.279)، (0.366)، (0.236)، و (0.366) على التوالي، وجد ارتباط عكسي والهيموجلوبين وخلايا الدم الحمراء والألبومين ومخرجات البول (-0.315)، (- NGAL بين Cystatin c مع NGAL 0.166)، (-0.508)، و (-0.727) على التوالي وارتباط طردي بين

واليوريا والكرياتينين والنيتروجين في الدم (0.312)، (0.456)، (0.231)، و (0.455) على التوالي، وجد ارتباط عكسي بين سيستاتين سي والهيموجلوبين والألبومين ومخرجات البول (-0.299)، (-0.453)، و (-0.675) على التوالي وارتباط طردي بين سيستاتين سي مع البروتين التفاعلي سي واليوريا والكرياتينين والنيتروجين في الدم (0.278)، (0.366)، (0.214)، و (0.366) على التوالي.

ومعدل الترشيح الكبيبي sCr أظهرت نتائج الدراسة أن معدل الترشيح الكبيبي المقدر بواسطة Cys C ومعدل الترشيح الكبيبي بواسطة Cyc Cو sCr بواسطة $P \leq$ أظهر انخفاضًا كبيرًا عند (49.60 ± 27.64) و (20.68 ± 7.85) و (7.32 ± 2.84) على التوالي في جميع 0.001 المرضى مقارنةً بمجموعه السيطره (109.71 ± 13.06) و (50.30 ± 7.73) و (16.30 ± 5.07 Cystatin C) على التوالي، وأعلى انخفاض في معدل الترشيح الكبيبي الموجود في لجميع KIM-1 و المراحل. وفي الختام، أظهرت الدراسة الحالية أن المؤشرات الحيوية التي تحتوي على NGAL و Cystatin C لـ AKI. كان لها أفضل ضمان تنبؤي وكشفت الدراسة أن معدل يعكس انخفاضًا في معدل الترشيح الكبيبي مع تفاقم Cystatin C الترشيح الكبيبي القائم على بشكل أفضل من معدل الترشيح الكبيبي القائم على الكرياتينين مما يعني علامة أفضل AKI لوظائف الكلى في المرحلة المبكرة من AKI.

بسم الله الرحمن الرحيم



صدق الله العظيم

المجادله ايه (11)

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم المختبرات السريرية

تقييم مستويات الليبوكالين المرتبط بالجيلاتيناز NGAL وجزيء إصابة الكلى -1 والسيستاتين- C للتنبؤ المبكر بمضاعفات إصابة الكلى الحادة (AKI) للمرضى الاطفال الذكور في محافظة كربلاء

رسالة مقدمة

الى مجلس كلية العلوم الطبيه التطبيقيه-جامعه كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

بواسطة

هاله رياض حسن

بكالوريوس تقنيات التحليلات المرضية (2017)

بأشراف

ا.د.عبير جياد يوسف الفتلاوي **د. قحطان محمد علي العبيدي**

1446\ه **2024\م**