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Interleukin-18 and Tumor Necrosis Factor Gene Polymorphisms and other Biochemical Markers as Risk Factors for Contrast Induced Nephropathy

A thesis

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{Clinical Chemistry}

By

Hayder Mohammed Hussein Nassrallah

(B.Sc. in Chemistry, University of Al-Mustansiriyah, 2002-2003)

Supervised By

Professor

Dr. Fadhil Jawad Al-Tu'ma

Department of Chemistry and Biochemistry

Professor

Dr. Riyadh Muhi Al-Saegh

Department of Internal Medicine

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Dedication

To ...

My source of inspiration who helped and courage me in every stage throughout my life… My Father,

I dedicate this study to his soul Allah Almighty bless him....

To ….

My family: my mother, my wife, my sisters and my brother for supporting me spiritually throughout my life.

Respect and Love...

Hayder 2022

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Hayder M.Hussein

Supervisor's Certification

We certify that this M.Sc. thesis entitled:

{Interleukin-18 and Tumor Necrosis Factor Gene Polymorphisms and other Biochemical Markers as Risk Factors for Contrast - Induced Nephropathy}

was prepared by (**Hayder Mohammed Hussein Abbas**) under our supervision at the College of Medicine / University of Kerbala, as a partial requirement for the Master degree in **Clinical Chemistry**.

Prof. Dr. Fadhil Jawad Al-Tu'ma
Department of Chemistry and Biochemistry
University of Kerbala

Prof. Dr. Riyadh Muhi Al-Saegh
Department of Internal Medicine
University of Kerbala

In the view of the available recommendation, we forward the present thesis for debate by the examining committee.

Lect. Dr. Atheer Hameid Odda
Head of Department of Chemistry and Biochemistry
College of Medicine - University of Kerbala

Examining Committee Certification

We, the examining committee, certify that we have read this M.Sc. thesis entitled: -

{Interleukin-18 and Tumor Necrosis Factor Gene Polymorphisms and other Biochemical Markers as Risk Factors for Contrast Induced Nephropathy}

We have examined the postgraduate student **(Hayder Mohammed Hussein Abbas)** in its M.Sc. thesis content and, in our opinion; it meets the standard for the degree of master in **(Clinical Chemistry)** and it is adequate with **(Excellent).**

Signature:

Prof. Dr. Ahmed Hussein AL-Mayali
College of Medicine
University of Kerbala
Date: / / 2023

(Member)

Signature:

Assist. Prof. Dr. Rana. M. Hameed
College of Medicine
University of Kerbala
Date: / / 2023

(Member)

Signature:

Prof. Dr. Fadhil Jawad Al-Tu'ma
College of Medicine
University of Kerbala
Date: 1 / 1 / 2023

(Member / Supervisor)

Signature:

Prof. Dr. Riyadh Muhi Al-Saegh
College of Medicine
University of Kerbala
Date: / / 2023

(Member / Supervisor)

Signature:

Prof. Dr. Mufeed Jalil Ewadh
College of Medicine - University of Babylon
Date: / / 2023
(Chairman)

Approved by the College of Medicine - University of Kerbala

Signature:

Date: 9 / 1 / 2023
Prof. Dr. Riyadh Dayhood Al-Zubaidi
Dean of the College of Medicine

Summary

Contrast-induced nephropathy is a significant complication of angiographic procedures caused by contrast media injection, and considered as third most frequent cause of hospital-acquired acute renal failure which represents about 12% of the cases. Contrast-induced nephropathy is defined as an increase in serum creatinine of 0.5 mg/dL (44 μmol/L), or a relative 25 % increase from the baseline value, within a period of 24 hours following intravascular administration of contrast media. Despite being a widespread disease, the pathophysiology of contrast-induced nephropathy is still incompletely understood and developed at a rate of about 14.5%. In-hospital mortality for patients with contrast-induced nephropathy was 7.1% in those who did not require dialysis and 35.7% in those who did, compared to 1.1% for patients with no contrast-induced nephropathy.

The aim of the present study is to investigate the impacts of contrast media following therapeutic coronary angiography on interleukins-18 and tumor necrosis factor alpha serum levels. To investigate the role of both genotypes (rs1946518) interleukin-18 and (rs361525) tumor necrosis factor alpha gene polymorphisms as susceptible markers after percutaneous coronary intervention.

The study was conducted in cardiac catheterization unit at Karbala Center of Cardiology, and Al-Kafeel super specialty hospital, Karbala health directorates, Kerbala, Iraq. The study included 60 patients (47 males, 13 females) who underwent elective or emergency coronary angiography. Clinical examination and laboratory investigations were done before and 24 hours after interventions. These investigations include: serum creatinine, blood urea, estimated glomerular filtration rate, serum tumor necrosis factor alpha and serum interleukin-18. *Molecular analysis*: interluekine-18 tag SNPs (rs1946518) and tumor necrosis factor alpha tag SNPs (rs361525) were analyzed by Allele-specific PCR. Patients with incomplete data, those who underwent diagnostic coronary angiography only, and those who have other causes of acute kidney injury were excluded from this study.

The contrast agent has a risk to induce acute kidney injury represented by a significant rises in serum creatinine and blood urea ($p < 0.001$) 24 hours after coronary angiography. Past history of type 2 diabetes mellitus, and ischemic cardiac failure had a statistically significant adverse effect on the prevalence of contrast induced acute kidney injury ($p=0.001$, 0.003 and 0.008) respectively. There were a significant rising of interleukin-18 serum level after contrast media exposure ($p = 0.001$), while no significant deference was found in tumor necrosis factor alpha. When compared to (no contrast-induced nephropathy) controls, the (+ contrast-induced nephropathy) patients showed increased frequencies of CA+ AA (rs1946518) genotypes in interleukin-18 (OR =0.8 (1.020-622.737), $p = 0.048$; OR = 1.029 (1.026-1.029), $p = <0.001$, respectively). Significant different rates of serum creatinine and estimated glomerular filtration rate after percutaneous coronary intervention in (rs1946518) genotypes interleukin-18 ($p = 0.014$, 0.024, respectively).

In conclusion, there was an elevation of interleukine-18 after therapeutic coronary interventions as a key factor behind contrast agent induced acute kidney injury. Gene polymorphisms of (rs1946518) interleukin-18 is associated with contrast-induced nephropathy risk and renal outcome after percutaneous coronary intervention.

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List of Abbreviations

Abbreviations	Description
ACE	Angiotensin-converting Enzyme.
AKI	Acute kidney Injury.
ARDS	Acute Respiratory Distress Syndrome.
ATN	Acute Tubular Necrosis.
BMI	Body Mass Index.
CAG	Coronary Angiography.
CCAU	Coronary Catheterization Unit.
CI-AKI	Contrast-induced Acute kidney Injury.
CKD	Chronic kidney Disease.
CM	Contrast Media.
CMSC	Contrast Media Safety Committee.
CT	Computed Tomography.
Cys C	Cystatin C.
ESKD	End-stage Kidney Disease.
GBCA	Gadolinium-Based Contrast Agents.
GFR	Glomerular Filtration Rate.
GN	Glomerulonephritis.
IOCM	Isotonic Osmolality Contrast Media.
KDIGO	Kidney Disease Improving Global Outcomes.
LOCM	Low-osmolality Contrast Media.
MDRD	Modification of Diet in Renal Disease.
MRI	Magnetic Resonance Imaging.
MRS	Mehran CIN-Risk Score.
PCI	Percutaneous Coronary Intervention.
ROS	Reactive Oxygen Species.
RRT	Renal Replacement Therapy.
S.Cr	Serum Creatinine.
STEMI	ST-segment Elevation Myocardial Infarction.
UTI	Urinary Tract Infection.
SOD	Superoxide Dismutase.
GSH	Glutathione.
MDA	Malondialdehyde.

Chapter ONE

Introduction and Literature Review

1. Introduction.

The kidney is responsible for a wide variety of important functions, some of which are the regulation of homeostasis, blood pressure, and erythropoiesis, the facilitation of acid-base balance; the excretion of nitrogenous wastes, detoxification, and filtration. Any alteration to these physiological processes induces to kidney injury, which can lead to kidney disease. Diseases and conditions such as glomerulonephritis, polycystic kidney disease, infections of the urinary tract, and kidney stones are among the most serious **(Chmielewski, 2003)**. The underlying conditions of coronary disorders, diabetes, hypertension, malaria, or the infection aroused by viruses like HIV may also play a role in the development of kidney diseases. In addition, nephrotoxicity, also known as kidney damage, can be caused by exposure to high concentrations of environmental toxins, certain classes of drugs, or drug metabolites. This type of injury is commonly referred to as nephrotoxicity. **(Nderitu *et al*., 2013)**. Renal impairment is present in almost one-third of patients have coronary artery disease **(Neumann *et al*., 2019)**. It's a well-known fact, independent risk factor for adverse cardiovascular outcomes **(Mehran *et al*., 2009)**. Likewise, cardiovascular disease continues to be the most prevalent cause of death in people who have chronic kidney disease **(Tonelli *et al*., 2006)**. Importantly, a decline in renal function reflected by glomerular filtration rates (GFR) of less than 60.0 mL per minute per 1.73 m2 of body surface area has been linked to an increase in the incidence of cardiovascular disease **(Anavekar *et al*., 2004)**. A higher risk of hospitalization and long-term mortality results from this association **(Neumann *et al*., 2019)**. In individuals with acute coronary syndrome, even a moderate decline in renal function is prognostically significant **(Gibson *et al*., 2004)**. There are likely multiple factors involved in the poor outcomes of renal impairment

following acute coronary syndrome or percutaneous coronary procedures. Traditional risk factors like hypertension, diabetes, and dyslipidemia are present in both chronic renal disease and cardiovascular disease **(Said *et al.*, 2014)**. On the other hand, individuals with renal impairment were shown to have more aggressive and accelerated atherosclerosis, which made percutaneous coronary interventions (PCI) more difficult, with lower rates of procedural success and a higher proportion of coronary stenosis remaining after treatment **(Herzog *et al*., 2011)**. Previous studies have improved the adverse renal impairment prognosis following PCI and contrast media (CM) delivery **(Rihal *et al*., 2002)**. One of the cornerstones of modern diagnostic imaging is the administration of iodine-based CM, which enables precise visualization of minute anatomic structures and pathologic conditions during interventional procedures, conventional radiography, and computed tomography (CT) scanning in radiology **(Biondi-Zoccai *et al*., 2014)**. Additionally, CM are essential for a number of therapeutic processes. Contrast agents have still historically been viewed as potentially dangerous due to the possibility of adverse reactions **(Kwok *et al*., 2013)**. One of the more common untoward effects associated with CM administration is CIN. Contrast induced nephropathy is also known as contrast-induced acute kidney injury (CI-AKI) **(Solomon, 2008)**. In 1950s, it was first described in case reports of deadly acute renal failure following intravenous pyelography with patients who had renal disease arising from multiple myeloma **(Rear *et al*., 2016)**. The 2018 guidelines of the CM Safety Committee (CMSC) of the European Society of Urogenital Radiology (ESUR) **(van der Molen *et al*., 2018)** suggest a diagnosis for CI-AKI if there is an increase of serum creatinine (S.Cr) of at least 0.3 mg/dL (26.5 μmol/L) or at least 1.5 times above baseline within 48 to 72 h of intravascular administration of a CM. The Chinese expert consensus **(Chen *et al*., 2014)** defined CI-AKI as an increase in the S.Cr level of 25% (or 0.5 mg/dL) above

baseline 24 hours after administration of a CM. Contrast association acute kidney injury nephropathy (CA-AKI) is a general phrase used to describe patients whose acute kidney injury (AKI) developed shortly after they received iodinated CM. Iodinated (contain iodine atoms) CM is being commonly used for both therapeutic and diagnostic catheterization **(Tehrani *et al.*, 2013)**. An important cause of hospital acquired AKI is administration of radiography CM which increases mortality while hospitalized **(Tanaga *et al.*, 2012)**.Therefore, tremendous efforts have been made to create contrast agents with the least amount of danger of renal damage **(Nunag *et al.*, 2009)**.This has resulted in a switch from older agents with high osmolarity to newer agents with decreased osmolarity, as this chemical feature has been highlighted as a potentially significant determinant in renal safety **(Sendeski, 2011)**.

Inflammatory process has been identified as a major player in the pathophysiology of AKI in recent years **(Kinsey *et al.*, 2008 and Akcay *et al.*, 2009)**. Infiltration of inflammatory cells in the injured kidney has been noted. These cells are believed to play significant roles. Releasing oxygen radicals and vasoconstrictors, causing and maintaining kidney damage, and mediating direct endothelial injury can be caused by releasing endothelin and preventing the release of nitric oxide **(Akcay *et al.*, 2009)**. Given that inflammation plays a significant role in the onset of AKI, polymorphisms in inflammation-related individual's susceptibility to AKI may be influenced by genetics. Genetic factors have been suggested that played a role in the inter-individual variations in AKI susceptibility. Numerous Studies have been carried out to determine the association between different genetic polymorphisms and risk of AKI **(He *et al.*, 2018)**. The majority of AKI genetics studies involve hypothesis-driven (as

opposed to hypothesis-generating) candidate gene investigations that have consistently failed to uncover contributing variants. Although only a few unbiased, massive studies have been conducted, there is still a serious need for more genetic studies **(Larach *et al*., 2018)**. Genetic factor have been considered as potentially responsible for AKI susceptibility and severity, in attempt to explain how only particular patients are related to AKI, and why different patients respond differently to treatment **(Weinstein *et al*., 2013)**.

Studies were searched up until the year 2007 by Lu, Coca *et al.*, and up until 2011 by Cardinal-Fernández *et al*. Both came to the conclusion that there is currently no direct evidence linking a particular genetic variation to AKI **(Lu *et al*., 2009 and Cardinal-Fernández *et al*., 2012)**.

1.1. Kidney Diseases.

Pathologically, several types of renal diseases can differ greatly with factors such as patients, ethnicity, sex, age group, and geographical location/origin **(Rafique *et al*., 2022)**. The various nephron components are anatomically closely related to one another and depend on a common blood supply. All parts of the nephrons are affected to some extent by renal dysfunction of any kind, though occasionally either glomerular or tubular dysfunction is predominate. The net effect of renal disease on plasma and urine is determined by the ratio of damaged glomeruli to tubules and the number of nephrons **(Martin *et al*., 2012)**. Increasing urine albumin and excess fluids and (or) impaired GFR a potentially significant marker for renal dysfunction **(Levey *et al*., 2013)**. Broadly the kidney injury or nephrotoxicity is classified into two classes: AKI and chronic kidney injury (CKD). According to studies, AKI is one of the severe types of kidney injury that affects 13 million people across the globe which has a population of 85% population includes from developing countries. Acute kidney injury is present in 21% of adults worldwide, with a mortality incidence of 13.8% (Sri

Laasya, Thakur *et al*. 2020). The final and most lethal form is CKD, which affects 10.4% of male and 11.8% of female worldwide. Kidney stones, which afflict 2% to 3% of the world's population, are another type of kidney damage **(Mear *et al*., 2017)** Glomerulonephritis (GN) linked with 10% cases of CKD and finally the Urinary tract infections (UTIs) **(Sri Laasya *et al*., 2020)**.

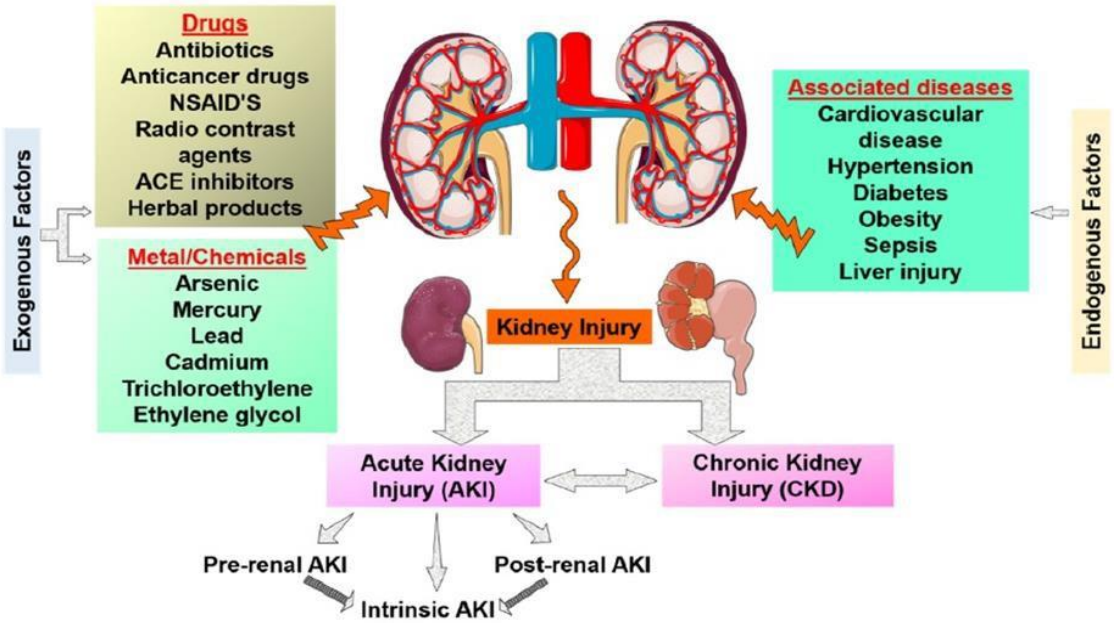


Fig. (1-1): Illustrative Representation of Various Factors Involved in Kidney Injury and the Correlation between AKD and CKD Involving Pre-and Post-renal AKI (Fevrier-Paul *et al*., 2018).

The average GFR of a healthy adult individual is 100–125 mL/min. An increase or decrease in the GFR can lead to a variety of kidney injury symptoms and eventually to renal failure. The failure could cause hemodynamic abnormalities, direct cellular and tissue damage, and obstruction of renal excretion, which would cause nephrotoxicity.

This toxicity is also brought about by instances of kidney-specific detoxification and failure, as well as inefficient excretion, which can be brought

on by underlying illness conditions or the presence of toxicants, as illustrated in Figure: 1.1 **(Fevrier-Paul *et al*., 2018)**.

1.1.1. Acute Kidney Injury.

Acute kidney injury is a highly complex clinical disease that is characterized by a rapid rate of decreased GFR, manifested by an increase in S.Cr concentration or oliguria, or both **(Manohar *et al*., 2019)**. Acute kidney injury is prevalent among hospitalized patients, affecting approximately 10%–20% of hospitalized patients, of whom 10% require renal replacement therapy (RRT) **(Thongprayoon *et al*., 2018)**. The incidence of AKI among seriously ill patients has been estimated to be as high as 45–50% **(Tao *et al*., 2013 and Ronco *et al*., 2019)**. Acute kidney injury is linked to high morbidity and mortality, additional costs incurred during the hospitalization process, longer hospital stays, and long-term consequences such as CKD and end-stage kidney disease (ESKD) **(Cerdá *et al*., 2015 and Thongprayoon *et al*., 2015)**. At 30 days after AKI, the overall mortality rate is as high as 24%. Around 1.7 million people worldwide are estimated to pass away from AKI each year **(Mehta *et al*., 2015)**. Conventionally, AKI is measured by the dynamic change in S.Cr. Cystatin C (CysC) appears to be a more precise biomarker for evaluating kidney function **(Hong *et al*., 2022)**. Dialysis independence is often acquired in people who survive their illness and do not have underlying chronic kidney disease. However, Research indicates that people who have experienced AKI are more likely to develop CKD in the future **(Bellomo *et al*., 2012)**.

According to the International Ascites Club (ICA) consensus in 2015, AKI was defined as an increase in S.Cr level ≥0.3 mg/dL (26.5 μmol/L) within 48 h or

≥50% over baseline within the first 7 days **(Hong *et al.*, 2022)**. In 2012, the Kidney Disease Improving Global Outcomes (KDIGO) **(Kellum, 2012)** gave out guidelines on the management of AKI to make the diagnosis process of the condition more standardized, and the severity of the disease was determined based on absolute or relative increases in S.Cr and further progressive extent of oliguria, baseline S.Cr is crucial in AKI diagnosis and classification **(Acosta-Ochoa *et al.*, 2019)**. Inaccurate establishment of baselines S.Cr can contribute to an incorrect diagnosis of AKI and can also have an effect on the overall prognosis of the outcomes associated with AKI **(Siew *et al.*, 2012)**. Acute kidney injury can be divided into three distinct groups based on its causes **(Hilton, 2007)**:

A. Decreased renal blood flow (40-80% of cases, pre-renal).

B. Parenchymal renal damage (35-40% of cases, intrinsic renal)

C. Obstructed urine flow (2-10% of cases, post-renal).

1.1.1.1. Pre-renal Failure.

Pre-renal renal failure is due to insufficient nephron perfusion, which in ultimately decreases the GFR. Essentially, it is caused by an imbalance in the flow of nutrients and oxygen to the nephrons during times of elevated energy demand. Therefore, any action that alters systemic circulation or reduces renal perfusion can impair GFR. These auto-regulatory systems may not be sufficient to support the GFR in people who have a history of CKD. Pre-renal AKI can be caused by, but is not limited to, a variety of factors; intravascular volume depletion, cirrhosis, sepsis, heart failure, over diuresis, shock, hypotension, bilateral renal artery stenosis/solitary functioning kidney It is made much worse by angiotensin-converting enzyme (ACE) inhibitors, and by other medications as well (i.e., non-steroidal anti-inflammatory drugs (NSAIDs), calcineurin inhibitors, diuretics, etc.) **(Manzoor *et al.*, 2020)**.

1.1.2. Contrast Induced Nephropathy.

Contrast-induced nephropathy is a pathological condition that typically arises 48 hours following iodine-based CM exposure, characterized by an increase in S.Cr levels of more than 44.2 μmol/l (0.5 mg/dl) or 25%, compared to the baseline S.Cr level. The prevalence of CIN is increasing due to the widespread use of iodine contrast agents in the field of radiation diagnosis and interventional therapy. Contrast induced nephropathy is the third cause of AKI at the moment **(Zhang *et al*., 2020)**. The European Society of Urogenital Radiology defines CI-AKI as impairment in renal function (an increase in S.Cr by >0.5 mg/dl or >25% within 3 days following intravascular administration of contrast medium, without an alternative etiology). The AKI Network definition is a rise in S.Cr ≥0.3 mg/dl with oliguria, which is compatible with previous definitions and may be a new standard to follow **(McCullough Peter, 2008)**.

1.1.2.1. Incidence of Contrast Induced Nephropathy.

With the rapidly increasing use of contrast agents in medical imaging, contrast induced nephropathy has established as one of the primary causes of iatrogenic renal insufficiency **(Nash *et al*., 2002)**. Due to the lack of consistent diagnostic criteria and the inclusion of patients with diverse risk profiles, the findings of current epidemiology surveys on the incidence of CIN are inconsistent **(Zhang *et al*., 2020)**. In addition, the occurrence and severity of CIN are correlated with number of risk factors and their degree of severity. In a previous large retrospective study including 985,737 patients who underwent PCI report that the incidence of CIN was strongly correlated with the severity of baseline CKD and S T -segment elevation myocardial infarction (STEMI) presentation. Regarding patients with normal renal function (GFR ≥60 ml/min/1.73 m2), the occurrence of

CIN was 5.2%, and dialysis infrequently necessary in these cases (0.07%) **(Tsai *et al*., 2014)**. Unlike, patients with GFR ≤30 ml/min/1.73 m2 suffered from a morbidity of CIN as high as 26.6%, of which 4.3% demand for dialysis. Furthermore, an even higher incidence of CIN (36.9%) and dialysis requirement (7.2%) presented in severe CKD with STEMI **(Zuo *et al*., 2016) (Gurm *et al*., 2011)**. Other studies showed no difference in the incidence of AKI between patients with and those without intravascular contrast administration **(Wilhelm-Leen *et al*., 2017)**, suggesting that contrast agents may not affect the kidneys, and that the incidence of CIN is indeed underreported. The patients who undergo intravascular contrast administration have a lower risk of CIN and are managed more intensively than those who do not, which is a reasonable explanation for this. The true incidence of CIN is therefore unknown until uniform diagnostic criteria and a suitable control group have been developed to prevent confounding factors for patients undergoing procedures with intravascular contrast administration **(Zhang *et al*., 2020)**.

1.1.2.2. Pathogenesis of Contrast Induced Nephropathy.

The pathophysiological mechanisms of CIN have not been completely understood. At this time, several different mechanisms including direct effect, indirect effect, and formation of reactive oxygen species (ROS) have been suggested **(Kusirisin *et al*., 2020)**. In direct effects, high osmolality CM can directly cause cytotoxicity in nephrons, including renal tubular epithelial cells and endothelial cells, which can then lead to mitochondrial malfunction, cellular apoptosis or necrosis, and interstitial inflammation. While in indirect effects, CM can alter renal hemodynamics, leading to intrarenal vasoconstriction contributing to medullary hypoxia **(Mehran *et al*., 2019)**. In terms of ROS

formation, CM can either enhance ROS production excessively or decrease antioxidant enzyme activity, which causes an increase in oxidative stress and impairs renal function. Additionally, medullary hypoxia causes an increase in ROS, which in turn causes oxidative stress in the mitochondria and mitochondrial malfunction. This process is mediated by an increase in vasoconstrictive mediators, such as renin, angiotensin II, and endothelin, and a decrease in vasodilatory mediators, including nitric oxide and prostaglandin I2 (PGI2). Lastly, CM can produce ROS and also lower antioxidant enzyme activity as a result of numerous complicated pathways which result in oxidative stress, leading to progression of damage renal function **(Kusirisin *et al*., 2020)**. Overall, it is clear that oxidative stress and mitochondrial function are important factors in the pathogenesis of CIN **(Heyman *et al*., 2010)**. Therefore, methods that protect mitochondrial malfunction as well as reducing oxidative stress could be used to prevent CIN. However, the precise pathophysiology of CIN is unknown, and a number of factors, including renal ischemia, especially in the medulla, the formation of ROS, a decrease in the production of nitric oxide, and tubular epithelial and vascular endothelial injury, may be involved, see Figure: 2.1 **(Mamoulakis *et al*., 2017)**.

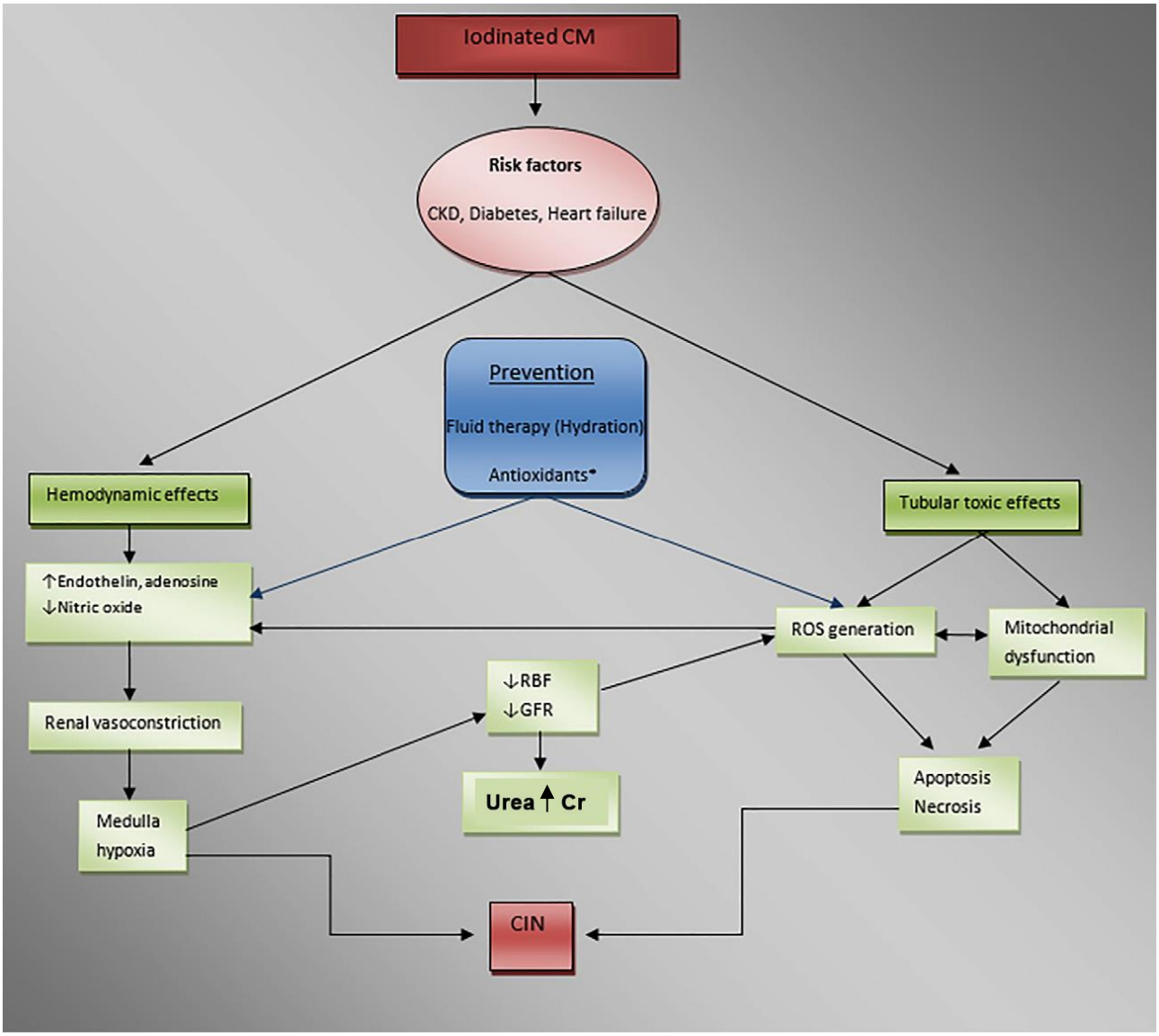


Fig. 1.2: Pathophysiology and risk factor of CIN. Several factors, such as renal ischemia, particularly in the medulla, ROS formation, reduction of nitric oxide production, tubular epithelial and vascular endothelial injury are interconnected. CM: contrast media, CKD: chronic kidney disease, RBF: renal blood flow, GFR: glomerular filtration rate, S.Cr: serum creatinine, S.Urea: serum urea, ROS: reactive oxygen species (Mamoulakis *et al*., 2017).

1.1.2.3. Contrast Media.

Contrast media (as well as called contrast agents or dye) are chemical substances frequently used in medical X-ray, CT, magnetic resonance imaging (MRI), angiography, and sometimes ultrasound imaging. Contrast media improve and enhance the images quality (or pictures), therefore, that the radiologist (a specialist doctor trained to examine the images and deliver a written report to the patient‘s own doctor or specialist) can provide a more accurate report on your body's functioning and whether any diseases or abnormalities are present **(Goergen *et al*., 2015)**. Iodinated and gadolinium-based CM are used on a daily basis in most radiology practices. These type of agents are essential to providing precise diagnoses, and almost always safe and effective when used properly. However, reactions to CM do happen and some of them can be life-threatening. Therefore, it is important for faculty to know how reactions to contrast agents manifest and how to deal with them promptly **(Beckett *et al*., 2015)**. Iodine-based contrast agents could be classified according to iconicity (ionic or nonionic), osmolarity (high, low, or iso), and benzene rings number (monomer or dimer), contrast agents type of nonionic cause less discomfort and fewer adverse reactions compared with ionic agents **(Thomsen, 2011)**. Acute reactions are much less likely to happen with low-osmolar contrast agents than with high-osmolar agents. Since the late 1980s, gadolinium chelates have been approved for intravascular use in MR imaging, and they have been well tolerated. Gadolinium-Based Contrast Agents (GBCA) are classified based on their iconicity (ionic or nonionic), the chelating ligand (macrocyclic or linear), their pharmacokinetics (extracellular or organ specific) and their potential for causing nephrogenic systemic fibrosis **(Thomsen, 2011)**. Both ionic and nonionic GBCAs can be administered for intravascular injection in comparison to iodine-based contrast agents with minimal to no difference in acute reactions **(Thomsen, 2011)**.

1.1.2.4. Mechanism of Contrast Induced nephropathy.

Contrast-induced nephropathy is among of adverse side effects of iodinated CM. The pathogenesis of CIN is associated to hemodynamic alteration caused by vasoconstriction, which results in a decrease in GFR in addition to renal ischemia. Direct cytotoxicity to renal tubular cells is an additional mechanism that leads to kidney injury. Direct cytotoxicity to renal tubular cell and medullary ischemia are two basic mechanism to result in CIN **(Yang *et al*., 2018)**. Medullary ischemia is a complicated outcome of vasoconstriction, lower oxygen delivery and higher oxygen demand. Contrast-induced nephropathy is caused by three mechanisms, including an increase in oxidative stress, an increase in renal vasoconstriction, and the induction of tubular cell **(Lameire, 2006)**. Numerous factors such a renal ischemia, particularly in the medulla, ROS generation, degreasing of nitric oxide production, tubular epithelial and vascular endothelial injury could be responsible to CIN. Many research suggested that iodinated CM exert cytotoxic effects and renal tubular epithelial cells present severe cell death by autophagy and/or apoptosis **(Mamoulakis *et al*., 2017)**. Iodinated CM result in renal vasoconstriction by increase of endothelin and adenosine, and shift the blood flow from the medulla to the cortex and GFR are decreased. Reduced renal blood flow can cause oxidative stress to generate more ROS. Iodinated CM directly induced osmotic necrosis or vacuolization in tubular cells, which resulted in acute tubular cell death **(Schräder 2005 and Wong *et al*., 2016)**. Numerous antioxidant compounds have been documented prevention effects by CIN, including N-acetylcysteine (NAC), sodium bicarbonate, statins, ascorbic acid , and later, phosphodiesterase type 5 inhibitors **(Prezzi, Khan *et al*., 2015)**.

1.1.2.5. Diagnosis of Contrast Induced Nephropathy.

In general CIN is a reversible form of acute renal failure that begins soon after iodinated CM administration following angiographic or other procedures such as urography, and it is identified as an increase in S.Cr level that mostly record within the first 48 hours after CM administration and peaks within 5 days next **(Wi *et al*., 2011)**. There are no established diagnostic standards for CIN or post-contrast AKI; however, S.Cr has been utilized extensively in the past, either as an absolute elevation of 0.5 to 2.0 mg/dL from baseline or as a 25% to 50% increase of baseline S.Cr values **(Mamoulakis *et al*., 2017)**. However, S.Cr is not a real-time indicator of altering renal function, though. It increases gradually in relation to how much filtration function is lost in contrast- induced nephropathy, delaying diagnosis by an average of 48–72 hours. Alteration in S.Cr level are not sensitive or specific for small alterations. Therefore, earlier biomarkers of acute kidney damage following coronary angiography are crucial to ensuring early intervention of renal damage development.

1.1.2.6. Prevention and Management of Contrast Induced Nephropathy.

Significant preventive strategy and intensive management are essential to reduce CIN incidence **(Zhang *et al*., 2020)**.

1.1.2.6.1. Proper Use of Contrast Media.

Because direct chemical toxicity is mostly determined by the physicochemical features of the CM, physicians should tread carefully when selecting a contrast agent, especially for patients with high-risk. The principles of contrast application include selecting a less-toxic iodinated CM and limiting contrast volume **(Zhang *et al*., 2020)**. Any iodinated CM can lead to CIN. However, the renal toxicity of different iodinated contrast agents is wildly variable. In present, low-osmolality CM (LOCM, e.g. 600–800 mOsm/ kg) and isotonic osmolality CM (IOCM, e.g.

290 mOsm/kg) have been frequently used for coronary angiography and PCI, while high- osmolality contrast media (e.g. 1500–1800 mOsm/kg) because of their higher nephrotoxicity compared with other iodinated contrast agent should progressively phased out of use **(Zhang *et al*., 2020)**. It could be predicted that a high osmolality of the CM would be correlated with increased renal deleterious effects. However, as recently reportedly, IOCM isn‘t preferable than LOCM. In actuality, there is little difference between LOCM and IOCM‘s nephrotoxicity levels **(Azzalini *et al*., 2018)**. This may be attributed to the higher viscosity of IOCM compared with LOCM **(Persson *et al*., 2005).** Interesting investigations have shown that in high-risk patients with renal insufficiency and diabetes, the nephrotoxicity of IOCM is weaker than that of LOCM **(Aspelin *et al*., 2003)**. For these reasons above, the condition of the patient should be taken into consideration when choosing the optimal type of contrast media.

1.1.2.6.2. Personalized Hydration.

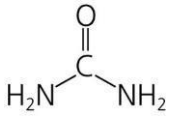
Recent research suggests that per-procedural hydration is the most effective preventive treatment for people at risk of CIN. It is recommended that non-dehydrated individuals should take 500 mL of water before the contrast procedure. In addition, 2500 ml of saline should be administered intravenously within 24 h following contrast exposure to keep a urine formation rate over 1 ml/kg/h. Saline should be given to high-risk patients for a further 12-24 hours after the contrast is given at a rate of 1 ml/kg/h in the absence of any signs of heart failure **(Andreucci *et al*., 2014)**. However, it has been questioned

if the standardized hydration volume is sufficient to reduce the incidence of CIN. Additionally, prophylactic fluids in high-risk patients may increase their risk of heart failure, arrhythmias, and short-term death **(Nijssen *et al*., 2020)**.

1.2. Biomarker for investigate CIN.

1.2.1. Urea.

The kidneys excrete urea, a waste product of metabolism, as urine. Reduced urea excretion and a corresponding increase in blood concentration are signs of kidney disease. Protein-derived amino acids are converted to ammonia via the process of deamination. The liver's enzymes then convert ammonia into urea. As a result, the body's ability to break down protein, the amount of urea that is properly excreted by the kidneys, and protein intake all affect the concentration of urea **(Salazar, 2014)**. Urea is a good analyte to assess renal function due to the body's dependency on the renal system to excrete urea. Urea is a small organic molecule (MW 60) consisting two amino (NH_2) groups and a linked carbamyl (C-O) group:



It is the principal nitrogenous end product of protein and amino acid catabolism. Proteins are firstly degraded to constituent amino acids, which are in turn degraded (deaminated), with production of ammonia (NH_3), which is toxic. By a set of five enzymatically regulated reactions, as known collectively as the ‒urea cycle‖, toxic ammonia resulting from protein degradation is converted to non-toxic urea **(Kazory, 2010)**. A small amount of urea (<10 %) is eliminated via gut and the sweat, but most of urea that produced in the liver is transported in blood to the kidneys where it is excrete from the body as urine **(Kazory, 2010)**. The normal kidney able excrete large amounts of urea. In case the rate of formation

exceed the rate of clearance, plasma concentration rise. The production rate is accelerated by:

A. Diet with high-protein.

B. Absorption of amino acids and peptides from digested blood after hemorrhage into the gastrointestinal lumen or soft tissues.

C. Due to starvation increased catabolism, sepsis, and tissues damage or steroid treatment **(Crook, 2013).**

The causes of increased and decreased plasma/serum urea **(Higgins, 2016)**.

A. Causes of increased plasma / serum urea concentration (i.e. urea > 7.8 mmol/l).

1. Renal diseases / failure (AKI or CKD)

2. Dehydration due to:

a. Low fluid intake

b. Excessive fluid loss (sweating, vomiting)

c. Diarrhea, diuretic drugs …. etc.

B. Decreased renal perfusion due to:

1. Heart failure

2. Hypovolemic shock

3. Severe hypotension

C. Gastrointestinal bleeding

D. Aging

E. High-protein diet

F. Catabolic states:

a. Trauma

b. Severe infections

c. Starvation

G. Some drugs with catabolic effects such as using corticosteroids.

1.2.2. Creatinine.

Creatinine, is a non-protein nitrogenous waste product, is formed from the degradation of creatine and phosphocreatine and can even act as an indicator of renal function. Creatine is synthesized in the liver, pancreas, in addition to kidneys from the transamination of the amino acids arginine, glycine, and methionine. Creatine then circulates throughout the body and is turned to phosphocreatine by the process of phosphorylation in the skeletal muscle and brain **(Salazar, 2014)**. The most of the creatinine is produced in the muscle. As a result, the concentration of plasma creatinine is effected by the muscle mass of patient. Compared to blood urea nitrogenous, creatinine is less influenced by diet and more precise as an indicator of renal function **(Salazar, 2014)**. Measurement of blood creatinine and serum urea nitrogen levels is extremely valuable for determining renal function in the clinical setting. Measurement of these two biomarkers are best observed in concert, evaluating their absolute levels as well as their correlation to one another. Serum creatinine level more accurate test because it is less affected by extra renal factors than the serum urea nitrogen level. Reproducibility of measurement is within 2% **(Lyman, 1986)**. The equations of S.Cr prediction are commonly used in screening and clinical settings in order to estimate GFR **(Drion *et al*., 2012)**. Creatinine has been used as a GFR indicator since the 1920s. It is an excellent marker of glomerular filtration since it is not protein-bound, is not metabolized in the kidney, and is freely filtered in the glomerulus. 10% to 20% of creatinine's excretion occurs by proximal tubular secretion, which causes overestimation of the true GFR, especially in patients with CKD **(McMahon and Waikar, 2013)**. Due to the intake of creatine during meals, the plasma creatinine concentration differs more than the urea concentration throughout the day. The plasma concentration of creatinine may be affected by sustained high-protein diets and catabolic states, but less so than the

plasma concentration of urea. However, the assay precise may be less than that of urea **(Royakkers, 2014)**. Increasing ingestion of cooked meat can be result in increase in S.Cr (which the heating of meat cooking could convert creatine to creatinine) or increased intake of protein and creatine food supplements, in excess of the recommended dosage. Creatine is found in the organs, muscles, and animals as body fluids of serum Creatine supplements are used to enhance sports performance because they promote protein synthesis and provide a rapid supply of energy for muscular contraction. In addition, hard exercise raises creatinine by accelerating muscle breakdown **(Samra and Abcar, 2012)**.

1.2.3. Glomerular Filtration Rate.

Glomerular filtration rate is determined as the urinary (or plasma clearance) of an optimal filtration marker **(Stevens *et al*., 2006)**. The process of glomerular filtration has been known for more than 160 years since Ludwig initially suggested in 1844 that the renal glomeruli produce a protein-free ultra-filtrate through physical and mechanical processes **(Smith, 1951)**. The measurement of the rate of formation of the GFR wasn‘t establish for real until 1926 and real precision was obtained as a result of the valuable researches of A.N Richards and Homer Smith **(Smith, 1951)**. Researches who described the inulin (a polysaccharide neither secreted nor reabsorbed by the renal tubules and which is filtered freely at the glomerulus) as a chemical substance for measuring GFR using the clearance method independently. The constant infusion of Inulin, combined with timed urine collection and plasma samples, with calculation of the Inulin Clearance (Cin), has become the "gold standard" for measuring GFR. Since then, various methods for estimating GFR have been developed that approximate the Cin. One of these is the clearance of endogenous creatinine (Ccr), which is a substance produced in muscle by the non-enzymatic dehydration of creatine. Due

to the fact that creatinine is normally secreted by the renal tubule, so The Ccr overestimates the Cin by about 22% **(Glassock and Winearls 2009)**. Determining GFR is essential in detecting kidney impairment disease, monitoring its progression, and making decision for treatment **(Pavkov, Nelson 2014)**. The normal values are approximately 120 ml minute per 1.73 m^2 in young women and 130 ml per minute per 1.73 m^2 in young men **(Stevens *et al*., 2006)**.

1.2.3.1. Measurement of Glomerular Filtration Rate.

Glomerular filtration rate cannot be readily identified in clinical practice, but it can be estimated using an equation based on S.Cr level, gender, race, age, and body size **(Levey *et al*., 2009)**. Equation for estimation of Ccr from serum creatinine measurements had been in use for many years **(Hogg *et al*., 2003)** mostly for assisting in drug dosing of patient that were nephrotoxic or those which were eliminated greatly by GFR the formula established in 1999 by Levey and co-workers **(Levey *et al*., 1999)** was one of the first comprehensive attempts to formulate an equation for estimating GFR (eGFR) from the direct measurement of a serum creatinine level.

Estimated GFR (eGFR) in ml/min/1.73 m^2 = 186 × (Serum creatinine in mg/dl) − 1.154 × (Age in years) − 0.203 × (0.742 if female and 1.21)

This equation was constructed using data from patients who participated in the Modification of Diet in Renal Disease (MDRD study), all of whom had established (non-diabetic) CKD and who had measurements using iothalamate clearance (Cio) as a surrogate for true GFR **(Glassock *et al*., 2009)**. The values of eGFR (in ml/min/1.73 m^2) are calculated from the S.Cr concentration (in mg/dL) using the modifier variables of age in years, ancestry (black vs non-

black) and sex as surrogates for endogenous creatinine production and excretion. When the Kidney Disease Outcomes Quality Initiative (KDOQI) recommendations for the assessment, classification, and staging of CKD were published by the National Kidney Foundation in 2002, the issue with eGFR measurement started to have far more significant clinical implications **(Hogg *et al*., 2003)**.

1.3. Interleukin-18.

It is a pro-inflammatory cytokine triggered by an inactive form of caspase-1, mononuclear cells, macrophages and non-immune cells including proximal tubule cells, produced it (Figure: 3.1) **(Charlton *et al*., 2014)**. Interleukin-18 is also a sensitive and specific marker that helps to determine the incidence of AKI, it is tested in serum and urine as well. This kind of cytokine is secreted by many cells in the human body, such as dendritic, small intestine cells, keratinocytes, cells osteoblasts, and epithelial cells of renal tubules **(Edelstein, 2016)**. When proximal tubular cells are exposed to nephrotoxic agents, hypoxia, ischemia, and AKI occur, there is an increase in IL-18 **(Solomon *et al*., 2006)**. It can be detected in urine quickly and accurately using commercially available ELISA and microbead-based assays. Urine IL-18 level rise within the first 6 hours after renal injury and reaches peak at 12-18 Hours. Urine IL-18 predicted the development of AKI in patient with Acute Respiratory Distress Syndrome (ARDS) network trial, even before 24 and 48 hours and the death of patient who have ARDS in ICU **(Shingai *et al*., 2014)**.

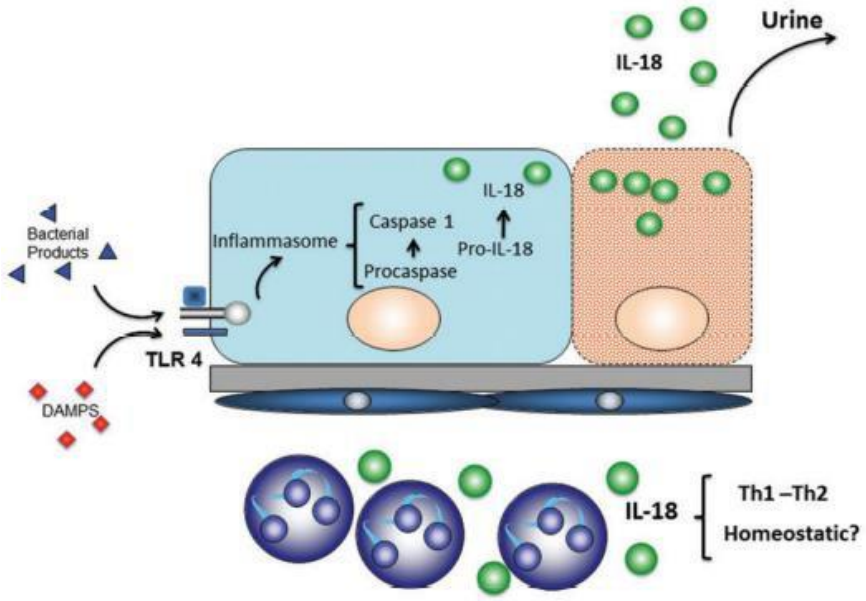


Figure: 1.3: IL-18. It is produced by immune cells and by active epithelial cells. Following activation of toll like receptor 4 (TLR4), activation of inflammasome leads to cleavage of pro-caspase 1 to caspase-1. This in turn cleaves pro-IL-18 into the active IL-18 molecule. IL-18 has prion-flammatory properties or may have homeostatic properties. (Charlton *et al*., 2014).

1.3.1. Structure of Interleukin-18.

With the exception of the segment between residues 34 and 43, the general structure of IL-18 is well known. 12 strands (S1-S12) of IL-18 combine to form three twisted four-stranded sheets, each of which has one short -helix (H1) and one 310-helix (H2). The three β-sheets are packed against each other to form a β-trefoil fold. The general architecture of IL-18 shows very similar to that of such IL-1 family members as IL-1β but IL-18 and IL-1 differ significantly in the length and shape of numerous segments between the strands, S3–S4, S4–S5, S7–S8 and S11–S12 **(Kato *et al*., 2003).**

1.3.2. Biological Function of Interleukin-18.

Interleukin-18 is a pro-inflammatory cytokine with a 22-kDa molecular weight that becomes more active during inflammatory processes that are involved in the activation and differentiation of various T cell populations (Figure: 4.1) **(Kaplanski, 2018)**. It mediates the effects of hypoxia on tissue. Urine IL-18 is an early, rapid, and inexpensive marker provides for the early diagnosis of kidney injury brought on by ischemia or nephrotoxins. But a little is understood about the serum IL-18's potential as an AKI biomarker **(Zdziechowska *et al*., 2020).** Renal tubular cells and macrophages both produce the cytokine IL-18, which is triggered by caspase-1. It has been demonstrated in animal experiments to be a mediator of acute tubular injury. In a variety of circumstances, including ischemia-reperfusion injury, sepsis, and cancer, IL-18 is produced in the proximal tubules of the human kidney and released into the urine. It can be rapidly and effectively measured in urine by commercially available ELISA and microbead-based assays. Urine IL-18 concentration raise within the first 6 h following renal injury and reaches peak at 12-18 hour **(Teo *et al*., 2017)**. Urine IL-18 was found to have intermediate diagnostic accuracy in a recent systematic evaluation of 11 trials involving 2796 individuals and was thought to offer potential as a biomarker for the early detection of AKI **(Lin *et al*., 2015)**.

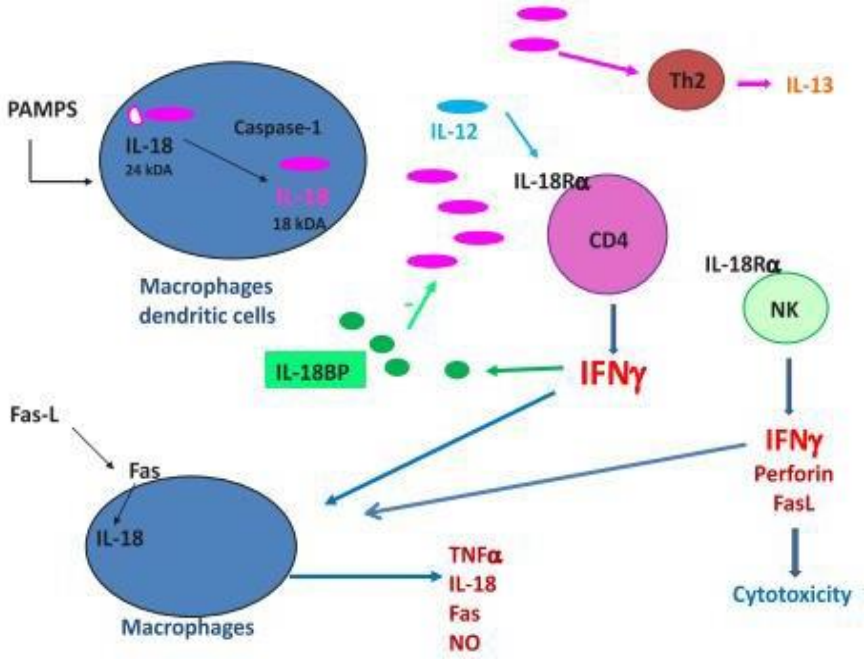


Figure: 1.4: Biological functions of IL-18. Activation of dendritic cells (DC) or macrophages may induce IL-18 precursor transcription, but IL-18 precursor is also constitutively present in the cells. Upon activation of NLRP3, pro-IL-18 is processed by caspase 1 and released in its 18 kDa mature form. In association with IL-12 or IL-15 which increase IL-18Rβ expression on T cells, IL-18 induces IFNγ production by CD4 T cells. IFNγ in turn, activates macrophages to produce inflammatory cytokines. IL-18 can also activate macrophages directly to induce chemokine secretion and NK cells to induce IFNγ secretion or to stimulate perforin- and FasL-mediated cytotoxicity. In macrophages, the interaction of FasL with Fas induces IL-18 processing by caspase 8. Alternatively, in the absence of IL-12 or IL-15, IL-18 activates Th2 CD4 lymphocytes to produce IL-13 and IL-4 (Kaplanski, 2018).

1.3.3. Interleukin -18 Level after Percutaneous Coronary Intervention Angiography.

In 150 patients undergoing coronary angiography, IL-18 was evaluated in a research. At 24 hours after the procedure, 13 patients (8.7%) developed AKI and a substantially higher IL-18 level compared to controls with an AUC of 0.75 (P = 0.011, odds ratio: 10.7). Additionally, this investigation demonstrated urine IL-18 to be an independent prognostic marker for late significant cardiac events [relative

risk: 2.09; P 0.01] 24 hours after contrast administration **(Connolly *et al*., 2015)**. Another research found that IL-18 significantly elevated within 2 hours of contrast angiography (P 0.05) and reached its peak at 24 hours later (P 0.01) **(Malyszko *et al*., 2009)**.

1.3.4. Interleukin-18 Promoter Polymorphism.

The IL-18 gene contains six exons and is found on chromosome 11q22.2-22.3. A single nucleotide polymorphism (SNP) in the IL-18 gene's promoter region, such as the SNP rs187238 (-137G/C), promote altered transcriptional activity of the gene in vitro and is related to serum IL-18 in vivo. It has also been linked to a number of illnesses, such as ischemic stroke, diabetic nephropathy, and cardiovascular mortality **(Opstad *et al*., 2011)**. A previous study in neonatal term infant showed that urine IL-18 (uIL-18) shown to be a highly sensitive predictor of AKI in neonatal term infants more than 90% **(Li *et al*., 2012), the** sensitivity and specificity of another research in critically ill neonates were 64% and 92%, respectively **(Oncel *et al*., 2016)**. Additionally, it is known that polymorphisms in the IL promoter influence the levels of cytokine expression. Five single nucleotide polymorphisms (SNPs) have been detected in the IL- 18 gene promoter and exon 1, i.e. -137 G > C, -607C > A, -656 G > T, þ113 T > G and +127C > T, with +137 G > C (rs187238),-607C > A (rs1946518), and -656 G > T (rs1946519) IL-18 expression is affected **(Thompson, 2007)**. Although there is good evidence suggesting that the IL-18 protein may play a role in the progression and stability of atherosclerosis, there is limited evidence that the IL18 gene itself is important. As previously noted, Tiret *et al*. **(Tiret *et al*., 2005)** used the Athero-Gene study to demonstrate how IL18 variation could affect protein synthesis. Using those same haplotypes, they also demonstrated that IL18 haplotypes were related to cardiovascular mortality during follow-up.

Although no single SNP was found to be associated with cardiovascular mortality, using a haplotype-based approach, haplotypes were found to be significantly worldwide associated with the probability of future cardiovascular mortality (P ¼ 0.045) **(Thompson *et al.*, 2007)**. Excluding deaths after four years and adjustment for cardiovascular risk variables enhanced the link (P 14 0.006). Using the most prevalent haplotype as a reference, the identical haplotype related with lower IL-18 levels was also related with a lower risk of cardiovascular death. Only after adjusting for IL-18 levels was this association attenuated, supporting the view that the genotype associated risk was acting via its effect on IL-18 levels **(Thompson *et al.*, 2007)**.

1.4. Tumor Necrosis Factor Alpha.

Tumor necrosis factor alpha (TNF-α), a pleiotropic cytokine, has significant inflammatory function in renal disorders like lupus nephritis, anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis and renal allograft rejection **(Ernandez *et al.*, 2009)**. However, TNF-α also plays essential immunoregulatory roles that are demanded to maintain immune homeostasis. These complicated biological functions of TNF-α are control by its two receptors, TNFR1 and TNFR2. For example, in an animal model of immune complex-mediated glomerulonephritis, TNFR2 induce leukocyte infiltration and tissue damage. In contrast, TNFR1 serves an immunoregulatory role in a mouse lupus model, where a deficiency this receptor results in more severe autoimmune symptoms. Tumor necrosis factor alpha's proinflammatory and immunoregulatory effects in humans are dramatically demonstrated in patients using anti-TNF medications: These medications are extremely useful in certain inflammatory disorders such as rheumatoid arthritis, but they have also been linked to the induction of autoimmune lupus-like syndromes and increased autoimmunity in patients with multiple sclerosis **(Ernandez *et al.*, 2009)**. However, Tumor

necrosis factor alpha is primarily produced by activated macrophages, is frequently associated with proinflammatory properties and plays a significant role in innate as well as adaptive immunity, especially when it relates to host defenses mechanism against intracellular bacteria **(Hehlgans *et al.*, 2005)**.

1.4.1. Structure of Tumor Necrosis Factor Alpha.

Tumor necrosis factor alpha is homotrimeric existence and coupling by edge-to-face association of the antiparallel sandwich structure of the wedge-shaped monomers. Is typically generated as a type II transmembrane protein that is 233 amino acids long and organized in stable homotrimers **(Tang *et al.*, 1996)**. The metalloprotease TNF-α converting enzyme releases the soluble homotrimeric cytokine (sTNF) through proteolytic cleavage from this membrane-integrated form (TACE, also called ADAM17) **(Black *et al.*, 1997)**. At doses below the nanomolar range, the soluble 51 kDa trimeric sTNF has a tendency to dissociate and lose its bioactivity. Human TNF-α which has been secreted has a triangular pyramid shape and a mass of about 17 kDa. Both the secreted and membrane bound versions are physiologically active, although their specific activities are debatable. However, these types have biological activities that are both overlapping and unique **(Black *et al.*, 1997)**.

1.4.2. Tumor Necrosis Factor Alpha in Inflammatory Kidney Diseases.

Tumor necrosis factor alpha has a major role in renal inflammation and glomerular damage caused by immune complex deposition. Tumor necrosis factor alpha expression in the kidney is increased in both mice and humans with glomerulonephritis **(Neale *et al.*, 1995)**, and associated with a raise in serum levels of TNF-α as well as urine **(Ozen *et al.*, 1994)**. Tumor Necrosis Factor Alpha has been shown to play a functional proinflammatory role in experimental models of immune complex-mediated glomerulonephritis, where TNF-α

deficiency or blocking treatments clearly give protection against glomerular damage. For instance, systemic administration of TNF-α enhanced glomerular injury in rats using the well-known accelerated nephrotoxic serum nephritis model. A newer publication provides new thoughts on the respective role of each TNFR in immune complex-mediated glomerulonephritis **(Vielhauer *et al*., 2005)**. When TNFR1- and TNFR2-deficient mice were treated to the accelerated nephrotoxic nephritis model and renal damage was compared to wild type animals, TNFR1-deficient mice displayed a delay in disease that was associated with a decrease in the adaptive humoral response. As previously reported, TNFR1 is required for T-cell priming and the delayed type hypersensitivity response, therefore this could be explained by impaired T-cell priming required for B-cell activation and proliferation **(Ernandez *et al.*, 2009)**. However, at a later time point, TNFR1-deficient mice showed similar levels of glomerular damage to wild-type mice, along with a noticeably higher lymphocytic infiltration in renal tissue. This may help to explain why glomerular damage developed in these animals despite their impaired adaptive immune response. This was associated with a reduction in T-cell apoptosis, which may be mediated by TNFR1 as shown by other groups **(Ernandez *et al.*, 2009)**.

1.4.3. Tumor Necrosis Factor Alpha Gene Polymorphism.

The human TNF-α gene has four exons and is located on chromosome 6p21.3. More than 600 SNPs in the TNF-α gene have been found recently, with some of them (-238 G/A, 244 A/G, 308 G/A, 376 A/G, 575 A/G, 857 C/T, 863 C/A, 1031T/C, 1125 G/C, and 1196 C/T) positioned in the promoter and able of altering protein synthesis **(Baena *et al*., 2002 and Heesen *et al*., 2004)**. Human TNF-α gene polymorphisms were studied in Iranian pulmonary tuberculosis patients as an infectious disease. They discovered that polymorphisms in the TNF\238 gene were associated to an increased risk of development pulmonary

tuberculosis. Studies in Caucasians demonstrated a correlation between the (-238A) allele and persistent HBV infection **(Zum BÜschenfelde, 1998)**. The TNF-ß coding gene, also known as lymphotoxin-·, located next to the TNF-α· gene. Single nucleotide polymorphism at position +1069 (G to A) in the first intron of the TNF-ß gene has been characterized. The _A‘ variant is the TNF-ß2 allele whereas the less frequent ‘G‘ variant known as the TNF-ß1 allele. The TNF-ß1 allele is correlated with the presence of a digestion site for the DNA-sequence-specific restriction enzyme **(Tabrizi *et al*., 2001).**

There has been increased interest in determining the function of genetic polymorphisms as potential predictors of adverse outcomes in AKI patients **(Perianayagam *et al*., 2012)**. Proinflammatory cytokines are produced as a result of ischemia-reperfusion and nephrotoxic injury in experimental settings, and these cytokines alter the morphology and function of glomerular endothelial and tubular epithelial cells **(Akcay *et al*., 2009)**. Moreover, distant organ injury can also be controlled by cytokines **(White *et al*., 2012)**. High circulating levels of TNF-α have been correlated with adverse clinical outcomes in patients with AKI. Functionally relevant polymorphisms in the TNF-α gene promoter that influence transcriptional activity **(Jaber *et al*., 2004)**.

1.5. Role of genetic studies in clinical diagnosis.

Genetic science has advanced at an exponential rate in recent years, most notably in the mapping of the human genome. As several recent reviews have highlighted, this has many implications for primary healthcare practice and will proceed in the future **(Vrijenhoek *et al*., 2015)**. Genetics has been marked by some as the new ‘‘revolution‘‘ in medicine, proposing a rapid, fundamental change to, or overthrow of, existing health paradigms **(Abbott, 2003)**. Common

diseases typically defined more on their clinical symptoms than their biological mechanisms. The tools required to categorize diseases based on their mechanisms may be made available by molecular genetics. This is likely to have a significant impact on clinical decisions, such as treatment selection, as well as our capacity to more precisely characterize the course of disease and causal environmental factors **(Bouvier *et al.*, 2019)**. In medicine, molecular genetics was initially used to map and identify major single gene disorders such as cystic fibrosis **(Dekkers *et al*., 2013)** and polycystic kidney disease **(Porath *et al*., 2016)**. These illnesses exhibit conventional Mendelian inheritance patterns and are brought on by highly penetrant mutations in single genes **(Rafi *et al*., 2018)**. A precise definition of disease will result from understanding the biological processes and pathways that genetics has identified as contributing to disease. The majority of patients might be managed from this point forward **(Bell, 1998)**. Although they only affect a small percentage of those who are impacted, these offer significant insights into the mechanisms of disease **(Bell, 1998)**. The majority of genetic research and clinical work has focused mainly on relatively rare genetic disorders like cystic fibrosis and phenylketonuria. These illnesses exhibit conventional Mendelian inheritance patterns and are brought on by highly penetrant mutations in single genes **(Andermann *et al*., 2008)**. Genetic information is likely to play a crucial role in clinical diagnosis in the future given the rising trend toward focusing healthcare resources so that they are used as efficiently as possible, developing accurate disease definitions to predict their clinical course, focusing other forms of screening, and selecting the best treatments. It is already possible to recognize those who are most at risk for breast or colon cancer and target screening or early interventional care for these individuals **(Bell, 1998)**.

1.6. Genetic Susceptibility to Acute Kidney Injury.

Acute kidney injury is a widely held fears since it has a significant impact on morbidity, mortality, and healthcare system expenditure. AKI is a complicated conglomeration of symptoms that frequently occurs in conjunction with other syndromes in its broad clinical spectrum, and not a straightforward illness **(Ortega-Loubon *et al*., 2021)**. It has been suggested that genetic factors may contribute to the susceptibility and severity of AKI, describing why certain patients are more likely to develop the condition and why different patients respond to treatment in various ways **(Chang, *et al*., 2013 and Karimi, *et al*., 2012)**. This fact has given rise to a new branch of exercise treatment called as personalized medicine, whose goal is to treat patients as unique individuals rather than as a group **(Lazăr *et al*., 2019)**. A more individualized medical approach could result from a better understanding of how gene interaction affects health or disease, enabling the construction of a specific individual genetic print on which medical decisions should be made. Numerous reports have examined the relationship between various SNPs and the susceptibility for AKI in diverse clinical contexts **(Cardinal-Fernández *et al*., 2012)**. Case-control and observational studies are the most common types of polymorphism association studies, which compare the occurrence of a genetic variation in a subset of people with a particular illness to that occurrence in a group of healthy controls. Additionally, despite the fact that many polymorphisms have been explored, the majority of studies frequently use relatively small homogeneous sample sizes, which severely limits the ability to draw conclusions from the results of the general population and frequently yields conflicting results with mainly no significant findings. The outcomes are typically irregular and inconsistent across all of the analyses (**Lu *et al*., 2009)**. The relationship between genetic

polymorphisms and the risk of AKI often varies across ethnic groups, demographics, and geographic borders **(Chang *et al*., 2013)**. This is crucial because some genetic mutations are rare in some ethnic groups but not in others, depending on the group. The third most frequent cause of AKI, CI-AKI, is mentioned in most articles' clinical settings, along with patients undergoing heart surgery and those who are severely ill **(Ortega-Loubon *et al*., 2021)**.

1.7. Gene Polymorphisms.

Polymorphism association studies are often comprised of case-control and observational studies that compare the occurrence of a genetic variant in certain individuals suffering from a specific disease to the occurrence in a healthy control population. Furthermore, while several polymorphisms have been identified, most studies often use relatively small homogeneous sample size populations, which severely limits the ability to draw conclusions from general population results and frequently yields conflicting results with mostly non-significant findings. In general, the outcomes of the various analyses are irregular and frequently variable **(Lu *et al*., 2009)**. Although a person's genotype is the mix of their parents' genotypes, two different people share 99.9% of their DNA sequences **(Venter *et al*., 2001)**. Observed variations on the left gene polymorphisms, which make up 0.1% of the human genome, are the focus of extensive research. Indeed, these variations serve as biological variety markers, and some genotypic polymorphisms have been related to specific phenotypes of human disease. It is unknown if any of these genetic polymorphisms play a role in the development of certain diseases because they may be located near other pathogenic genetic factors, a condition known as linkage disequilibrium **(Jaber *et al*., 2004)**. Variants may appear in one or more of the locations listed below: (1) the

promoter region, (2) the exon(s) or the gene coding region, (3) the intron(s) or the gene intervening sequences and (4) the 30-untranslated (30-UTR) region (Figure 1-5) **(Ortega-Loubon *et al*., 2021)**.

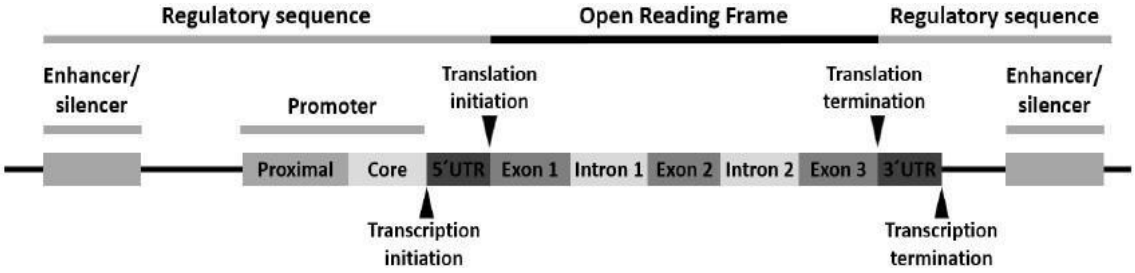


Fig. 1.5: Schematic Arrangement of A typical Human Gene with Locations for Gene Polymorphism.

1.8. Interleukin-18, Tumor Necrosis Factor Alpha Polymorphisms and Contrast Induced Acute Kidney Injury.

Although the role of susceptibility genetic factors in the development and severity of CIN following ischemic or nephrotoxic injury has remained unexplored, both TNF-α and IL-18 gene polymorphisms are associated with risk of mortality in patients with acute renal failure requiring dialysis and AKI pathogenesis **(Chang *et al*., 2013 and Ortega-Loubon *et al*., 2021)**. The study of the polymorphism of these genes may enhance the understanding of the pathogenesis in CIN, identify potential markers of susceptibility, severity and clinical outcomes, identify targets for therapeutic intervention and improve the strategies to prevent CIN **(Chang *et al*., 2013)**. It has been reported that IL-18 is associated with AKI, with adults having an increase in IL-18 after 24 hours of renal ischemia or decreased perfusion, especially in the proximal tubule **(Boyle *et al*., 2017)**. Furthermore, IL promoter polymorphisms are known to affect cytokine expression levels. Five single

nucleotide polymorphisms (SNPs) in the promoter area and exon 1 of IL-18 gene have been identified, i.e. -137 G > C, -607C > A, -656 G > T, +113 T > G and þ127C > T, with -137 G > C (rs187238), -607C > A (rs1946518), and -656 G > T (rs1946519) affecting IL-18 expression **(Davran *et al*., 2016)**.

Chang, Lu *et al*. concluded that high-transcription TNF-α genotypes are associated with higher CIN risk. In the meantime, we also demonstrated that the higher production genotype of GA+AA (rs1800629) in TNF-α had a higher rate of renal function decline **(Chang *et al*., 2013)**. High circulating levels of TNF-α have been associated with adverse clinical outcomes in patients with AKI. Functionally relevant polymorphisms within the promoter region of the TNF-α gene, which affect transcriptional activity **(Jaber *et al*., 2004)**. Human TNF-α gene is located on chromosome 6p21.3 and has 4 exons. Nowadays, more than 600 SNPs in the TNF-α gene have been identified, which some of them (- 238 G/A, 244 A/G, 308 G/A, 376 A/G, 575 A/G, 857 C/T, 863 C/A, 1031 T/C, 1125 G/C, and 1196 C/T) are located in the promoter and can change the production of protein **(Baena *et al*. 2002 and Heesen *et al*. 2004).**

1.9. Mehran Score for Post-PCI Contrast Nephropathy.

The Mehran CIN-Risk score (MRS) was established and initially tested in patients undergoing elective PCI for the prediction of CIN **(Mehran *et al*., 2004)**. Eight clinical and procedural criteria make up this score: age >75 years, hypotension, congestive heart failure, intra-aortic balloon pump, diabetes, anemia, and volume of contrast. Four categories of risk have been established using the distribution of the risk score (7.5%, 14.0%, 26.1%, and 57.3% of probability to develop CIN). Numerous risk factors have been described for CIN **(Marenzi *et***

***al*., 2004)**. In 2004, Mehran *et al*. published a risk score for the prediction of CIN following PCI **(Mehran *et al*., 2004)**. That risk score includes hypotension (5 points, if systolic blood pressure <80 mmHg for at least 1 h requiring inotropic support), use of intra-aortic balloon pump (5 points), congestive heart failure (5 points, if class III/IV by New York Heart Association classification or history of pulmonary edema), age (4 points, if >75 years), anemia (3 points, if hematocrit <39% for men and <36% for women), diabetes mellitus (3 points), CM volume (1 point per 100 mL), and eGFR (GFR in mL/min per 1.73 m^2 ; 2 points, if GFR 60 to 40; 4 points, if GFR 40 to 20; 6 points, if GFR <20). The corresponding scores for the 8 prognostic variables that make up the final Mehran CIN risk score were used to calculate it for each patient. The cut-off points and intervals defined by Mehran *et al*. were used to establish four categories of CIN risk. As follows: low, 5 points; moderate, 6 to 10; high, 11 to 15; and very high,>15.

Aims of The Study:

The current objectives include the following:

- To investigate the impacts of CM following therapeutic coronary angiography on IL-18 and TNF-α serum levels.
- The role of anti-inflammatory cytokine IL-18 and pro- inflammatory cytokine TNF-α gene polymorphisms as CIN susceptibility markers after PCI.
- Asses the incidence of CI-AKI Following Therapeutic Coronary Angiography in Karbala Cardiac Catheterization Center unit at Al-Hussein Medical City.
- Asses Mehran risk score to predict the risk of CI-AKI incidence with people they planned or emergency underwent to PCI.

Chapter Two

Materials and Methods

2. Materials and Methods.

2.1. Subjects.

This is a cross over study included patients before and after coronary angiographic intervention. All patients were collected at Karbala heart center, coronary catheterization unit (CCAU), Karbala Health Directorate, and Al-Kafeel sub-specialty hospital cardiac catheterization unit, holly Karbala, Iraq. Bio investigations were done in Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala. The study started from Nov., 2021 to Sep., 2022.

2.1.1. Patients.

Sixty patients who attended the CCAU for elective or emergency therapeutic coronary angiography and interventions were collected in this study.

2.1.1.1. Inclusion criteria.

All patients underwent therapeutic coronary angiography with or without stenting were included in this study. Patients data: age, gender, diabetes mellitus (DM), type of treatment, history of renal disease, history of any other diseases, height, weight, body mass index (BMI), blood pressure before and during angiography were collected.

Contrast induced nephropathy is defined as an increase in S.Cr of 0.5 mg/dL (44umol/L) **(Cely, *et al*. 2012)**, or a relative 25 % increase from the baseline value, within a period of 24 hours following intravascular administration of CM **(McCullough Peter, 2008 and Mehran *et al*., 2006)**.

2.1.1.2. Exclusion criteria.

Patients with following characteristics were excluded:

- Patients with incomplete data.
- Patients who underwent diagnostic coronary angio only.
- Patients who have other causes of AKI.

2.1.2. Approval of the ethical committee.

The Ethical Committees approved this study protocol are: Committee of College of Medicine, University of Kerbala and Department of Medicine and committee of Karbala Heart Center, coronary catheterization unit in Karbala Health Directorate / Kerbela - Iraq.

2.1.3. Blood collection.

Five milliliters of blood were drawn by vein puncture from all individuals participated in this study before and after 24 hours. The blood was divided into two parts: The first part was used for gene analysis. It included two milliliters of blood collected in EDTA containing tube and used for DNA extraction, then were analyzed directly to obtain high purity of DNA. The second part included three milliliters of blood placed in serum tube. It was left fifteen minutes at room temperature for coagulation. Blood was centrifuged for 15 minutes at 4000 rpm. Serum was collected then stored at -60 C till analyses for measuring the blood urea, S.Cr, serum IL-18 and serum TNF-α.

2.1.4. Body Mass Index.

Body mass index (BMI) was calculated by dividing the body weight (kg) by the square of height (m^2) **(Garrow *et al.*, 1985)**. According to the following equation:

BMI = Weight (kg) / Square Height (m^2).

Normal BMI level was ranged between (20-24.9) kg/m2 while for overweight was ranged between (25-29.9) kg/m2 and when BMI ≥ 30kg / m2, is considered as obese **(Robertson, 2019)**.

2.1.5. Glomerular Filtration Rate.

Value of GFR was calculated by using age and S.Cr by using the following MDRD equation **(Levey *et al.*, 1999): eGFR (ml/ min/1.73 m^2) = 186 × (S.Cr) $^{-1.154}$ × (age) $^{0.203}$ × (0.742 if female) × (l.212 if black American).**

2.1.6. Diabetes Mellitus.

Diabetes mellitus (DM) is the general term for a group of metabolic disorders characterized by chronic hyperglycemia. The cause is either a disruption in insulin secretion (Type 1) or a disruption in insulin effect (Type 2), or both **(Petersmann *et al*., 2018)**.

Diagnostic Criteria of Diabetes Mellitus

- Occasional plasma glucose value of ≥ 200 mg/dl (≥ 11.1 mmol/l).
- Fasting plasma glucose of ≥ 126 mg/dl (7.0 mmol/l) (fasting time 8–12 h).
- Oral glucose tolerance test (OGTT) 2-h value in venous plasma ≥ 200 mg/dl (≥11.1 mmol/l).

2.1.7. Heart Failure.

Is a complex clinical syndrome caused by any structural or functional dysfunction of ventricular filling or blood ejection. The primary symptoms and diagnosis of heart failure (HF) include dyspnoea and exhaustion, which can restrict exercise tolerance, as well as fluid retention, which can cause pulmonary and/or splanchnic congestion and/or peripheral oedema **(Bozkurt *et al*., 2021)**.

2.1.8. Cardiac Angiography Procedures.

Coronary angiography is a diagnostic procedure that employs contrast and specialized X-rays to visualize the coronary arteries. Diagnostic and therapeutic procedures are the two main varieties of this procedure. All patients involved in this study were given low-osmolar non-ionic contrast media Omnipaque (Iohexol) 350 mg/1ml), the osmality is 844 mOsm/1) **(Barrett *et al*., 2006)**. All patients received amount of CM at range of (50-500) ml. A one way valve sheath was inserted via Salinger‘s technique through the right or left femoral or radial arteries. A coronary artery catheter was selected according to patient‘s arterial size and right or left artery. The contrast dye then is injected through the catheter into the

coronary arteries. By looking at the fluoroscopic X-ray screen, the doctor can see the flow of blood through the arteries and determine the site/s of lesions **(Ivar Seldinger, 2008)**.

2.2. Materials.

The instruments and kits, specific chemicals used in this study with their suppliers and manufacture origin are listed in tables (2-1) and (2-2). The primers which used in the amplification analysis of both IL-18 and TNF-α SNPs were; Forward (F) and Reveres (R1, R2), as shown tables (2-4), (2-5).

2.2.1. Instruments.

All the instruments and tools which are used in this study are shown below in table 2.1.

Table (2.1) Instruments used in this study.

Instruments and tools	Suppliers
Vortex mixer.	(karlkole) -U.K.
Centrifuge.	(kokusan)- Japan.
Deep Freezer.	Arctiko-Austria.
Incubator.	Germany.
Micropipette (100-1000) pL.	Germany.
Micropipette (10-100) pL.	Germany.
Multichannel pipette.	Germany.
Printer.	Epson-U.S.A.
Elisa washer.	ELX800-U.S.A.
Elias reader.	ELX800-U.S.A.
Water bath.	Grant – England.
Disposable syringes (5 ml).	Medical jet (Syria).
EDTA tube.	Plastilab (China).

Gel tube.	Al.Malak (China).
Gel electrophoresis.	Biometra (Germany).
Hood.	C.B.S scientific (USA).
Nanodrop.	Bio Drop (England).
PCR.	Biometra (Germany).
Photo documentation.	UVP (UK).
Eppendrof tube (0.5 and 1 ml).	Plastilab (China).
Balance.	Precis / Switzerland.
Digital camera.	Canon/ England.

2.2.2. Chemicals.

The chemicals and kits with their suppliers which were used in this study are listed in the table 2.2.

Table 2.2: Chemicals and kits used in this study

Chemicals	Source
Human IL-18 Elisa Kit.	PARS BIOCHEM (China).
Human TNF-α Elisa Kit.	PARS BIOCHEM (China).
Urea Kit.	Randox, U.K.
Creatinine Kit.	Randox, U.K.
Genomic DNA Extraction Kit.	Add Bio (Korea).
Agarose.	BDH/ England.
Primers.	Bonier/ KOREA.
TBE Buffer.	Bio basic/ Korea.
DNA Leader.	Bioneer/ KOREA.
Ethidium Bromide.	Sigma / USA.
Ethanol 95%.	System/ Malaysia.
PCR Premix.	Bonier/ KOREA.

2.3. Methods.

2.3.1. Measurement of serum urea.

Principle.

Kinetic, enzymatic method with urease and glutamate dehydrogenase

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} CO_2 + NH_3$$

$$NH_4^+ + \text{2-Oxoglutarate} + NADH + H^+ \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

The rate of absorbance changing at 2=340 nm is proportional to the urea concentration (**Mohan *et al.*, 2021)**.

REAGENTS.

1-Reagent.	**2 x 30 ml**
2-Reagent.	1x 15 ml

The reagents, stored at 2-8°C are stable up to expiry date printed on the package. Stability on board of the analyzer at 2-10°C is 12 weeks.

Concentrations in the reagent.

1-Reagent.

2-Reagent.

2-Oxoglutarate.	**≤ 48.6 mmol/1**
NADH.	**≤ 1.6 mmol/l**
Buffer, preservative.	

Specimen requirement.

Serum at room temperature 10-20 min, centrifugation 20 min at the speed of 2000-3000 r.p.m.

Procedure:

1-Reagent and 2-Reagent were ready to use. Deionized water is recommended as a reagent blank .

REFFRENCECE VALUES:

	mg/dl	mmol/l
Serum/ Plasma	< 50	< 8.3

1 mg of urea corresponds to 0.467 mg of urea nitrogen. It is recommended for each laboratory to establish its own reference ranges for local population.

Calculation:

The results have been obtained using the automatic analysers ACCENT-200 and/or ACCENT MC240. Results may vary if a different instrument or a manual procedure is used.

2.3.2. Measurement of serum creatinine.

Principle.

Fixed time analysis. Creatinine reacts in alkaline environment with pirate to give a colored compound whose intensity is proportional to the creatinine concentration in the sample **(Delanghe *et al.*, 2011)**.

Reagents.

R1	Lithium hydroxide	120.0 mmol/l
	Boric acid.	80.0 mmol/l.
R2	Picric acid.	67.0 mmol/l.

Procedure

Wavelength.	1: 510 (500-550) nm.
Working Temperature.	37°C.
Optical Path.	1 cm.
Reaction.	Fixed time.

Reference Values.

Serum – plasma
Man 0.9 - 1.3 mg/dl (80 -115 µmol/1)
Man Urine 800 - 2000 mg/24h (7.1 -17.7mmo|/24h)
Woman 0.6 - 1.1 mg/dI (53 - 97 µmol/I)
Woman Urine 600 - 1800 mg/24h (7.1 -15.9 mmo|/24h)

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

Calculation:

Creatinine [mg/al] or [µmol/l] = (E2C - E1C) X Conc. STD

The reagent performances are related to 37°C, 1 cm and 510 nm

2.3.3 Measurement of serum IL-18.

Human IL-18 was measured using ELISA assay (PARS BIOCHEM, Cat NO. PRS-00857hu) for the quantitative determination of human IL-18 concentration in serum.

Principle of the assay.

The kit assay human IL-18 level in the sample, used purified human IL-18 antibody to coat microliter plate wells, made solid-phase antibody, then added IL-18 to wells, combined IL-18 antibody which with HRP labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, added TMB substrate solution, TMB substrate becomes blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of IL-18 in the samples is then determined by comparing the O.D. of the samples to the standard curve (www.pariselements.com).

Table (2.3): Materials and Chemicals.

Standard: 135ng/L	0.5ml x I bottle
Standard diluent.	1.5ml x 1 bottle
HRP-Conjugate reagent.	6ml x1bottle
Sample diluent.	6ml x1bottle
Chromogen Solution A.	6ml x1bottle

Chromogen Solution B.	6ml x1bottle
Stop Solution.	6ml x1bottle
Wash solution.	(20ml × 30 fold) xl bottle

Specimen requirement.

Serum coagulation at room temperature 10-20 min, centrifugation 20 min at the speed of 2000-3000 r.p.m.

Procedure:

1. Diluted and sample were added to standard: set 10 Standard wells on the ELISA plates coated, standard 100µl was added to the first and the second well, then standard dilution 50µl added to the first and the second well, mixed; taken out 100µl form the first and the second well then was added it to the third and the forth well separately. Then Standard dilution 50µl was added to the third and the forth well, mixed; then taken out 50µl from the third and the forth well discard, added 50µl to the fifth and the sixth well, then standard dilution 50µl was added to the fifth and the sixth well, mixed; taken out 50µl from the fifth and the sixth well and added to the seventh and the eighth well ,mixed ; taken out 50µl from the seventh and the eighth well and was added to the ninth and the tenth well, standard dilution 50µl was added to the ninth and the tenth well, mixed , taken out 50µl from the ninth and the tenth well discard (sample 50µl was added to each well after diluting, (density: 90ng/L, 60 ng/L,30 ng/L, 15 ng/L, 7.5 ng/L).

2. Added sample: set blank wells separately. Was added sample dilution 40µl to testing sample well, then was added testing sample 10µl (sample final dilution is 5-fold), was added sample to wells, and gently mixed.
3. Incubate: After closing plate with closure plate membrane, was incubated for 30 min at 37°C.

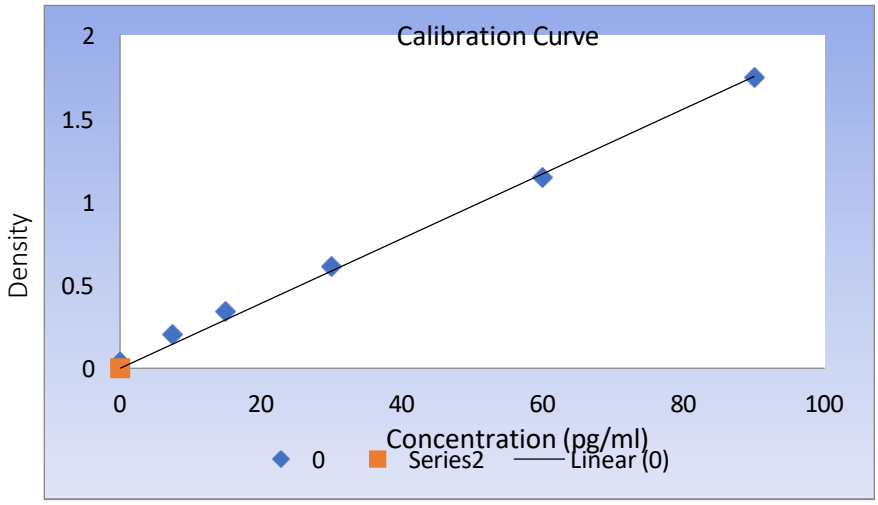
4. Configured liquid: 30-fold (or 20-fold) wash solution was diluted 30-fold (or 20- fold) with distilled water and reserved.
5. Washing: Uncovered closure plate membrane, discard liquid, dried by swing, washing buffer was added to every well, still for 30s then drain, repeated 5 times, and was dried by pat.
6. Added enzyme: was add HRP-Conjugate reagent 50µl to each well, except blank well.
7. Incubate: Operation with 3.
8. Washing: Operation with 5.
9. Color: Chromogen solution A 50µl was added and Chromogen solution B to each well, evaded the light preservation for 15 min at 37°C.
10. Reaction has been stopped: Stop Solution50µl was added to each well, blue color change to yellow color.
11. Assay: taken blank well as zero, absorbance at 450nm after Adding stop solution has been read within 15 min.

Assay range.

5 ng/L – 100 ng/L

Calculation.

Standard density was taken as the horizontal, the OD value for the vertical, drawn the standard curve on graph paper, corresponding density according to the sample OD value by the sample curve, multiplied by the dilution multiple.



2.3.4 Measurement of serum TNF-α.

Human TNF-α was measured using ELISA assay (PARS BIOCHEM, Cat NO. PRS-01568hu) for the quantitative determination of human TNF-α concentration in serum.

Principle of the assay.

The kit assay human TNF-α level in the sample, was used purified human TNF-α antibody to coat microtiter plate wells, made solid-phase antibody, then added TNF-α to wells, combined TNF-α antibody which With HRP labeled, become antibody-antigen- enzyme-antibody complex, after washing completely, added TMB substrate solution, TB substrate becomes blue color at HR enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of TNF-α in the samples is then determined by comparing the O D. of the samples to the standard curve (www.pariselements.com).

Materials and Chemicals.

Standard: 135ng/L.	**0.5ml x I bottle.**
Standard diluent.	1.5ml x 1 bottle.
HRP-Conjugate reagent.	6ml x1bottle.
Sample diluent.	6ml x1bottle.
Chromogen Solution A.	6ml x1bottle.
Chromogen Solution B.	6ml x1bottle.
Stop Solution.	6ml x1bottle.
Wash solution.	(20ml × 30 fold) xl Bottle.

Specimen requirement

Serum coagulation at room temperature 10-20 min, centrifugation 20 min at the speed of 2000-3000 r.p.m.

Procedure:

1. Dilute and sample were added to standard: set 10 standard wells on the ELISA plates coated, added standard 100µl to the first and the second well, then add standard dilution 50µl to the first and the second well, mixed; taken out 100µl form the first and the second well then added it to the third and the forth well separately. Then was added standard dilution 50µl to the third and the forth well ,mixed; then taken out 50µl from the third and the forth well discard, added 50µl to the fifth and the sixth well ,then standard dilution were added 50µl to the fifth and the sixth well, mixed; taken out 50µl from the fifth and the sixth well and added to the seventh and the eighth well, then standard dilution were added 50µl to the seventh and the eighth well, mixed ; taken out 50µl from the seventh and the eighth well and added to the ninth and the tenth well, added standard dilution 50µl to the ninth and the tenth well, mix , taken out 50µl from the ninth and the tenth well discard (added sample 50µl to each well

after diluting, (density: 300ng/L, 200ng/L 100 ng/L, 50 mg/L, 25ng/L).

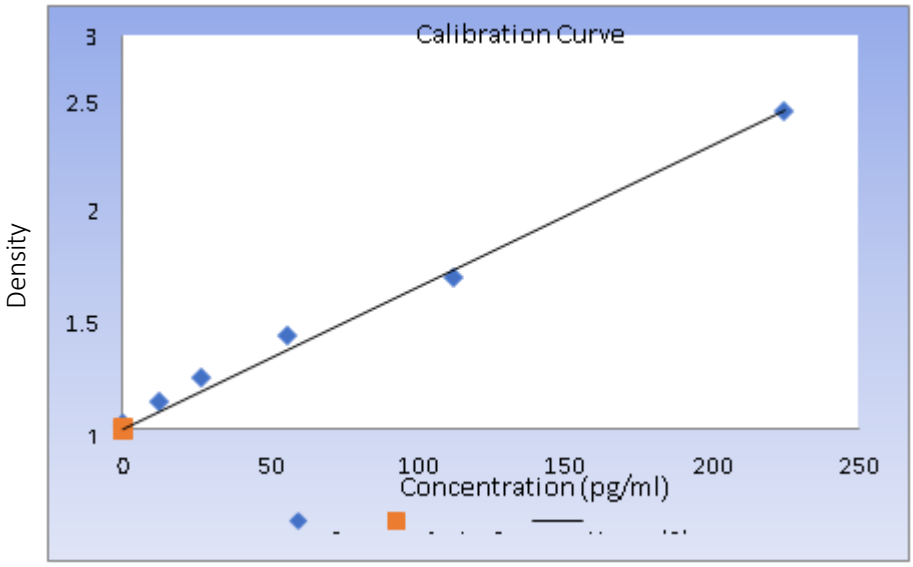
2. Added sample: Set blank wells separately. Was added Sample dilution 40µl to testing sample well, then was added testing sample 10µl (sample final dilution is 5-fold), was added sample to wells, didn‘t touch the well wall as far as possible, and gently was mixed.
3. Incubate: After closing plate with closure plate membrane, was incubated for 30 min at 37°C.
4. Configured liquid: 30-fold (or 20-fold) wash solution was diluted 30-fold (or 20- fold) with distilled water and reserve.
5. Washing: Uncovered closure plate membrane, discard liquid, dried by swing, was added washing buffer to every well, still for 30s then drain, repeated 5 times, dried by pat.
6. Add enzyme: was add HRP-conjugate reagent 50pl to each well, except blank well.
7. Incubate: Operation with 3.
8. Washing: Operation with 5.
9. Color: were added chromogen solution A 50µl and chromogen solution B to each well, evade the light preservation for 15 min at 37°C.
10. The reaction was stopped: Added stop solution 50µl to each well, stop the reaction (the blue color change to yellow color).
11. Assay: taken blank well as zero, read absorbance at 450nm after adding stop solution and within 15 min.

Assay range.

20 ng/L – 400 ng/L

Calculation.

Standard density was taken as the horizontal, the OD value for the vertical , drawn the standard curve on graph paper, corresponding density according to the sample OD value by the sample curve, multiplied by the dilution multiple.



2.4. Molecular Studies.

2.4.1. DNA Extraction.

The DNA extraction was carried out at Department of Chemistry and Biochemistry, College of Medicine, in Kerbala city. DNA Genome was extracted from blood sample according to protocol AddPrep Genomic DNA Extraction Kit.

Principle.

Add Prep Genomic DNA Extraction Kit is effective method for isolating genomic DNA from Blood, Tissue and Plant. In case of extraction from blood, this kit is designed for the rapid preparation of genomic DNA from up to 200 µl of a blood sample like a whole blood, plasma, serum, buffy coat and body fluids, and is suitable to use with whole blood treated with either citrate or EDTA. In case of extraction from tissue, this kit is designed for isolating genomic DNA from mammalian cells, mouse tail, hair root, variable tissues and Gram negative bacteria cells. In case of extraction from plant, this kit is designed for isolating genomic DNA from plant tissue like leaf, fruits and root, and processed food for GMO detection.

Procedure.

1. Were added 20 µl of Proteinase K solution (20 mg/ml) to a 1.5 ml micro-centrifuge tube.
2. Transferred 200 µl of sample to the 1.5 ml micro-centrifuge tube with proteinase K solution: If the sample volume is less than 200 µl, we were added the appropriate volume of PBS.
3. Then was added the 20 µl of RNase A solution (10 mg/ml).
4. Added 200 µl of Binding Solution to the sample tube, and mixed well by pulse-vortexing for 15 sec.

5. Incubated at 56℃ for 10 min: longer incubation times have no effect on yield or quality of the purified DNA.
6. Added 200 µl of absolute ethanol and mixed well by pulse-vortexing for 15 sec: After this step, briefly spin down to get the drops clinging under the lid.
7. Carefully transferred the lysate into the upper reservoir of the spin column with 2.0ml collection tube without wetting the rim.
8. Centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
9. Added 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow through and assemble the spin column with the 2.0 ml collection tube.
10. Added 500 µl of Washing 2 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow through and assemble the spin column with the 2.0 ml collection tube.
11. Dried the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.
12. Then transferred the spin column to the new 1.5 ml micro-centrifuge tube.
13. Added 100 ~ 200 µl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 min.
14. Eluted the genomic DNA by centrifugation at 13,000 rpm for 1 min.

2.4.2 Determination the Concentration and Purity of the Extracted DNA.

Spectrophotometric methods were used to estimate the concentration and purity of extracted DNA **(Adams, 2003)**.

2.4.2.1 Spectrophotometric Methods.

Principle:

The purity and concentration of DNA were measured by absorbance method using the Nano drop instrument. The absorbance readings were done at 260 nm and at 280 nm. At 260 nm the DNA strongly absorbs light while at 280 nm the protein absorbs light most strongly. DNA purity was measured by theA260/A280 ratio. The A260/A280 ratio 1.8-2.0 is commonly accepted **(Tataurov *et al*., 2008) (Adams, 2003)**.

Procedure:

Initially 1 µL of nuclease free water was smeared on the highly sensitive micro detector of nano-drop as blank. The micro detector was cleaned up from blank. Then 1 µL of DNA sample was applied on the micro detector of nano-drop. The concentration and A260/A280 ratio of DNA were documented from the instrument **(Scientific, 2008)**.

2.4.3. Amplification of DNA.

The allele-specific PCR, which is also known as an ARMS- PCR (amplification refractory mutation system) or PASA (PCR amplification of specific alleles) or AS-PCR, was used to detect the SNPs **(Darawi *et al*., 2013)**. Allele- specific PCR reaction protocol was used for SNPs detection gene of IL-18 (rs1946518) [A/C] and TNF-α (rs361525) [G/A]. The ALLEL SPECIFIC -PCRreactions were performed in 25 µl volumes in PCR tubes under sterile conditions, all the volume of the reaction mixture was completed to 25 µl with using DDH2O and the master mix which contained optimum concentrations of reaction requirements (MgCl2 1.5 mM, Taq polymerase 1 U, each dNTPs 200 µM) has been used, table (2-4).

Table (2-4). Components of master mix for detection of Il-18 and TNF-α gene

No.	Material	Volume(µl)
1	Master Mix	12.5
2	Forward	1.5
3	Reverse	1.5
4	Template DNA	5
5	Deionized H_2O	4.5
Total		25 µl

2.4.3.1. Primers for PCR.

A primer is a short single strand of DNA fragments consisting of (18-22) bases known as oligonucleotides that have a sequence that is complementary to the target DNA region. Without the use of primers, the amplification process cannot begin on a single DNA molecule. Thus, it should first be annealed to the single strands that result from the denaturation of the double stranded DNA **(Chaitanya, 2013)**. Polymerase chain reaction was performed using a specific primer pairs designed for IL-18 and TNF-α gene. Based on NCBI database, all gene information and SNPs detail, were collected using Genius software designed.

Preparation of the Primers in the Following Steps:

Materials: Lypholized primers, sterile dH_20

1. The tube was spin down before opening the cap.

2. Prepareing master stock, pmoles/µl, the desired amount of sterile dH_20 was added according to the manufacturer to obtain a 100 pmoles/µl (Master Stock).
3. The tube was mixed properly to re-suspend the primers equally.
4. Preparing working stock, 10 pmoles/µl, ten microliters of the master stock were transferred to a 0.5 ml eppendorf tube that contains 90µl of sterile dH_20 to obtain a 10 pmoles/µl (Working Stock).
5. The master stock was stored at -20 C^o.

The sequence of primers used for PCR amplification of IL-18 gene (rs1946518) and TNF-α gene (rs361525) was illustrated in Tables (2-4) and (2-5) respectively.

Table (2-5). Specific Primers of IL-18 gene (rs1946518)

Primer	Sequence (5´ –3´)	Allele	Size(bp)	Company
F1	GCTGTATCAGATGCAAGCCACA	A	186	BIONEER/ Korea
F2	GCTGTATCAGATGCAAGCCACC	C	186	BIONEER/ Korea
R	GGTCAGTCTTTGCTATCATTCCAG G		186	BIONEER/ Korea

Table (2-6). Specific Primers of TNF-α gene (rs361525)

Primer	**Sequence (5´ –3´)**	**Allele**	**Size(bp)**	**Company**
F1	TCACACTCCCCATCCTCCCTGCTC C	G	349	BIONEER / Korea
F2	TCACACTCCCCATCCTCCCTGCTC T	A	349	BIONEER / Korea
R	AGCCTTTCCTGAGGCCTCAAGCC		349	BIONEER / Korea

2.4.3.2. Polymerase Chain Reaction.

The polymerase chain reaction, also known as PCR, has rapidly emerged as one of the most essential methods in modern biological and medical research **(Wang, Wang *et al*., 2017)**. It amplifies a specific region of a DNA strand to generate thousands to millions of copies of a particular DNA sequence **(Guo *et al*., 2011)**.

A PCR basically requires the following:

1. DNA template containing the target DNA region.
2. Two primers to initiate DNA synthesis.
3. A thermostable DNA polymerase to catalyze DNA synthesis.
4. Deoxynucleoside triphosphates (dNTPs, the building blocks of new DNA strand).
5. Buffer including bivalent cations, usually Mg2+.

There are three steps of a PCR that are cycled about 25-35 times (Jones and Winistorfer 1993) this steps including the following:

1. **Denaturation:** This step includes separation of the double DNA strands into two single strands are accomplished by heating for about 94-95°C.
2. **Annealing:** At lower temperature (55-65°C), DNA primers (which are short single strand DNA fragments) attach to the ends of each strands of the DNA and initiates the reaction.

- **Extension:** This step occurs at 72-74°C, where each primer binding to the DNA template will be extended complementary to the template DNA. This process is carried out in the presence of the Taq DNA polymerase, because of its ability to operate efficiently at high temperatures.

2.4.3.3. Optimization of PCR Conditions.

Different volumes of primer (0.5 µl, 1 µl, 1.5 µl,) with different volumes of template DNA (1 µl, 2 µl, 3 µl, 4 µl, 5 µl, 6 µl) and different experiments of the reaction conditions were trailed in order to optimize the conditions of the reaction. PCR tube centrifuged for 30 seconds at 2000 xg in a micro-centrifuge in order to mix solutions well at room temperature then tubes are placed in the thermocycler to start the reaction. Programs of the PCR protocol reaction for IL-18 (rs1946518) table (2-6) and TNF-α (rs361525) table (2-7).

Table (2-7). Allele Specific –PCR Program for Detection of IL-18 (rs1946518) SNP

No.	Stage	Cycle	Step	Temp.	Time
1	Initial Denaturation	1	1	92 °C	2min.
2	Denaturation	45	1	92 °C	30 sec.
3	Annealing	35	2	45 °C	30 sec.
4	Extension	45	3	72 °C	55 sec.
5	Final Extension	1	1	72 °C	5 min.
6	Hold Phase			10 °C	

Table (2-8). Allele specific –PCR Program for Detection of TNF-α (rs361525) SNP.

No.	Stage	Cycle	Step	Temp.	Time
1	Initial Denaturation	1	1	92 °C	2min.
2	Denaturation	45	1	92 °C	30 sec.
3	Annealing	35	2	45 °C	30 sec.
4	Extension	45	3	72 °C	55 sec.
5	Final Extension	1	1	72 °C	5 min.
6	Hold Phase			10 °C	

2.4.4. DNA Electrophoresis.

According to the Harisha method **(Harisha, 2008)**, agarose gel electrophoresis was performed, and (2–3 µl) of ethidium bromide staining was applied. Which can bind with DNA by forming close van der Walls contacts with the base pairs and that's why it binds to the hydrophobic interior of the DNA molecule. Molecules that bind in this manner are called intercalating agents because they intercalate into the compact array of stacked bases, Using the same procedure, agarose at a concentration of 2% was produced.

2.4.4.1. Agarose Gel Electrophoresis.

In this method of separation, an electric current is used to move the biomolecules through a porous gel matrix at a pace proportional to the charge, size, and shape of the particles **(Sanderson *et al*., 2014)**.

Procedure: One hundred milliliters of a 2% agarose solution were prepared by the following steps **(Sofronjuk, 2019)**.

A. Preparation of solution: 1X TBE buffer (tris borate EDTA) was prepared by diluting 10 TBE buffer with deionized water (one volume of 10X TBE buffer with 9 volume of deionized water 1:10 dilution).

B. Preparation of the agarose gel.

1. Two grams of agarose were weighted and placed inside a conical flask and 100 mL of 1x buffer (TBE) was added with gentle mixing.
2. The solution was placed in the microwave for one minute until the agarose was completely dissolved and the solution became clear and then allowed to cool to about 55 °C before pouring.
3. Ethidium Bromide dye solution (2µl) was added to the solution.
4. The gel tray ends were closed with tape.

5. The comb was placed in the gel tray about 1 inch from one end of the tray.
6. The gel solution was poured into the chamber and allowed to solidify for 30 minutes at room temperature.
7. The comb was removed from the gel and the chamber was placed in a horizontal electrical system and was covered (only until the wells were flooded) with the same buffer TBE used to prepare the gel.
8. About (6 µl) from the samples were loaded on each well with extreme care to avoid damage to wells and contamination of adjacent wells.
9. Cathode pole was connected to the well side of the unit and the anode to the other side.
10. Electrophoresis was performed at 75 V, for 60-100 min or until the dye markers were migrated at an appropriate distance, depending on the size of the DNA to be visualized.

2.4.4.2. Photo Gel Documentation.

The agarose gel was placed over of an ultraviolet transilluminator device and exposed to ultraviolet light. The photos were collected using a digital camera and nceptualized by a computer that was connected to the transilluminator.

2.5. Statistical Analysis.

Information from the questionnaire from all participants were entered a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020) (1). Descriptive

statistics was performed on the participants‘ data of each group. Values were illustrated by n (%) for categorical, Scale variables were presented by mean ± standard deviation for normal data while non-normal data. The distribution of the data was checked using Shapiro-Wilk test as numerical means of assessing normality.

ANOVA design was used to analyze data, where the requirement was to assess the effect of the before and after laparoscopic ovarian cystectomy repeated measurements on the level of urea and creatinine ،IL18 ،TNF-α and eGFR in patients. Significant differences variables among the parameters were confirmed through analytical statistical tests. Chi square was used to measure the association between categorical variables. Fisher‘s exact test was used as an alternative when the chi square was inapplicable. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

The association between the analyzed factors which was estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a non-conditional logistic regression.

Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

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Chapter Three

Results and Discussion

3. Results and Discussion:

Our study included 60 patients who underwent elective and emergency therapeutic PCI, the gender distribution of patients was 78.3% males and 21.7% females and mean age of the patients was 56.3 ± 9.2 years. Their mean BMI was (29.91 ± 3.94). Patient‘s age of this study was divided into three group as shown in figure (3.1).

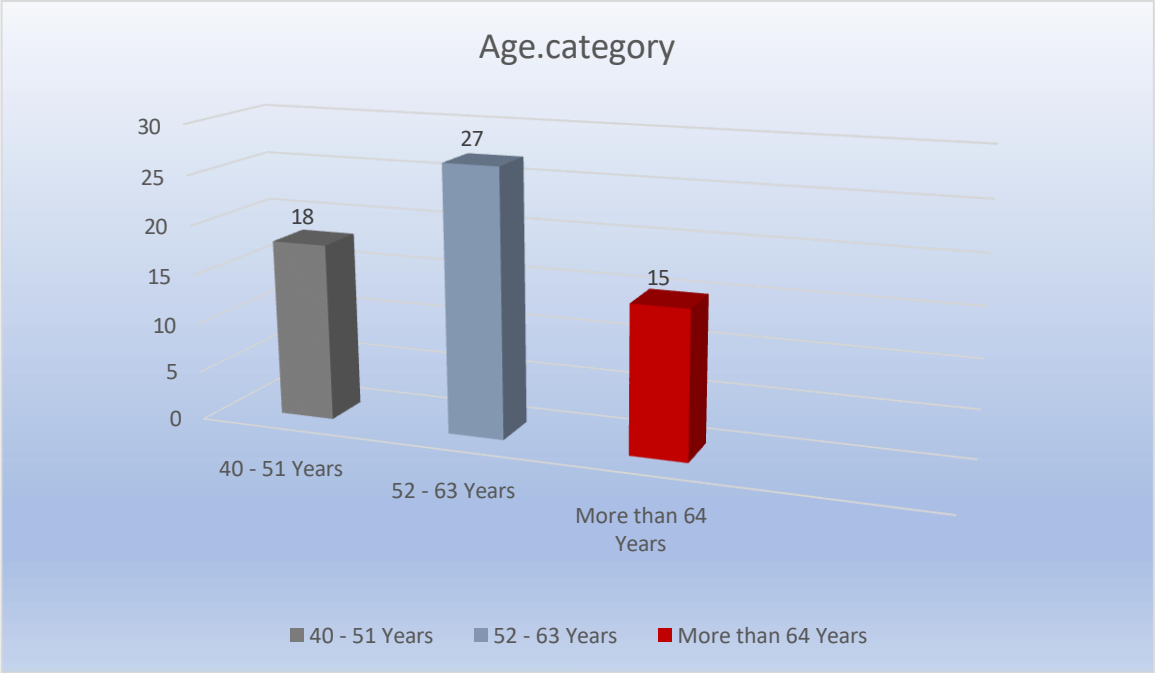

Fig. (3.1) Age groups of the patients in percentages.

The results of the current study showed that obesity accounted for 48.3%, history of hypertension 48.3%, and history of DM 35% as shown in table (3-1).

Table (3-1) Demographic characteristics of the patients.

Variable	Group	Frequency	Percentage
Age Category	40 - 51 Years	18	30%
	52 - 63 Years	27	45%
	More than 64 Years	15	25%
BMI Category	Normal weight	7	11.7%
	Over weight	24	40%
	Obesity	29	48.3%

Hypertension	Yes	29	48.3%
	No	31	51.7%
Diabetes Mellitus	Yes	21	35%
	No	39	65%
Herat failure	Yes	11	18.3%
	No	49	81.7%
Occupation	House Wife	13	21.7%
	Employee	14	23.3%
	Free Work	20	33.3%
	Retired	7	11.7%
	Soldier	6	10%

Descriptive statistics (Frequency & percentage)

There was significant statistical association between history of DM, history of taking metformin, and history of heart failure (p=0.001, 0.003, and 0.008 respectively), as shown in table (3-2).

Table (3-2): Cross tabulation between demographic data and catheterization.

Variable		**Catheterization**		**p value**
		CIN – N = 54	**CIN + N= 6**	
Gender	Male	42(77.8%)	5(83.3%)	0.754 [NS]
	Female	12(22.2%)	1(16.7%)	
Residence	From Karbala	40(74.1%)	4(66.7%)	0.697 [NS]
	Other governorate	14(25.9%)	2(33.3%)	
Age groups	40-51 years	17(31.5%)	2(33.3%)	0.86 [NS]
	52-63 years	25(46.3%)	2(33.3%)	
	More than 64 years	12(22.2%)	2(33.3%)	

BMI categories	Normal weight	6(11.1%)	1(16.7%)	0.58 [NS]
	Overweight	21(38.9%)	3(50%)	
	Obese	27(50%)	2(33.3%)	
Occupation	Housewife	12(22.2%)	1(16.7%)	0.883 [NS]
	Employee	12(22.2%)	2(33.3%)	
	Free work	18(33.3%)	2(33.3%)	
	Retired	6(11.1%)	0(0%)	
	Soldier	6(11.1%)	18(34.6%)	
History of DM	Present	15(27.8%)	6(100%)	**0.001 [S]**
	Not Present	39(72.2%)	0(0%)	
History of HT	Present	25(46.3%)	4(66.7%)	0.417 [NS]
	Not Present	29(53.7%)	2(33.3%)	
History of HF	Present	7(13%)	4(66.7%)	**0.008 [S]**
	Not Present	47(87%)	2(33.3%)	

Results are presented as n= number of subjects and percentage, $p<0.05$ considered significantly different, [S]= Significant, [NS]= Non significant. BMI: Body Mass Index , HT : Hypertension , DM : diabetes, HF: Herat failure.
Chi-square test

There were a non-significant differences between both group (CIN -) and (CIN +) in most demographic characteristics of this patient‘s study regarding gender, age group, BMI and history of hypertension. In contrast there are significant differences in demographic characteristics in history of HF and DM history, and that matched with Mehran *et al*. results obtained regarding association of baseline, clinical, and CIN incidence after PCI **(Mehran *et al*., 2004)**.

Our data shows that there was significant association between those taking metformin.

Metformin	Present	4(7.4%)	4(66.7%)	**0.003 [S]**
	No Present	50(92.6%)	2(33.3%)	

Although several studies showed that metformin (+) and metformin (-) groups had no difference in the terms of CIN or AKI after contrast exposure **(Oktay *et al*., 2017 and Zeller *et al*., 2016)**, the study demonstrated significant differences between the two concerned groups, however patients with T2DM are more likely to develop CIN than those without DM **(Kirpichnikov *et al*., 2002)**. Metformin is eliminated from the body through the kidneys, and diagnostic procedures involving a contrast agent may result in lactic acidosis (LA) and CIN nephropathy **(Senoo *et al*., 2010 and Eppenga *et al*., 2014)**

3.1. Incidence of Contrast Induced Nephropathy.

The incidence of CIN was occurred in 6 patients (10 %) through 24 hours after the procedure as shown in Figure (3.2). Even people with normal kidney function have a CIN after being exposed to CM, according to the results.

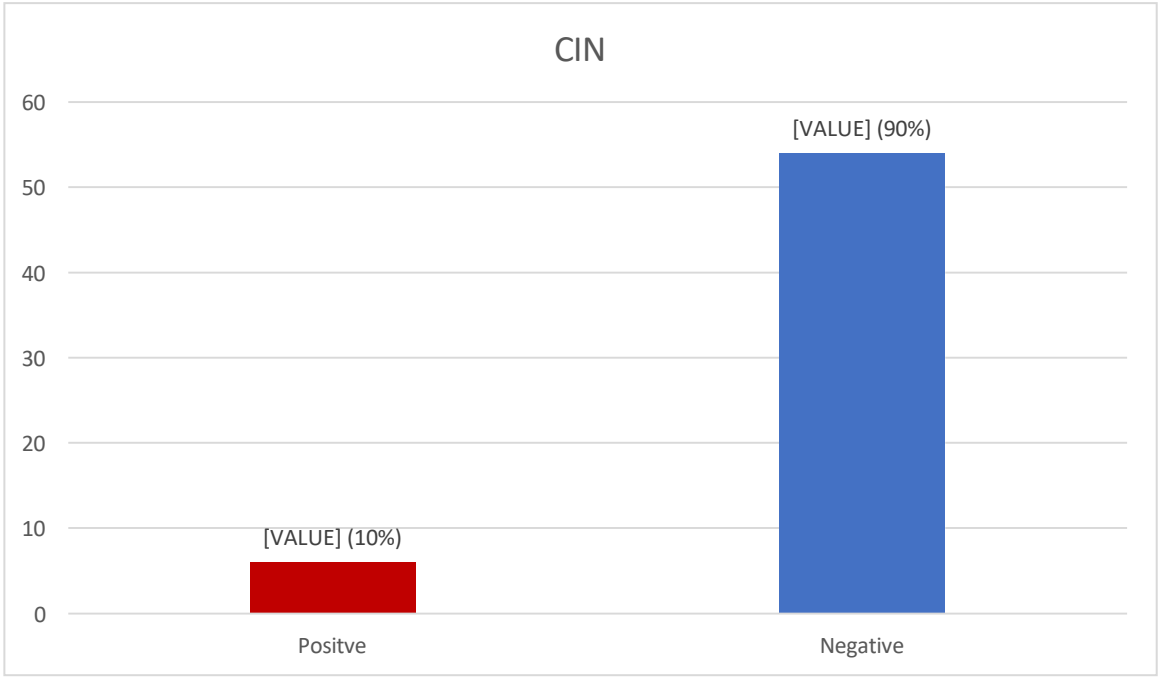

Fig. (3.2) Incidence of contrast induced nephropathy

This study found that even patients with normal kidney function have CIN after CM administration; this finding is consistent with previous study by Assareh *et al.* **(Assareh *et al*., 2016)**, while another study conducted by Sato *et*

al. found that CIN was an independent predictor of subsequent renal events in patients who underwent cardiac catheterization **(Sato *et al.*, 2017)**.

Numerous investigations and researches yield different findings regarding the incidence of CIN, for example in other study included 490 patient underwent angiography procedure the CIN incidence was 20.2% and they had a higher serum creatinine after 2-3 days and lower GFR [96.54(19.57) vs. 77.24 (21.31)] **(Mujtaba *et al*., 2020)**. Likewise high rates (23 to 31 %) have been observed in several Iraqi cities, including Karbala and Baghdad **(Al-Tu'ma *et al*., 2017 and Rasheed *et al*., 2017)**. Other study included 42 individuals with type 2 diabetes who developed CIN after PCI, 48 hours after the procedure; they observed that the incidence of CIN in diabetic patients was roughly 2.6 times than that of non-diabetic patients **(Nassir, 2014)**. A previous study was performed by Wang *et al*. that included 300 patients who underwent coronary angiography ,29 patients (9.6%) of them developed CIN 48 hours after the procedure **(Wang *et al*., 2016)**. While others prospectively evaluated 166 patients their S.Cr and CysC measured at baseline and at 12, 24 and 48 hours after exposure to CM. He found that CIN incidence occurred in 30 patients (18%) **(Ribichini, Gambaro *et al*. 2012)**. But another study which includes 100 patients with CKD history undergoing coronary angiography. Frequency of CIN was 11% and 1 patient required dialysis **(Alharazy *et al*., 2014)**.

There are a much of reasons to make these differences in rate incidence of CIN among studies and researchers such as specimen number of study which some have large and others have a small as well as the procedure of CIN occurrence that researchers depend on after 24 hours of angiography while others taken 48 hours. Also studies found a higher incidence among PCI group with a p value of (<0.05) then Coronary Angiography (CAG).

This study showed the incidence was higher and reported of CIN after PCI procedure was 10% (6 out 60). Ari *et al*. conducted a recent study showed CIN occurred in 13.3% (280 of 2108 patients) of CAG patients, 13.08% (50 of 382 patients) of elective PCI patients, and 21.49% (25 of 114 patients) of emergency PCI patients. CIN rates were significantly higher in the emergency PCI group than in the diagnostic CAG ($p = 0.009$) and elective PCI ($p = 0.02$) groups when compared to the other two groups **(Hasan *et al*., 2017)**. This may be explained by a larger amount of CM used in PCI group **(Schiffrin *et al*., 2007)**. In addition to differences in the definitions of CIN (absolute or relative) **(Mohammed *et al*., 2013)**. Diabetes, being over the age of 70, anemia, dehydration, and hyperlipidemia are all risk factors for CIN. The amount and type of CM used are procedure-relative risk factors **(Pistolesi *et al*., 2018)**. Thus, a risk factor for CIN may be identified as a contrast volume >100 ml **(Sun *et al*., 2019)**. All of the study participants received the low-osmolar contrast agent Omnipaque (Iohexol) 350 mg/1mI), which has an osmality of 844 mOsm/1. When compared to high-osmolar CM, the incidence of CIN is lower with low-osmolar and iso-smolar CM. Pandya *et al*. discovered that in patients with CKD stage 3 and above undergoing diagnostic CAG, the use of iso-osmolar CM showed no statistically significant difference in the incidence of CIN compared to low-osmolar CM **(Pandya *et al*., 2017)**. As there are no indications for using high-osmolar CM today, it has almost entirely been replaced by low-osmolar CM and iso-osmolar, which have less of an impact on creation of CIN **(Bartholomew *et al*., 2004 and Tanaga *et al*., 2012)**.

There was a significant difference in the mean of all laboratory parameters ($p < 0.001$). These results suggest that contrast really does have a risk to induce AKI, as shown in (table3-3).

Table (3-3) Difference in mean of laboratory parameters pre and post contrast

Laboratory Parameters		Mean ± 2SD	Paired t-test p-value
Urea (mg/dl)	PreTest	38.76±15.59	**< 0.001[S]**
	PostTest	45.59±20.98	
Creatinine (mg/dl)	PreTest	0.858±0.202	**< 0.001[S]**
	PostTest	1.01±0.28	
IL18 (pg/ml)	PreTest	44.99±11.82	**< 0.001[S]**
	PostTest	60.71±11.31	
TNF-α (pg/ml)	PreTest	29.75±10.28	**< 0.001[S]**
	PostTest	49.62±9.98	
eGFR (ml/min/1.73m^2)	PreTest	99.60±33.07	**< 0.001[S]**
	PostTest	83.78±30.13	

Results are presented as mean ± SD, $p<0.05$ considered significantly different, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration.
Paired T– test.

Both clinical trials as well as animal experiments revealed that CM had a toxic effect on renal tubules **(Golshahi *et al.*, 2014)**. Romano *et al*. demonstrated that iso-osmolar and low-osmolar CM induced apoptosis in human embryonic kidney, porcine proximal renal tubular, and canine Madin-Darby distal tubular renal cells **(Romano *et al*., 2008 and Kolyada *et al*., 2008)**. However several factors have been linked to the pathogenesis of CIN, including hemodynamic changes in renal blood flow, which causes hypoxia in the renal medulla, and the direct toxic effect of contrast media on renal cells **(Golshahi *et al*., 2014)**.

A significant difference was found between the means of urea pre and post contrast exposure, (p = 0.001, 0.001), creatinine in post contrast (p = 0.001),

And IL-18 post contrast (p = 0.001), and eGFR post contrast exposure (p = 0.002). No significant difference was found between means of other laboratory parameters, (p > 0.05), as shown in Table (3-4).

Table (3-4) Difference between means of laboratory Parameters before and after exposure to contrast material.

Laboratory Parameters		Catheterization		P value
		CIN – N = 54	CIN + N= 6	
Urea (mg/dl)	PreTest	36.14 ± 12.88	62.37 ± 19.22	**0.001[S]**
	PostTest	42.35 ± 17.92	74.83 ± 25.49	**0.001[S]**
Creatinine (mg/dl)	PreTest	0.86 ± 0.19	0.87 ± 0.31	0.913[NS]
	PostTest	0.96 ± 0.23	1.49 ± 0.26	**0.001[S]**
IL18 (pg/ml)	PreTest	44.58 ± 11.99	48.82 ± 10.25	0.409[NS]
	PostTest	58.64 ± 9.35	79.37 ± 10.94	**0.001[S]**
TNF-α (pg/ml)	PreTest	30.78 ± 10.29	20.53 ± 3.05	**0.019[S]**
	PostTest	49.55 ± 10.35	50.25 ± 6.24	0.872[NS]
eGFR (ml/min/1.73m^2)	PreTest	99.04 ± 30.85	104.72 ± 52.74	0.693[NS]
	PostTest	87.71 ± 28.57	48.43 ± 19.87	**0.002[S]**

Results are presented as mean ± SD, p<0.05 considered significantly different, [S] = Significant, [NS] = Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration, CIN –: patient with no contrast induced nephropathy, CIN +: patient with contrast induced nephropathy.
Paired T– test

There is significant increment in serum creatinine and urea while decrease in eGFR, 24 hours after angiography (p<0.001, 0.002), and there‘s a significant increment in serum level of IL-18 (p<0.001).This is consists with Mahran *et al*. **(Mehran *et al*., 2004)** and Kato *et al*. **(Kato *et al*., 2008)**. Ling *et al*. evaluate IL-18 in 150 patients undergoing CAG, thirteen

patients (8.7%) developed AKI and had a significantly raised IL-18 level at 24 hours post-procedure compared with controls with an AUC of 0.75 (P = 0.011, odds ratio: 10.7) **(Ling *et al*., 2008)**. Malyszko *et al*. reported that IL-18 levels increased significantly within 2 hours of contrast angiography (P 0.05) and peaked 24 hours after the procedure (P 0.01) **(Malyszko *et al*., 2009)**. However, a study of PCI patients by Bulent Gul *et al*. found no substantial difference between IL-18 levels in CIN patients and non- CIN controls at 24 or 72 hours post-contrast (P > 0.05) despite a CIN prevalence of 9.5% **(Gul *et al*., 2008)**.

Interluekin-18 is another promising marker for earlier detection of AKI. It is a mediator of tissue ischemia-induced inflammation and damage in many organs. Interluekin-18 is released by the damaged cells of the proximal tubule after being activated by caspase-1, according to studies of ischemic AKI in animal models, and as a result, its concentration level rises **(Gao *et al*., 2013)**. Additionally, we hypothesized that ischemia, hypotension, hypoperfusion, and the release of cytokines were linked to noticeably higher serum IL-18 levels in AKI **(Zdziechowska *et al*., 2020)**.

There was highly significant increment in serum level of TNF-α after exposure to CM of procedure, but comparing between two study groups (CIN+, CIN-) there is no significant differences and this is agreed by Banda J *et al* who reported elevated baseline serum concentrations of TNF-α ($p < 0.001$) and concluded that serum IL-18 and TNF-α demonstrated prognostic significance with mortality in CIN+ patients, despite the poor diagnostic discrimination performance for CIN **(Banda *et al*., 2020)**. Saritemur *et al.* demonstrated that suppressing the increased levels of pro-inflammatory cytokines and a decreased the levels of apoptotic proteins such as Caspase 3, had a positive effect on the irregular course of antioxidant/oxidative components such as superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) and major serum parameters such as creatine, which played a role in renal tubular damage

.He concluded that there's inhibitory effect of TNF-α in CIN injury **(Saritemur *et al*., 2015)**. However, more of clinical and experimental studies are needed to discover the effect of CM administration on human TNF-α activity and how far is correlated with CIN.

By performing a one-way ANOVA test to compare the mean of: urea, creatinine, IL-18, TNF-α, and eGFR. a significant difference was found between mean of urea pre contrast, (p = 0.008).Post hoc testing using LSD adjustment showed that the mean urea level for the age group more than 64 years (49.72±17.24) is significantly higher than that age groups 40-51 years and 52-63 years. The mean eGFR levels also shows significant differences in pre and post contrast (p = 0.005, and 0.016).Post hoc testing using LSD adjustment showed that the mean eGFR level pre contrast for the age group 40-51 years (117.58±28.72) is significantly higher than that age groups 52-63 years and more than 64 years. Also post hoc testing using LSD adjustment showed that the mean eGFR level post contrast for the age group 40-51 years (96.77±27.14) is significantly higher than that age groups more than 64 years. No statistically significant difference was found among other mean of laboratory parameters.

Table (3-5) Difference between mean of laboratory parameters levels in relation to age groups before and after exposure to contrast material.

Lab. Parameters		Age Group			P value
		40 -51 Years N= 18	52 – 63 Years N= 27	> 64 Years N= 15	
Urea (mg/dl)	PreTest	34.05±11.06	36.39±15.22	49.72±17.24	**0.008[S]**
	PostTest	41.10±19.63	42.85±21.21	57±19.49	0.062[NS]
Creatinine (mg/dl)	PreTest	0.79±0.15	0.89±0.23	0.89±0.19	0.208[NS]
	PostTest	0.96±0.24	1.02±0.3	1.08±0.29	0.472[NS]

IL18 (pg/ml)	**PreTest**	45.25±11.23	44.44±13.83	45.73±8.71	0.943[NS]
	PostTest	61.51±12.25	60.07±10.84	60.87±11.68	0.915[NS]
TNF -α (pg/ml)	**PreTest**	29.52±11.34	30.52±9.7	28.58±10.49	0.847[NS]
	PostTest	50.75±10.87	49.47±9.88	48.37±9.48	0.796[NS]
eGFR (ml/min/1.73m^2)	**PreTest**	117.58±28.72	96.29±33.05	81.6±28.14	**0.005[S]**
	PostTest	96.77±27.14	83.38±31.5	66.9±23.69	**0.016[S]**

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration
ANOVA test

By performing a one-way ANOVA test to compare the mean urea, creatinine, IL18, TNF-α, and eGFR. No significant difference was found between mean of laboratory parameters pre and post contrast and BMI groups, (p > 0.05).

Table (3-6) Difference between mean of laboratory Parameters levels in relation to BMI groups before and after exposure to contrast material.

Lab. Parameters		**BMI categories**			**P value**
		Normal weight N= 7	**Over weight N= 24**	**Obese N= 29**	
Urea (mg/dl)	**PreTest**	39.86±20.02	37.3±16.79	39.7±13.86	0.844[NS]
	PostTest	46.43±31.03	42.58±18.93	47.89±20.34	0.66[NS]
Creatinine (mg/dl)	**PreTest**	0.93±0.26	0.82±0.19	0.87±0.2	0.443[NS]
	PostTest	1.09±0.41	0.98±0.25	1.02±0.28	0.655[NS]
IL18 (pg/ml)	**PreTest**	48.02±9.29	43.96±11.53	45.13±12.79	0.731[NS]
	PostTest	58.88±12.83	61.93±9.94	60.15±12.28	0.772[NS]
TNF-α	**PreTest**	25.08±6.76	28.79±9.19	31.68±11.54	0.267[NS]

(pg/ml)	**PostTest**	48.71±11.74	49.89±7.59	49.62±11.52	0.964[NS]
eGFR (ml/min/1.73m^2)	**PreTest**	88.19±32.09	103.98±32.96	98.74±33.78	0.537[NS]
	PostTest	75.6±37.08	85.99±27.94	83.92±30.92	0.731[NS]

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration.
ANOVA test

Pearson correlation was performed to show the correlation of the age with urea, creatinine, IL-18, TNF-α, and eGFR. The results revealed significant positive correlation of the age with urea pre contrast (R = 0.3, P=0.016), and negative correlation with eGFR pre (R = -0.4, P=0.001), and post (R = -0.4, P=0.003), contrast as shown in table (3-7).

Table (3-7) Correlation of the laboratory parameters with age.

Laboratory Parameters versus age		**Correlation coefficient (r)**	**P value**
Urea(mg/dl)	PreTest	0.3	**0.016[S]**
	PostTest	0.2	0.065[NS]
Creatinine(mg/dl)	PreTest	0.2	0.13[NS]
	PostTest	0.1	0.281[NS]
IL18 (pg/ml)	PreTest	0.1	0.599[NS]
	PostTest	0.1	0.549[NS]
TNF-α(pg/ml)	PreTest	0.1	0.914[NS]
	PostTest	- 0.1	0.916[NS]
eGFR(ml/min/1.73m^2)	PreTest	- 0.4	**0.001[S]**
	PostTest	- 0.4	**0.003[S]**

p<0.05 considered significant, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration
Correlation (Bivariate)

Pearson correlation coefficient revealed no significant correlation of the BMI with laboratory parameters as shown in table (3-8).

Table (3-8) Correlation of the laboratory parameters with BMI.

Lab. parameters vs. BMI		Correlation coefficient (r)	P value
Urea(mg/dl)	**PreTest**	- 0.1	0.57[NS]
	PostTest	- 0.1	0.646[NS]
Creatinine(mg/dl)	**PreTest**	- 0.1	0.288[NS]
	PostTest	- 0.2	0.217[NS]
IL18 (pg/ml)	**PreTest**	- 0.1	0.702[NS]
	PostTest	- 0.1	0.858[NS]
TNF-α(pg/ml)	**PreTest**	0.2	0.227[NS]
	PostTest	- 0.1	0.721[NS]
eGFR(ml/min/1.73m^2)	**PreTest**	0.2	0.214[NS]
	PostTest	0.2	0.093[NS]

p<0.05 considered significant, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration.
Correlation (Bivariate)

Pearson correlation was performed to show the correlation of the (IL18) before contrast with urea, creatinine, IL18, TNF-α, and eGFR and after. The results revealed significant positive correlation of the (IL18) before contrast with Creatinine before procedure, Creatinine after procedure, IL18 after procedure, and TNF-α before procedure. Also negative correlation with eGFR pre and post contrast as shown in table (3-9).

Table (3-9) Correlation Coefficient between IL-18 level and Biochemical parameters in study groups before and after exposure to contrast material.

Biomarkers	*IL18.Before* (pg/ml)	
	Correlation coefficient (r)	***P (2-tailed)***
Urea before procedure (mg/dl)	**0.2**	**0.15 [NS]**
Urea after procedure (mg/dl)	**0.2**	**0.23[NS]**
Creatinine before procedure (mg/dl)	**0.4**	**0.004[S]**
Creatinine after procedure (mg/dl)	**0.5**	**<0.001[S]**
Il18 after procedure (pg/ml)	**0.5**	**<0.001[S]**
TNF-α before procedure (pg/ml)	**0.3**	**0.041[S]**
TNF-α after procedure (pg/ml)	**0.3**	**0.83[NS]**
eGFR before procedure (ml/min/1.73m^2)	**-0.4**	**0.002[S]**
eGFR after procedure (ml/min/1.73m^2)	**-0.5**	**<0.001[S]**

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration
Correlation (Bivariate)

Pearson correlation of the IL-18 with urea, creatinine, IL-18 after contrast exposure, TNF-α, and eGFR: revealed significant positive correlation of the (IL18) with Creatinine, and negative correlation with eGFR post contrast as shown in table (3-10).

Table (3-10) Correlation coefficients between *IL18 levels* and biochemical parameters in in study groups after exposure to contrast material for diagnostic coronary angiography.

Biomarkers	IL18.After (pg/ml)	
	Correlation coefficient (r)	P (2-tailed)
Urea before procedure (mg/dl)	0.2	0.236[NS]
Urea after procedure (mg/dl)	0.2	0.147[NS]
Creatinine before procedure (mg/dl)	0.1	0.562[NS]
Creatinine after procedure (mg/dl)	0.4	**0.003[S]**
Il18 before procedure (pg/ml)	0.4	**0.005[S]**
TNF-α before procedure (pg/ml)	-0.1	0.870[NS]
TNF-α after procedure (pg/ml)	0.1	0.498[NS]
eGFR before procedure (ml/min/1.73m2)	-0.1	0.901[NS]
eGFR after procedure (ml/min/1.73m2)	-0.4	**0.002[S]**

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration.

Correlation (Bivariate)

The logistic analysis of the patients concluded that the level of urea after contrast exposure is significant (OR = 1.064, 1.034, p = 0.007, p= 0.025), the level of creatinine after contrast exposure is significant (OR = 48.241, p =

0.018), the level of TNF-α before contrast is significant (OR = 0.872, p = 0.035), and lastly the level of eGFR after procedure is significant (OR = 0.929, p = 0.011). No other significant effect on other parameters p >0.05.

Table (3-11) the odds ratios of laboratory parameters before and after PCI.

Biomarkers	Angiography Intervention	
	OR (95% CI)	p value
Urea before procedure (mg/dl)	1.064(1.017 – 1.113)	**0.007[S]**
Urea after procedure (mg/dl)	1.034(1.004 – 1.066)	**0.025[S]**
Creatinine before procedure (mg/dl)	1.724(0.126 – 23.672)	0.684[NS]
Creatinine after procedure (mg/dl)	48.241(1.956 – 1189.812)	**0.018[S]**
Il18 before procedure (pg/ml)	1.024(0.959 – 1.094)	0.480[NS]
Il18 after procedure (pg/ml)	1.413(<0.0001)	0.922[NS]
TNF-α before procedure (pg/ml)	0.872(0.767 – 0.991)	**0.035[S]**
TNF-α after procedure (pg/ml)	1.007(0.925 – 1.097)	0.869[NS]
eGFR beore procedure (ml/min/1.73m^2)	1.005(0.980 – 1.030)	0.721[NS]
eGFR after procedure (ml/min/1.73m^2)	0.929(0.877 – 0.983)	**0.011[S]**

Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence IntervalIL18: Interleukins 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration
Regression (Multinomial Logistic)

3.2. ROC analysis

ROC analysis curve of IL18 levels in patients before exposure to contrast material showed the sensitivity and specificity: 66.7%, 57.4% respectively at the threshold point (45.25). The IL18 levels were not statistically differed before exposure to contrast material (p value =0.48).

Table (3-12) AUC, optimal threshold, Sensitivity and specificity of IL18 levels before exposure to contrast material.

Test Variable	AUP	Sensitivity %	Specificity %	Cut-off points	CI (95%)
IL-18 (pg/ml) (Before)	0.588	0.667	0.574	45.25	0.363 - 0.813

AUP: Area under pick, CI: con confidence interval

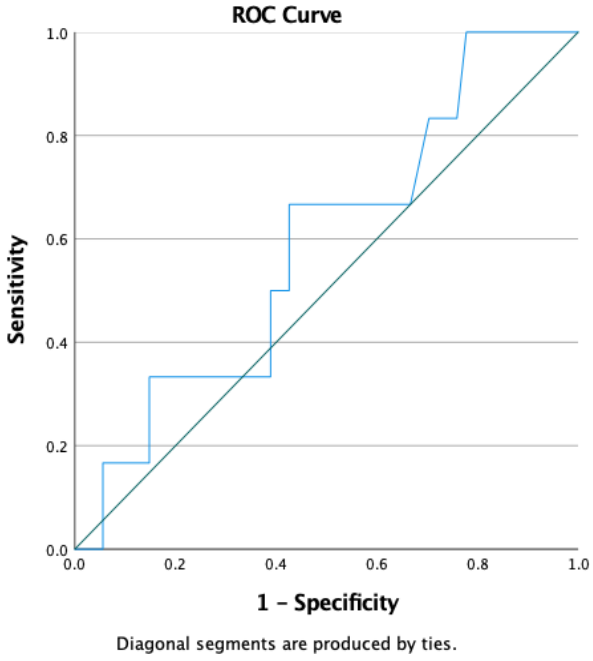

Fig. (3-3) Receiver operating characteristics (ROC) curve analysis of IL18 level before exposure to contrast material of conventional coronary angiography.

The ROC analysis curve of TNF-α levels in patients before exposure to contrast material was shown the sensitivity and specificity of: 79.6%, 66.7% respectively at the threshold point (22). The TNF-α levels were statistically differed before exposure to contrast material (p value =0.01).

Table (3-13) AUC, optimal threshold, sensitivity and specificity of TNF –α levels (Before) exposure to contrast material.

Test Variable	AUP	Sensitivity %	Specificity %	Cut-off points	CI (95%)
TNF-α (pg/ml) (Before)	0.818	0.796	0.667	21.8942	0.704 - 0.932

AUP: Area under pick, CI: con confidence interval

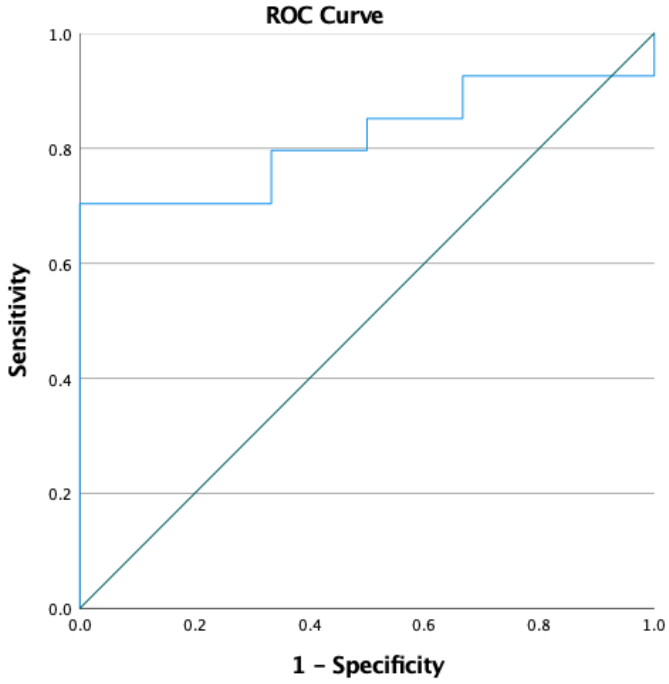

Fig. (3-4) Receiver operating characteristics (ROC) curve analysis of TNF-α level before exposure to contrast material of conventional coronary angiography.

The ROC analysis curve of TNF-α levels in patients after exposure to contrast material was shown the sensitivity and specificity to be 66.7%, 59.3% respectively at the threshold point (51.9373). The TNF-α levels were not statistically differed after exposure to contrast material (p value =0.8).

Table (3-14) AUC, optimal threshold, sensitivity and specificity of (TNF-α) levels after contrast material EXPOSURE.

Test Variable	AUP	Sensitivity %	Specificity %	Cut-off points	CI (95%)
TNF-α (pg/ml) (After)	0.525	0.667	0.593	51.9373	0.347 - 0.702

AUP: Area under pick, CI: con confidence interval

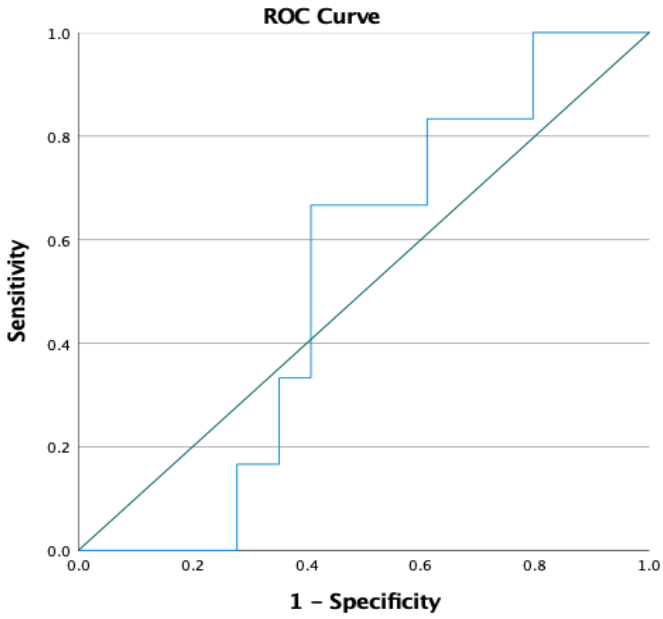

Fig. (3-5) Receiver operating characteristics (ROC) curve analysis of TNF-α level after exposure to contrast material of conventional coronary angiography

3.4. Mehran Risk Score

The CIN after percutaneous endovascular procedures has been linked to a number of baseline patient characteristics and procedural parameters, as well as an unfavorable outcome. The risk of CIN and its adverse outcomes has been shown to exist in both patients with and without chronic kidney disease, and to

increase in diabetic patients **(Iakovou *et al*., 2003)**. Patients with chronic kidney disease (CKD), diabetes mellitus, congestive heart failure, older age, hypotension, and anemia are at particular risk of CI-AKI, and Mehran *et al*. devised a simple risk score for prediction of CI-AKI after PCI. The Mehran risk score (MRS) is a scoring system based on comorbidities and procedural risk factors, including hypotension, intra-aortic balloon pumping, heart failure, age >75 years, anemia, diabetes mellitus, volume of contrast, and renal function. Classification is of <5 (low-risk), 6–10 (medium-risk), 11–15 (high-risk), and >16 (very high-risk) **(Mehran *et al*., 2004)**. Table (3-15) shows the difference in mean levels of biomarkers between groups of Mehran score pre and post contrast material for conventional coronary angiography conditions. There is significant difference between mean levels pre and post of urea, post test of Creatinine, posttest of IL-18, and posttest of eGFR as shown below.

Table (3-15) Difference between mean of laboratory parameters in relation to Mehran score groups before and after exposure to contrast material.

Lab. parameters		Mahran. Secore			P value
		Low N= 54	Moderate N= 3	High Risk N= 3	
Urea (mg/dl)	**PreTest**	**36.69±14.73**	**77.40±49.68**	**64.00±21.07**	**<0.001[S]**
	PostTest	43.65±21.54	119.33±101.91	71.00±24.27	**<0.001[S]**
Creatinine (mg/dl)	PreTest	0.88±0.269	1.13±0.58	0.73±0.25	0.224[NS]
	PostTest	0.98±0.29	2.19±1.48	1.45±0.25	**<0.001[S]**
IL18 (pg/ml)	PreTest	44.98±12.94	53.15±13.78	44.49±4.08	0.55[NS]
	PostTest	59.71±11.396	107.78±6.67	99.82±0.89	**<0.001[S]**
TNF-α (pg/ml)	PreTest	31.15±11.18	20.76±3.44	20.31±3.34	0.08[NS]
	PostTest	49.55±10.35	48.71±8.02	51.79±5.08	0.92[NS]
eGFR	PreTest	99.59±31.502	85.63±37.94	123.81±66.49	0.36[NS]

(ml/min/1.73m^2)	PostTest	88.26±30.03	45.43±24.53	51.43±18.94	**0.01[S]**

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration
ANOVA test

Analyses were conducted to assess the association between the laboratory parameters with the moderate and high-risk Mehran score according to logistic regression, there was significant association between different groups of Mehran score (low risk is a reference variable) in urea pre and post moderate risk (OR: 1.07(1.02–1.13), OR:1.04(1.01–1.08) respectively, while only in pre in high-risk group. Also, there is significant association between posttest of creatinine in moderate and high-risk groups. And finally, there is significant association in pre and posttest of eGFR in moderate and high-risk groups as shown in table (3-16) below.

Table (3-16): Logistic regression to show association between groups of Mehran score in relation to mean levels of laboratory Parameters

Variables		Moderate		High Risk	
		Odd Ratio 95% CI (Lower-Upper)	**P - value**	**Odd Ratio 95% CI)Lower-Upper)**	**P - value**
Urea (mg/dl)	PreTest	1.070(1.015 – 1.128)	**0.012[S]**	1.058 (1.006 – 1.112)	**0.03[S]**
	PostTest	1.041(1.006– 1.077	0.02[NS]	1.029(0.995 – 1.064)	0.09[NS]
Creatinine (mg/dl)	PreTest	6.634(0.420–104.86)	0.17[NS]	0.02(9.155 – 41.833	0.31[NS]
	PostTest	82.20(2.417-2795.95)	**0.014[S]**	33.125(1.191–921.27)	**0.04[S]**
IL18 (pg/ml)	PreTest	1.050(0.961 – 1.147)	0.28[NS]	997 (0.907 – 1.095)	0.94[NS]
	PostTest	3.408(<0.001)	0.93[NS]	3.974(<0.001)	0.92[NS]
TNF-α	PreTest	0.875(0.740 – 1.035)	0.11[NS]	0.868(0.731 – 1.030)	0.10[NS]

(pg/ml)	PostTest	0.991(0.88-1.116)	0.88[NS]	1.023 (0.910 – 1.150)	0.19[NS]
eGFR	PreTest	0.986 (0.948– 1.025)	0.47[NS]	1.021 (0.987 – 1.055)	0.233[NS]
(ml/min/1.73m^2)	PostTest	0.920(0.854 – 0.992	**0.03[S]**	0.936(0.874 – 1.001	**0.05[S]**

Results are presented as OR: odd ratio , p<0.05 considered significantly different, [S]= Significant, NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR:Estimated glomerular filtration
Regression (Multinomial Logistic)

Incidence of CIN in the study group according to Mehran risk score system. The bars show the numbers of patients with a (+ CIN) and those without (- CIN) in low, moderate and high risk patients according to Mehran risk score. There was significant difference in the incidence of CIN between the 3 groups ($p < 0.001$).

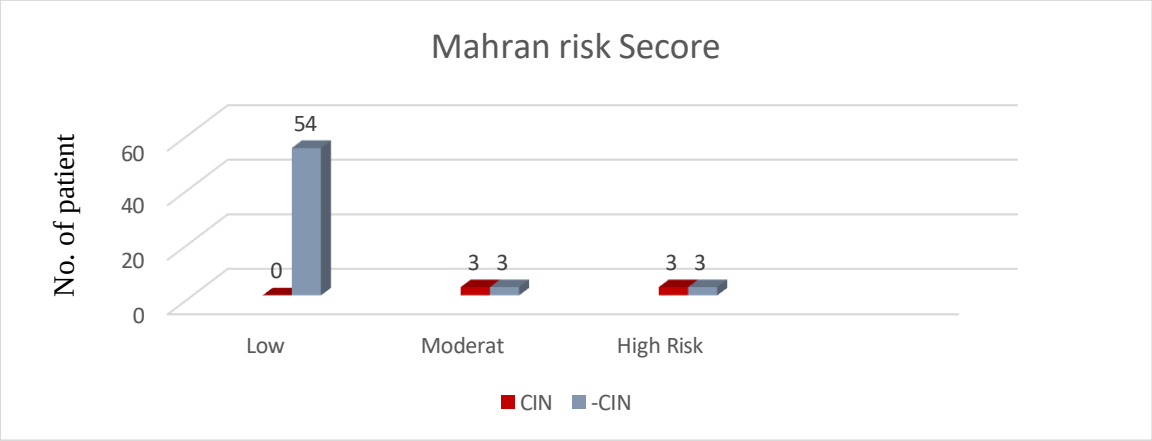

Fig. (3-6): Classification of patients according to Mehran risk score.

Correlation coefficients between Mehran score levels and biochemical parameters in in study groups before and after exposure to contrast material for conventional coronary angiography.

Table 3.17: Logistic regression shows association between groups of Mehran score in relation to mean levels of laboratory parameters.

Lab. parameters vs. Mehran score		Correlation coefficient (r)	P value
Urea(mg/dl)	PreTest	0.4	**0.001[S]**
	PostTest	0. 3	**0.014[S]**
Creatinine(mg/dl)	PreTest	-0.1	0.687[NS]
	PostTest	0.4	**0.005[S]**
IL18 (pg/ml)	PreTest	0.1	0.807[NS]
	PostTest	0.7	**<0.001[S]**
TNF-α (pg/ml)	PreTest	-0.3	**0.044[S]**
	PostTest	0.1	0.754[NS]
eGFR(ml/min/1.73m^2)	PreTest	0.1	0.348[NS]
	PostTest	-0.3	**0.011[S]**
BMI		-0.1	0.521[NS]
Age		0.4	**<0.001[S]**

p<0.05 considered significant, [S]= Significant, [NS]= Non significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration. Correlation (Bivariate)

3.5. Molecular Results Obtained

Genetic factors have been identified as involved in the issue for AKI susceptibility and incidence, explaining why only certain patients are more susceptible to AKI and why different patients respond differently to treatment **(Karimi *et al.*, 2012 and Weinstein *et al.*, 2013)**. A more individualized medical approach could result from a better understanding of how gene interaction affects health or disease, enabling the creation of a specific individual genetic print on which medical decisions should be based. Various reports have investigated the connection between diverse polymorphisms and

the potential for AKI in numerous clinical contexts **(Cardinal-Fernández *et al*., 2012 and Haase-Fielitz *et al*., 2007)**.

Tumor necrosis factor alpha gene mutations may influence the proinflammatory cytokine response to stressful stimulation. This has huge implications in the demonstration of AKI, because the intensity of proinflammatory reactions may determine the severity of AKI and, thus, the need for renal replacement therapy and in-hospital mortality **(Jaber *et al*., 2004)**. The TNF-α gene rs1800629 and gene rs361525 are most frequent studied polymorphism in AKI **(Hashad *et al*., 2017 and Ortega-Loubon *et al*., 2021)**. Figure (3-7) show agarose gel electrophoresis showed genotyping of TNF-α (rs361525) Polymorphism that analyzed in this study.

Fig. (3-7): Agarose Gel Electrophoresis Showed Genotyping of TNF-α (rs361525) Polymorphism, Lan M: DNA molecular, Lan 1, 2 Represented GG Genotype, Lan 3, 4 Represented AA Genotype, Lan 5, 6 Represented GA Genotype, Lan 7, 8 Represented GA Genotype.

Promoter polymorphisms in the IL18 gene may impact cytokine levels. The IL18 polymorphisms rs1946518 and rs187238 are two examples **(Ortega-Loubon *et al*., 2021)**. Interleukin-18 is also implicated in the pathogenesis of AKI. And has been linked to AKI, causing acute tubular necrosis in the kidney **(Lin *et al*., 2015 and Awad *et al.*, 2011)**. As a result, a low level of interleukin-18 may be a risk factor for AKI.

The results of TNF-α gene polymorphism (rs361525) were a clear band with a molecular size 349 bps. (Figure (3-7)) .The size of amplicon was determined by compare with DNA ladder 100 - 1500 bp.

Genetic polymorphism of TNF-α (rs361525) which were classified into three genotypes:

1. The major genotype group (GG) homozygous for the allele G.
2. The minor genotype group (AA) homozygous for the allele A.
3. Heterozygous (GA).

The distribution of genotyping groups of patients shows in table (3-18).

Table (3-18) Distribution of TNF-α Polymorphism (rs361525) in studied patients.

Variable	Group	Frequency	Percentage
Genotype	***GG (wild)***	52	86.7%
	GA (hetero)	5	8.3%
	AA (homo)	3	5%
Data Presented by numbers and percentage			

Examination the Hardy–Weinberg equilibrium for TNF-α (rs 361525) in patient underwent coronary angiography. The result of comparison between observed and anticipated values for SNP with TNF-α polymorphism rs 361525 in the tested patient were shown in figure (3-8), and table (3-19). The distribution and percentage of individuals having rs 361525 differ from those

expected under Hardy–Weinberg equilibrium {number of observed vs expected, which were: GG (54, 49.5); AA (2, 0.5); GA (4, 10) (goodness-of-fit $\chi 2$ for rs 361525 15.121, P = 0. 865} and therefore it was statistically not significant.

Table (3-19): Hardy–Weinberg equilibrium for TNF-α rs 361525 genotype in patients

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			G	A	
Symbol	**Frequency**	**%**	0.908	0.092	15.121 **P= 0.** 865
GG	52	86.7			
GA	5	8.3			
AA	3	5			
Total	60				

Cardinal-Fernández *et al*. have been identified 12 relevant studies that included 4835 patients. Eleven genes showed polymorphisms related to AKI susceptibility or severity. They were related to cardiovascular regulation and inflammatory response such as TNF-α, which agreed with our study findings that demonstrated TNF-α gene rs361525 has not been associated with AKI **(Cardinal-Fernández *et al*., 2012 and Ortega-Loubon *et al*., 2021)**. Chang, Lu *et al.* enrolled 508 patients in their study, three tag SNPs in TNF-a (rs1800629, rs1799964, and rs1800630) were selected, of which 53 (10.43%) had CIN according to the CIN definition. The main findings of the study had been: specific genotype polymorphisms of cytokines (GA+AA (rs1800629) in TNF-a, was associated with CIN risk and poor long-term renal outcomes after PCI **(Chang *et al*., 2013)**. In an allele-based analysis of CIN risk, the CIN patients had higher frequencies of the TNF-α A allele (rs1800629) and IL-10 G allele (rs1800896) than the controls (OR = 2.01 (1.13-3.55) and OR

= 2.58 (1.40-4.76), respectively). The other SNPs did not significantly correlate with CIN **(Chang *et al*., 2013)**.

Laboratory studies using animal models of ischemia-reperfusion, sepsis, endo toxemia, and nephrotoxic models have demonstrated that inflammation played a significant role in the pathogenesis of AKI **(Akcay *et al*., 2009)**. The recruitment and migration of circulating inflammatory cells into sites of inflammation is facilitated by vascular endothelial cells. Due to direct contact with harmful agents, the endothelial cells in the injured kidney start early inflammatory responses. The injured kidney is then infiltrated by leukocytes, including neutrophils, macrophages, natural killer cells, and lymphocytes **(Chang *et al*., 2013)**. TNF-α is essential for the gene expression of a neutrophil chemotactic factor in endothelial cells.TNF-α and other pro-inflammatory cytokines can be produced by renal tubular cells. TNF-α expression in early renal tissue plays an important role in mediating neutrophil infiltration and tissue injury following ischemia-reperfusion **(Donnahoo *et al*., 1999)**.

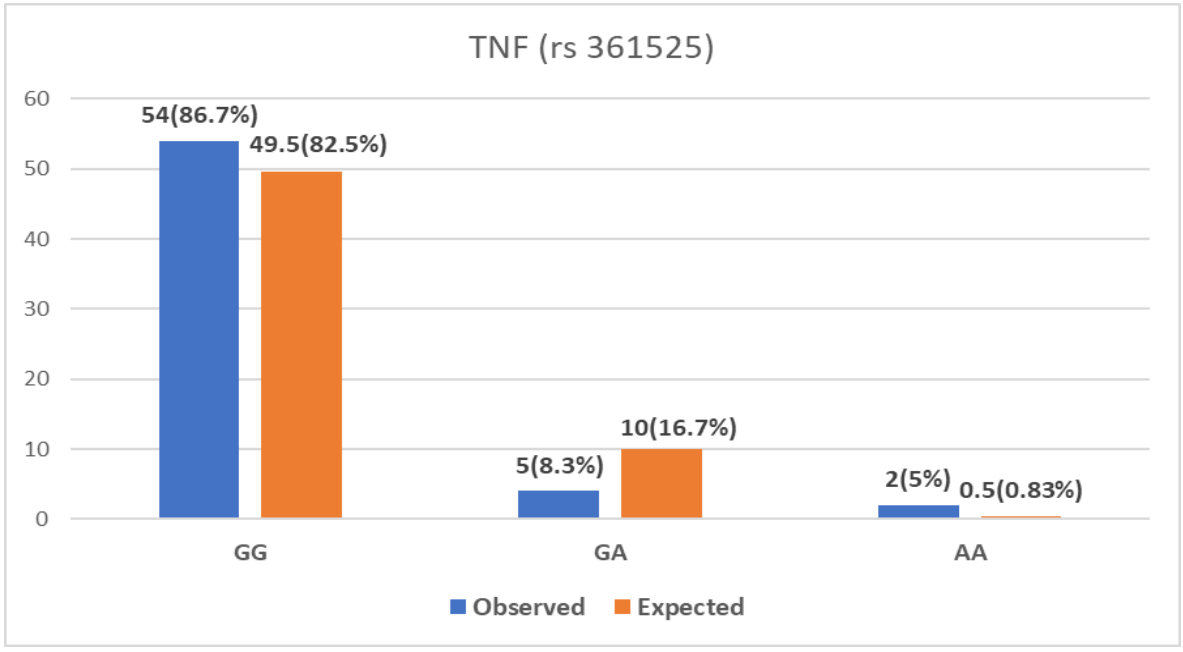

Fig. (3-8): Observed versus expected genotype frequencies of TNF-α polymorphism (rs361525) .TTF:Tumour Necrosis Factor Alpha

To show the difference between demographic characteristics and TNF-α Polymorphism (rs361525) table (3-20), by performing a one-way ANOVA test to compare the mean age, weight, height, BMI. No statistically significant difference was found among mean of demographic characteristics and TNF-α polymorphism (rs361525).

A cross-tabulation by a fisher‘s exact test was conducted between TNF-α Polymorphism (rs361525) groups and gender, residence, occupation, history of DM, history of HT, history of HF, and catheterization. There was no statistically significant association between them $p > 0.05$.

Table (3-20) difference in mean of demographic characteristic between TNF-α Polymorphic (rs361525) genotypes

Demographic parameters		Patient Genotype (N=60)			Statistical Analysis	P value
		GG (N=52)	AG (N=5)	AA (N=3)		
Age (years)		56.23 ± 10.1	63.6 ± 12.38	62 ± 5.19	**ANOVA** F= 1.563 *df* = 2	0.218 [NS]
Height (cm)		167.29 ± 4.89	166 ± 6.52	168.33 ± 2.9	**ANOVA** F = 0.23 *df* = 2	0.795 [NS]
Weight (kg)		84.25 ± 13.67	81 ± 9.19	83.67 ± 5.51	**ANOVA** F = 0.139 *df* = 2	0.871 [NS]
BMI (kg/m$^{2)}$		29.98 ± 4.15	29.37 ± 2.74	29.53 ± 1.74	**ANOVA** F = 0.068 df = 2	0.935 [NS]
Gender	**Male**	41(78.8%)	3(60%)	3(100%)	Fisher‘s exact = 1.599 df =2	0.382 [NS]
	Female	11(21.2%)	2(40%)	0(0%)		
Residence	**Kerbala**	38(73.1%)	4(80%)	2(66.7%)	Fisher‘s exact = 0.482 df =2	0.912 [NS]
	Outside Kerbala	14(26.9%)	1(20%)	1(33.3%)		

History of DM	**Yes**	16(30.8%)	3(60%)	2(66.7%)	Fisher's exact = 3.212 df =2	0.083 [NS]
	No	36(69.2%)	2(40%)	1(33.3%)		
History of HT	**Yes**	25(48.1%)	2(40%)	2(66.7%)	Fisher's exact = 0.69 df =2	0.462 [NS]
	No	27(51.9%)	3(60%)	1(33.3%)		
Occupation	**Housewife**	11(21.2%)	2(40%)	0(0%)	Fisher's exact= 4.194 *df* =8	0.937 [|NS]
	Employee	12(23.1%)	1(20%)	1(33.3%)		
	Free work	17(32.7%)	2(40%)	1(33.3%)		
	Retired	6(11.5%)	0(0%)	1(33.3%)		
	Soldier	6(11.5%)	0(0%)	0(0%)		
History of HF	**Yes**	8(15.4%)	1(20%)	2(66.7%)	Fisher's exact = 4.383 *df* =2	0.12 [NS]
	No	44(84.6%)	4(80%)	1(33.3%)		
Catheterization	**Negative**	48(92.3%)	4(80%)	2(66.7%)	Fisher's exact= 3.599 *df* =2	0.178 [NS]
	Positive	4(7.7%)	1(20%)	1(33.3%)		

Results are presented as mean ± SD, or n= number of subjects and percentage, $p<0.05$ considered significantly different, [S]= Significant, [NS]= non-significant.

Table(3-21) to show the differences mean of between clinical parameters and TNF-α Polymorphic (rs361525) genotypes, by performing a one-way ANOVA test to compare the mean of urea, creatinine, IL18, TNF-α, and eGFR pre and post contrast, no significant difference was found between mean ± SD of parameters and TNF-α gene polymorphic (rs361525) genotypes ($p> 0.05$).

Table (3-21) Difference in mean of lab. Parameters pre and post contrast with TNF-α Polymorphic (rs361525) genotypes.

Demographic parameters		Patient Genotype (N=60)			Statistical test	P value
		GG (N=52)	AG (N=5)	AA (N=3)		
Urea (mg/dl)	Pre	37.26±13.36	47.46±25.72	50.33±29.16	ANOVA F= 1.901 *df* = 2	0.159[NS]
	Post	44.09±20.01	53.80±27.36	58.00±28.58	ANOVA F= 1.041 *df* = 2	0.36[NS]
Creatinine (mg/dl)	Pre	0.87±0.21	0.78±0.13	0.77±0.12	ANOVA F= 0.779 *df* = 2	0.464[NS]
	Post	1.01±0.27	0.98±0.41	1.08±0.31	ANOVA F= 0.114 df = 2	0.893[NS]
IL18 (pg/ml)	Pre	45.33±12.56	40.18±3.53	47.33±2.75	ANOVA F= 0.486 *df* = 2	0.617[NS]
	Post	60.72±11.38	58.88±11.21	63.67±14.15	ANOVA F= 0.163 *df* = 2	0.85[NS]
TNF –α (pg/ml)	Pre	30.18±10.75	24.67±2.94	30.83±9.00	ANOVA F= 0.666 *df* = 2	0.518[NS]
	Post	49.81±10.56	48.70±4.85	47.85±6.39	ANOVA F= 0.76 df = 2	0.927[NS]
eGFR (ml/min/1.73m^2)	Pre	99.28±34.44	102.25±29.18	100.78±17	ANOVA F= 0.02 *df* = 2	0.981[NS]
	Post	84.28±30.71	86.50±31.83	70.63±20.50	ANOVA F= 0.306 df = 2	0.738[NS]

Results are presented as mean ± SD, $p<0.05$ considered significantly different, [S]= Significant, [NS]= Non-significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration.
Chi-square test & ANOVA test

The odds ratios of the detected genotypes of TNF-α polymorphism (rs361525) in table (3-18) shows that no significant effect of all genotypes association with CIN.

Table (3-22) Association between the TNF-α gene polymorphisms with CIN after PCI

TNF-α rs361525	CIN -	CIN +	OR (95% CI)	p value
GG	48(88.9%)	4(66.7%)	1a	-
AG	3(5.6%)	2(33.3%)	0.421 (0.023-7.593)	0.558 [NS]
AA	3(5.6%)	**0(0.0%)**	1.185 (0.115-12.169)	0.448 [NS]

Results are presented as numbers and percentage, $p<0.05$ considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, a; reference ategory, CIN-: patient with no contrast induced nephropathy, CIN+: patient with contrast induced nephropathy.
Regression (Multinomial Logistic)

The results of Interleukin 18 polymorphism (rs1946518) were a clear band with a molecular size 186 bps. Figure (3-9) the size of amplicon was determined by compare with DNA ladder 100 - 1500 bp. Figure (3-9): show agarose gel electrophoresis genotyping of IL-18 (rs1946518) polymorphism that analyzed in this study.

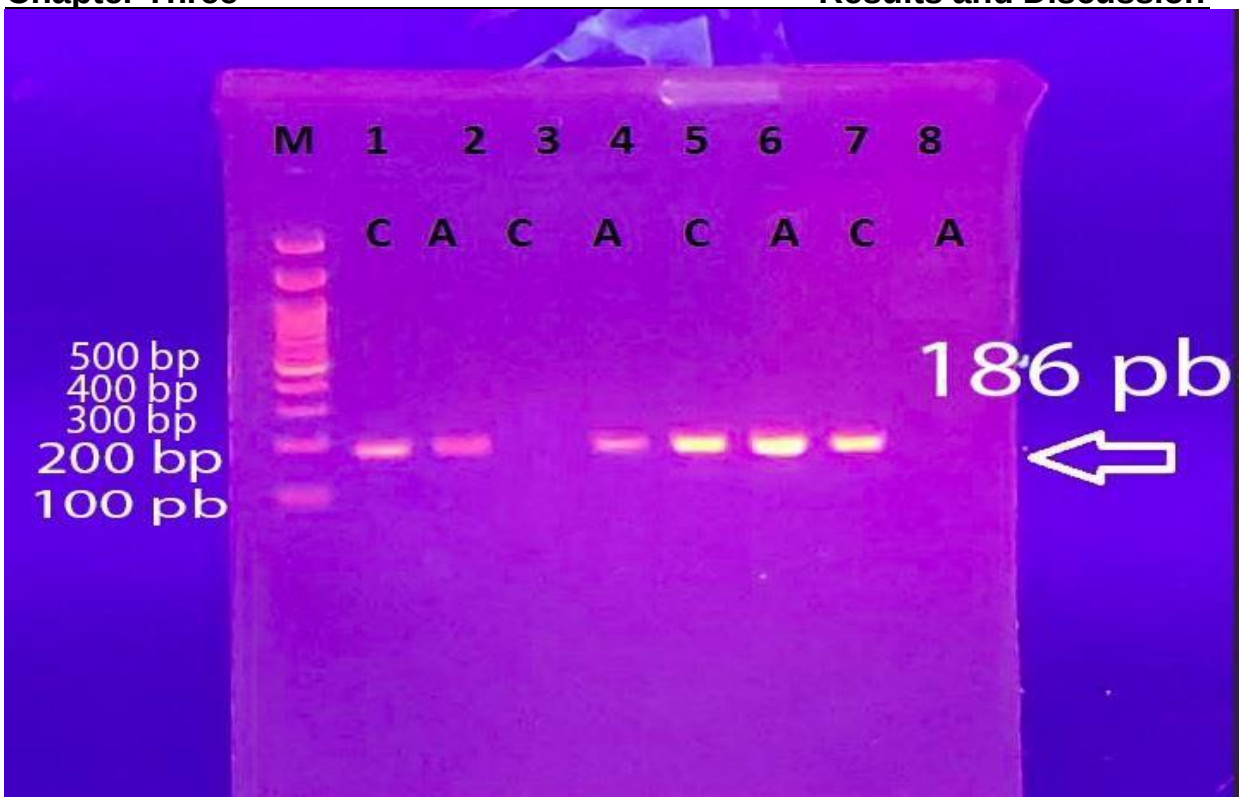

Fig. (3-9): Agarose Gel Electrophoresis Showed Genotyping of Interleukin-18 (rs1946518) polymorphism, Lan M: DNA molecular, Lan 1, 2 Represented CA Genotype, Lan 3, 4 Represented AA Genotype, Lan 5, 6 Represented CA Genotype, Lan 7, 8 Represented CC Genotype.

Genetic polymorphism of Interleukin 18 polymorphism (rs1946518) which observed was classified into three genotypes:

1. The major genotype group (CC) homozygous for the allele C.
2. The minor genotype group (AA) homozygous for the allele A.
3. The heterozygous (CA).

Table (23) summarizes the distribution of genotypes groups of Interleukin 18 polymorphism (rs1946518) in patients.

Table (3-23): Percentages of Interleukin-18 Polymorphic (rs1946518) Group patients

Variable	Group	Frequency	Percentage
Genotype	CC (wild)	20	33.3%
	CA (hetero)	31	51.7%
	AA (homo)	9	15%
Data Presented by numbers and percentage			

Examination the Hardy–Weinberg equilibrium of IL 18 (rs 1946518) in patients who underwent coronary angiography. Result of comparison between observed and anticipated values for SNIP with IL-18 polymorphism rs 1946518 in the tested population were shown in Figure (3-10), and Table (3-19). The distribution and percentage of individuals having rs 1946518 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected, which were: CC (20, 21); AA (9, 10); CA (31, 29) (goodness-of-fit $\chi 2$ for rs 1946518 0.289, $P < 0.003$} and therefore it was statistically significant.

Table (3-24): Hardy–Weinberg equilibrium for rs 1946518 genotype in patient Studies.

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			C	A	
Symbol	Frequency	%	0.592	0.408	0.289 P= 0.003
CC	20	33.3			
CA	31	51.7			
AA	9	15			
Total	60				

The main findings of this study were that specific genotype polymorphisms of IL-18 (rs1946518) aleles in were related to the risk of CI-AKI following PCI; this finding is consistent with earlier studies that linked the pathogenesis of AKI to disrupted levels of IL-18 and inducing acute tubular necrosis, which may act as a risk factor for AKI. The IL18 gene's promoter polymorphisms may affect the cytokine's level. The rs1946518 and rs187238 polymorphisms are two examples of such IL18 polymorphisms **(Ortega- Loubon *et al*., 2021 and Lin *et al*., 2015)**. Polymorphisms in genes associated with the inflammatory process may affect the degree of inflammation in the body, which may influence an individual's risk or susceptibility to AKI **(He *et al*., 2018)**.

A case control study recruited 112 patient by Kadi *et al.* demonstrated the association of -137G > C, -607C > A and -656G > T polymorphisms with serum IL-18 while urinary IL-18 levels showed that only -137G > C and - 656G > T polymorphisms significantly associated with AKI **(Kadi *et al*., 2021)**.

He *et al.* research failed to find an association of the interleukin gene polymorphisms (IL6 rs1800795, IL6 rs1800796, IL6 rs1800797, IL10 rs1800896, IL10 rs3021097, IL18 rs1946518, IL18 rs187238) with AKI risk and only suggests that these gene polymorphisms may play limited roles in susceptibility to AKI **(He *et al*., 2018)**.

The differences between our findings and those of previous research could be justified by four limitations. First, the relationship between genetic polymorphisms and disease risk typically varies throughout populations **(Karban *et al*., 2004)**, and this study is the first one that investigated the association of IL18 rs1946518 polymorphism with CI-AKI risk in Iraq. Second, due to limitation of our study regarding time and budget the sample sizes that used was low which could lead to bias or inaccurate results obtained. Third, our study was aim to identify the genetic polymorphisms which could predict risk for CI-AKI only, since other studies investigated the injury regardless of its cause. Finally, this research focused on adult population, while previous works focused on pediatric and children population.

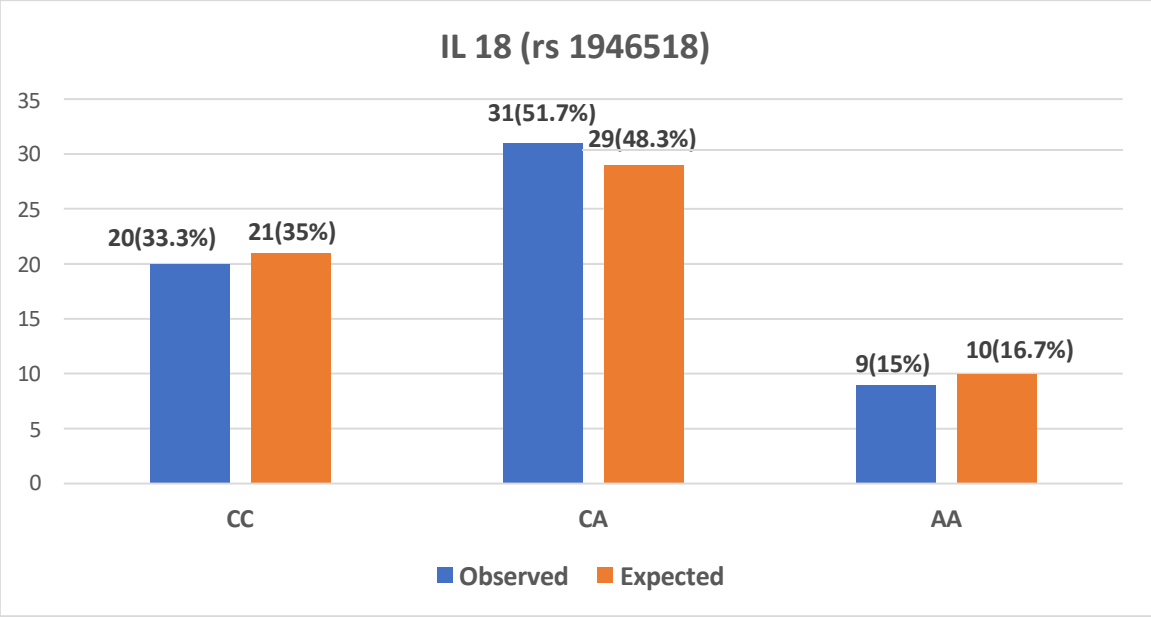

Fig. (3-10): Observed versus expected genotype frequencies of IL-18 polymorphism (rs1946518). (IL-18: Interleukin-18)

Table (3-20) to show the difference between demographic characteristics and Interleukin 18 polymorphism (rs1946518), by performing a one-way ANOVA test to compare the mean of age, weight, height, and BMI. No statistically significant difference was found among mean of demographic characteristics and Interleukin 18 polymorphism (rs1946518).

A cross-tabulation by a fisher‘s exact test was conducted between IL-18 polymorphism (rs1946518) groups and gender, residence, occupation, history of DM, history of HT, history of HF, and catheterization. Found a statistically significant association between IL-18 polymorphism (rs1946518) and history of DM, occupation and catheterization ($p = 0.044, 0.014, 0.004$) respectively, while no significant association with other variables $p > 0.05$.

Table (3-25): Mean difference of demographic characteristic between different genotype of Interleukin 18 polymorphism (rs1946518)

Demographic parameters		Patient Genotype (N=60)			Statistical test	P value
		CC (N=20)	CA (N=31)	AA (N=9)		
Age (years)		56.6±10.69	56.32±10.38	61.11±8.88	ANOVA F= 0.796 *df* = 2	0.456[NS]
Height (cm)		166.75±5.60	167.13±5.05	168.67±2.18	ANOVA F= 0.479 *df* = 2	0.622[NS]
Weight (kg)		81.6±15.24	86.1±12.07	81.78±10.65	ANOVA F= 0.87 *df* = 2	0.424[NS]
BMI (kg/m$^{2)}$		29.06±4.33	30.79±3.65	28.74±3.67	ANOVA F= 1.696 df = 2	0.193[NS]
Gender	**Male**	14(70%)	25(80.6%)	8(88.9%)	Fisher‘s exact= 1.342 df =2	0.573[NS]
	Female	6(30%)	6(19.4%)	1(11.1%)		
Residence	**Karbala**	14(70%)	24(77.4%)	6(66.7%)	Fisher‘s exact= 0.777 df =2	0.783[NS]
	Outside Karbala	6(30%)	7(22.6%)	3(33.3%)		
History of DM	**Yes**	8(40%)	7(22.6%)	6(66.7%)	Fisher‘s exact= 6.072 df =2	**0.044[S]**
	NO	12(60%)	24(77.4%)	3(33.3%)		
History of HT	**Yes**	11(55%)	12(38.7%)	6(66.7%)	Fisher‘s exact= 2.669 df =2	0.277[NS]
	No	9(45%)	19(61.3%)	3(33.3%)		
Occupation	**Housewife**	6(30%)	6(19.4%)	1(11.1%)	Fisher‘s exact= 16.92 *df* =8	**0.014[S]**
	Employee	4(20%)	9(29%)	1(11.1%)		
	Free work	7(35%)	11(35.5%)	2(22.2%)		
	Retired	2(10%)	0(0%)	5(55.6%)		
	Soldier	1(5%)	5(16.1%)	0(0%)		
History of HF	**Yes**	3(15%)	4(12.9%)	4(44.4%)	Fisher‘s exact= 4.246 *df* =2	0.122[NS]
	No	17(85%)	27(87.1%)	5(55.6%)		
Catheteriza -tion	**Negative**	19(95%)	30(96.8%)	5(55.6%)	Fisher‘s exact= 9.533 *df* =2	**0.004[S]**
	Positive	1(5%)	1(3.2%)	4(44.4%)		

Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant
Chi-square test & ANOVA test

Table (3-26) to show the difference between clinical parameters (mean) and Interleukin 18 polymorphism (rs1946518), by performing a one-way ANOVA test to comparing the mean of urea, creatinine, IL18, TNF-α, and eGFR pre and post contrast. Significant difference was found between means of urea ($p = 0.043$). Post hoc testing using LSD adjustment showed that the mean urea level for the CC SNP (43.29±14.37) is significantly higher than that CA SNP (33.96±11.92). Significant difference was found between mean of level of creatinine ($p = 0.024$), post hoc testing using LSD adjustment showed that the mean creatinine level for the AA SNP (1.24±0.37) is significantly higher than that CC and CA SNP (0.99±0.26, 0.96±0.24) respectively. Also Significant difference was found between mean of IL18 ($p = 0.014$), post hoc testing using LSD adjustment showed that the mean IL18 level for the AA SNP (70.65±15.99) is significantly higher than that CC and CA SNP (59.45±9.70, 58.65±9.43) respectively. And lastly significant difference was found between mean of eGFR ($p = 0.024$), post hoc testing using LSD adjustment showed that the mean eGFR level for the AA SNP (59.04±30.40) is significantly lower than that CC and CA SNP (86.81±29.72, 89.01±27.59) respectively. No Significant difference was found between other mean of laboratory Parameters and IL-18 polymorphism (rs1946518) groups ($p > 0.05$).

Table (3-26) Difference in mean of laboratory parameters pre and post contrast with IL-18 polymorphism (rs1946518).

Demographic parameters		Patient Genotype (N=60)			Statistical test	P value
		CC (N=20)	CA (N=31)	AA (N=9)		
Urea (mg/dl)	**Pre**	43.29±14.37	33.96±11.92	45.24±24.14	ANOVA F= 3.335 *df* = 2	**0.0.43[S]**
	Post	48.90±17.73	41.29±18.78	53.11±31.85	ANOVA F= 1.504 *df* = 2	0.231[NS]
Creatinine (mg/dl)	**Pre**	0.84±0.23	0.85±0.16	0.92±0.27	ANOVA F= 0.568 *df* = 2	0.57[NS]
	Post	0.99±0.26	0.96±0.24	1.24±0.37	ANOVA F= 3.973 df = 2	**0.024[S]**
IL18 (pg/ml)	**Pre**	42.92±11.55	44.89±10.61	49.99±15.90	ANOVA F= 1.117 *df* = 2	0.334[NS]
	Post	59.45±9.70	58.65±9.43	70.65±15.99	ANOVA F= 4.617 *df* = 2	**0.014[S]**
TNF-α (pg/ml)	**Pre**	30.17±9.85	29.85±10.27	28.49±12.25	ANOVA F= 0.083 *df* = 2	0.92[NS]
	Post	50.51±10.61	48.50±10.39	51.50±7.18	ANOVA F= 0.425 df = 2	0.656[NS]
eGFR (ml/min/1.73m^2)	**Pre**	105.69±40.66	99.54±24.91	86.30±38.96	ANOVA F= 1.069 *df* = 2	0.35[NS]
	Post	86.81±29.72	89.01±27.59	59.04±30.40	ANOVA F= 3.964 df = 2	**0.024[S]**

Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non-significant, IL18: Inter Luekiens 18, TNF-α: Tumor Necrosis factor alpha, eGFR: Estimated glomerular filtration
ANOVA test

The odds ratios of the detected genotypes of IL-18 polymorphism (rs1946518) in table (12) shows that Interleukin 18 polymorphism (rs1946518) have significant effect on the CA, AA (0.8 (1.020-622.737), 1.029 (1.026-1.029) associated to CIN.

Table (3-27) Association between the IL-18 gene polymorphisms with CIN after PCI

IL-18 rs1946518	CIN -	CIN +	OR (95% CI)	p value
CC	19(35.2%)	1(16.7%)	1a	-
CA	27(50 %)	4(66.7 %)	0.8 (1.020-622.737)	**0.048 [S]**
AA	8(14.8%)	1(16.7%)	1.029 (1.026-1.029)	**<0.001 [S]**

Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, a; reference category, CIN - : Patient without contrast induce nephropathy , CIN + : Patient with contrast induce nephropathy.
Regression (Multinomial Logistic)

A cross-tabulation by performing a fisher‘s exact test (table 10) between TNF-α Polymorphism (rs361525) and IL-18 polymorphism (rs1946518); shows no statistically significant association between them (p > 0.05).

Table (3-28) Association between TNF-α Polymorphism (rs361525) with IL-18 polymorphism (rs1946518)

		Patient Genotype IL-18 polymorphism (rs1946518)(N=60)			Statistical test	P value
		CC (N=20)	CA (N=31)	AA (N=9)		
Patient Genotype TNF-α Polymorphism (rs361525) (N=60)	**GG (N=52)**	19(36.5%)	25(48.1%)	8(15.4%)	Fisher‘s exact= 3.386 *df* =4	0.452 [NS]
	AG (N=5)	1(20%)	4(80%)	0(0%)		
	AA (N=3)	0(0%)	2(66.7%)	1 (33.3%)		

Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, IL-18: Interleukin-18, TNF-α: Tumor Necrosis Factor Alpha.

Chapter Four

Conclusion and Recommendations

4. Conclusion and future work

4.1. Conclusions

According to the observed data, we can conclude the following:

- The percentage of CIN was 10%.
- A significant difference in serum IL18 and eGFR between two groups of patient (CIN- and CIN+) 24 hour after contrast media administration.
- A significant difference in serum level of TNF-α between CIN -and CIN+ patient‘s groups.
- According to Mehran risk score most of patients in this study have a low risk score (<5) of CIN.
- This study found that no association between gene polymorphisms of TNF-α (rs361525) and CI-AKI.
- Polymorphism of IL 18 (rs1946518) is associated with CI-AKI.

4.2. Recommendations:

- Monitoring the renal function before and after exposure to contrast media should be mandatory for patient who underwent PCI.
- Using Mehran risk score to predict the risk of CI-AKI incidence is recommended in coronary intervention.

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Chapter Five

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الخلاصة

يعتبر اعتلال الكلية الناجم عن التصوير التضليلي من المضاعفات الهامة لإجراءات تصوير الأوعية الدموية الناتجة عن الحقن بوسائط التباين، ويعتبر السبب الثالث الأكثر شيوعًا للفشل الكلوي الحاد المكتسب من المستشفى والذي يمثل حوالي 12٪ من الحالات. يتم تعريف اعتلال الكلى الناتج عن التصوير التضليلي على أنه زيادة في مصل اللحمينين بمقدار 0.5 مجم / ديسيلتر (44 مايكرومول / لتر) ، أو زيادة بنسبة 25 ٪ من قيمة الأساس ، خلال فترة 24 ساعة بعد إعطاء المادة التضليلية داخل الأوعية. على الرغم من كونه مرضًا واسع الانتشار ، إلا أن الفيزيولوجيا المرضية لاعتلال الكلى الناتج عن التصوير التضليلي لا تزال غير مفهومة تمامًا.

ان معدل اعتلال الكلى الناتج عن التصوير التضليلي حوالي 14.5 ٪ و معدل الوفيات داخل المستشفى هو 7.1% في أولئك الذين لم يحتاجوا إلى غسيل الكلى و 35.7 ٪ في أولئك الذين احتاجو لغسل الكلى ، مقارنة بـ 1.1 ٪ للمرضى الذين لم يحصل لديهم اعتلال الكلى.

تهدف هذه الدراسة الى التحقق من تأثير المادة التضليلة لتصوير الأوعية التاجية العلاجية على مستويات مصل عامل نخر ألفا الورمي. والتحقق من دور الأنماط الجينية لكل من الاشكال الجينية انترليكين-18 (rs1946518) ولعامل النخر الورمي (rs361525) كعلامات تكهنية لأعتلال الكلى الناتج عن التصوير التضليلي بعد التدخل التاجي عن طريق الجلد.

أجريت الدراسة في وحدة قسطرة القلب في مركز كربلاء للقلب ، وفي مستشفى الكفيل التخصصي في مديرية صحة كربلاء ،في كربلاء ، العراق. شملت الدراسة 60 مريضا (47 ذكرا و 13 إنثى) خضعوا لتصوير الأوعية التاجية الاختيارية أو الطارئة العلاجي. تم إجراء الفحص السريري والفحوصات المخبرية قبل وبعد 24 ساعة من التداخلات القسطارية. تشمل هذه الفحوصات: مصل اللحمينين ، يوريا الدم ، معدل الترشيح الكبيبي المقدر ، عامل النخر الورمي الفا في الدم. التحليل الجزيئي: تم تحليل علامة انترليكين-18 (rs1946518) وعلامة النخر الفا الورمي (rs361525) بواسطة تفاعل البوليميراز المسلسل الخاص بالأليل. تم استبعاد المرضى الذين لديهم بيانات غير كاملة ، والذين خضعوا لتصوير الأوعية التاجية التشخيصي فقط ، وأولئك الذين لديهم أسباب أخرى لـتضرر الكلى الحاد من هذه الدراسة.

لمادة التضليل خطر في اعتلال الكلى الحاد و الممثل بارتفاع كبير في اللحمينين في الدم واليوريا البولينا في الدم (عن قيمة P <0,001) بعد 24 ساعة من التداخل القسطاري للاوعية التاجية. ان للتاريخ السابق لمرض السكري من النوع الثاني والفشل القلبي تأثير ضار معتد به إحصائياً على انتشار إصابة الكلى الحادة الناجمة عن التصوير التضليل (عن قيمة P =1 ، 0.003 و 0.008) على التوالي. وكان هناك ارتفاع مؤثر في مستوى مصل الانترليكين -18بعد التعرض لوسائط التباين (p = 0.001) ، بينما لم يتم

العثور على إختلاف مؤثر في عامل النخر الورمي الفا. وأظهرت النتائج ترددات متزايدة من الانماط الجينية (CA+AA rs1946518) للأنترليكين-18 لدى المرضى المصابين باعتلال الكلى الناتج عن التصوير الضليلي. وكذلك فروق ذات دلالة إحصائية في معدلات اللحمينين و معدل الترشيح الكبيبي المقدر بعد التدخل التاجي عن طريق الجلد في الأنماط الجينية (rs1946518) للانترليكين-18 (P = 0.014 ، 0.024) على التوالي.

استنتجت الدراسة الى ان ارتفاع الانترلوكين-18 بعد التدخلات العلاجية للشرايين التاجية هو عامل رئيسي وراء إصابة الكلى الحادة التي يسببها وسائط التباين للتصوير الضليلي. ويرتبط تعدد الأشكال الجيني (rs1946518) للانترليكين-18و مخاطرعلى اعتلال الكلى بعد التدخل التاجي عن طريق الجلد. هناك حاجة إلى مزيد من الدراسات المستقبلية لتأكيد النتائج التي توصلنا إليها.

جمهوريـــــة العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربـــــلاء- كلية الطب
فرع الكيمياء والكيمياء الحياتية

الانترلوكين - 18 وتعدد الاشكال الجيني لعامل النخر الورمي ـ الفـــا مع دالات كيموحيويـة اخرى كعوامـل خطـورة لاصابـات الكلـيتين نتيجـة التعرض لمواد التصوير الضليلي

رسالة

مقدمة الى مجلس كلية الطب ـ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في (الكيمياء السريرية)

من قبل

حيدر محمد حسين نصرالله
(بكالوريوس علوم- كيمياء ـ الجامعة المستنصرية – 2002-2003)

إشراف

الاستاذ الدكتور فاضل جواد ال طعمة
فرع الكيمياء والكيمياء الحياتية

الاستاذ الدكتور رياض محي الصائغ
فرع الطب الباطنـي

كليـــة الطب ـ جامعـــة كـــربلاء

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