Study of Some Purine Metabolic Enzymes In Sera Of Patients with Renal Failure

A Thesis Submitted to the Committee of the College of Medicine/ Babylon University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Clinical chemistry

By

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November 2008 A.D **Dhilkada 1429 A.H**

بسم الله الرحمن الرحيم

((قالوا سبحانك لا علم لنا إلا ما علمتنا إنك
أنت العليم الحكيم))

صدق الله العلي العظيم

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DEDICATION

TO
MY PARENTS,

FATHER(THE LATE),MOTHER,

BROTHERS,

TO
MY HUSBAND (MOHAMAD)

ACKNOWLEDGEMENT

I would like to express my thanks to Hilla , Most gracious and most merciful. .
It is of my pleasure to express my deep appreciation to my supervisors professor **Dr. Sahib Ali Mahdi and Dr. Moaed Emran** for their valuable guidance during the progress of the present work .
. I am deeply indebted to Dr. Hatem Abdul- Latif for his precious guidance to carry out the statistical analysis.

I am extremely thankful to Dr. Ali Khair – Allah , the dean of College of Medicine, Babylon University for providing all the needed facilities which were essential for the successful completion of the present work. .

I would like to thank the staff of department of Biochemistry in the College of medicine in Babylon University.

I would like to express gratitude to the medical staff in mirjan teaching hospital especially the physicians and chemists of the hospital and Fatma Razak for helping me in the collecting samples. .

SAWSAN

Abstract

In this study , 107 patients (51 male and 56 females) suffering from renal failure who were admitted to Mirjan Teaching Hospital were included . Another , 20 healthy individuals were included as control group . Blood samples were obtained and the sera were separated and it was found that renal failure was more predominant among the patients age group ranging from 40 – 70 years old. .

Besides , it was found out that the mean values of some biochemical parameters important for the detection of the disease which were investigated in this study . It was found that urea and creatinine mean value for all patients were highly significant if compared to control group. .

Total protein and albumin levels decrease whereas globuline elevated. Adenosine deaminase was found to decrease in all patients significantly but there were no differences in its concentration between males and female. .

On the other hand , xanthine oxidase activities also reduced but not significantly. .

Alkaline phosphatase was also investigated . It was seen that this enzyme increased above the normal level .

Moreover, lipid peroxidation , cholesterol and triglyceride were also studied . It was noticed that high levels of lipid peroxidation were represented by the increase of malonyl dialdehyde in patients sera whereas cholesterol decreased and triglyceride was found to be higher than the normal. Iron which was estimated in (49) patients and it was seen that (33) patients had low concentration of iron whereas (16) patients had normal values of this trace element. .

Copper level , on other side , was found to be less than that of control group. .

Abbreviations

Abbreviation	Details
ART	Acute Renal Failure
AMP	Adenosine Monophosphate
ADA	Adenosine Deaminase
ATP	Adenosine Triphosphate
4-AA	4-Amino Antipyrine
cAMP	Cyclic Adenosine Mono Phosphate
Cu	Copper
Cr	Creatinine
CRF	Chronic Renal Failure
CHE	Cholesterol Esterase
CH	Cholesterole
EPo	Erythropoietin
EHSPT	Ethyl-Hydroxyl Sulfo Propyl methylaniline
GFR	Glomerrular Filtration Rate
GPO	Glycerol Phosphat Oxidase
GK	Glycerol Kinase
OH	Hydroxide
HIV	Human Immuno Deficiency
HDL	High Density Lipoprotein
KD	Kelo Dalton
IgG	Immuno globulin G
MDA	Malon Dialdehyde
M.W.	Molecular Weight
LPL	Lipoprotein Lipase
LDL	Low Density Lipoprotein
NSAIDs	Non Steroidal Anti- Inflammatory
NADP	Necotineamide Adenine Dinucleotide Phosphate

Abbreviation	Details
NAD	Necotineamide Adenine Dinucleotide
OD	Obtical Density
POD	Peroxidase
R-1-P	Ribose-1 -phosphate
RBC	Red Blood Cell
PNP	Purine Nucleoside Phosphorylase
P	Propability
TCA	Tri Chloroacetic Acid
TBA	Thio Barbuturic Acid
UN	Urea Nitrogen
VLDL	Very Low Density Lipoprotein
XOD	Xanthine Oxidase
EC	Enzyme commission

List of Contents

Subject	Page No.
Acknowledgement	I
Abstract	II
Abbreviations	III
List of contents	V
List of tables	VIII
List of figures	X
Chapter One : Introduction and Literature Review	
1.1. Introduction	1
1.2. Literature review of kidney disease	3
1.2.1 The kidney	3
1.2.2 Acute renal failure	7
1.2.2.1 pre- renal causes	9
1.2.2.2 post – renal causes	10
1.2.2.3 Intrinsic causes	10
1.2.3 Chronic renal failure (CRT)	12
1.2.4 Biochemistry of renal failure	15
1.2.4.1 Asotemia (uremia and creatinine elevation)	15
1.2.4.1.1- Pre – renal azotemia	16
1.2.4.1.2 Renal azotemia	16
1.2.4.1.3 Post – renal azotemia	17
1.2.4.2 Hypoproteinemia and protein urea	19
1.2.5. Lipid metabolism and renal failure	19
1.2.5.1 Lipid profile	19
1.2.5.2 Lipid peroxidation	21
1.2.6 Xanthine oxidase	23
1.2.6.1 Definition	23

Chapter One : Introduction and Literature Review	
1.2.6.2 Characteristics of xanthine oxidase	25
1.2.6.3 Clinical importance of xanthine oxidase	25
1.2.7 Adenosine deaminase	26
1.2.7.1 Definition	26
1.2.7.2 Properities	27
1.2.7.3 Clinical significance of ADA	29
1.2.8 Trace elements and renal failure	33
1.2.8.1 Coppr	33
1.2.8.2 Iron	35
Chapter Two : Materials and Methods	
2.1 Materials	38
2.1.1 Patients	38
2.1.2 Chemicals materials	39
2.1.3 Instruments	41
2.2 Methods	42
2.2.1 Determination of xanthine oxidase activity	42
2.2.2. Determination of adenosine deaminase activity	44
2.2.3. Determination of serum malondialdehyde (MDA)	48
2.2.4. Determination of triglycerides	50
2.2.5. Determination of cholesterol	53
2.2.6. Determination of phosphatase alkaline	56
2.2.7. Determination of urea	59
2.2.8. Determination of creatinine	62
2.2.9. Determination of total protein	64
2.2.10. Determination of albumin	66
2.2.11 Determination of iron	68
2.2.12 Determination of copper	71

Chapter Three : Results and Discussion	
3.1. Distribution of renal faiture cases according to age	74
3.2. Study some biochemical indicators of renal failure	76
3.2.1 Urea and creatinine concentration	76
3.2.2. Total protein, albumin and globuline concentratiu	78
3.3. Adenosin deaminase activity	80
3.4. Xanthine oxidase activity	83
3.5. Alkaline phosphatase	85
3.6. Urea and creatinin and their relationship with nucleotide metabolic enzymes	88
3.7. Cholesterol , triglyceride and lipid peroxidation and renal failure	93
3.8. Study of some trace elements in uremic patients	97
3.8.1. Iron	97
3.8.2. Copper	100
Conclusions & Recommendations	
Conclusions	103
Recommendations	104
References	
References	105

List of Table

Table No.	Title of the Table	Page No.
2.1.	The chemicals used throughout the study	39
2.2.	The equipments and apparatuses used in the study	41
3.1.	Mean values of urea and creatinine concentration in patients (P) with renal failure and control group	76
3.2.	Mean values of total protein ,albumin and globulin control in patient with renal failure and control group	78
3.3.	Mean values of adenosine deaminase in patients with renal failure and control group	80
3.4.	Mean values of adenosine deaminase activity in patients with renal failure and control group according to their sexes	82
3.5.	Mean values of Xanthine oxidase in patients with renal failure and control group	83
3.6.	Distribution of xanthine oxidase mean values in patients with renal failure and control group according to the sexes	85
3.7.	Mean values of alkaline phosphatase in patients with renal failure and control group	86
3.8.	Distribution of alkaline phosphatase mean values patients with renal failure and control group according to patients sexes	87

Table No.	Title of the Table	Page No.
3.9.	Mean values of MDA , cholesterol and triglyceride in patients with renal failure and control group	93
3.10.	Mean values of MDA in patients with renal failure and control group according to sex	96
3.11.	Mean values of iron in renal failure patients	98
3.12.	Mean values of PCV for patients with renal failure and control group	99
3.13.	Mean value of Copper in patients and control group	100
3.14.	Mean values of Copper in patients according to their sex	102

List of Figures

Figure No.	Title of the Figure	Page No.
1.1.	Classification of renal failure	1
1.2.	Human kidney	5
1.3.	Degradation of purine nucleotides (adenosine monophosphate)	31
1.4.	Degradation of purine nucleotides (adenosine)	32
2.1.	Schematic diagram for assessment lipid peroxidation via determination the byproduct; malondialdehyde	48
3.1.	Distribution of renal failure patients according to their ages	75
3.2.	Relationship between creatinine and adenosine deaminase activity	90
3.3.	Relationship between creatinine and malondialdehyde	91
3.4.	Relationship between creatinine and xanthine oxidase activity	92

Chapter One

Introduction
and
Literature Review

1.1. Introduction

Renal Failure is a loss of renal function ;characterized by uremia, the retention of nitrogenous wastes in the blood. Renal failure can broadly be divided into two categories (1) .

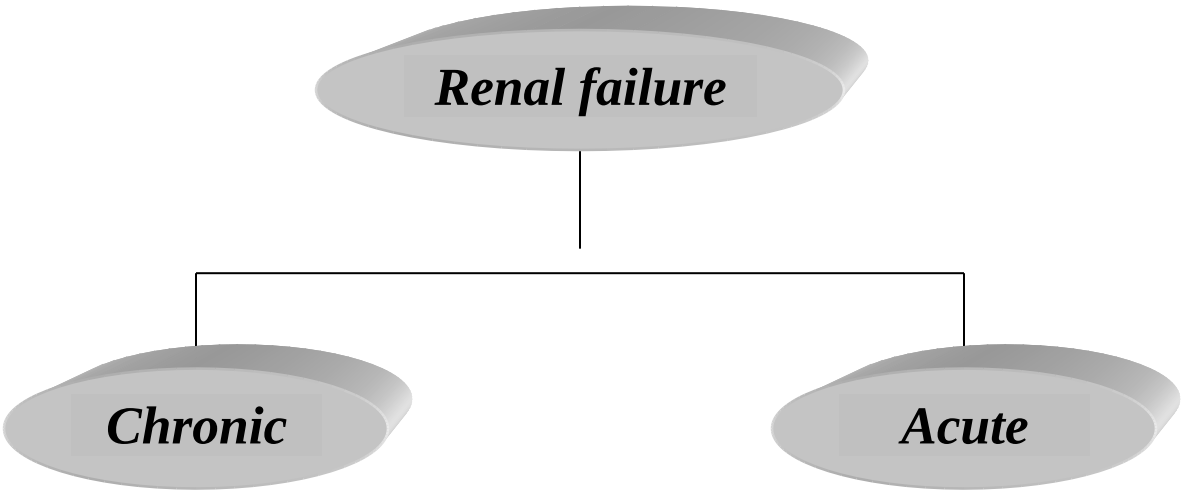


Fig. (1.1) Classification of renal failure

The type of renal failure (acute and chronic) is determined by the trend in the serum creatinine . Other factors which may help differentiate acute and chronic kidney disease include the presence of anemia and the kidney size on ultrasound . Long – standing .,i.e. chronic kidney disease generally leads to anemia and small kidney size (1). .

Chronic renal failure (CRF) is a pathophysiologic process with multiple etiologies , resulting in the inexorable attrition of nephron number and function and frequently leading to an irreversible deterioration in renal function (2 , 3) . It has been estimated that at least 6% of the adult U.S. population have chronic renal damage . .

Acute renal failure (ARF) complicates approximately 5% of hospital admissions and up to 30% of admissions to intensive care units (4) . Chronic renal failure is often accompanied by hypertension , proteinuria or anemia . Because of the wide spread effects of renal failure , symptoms and signs may develop in relation to almost everybody system . Patients may be presented with complaints which are not obviously renal in origin, such as tiredness or breathlessness. In the end –stage, renal failure patients appear ill and with anemia with cardiovascular, hematological, gastrointestinal abnormalities, neuropathy , and myopathy may also be present (5).

The study of the possible changes in the serum component in CRF is very important .The biochemical changes in CRF include water, electrolytes , endocrine and metabolic disorders and acid-base disturbances . In CRF, the generation

of ammonia decreases leading to metabolic acidosis which will lead to extra renal buffering mechanism (6) . Proteins, immunoglobulin G (IgG) , C3 , and C4 were measured in other renal disorders ; C3 and C4 levels were normal in acute tubular interstitial nephritis (7) . .

1.2. kidney disease :

1.2.1. The Kidney

The kidneys are a pair of first –sized organs located outside the peritoneal cavity on each side of the spine . Together with the skin and the respiratory system, the kidney is the body's primary excretory organs . The kidney is a highly specialized organ that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific body needs . The importance of urine formation and excretion as a life –sustaining function is highlighted in situations in which kidney function is suddenly lost . Without at least one functionin kidney, death can occur within a few days (8). .

The nephron is the kidney's primary function unit. Each kidney has about one million nephrons .Each nephron contains a filtering system known as a glomerulus and a tubule, through which the filtered liquid passes .Each glomerulus consists of a capillary network surrounded by a membrane called Bowman's capsul.The afferent arteriole carries blood from the renal artery into the glomerulus, where it divides to form a circulatory network. At the distal end of the glomerulus, the capillaries return to form the efferent arteriole through which blood leaves the glomerulus (9). .

A large amount of circulating blood flows through the kidneys. Approximately 25% of the cardiac output or 1200 ml of blood per minute is received by the kidney .One liter of urine

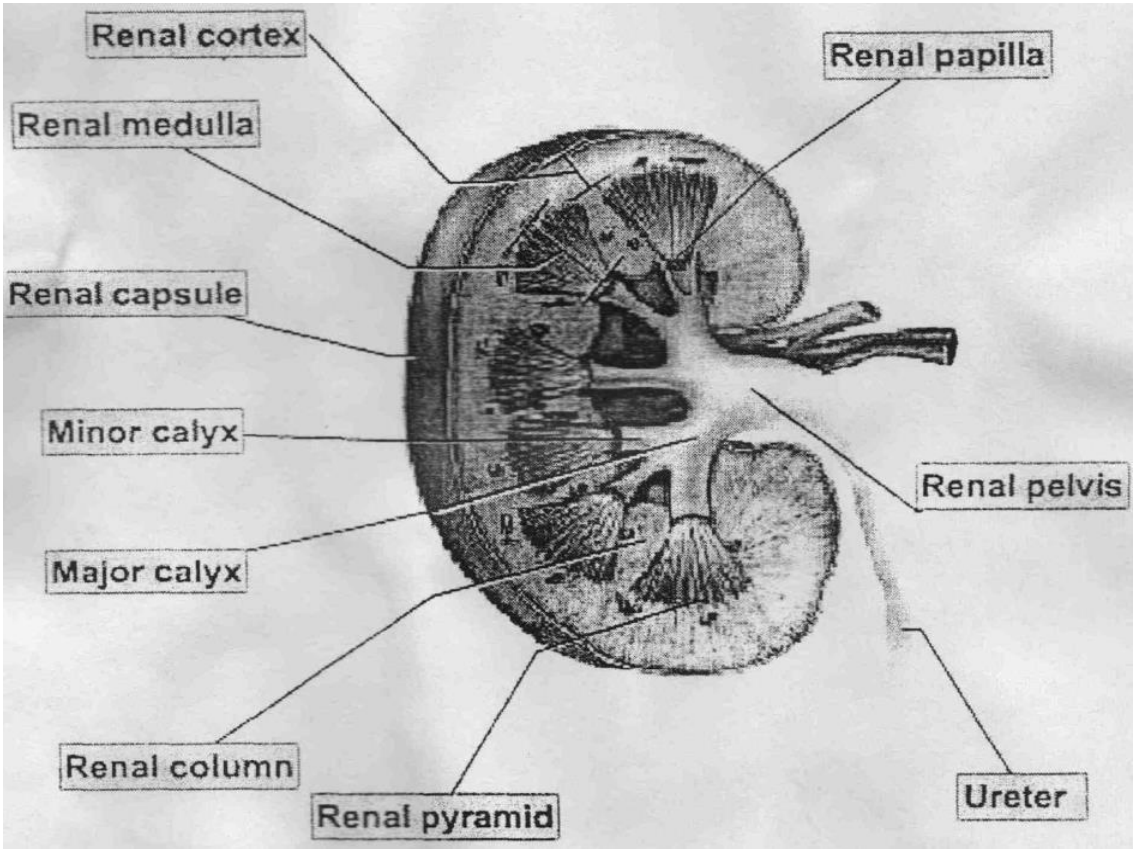


Fig.(1.2.) Human Kidney (9)

is the end product of more than 1000 liters of circulating blood processed through the kidney. Urine formation begins in the glomerular capillaries , with dissolved substances passing into the proximal tubule as a result of the force of blood pressure in the large afferent arteriole and pressure in Bowman's capsule (10) .

The renal tubule is responsible for reabsorption and secretion. Reabsorption is the process of moving solutes from the tubules and reabsorping or retuning them to the bloodstream. Some substances such as glucose and sodium are reabsorped until the plasma level reaches a specific concentration is known as the renal threshold. Secretion is the process of transporting solutes into the renal tubule so that they can be excreted in the urine . Decrease of substances such as hydrogen ions to be eliminated at a rate that exceeds glomerular filtration. Both reabsorption and secretion are controlled by the selective permeability of different areas of the renal tubule to water, sodium , and urea (a by –product of protein metabolism)and the response of the distal collecting tubules in the kidney to hormones such as aldosterone, antidiuretic hormone, and parathyroid hormone (11) . .

The kidney has a remarkable ability to dilute or concentrate urine, according to an individual's changing physiological needs, and to regulate electrolyte excretion . Impaired renal function has adverse effects on blood chemistry , blood pressure , fluid balance , nutrient intake , and the person's general state of health. When kidney function becomes compromised by disease, the processes of glomerular filtration and renal tubular reabsorption and secretion become affected . Blood and urine biochemical tests reflect the extent of this dysfunction . Renal function tests are used to screen for kidney disease, to help determine the cause of kidney disease, determine the extent of renal dysfunction (12). .

1.2.2. Acute renal failure

Acute renal failure is characterized by deterioration of renal function over a period of hours to days , resulting in the failure of the kidney to excrete nitrogenous waste products and to maintain fluid and electrolyte homeostasis (13). .

Acute renal failure can result from decreased renal perfusion without cellular injury ; an ischemic , toxic , or obstructive insult to the renal tubule ; a tubulo interstitial process with inflammation and edema; or a primary reduction in the filtering capacity of the glomerulus . If renal tubular and glomerular function is intact but clearance is limited by factors compromising renal perfunssion , the failure is termed pre -renal failure, or pre-renal azotemia . If renal dysfunction is related to obstruction of the urinary outflow tract it is termed post renal failure , or post-renal azotemia (14). .

Acute renal failure due to a primary intra- renal cause can be called intrinsic renal failure , or renal azotemia . Pre-renal failure and intrinsic renal failure due to ischemia and nephrotoxins are responsible for most episodes of acute renal failure. .

Pre-renal azotemia accounts for approximately 70 percent of community-acquired cases of acute renal failure (12) and 40 percent of hospital-acquired cases (15).

Sustained pre-renal azotemia is the most common factor that predisposes patients to ischemia –induced tubular necrosis(16). .

Hospital-acquired acute renal failure is often due to more than one insult (15). Frequently encountered combinations of

acute insults include exposure to amino-glycosides in the setting of sepsis (17) administration of radio-contrast in patients receiving angiotensin-converting-enzyme inhibitors, (18) or treatment with non-steroidal anti-inflammatory agents (NSAIDs) in the presence of congestive heart failure (17).

1.2.2.1. Pre-renal Causes

Pre-renal azotemia is rapidly reversible if the underlying cause is corrected. In outpatient setting, vomiting, diarrhea, poor fluid intake, fever, use of diuretics and heart failure are all common causes. Elderly patients are particularly susceptible to pre-renal azotemia because of their predisposition to hypovolemia and high prevalence of renal artery atherosclerotic disease (18). .

Among hospitalized patients , pre-renal azotemia is often due to cardiac failure , liver dysfunction ;or septic shock (19). In surgical patients, pre-renal azotemia is a common cause of perioperative and postoperative renal dysfunction(18). .

1.2.2.2. Post- renal causes

Acute renal failure occurs when both urinary outflow tracts are obstructed or when one tract is obstructed in a patient with a single function kidney. Obstruction is most commonly due to prostatic hypertrophy, cancer of the prostate or cervix, or retroperitoneal disorders (19) and is often present in the outpatient setting (20).

Post-renal causes are important to rule out quickly, since the potential for recovery of renal function is often inversely related to the duration of obstruction (17)

In addition , even in patients with advanced stages of cancer, ureteral stenting or percutaneous nephrostomy can relieve the obstruction and may improve short-term outcome(21).

1.2.2.3. Intrinsic causes

Intrinsic renal failure diseases that result in acute renal failure are categorized according to the primary site of injury: tubules , interstitium, vessels , or glomerulus . Injury to the tubules is most often ischemic or toxic in origin . Pre-renal azotemia and ischemic tubular necrosis represent a continuum ,

with the former leading to the latter when blood flow is sufficiently compromised to result in the death of tubular cells .

Many clinical conditions can lead to kidney ischemia as a result of either extra-renal or intra-renal factors that compromise renal blood flow . Although most cases of ischemic acute renal failure are reversible if the underlying cause is corrected , irreversible cortical necrosis can occur if the ischemia is severe , especially if the disease process include micro-vascular coagulation such as that may occur with obstetrical complication,snake bites,or the hemolytic uremic syndrome(22).

Acute renal failure due to acute interstitial nephritis is most often caused by an allergic reaction to a drug (21).

Other less frequent causes include autoimmune diseases (e.g.,lupus) , infiltrative disease (e.g.,sarcoidosis), and infectious agents (e.g legionnaire's disease and lantavirus infection) (23). Renal failure due to acute interstitial nephritis is often reversible after the withdrawal of the offending medication or treatment of the underlying disease. .

Corticosteroids may hasten the recovery of renal function during acute interstitial nephritis (21), but their role remains controversial because controlled studies are lacking and corticosteroids may be contraindicated in patients with underlying infection. .

Glomerulo nephritis can be present as sub-acute or acute renal failure . Serologic assays and immuno-pathological examination of the kidney can identify specific causes of rapidly progressive glomerulonephritis . It is important to diagnose glomerulonephritis quickly, since prompt use of immunosuppressive agents, plasma exchange, or both may be indicated to reduce the occurrence of life threatening complications and decrease the risk of end stage renal failure (23). .

1.2.3. Chronic renal failure (CRF)

Chronic renal failure is a clinical syndrome which evolves as kidney function gradually decreases .(CRF) reflects irreversible loss of renal function and may result from either primary renal diseases or secondarily due to renal involvement in a variety of systemic diseases including diabetes mellitus , hypertension , or collagen tissue diseases(25). .

Chronic renal failure is characterized by a persistently abnormal glomerular filteration rate (GFR) . It represents an evolving process that is initiated by various causes , all with the common end result of persistant and usually progressive damage of varying severity to the kidney (18). .

The main causes of chronic renal failure are as follows(26):

a-Vascular diseases such as renal artery stenosis , hypertensive nephro sclerosis and renal vein thrombosis by an allergic reaction to a drug (26). .

b-Primary glomerular diseases such as membranous nephropathy, rapid progressive glomerulonephritis. .

c-Secondary glomerular diseases which include diabetes mellitus systemic lupus erythematosus , rheumaoid arthritis, mixed connective tissue diseases , scleroderma , post-infection glomerulonephritis , HIV , neoplasia , haemolytic uremic syndrome and others (27). .

d-Tubulointeristitial disease : some drugs and infections play a role in renal failure , also , chronic hypokalemia, chronic hypercalemia , polycystic kidney , cystirosis , radiation nephritis , all and others may consider as a cause of chronic renal failure. .

e-Urinary tract obstruction as a result of urolithiasis , benign prostatic hypertrophy and tumors. .

Anemia occurs almost universally in patients with moderate to advanced chronic renal failure . .

The anemia of CRF is a hypoproliferative process due to decreased erythropoietin (EPo) biosynthesis,as well as to other factors, such as inhibitions of EPo, mild hemolysis, low grade blood loss and secondary hyper-parathroidism (26). Exessive destruction of red blood cells is also seen in advanced renal failure . Normally , red blood cells survive for about four months before being destroyed .This life span is reduced in renal failure , probably because of chemical effects of uremia and decreased flexibility of red blood cells . This haemolysis is usually mild and a person with a normal bone marrow would easily compensate for it by increasing all red blood cell production (25) . However , in renal failure , the bone marrow's capacity to compensate is diminished . Briefly , anaemia is a universal complication of chronic renal failure , it has multiple causes , the most important of which is decreased production of erythropoietin by the kidney (26). .

1.2.4. Biochemistry of renal failure

The diagnosis of kidney disease relies heavily upon observation of changes in the concentrations of urea nitrogen and creatinine in blood , abnormalities on urinalysis , and measurement of urine output . .

Although useful , these measurements indicate only existing renal insults, they do not give advance warning of pending renal damage , and may not adequately define the activity or reversibility of the disease process. Furthermore , because these measurements are indirect, they frequently do not indicate the anatomical site of the renal damage. There are many biochemical parameters which should be taken into account when renal failure is subjected for study (28). .

1.2.4.1. Azotemia (Uremia and creatinine elevation)

Azotemia is defined as an increase in urea nitrogen (UN) and creatinine and can result from a variety of disorders including , but not limited to, renal failure . Uremia is the term for the clinical syndrome of renal failure with azotemia and multi systemic problems such as polyuria , polydipsia,

mild non-regenerative anemia , vomiting , weight loss and other sequalae of inadequate renal failure . Azotemia can be due to pre-renal, renal or post-renal causes (29). .

1.2.4.1.1. Pre-renal azotemia

Pre-renal azotemia is due to a decrease in glomerular filteration rate (GFR) from circulatory disturbances causing decreased renal perfusion (28) , such as hypovolemia (shock , haemorrhage , vomiting) , cardiac disease or renal vaso constriction (30) . .

As urea levels in blood are dependent on flow rate through the renal tubules (decreased flow rate in pre-renal azotemia enhances renal nitrogen in blood) . Urea nitrogen may increase without any increase in creatinine level in early pre-renal azotemia (31). .

1.2.4.1.2. Renal azotemia

Renal azotemia results from decreased (GFR) when more than 3/4 of the nephrons are non-functional. Renal azotemia may be due to primary intrinsic renal diseases

(glomerulonephritis) or may be secondary to renal ischemia from pre-renal causes (32) .

Loss of most kidney functions usually follows concentrating defects in addition , there may be other evidence of renal tubular dysfunction in the urinalysis , such as protein urea, granular or cellular casts (33). Also, extracellular urea decreases cAMP production . The presence of urea in the blood has been held responsible for decreased affinity of oxygen for heamoglobin because of 2,3-diphosphoglycerate binding (30) . Besides , urea inhibits macrophage inducible nitric oxide synthesis at the post-transcription level (34) . .

At all stages of renal insufficiency , the serum creatinine is a much more reliable indicator of renal function than blood urea because the blood urea is far more likely to be affected by dietary and physiologic conditions , not related to renal function (29) . .

1.2.4.1.3. Post-renal azotemia

This results from obstruction (urolithiasis) or rupture of urinary outflow tracts (36). Regarding to urea , the main strategy that has been used up to now to decrease uremic solute

concentration is dialysis , but dialysis is nonspecific and removes essential compounds as well (37) . .

Moreover , uremic solutes accumulate not only in the plasma but also in the cells , where most of the biological activity is exerted (38) . .

The presence of urea in blood at high concentration will have many effects due to its toxicity .

Some investigators have found that urea which inhibits Nak_2cl cotransport in human erythrocytes (27) . The stages of renal failure have been defined according to the serum creatinine as follows. .

Serum creatinine 2.5 - 4.9 mg /dl : Moderate renal failure serum creatinine 5.0-9.9 mg /dl .Severe renal failure; serum creatinine 10 mg/dl or greater . End-stage renal failure. Like urea, creatinine distributes throughout total body water . Its concentration in serum is a function of the usually constant production and excretion rates . It may be slightly higher in the evening than in the morning , due, most likely, to dietary meat intake at day (36) . Unlike urea , creatinine is largely unaffected by gastero-intestinal bleeding or by catabolic factors such as , fever , and steroids . However, the ingestion of cooked meat can raise the serum creatinine because cooking converts the creatine in meal into creatinine (39). .

1.2.4.2. Hypoproteinemia and protein urea

It's known that both serum total protein and serum albumin are decreased in renal failure (Hypoproteinemia) . This may be attributed to the an increase of transcapillary escape rates of protein in uremic patient (41). .

In addition , proteinurea is associated with renal failure in which proteins can be detected in the urine of patients with renal failure (37) . .

Consistent with its role in pathophysiology , proteinuria is a strony predictor of clinical progression of renal disease .The rapidity of GFR decline is proportional to the severity of proteinurea (42). .

However , it's difficult to distinguish between glomerular proteins like albumin and tubular proteins like α-1 microglobulin . Hence it's difficult to report the origin of proteinuria because both types of proteins can be detected in urine samples of patients with renal failure (39,11).

1.2.5. Lipid metabolism and renal failure

1.2.5.1. Lipid profile

Lipid metabolism is also affected by renal failure .Profound

alterations of lipid metabolism occur in patients with acute renal failure . The triglyceride content of plasma lipoproteins , very low-density lipoprotein (VLDL) and low-density lipoprotein ones (LDL) increases while total cholesterol and in particular high density lipoprotein (HDL) decrease (43).

The same findings are seen with chronic renal failure, which show that serum lipids such as LDL, VLDL and triglyceride increase where as HDL decreases and cholesterol may remaine within the normal level or decrease . Moreover, some investigators have indicated that there is an alteration in lipoprotein metabolism in patients with renal failure (44,45).

The major cause of lipid abnormalities in renal failure is impairment of lipolysis . The activities of lipolytic systems , peripheral lipoproteinlipase and hepatic triglyceride lipase decrease in patients with acute renal failure to less than 50% of normal (46). Besides ,it has been shown that chronic renal failure results in profound lipid disorders which stem largely from dysregulation of high density lipoprotein (HDL) and triglyceride-rich lipoprotein metabolism (47). .

1.2.5.2. Lipid Peroxidation

Lipid peroxidation is a degenerative process that affects unsaturated membrane's lipids under conditions of oxidative stress. This complex process is believed to contribute to human diseases by disrupting the structural conformation, the packing of lipid components and the function of biological membranes(47). .

It has been pointed out that unsaturated phospholipids , glycolipids and cholesterol in cell membranes and other organized systems are prominent targets of oxidant attack . This can result in lipid peroxidation , a degenerative process that perturbs structure and function of the target system often with cytopathological consequences. .

Peroxidative modification of unsaturated phospho-lipid, glyco-lipids and cholesterol can occur in reactions triggered by:(48).

.

1- free radical species such as oxyl radicals proxyl radical , and hydroxyl radicals($OH^{\cdot}$); .

2- non-radical species such as singlet oxygen($O^{-)}$, ozone($O_{3)}$ and peroxynitrite generated by the reaction of super oxide with nitric oxide (44). Lipid peroxidation has been linked to a variety of disorders , and also plays a role in cytotoxic

effects of oxident based chemotherapeutic drugs.

It has been observed that there is a relationship between lipid peroxidation and renal failure . Lipid peroxidation increased in patients on dialysis and also in pre-dialysis adults with advanced chronic renal failure (49). .

Also, it has been pointed out that malonyl dialdehyde, which is considered as indicator of the occurance of lipid peroxidation has increased in patients with chronic renal failure (48) .

Besides, it has been seen that lipid peroxidation increased in haemodialyzed paitents with renal failure as a result of reaction oxygen specieces which affect on lipids and eventually lead to lipid peroxidation (50). .

On the other hand , lipid peroxidation was also increased in patients with acute renal failure .It has been observed that lipid peroxidation is high in acute renal failure and the main cause for this elevation belongs to the free oxygen radicals($O_2^{\cdot}$**)** which play as a causative agents of renal injury (51). .

In a study carried out on children with renal failure , it had been shown that lipid peroxidation is higher than the control group during the session of haemodialysis , the concentration of malonyldialdehyde remains at high levels (52). .

1.2.6. Xanthine Oxidase (EC. 1.1.3.22)

1.2.6.1. Definition

Xanthine oxidase activity is an enzyme which catalyzes the Oxidation of xanthine to uric acid . This enzyme is widely distributed , occurring in milk, small intestine , kidney and liver and also it is a flavoenzyme with an atom of molybdenum and four iron-sulfur centers in its prosthetic group (53). .

Under normal conditions, this enzyme exists as a dehydrogenase , converting hypoxanthine and xanthine into uric acid at the expense of NADP .When the tissues are exposed to metabolic stress, xanthine dehydrogenase undergoes selective proteolysis and is converted into xanthine oxidase .The latter also brings about conversion of hypoxanthine and xanthine into uric acid by using molecular oxygen (O_2) with the resultant generation of oxy free radicals (54). .

As a result of its ability to generate reactive oxygen species , the xanthine oxidase has received considerable attention as a pathophysiological cause of ischemia-perfusion injury and renal failure (55). .

It has been reported that there are many inhibitors which can affect on enzyme activity such as urea , metal ions , purine 6-aldehyde (54). .

Moreover , it has been proposed that iron plays a role in regulation of xanthine oxidase in vivo and it has been seen that ferric ions can form stable coordination with urate , thereby protecting the human body against iron-catalyzed oxidation(55).

Some authors report that this iron-urate complex inhibits the activity of xanthine oxidase and xanthine dehydrogenase (56). .

Xanthine oxidase exhibits a relatively wide temperature ranges and also variable pH optima depending on substrates used. Also, it has been seen that xanthine oxidase exhibits various specificity to various types of substrate . This may occur through its ability to change its substrate specificity when mutation occurs in the amino acids residues in the enzyme active site (57). .

Mutation of two amino acids residues in the active site of human xanthine oxidase for purine substrates results in conversion of the substrate preference to aldehyde oxidase . However both xanthine oxidase and aldehyde oxidase are

similar in protein structure and prosthetic group composition but differ in substrate preference (58). .

1.2.6.2. Characteristics of xanthine oxidase

Xanthine oxidase catalyzes the oxidation of xanthine to uric acid:(53)

$$Xanthine + O_2 + 2H_2O \text{ ----------} \rightarrow Urate + 2H_2O_2$$

Molecular Weight : 275,000

Optimum PH : 4.6

Specificity **:** The enzyme has a broad specificity catalyzing the reduction of O_2, cytochrome c, NO^{-3}, $Fe(CN)_6^{-3}$ and various quinines and dyes by aldehydes and purines .

1.2.6.3. Clinical importance of xanthine oxidase

Difficiency of xanthine oxidase activity causes build up of xanthine which propably precipitates in the urine (xanthineurea) (59). .

Although half the patients with this disorder are assymptomatic, about 10% have myopathy and about a third of them have nephrolithiasis as a consequence of dearangement in purine metabolism (55). .

Besides, this enzyme is also seen to increase in the blood of patients with myocardial infarction which indicates that myocardial ischemia has a definite correlation with xanthine oxidase activity and thus the measurements of xanthine oxidase activity may be used as a biochemical marker of myocardial infarction (60). .

During the ischemic period , ATP is catabolized to yield hypoxanthine. The hypoxic stress also triggers the conversion of NAD-reducing xanthine dehydrogenous to the oxygen-producing xanthine oxidase (58). .

Xanthine oxidase may also be related to the carcinosenic process as one of the enzyme systems involved in nucleoprotein metabolism . Also, xanthine oxidase causes profound natiuresis without affecting renal blood flow outoregulation (61) . .

1.2.7. Adenosine deaminase (adenosine aminohydrlase ; (EC. 3.5.4.4)

1.2.7.1. Definition

Adenosine deaminase activity was first noticed by Gyorgy and Rothler. The enzyme is widely distributed and is found in the intestinal mucosa , liver ,skeletal muscle , kidney ,

lymphocytes , leukocytes and erythrocytes (62). . Adenosine deaminase catalyses the irreversible hydrolytic deamination of adenosine to produce inosine and ammonia. Inosine is then further degraded to uric acid or salvaged at several points for the biosynthesis of purines .(Fig.1.3-1.4) Ammonia is detoxified either by conversion to urea or glutamine . .

1.2.7.2. Properities

Adenosine deaminase is a cytosolic enzyme which participates in the purine metabolism where it degrades either adenosine or 2-deoxyadenosine producing inosine or 2-deoxyinosine respectively. .

Moreover, adenosine deaminase may appear also on the cell surface which is called (ecto-adenosine deaminase) (membrance bound form). There is no difference in catalytic activity between cytosolic ADA and ecto-ADA have been found and also, ecto-ADA could have functions independent of its enzymatic activity (63). .

Adenosine deaminase from human tissue is heterogeneous with respect to M.W. The enzyme in erythrocytic is a single polypeptide chain of M.W. 26.000 – 38.000 . .

In other tissues , ADA is present either as a complex of M.W (230,000 - 440,000) depending on the source, or as a mixture of this larger form and a low M.W form of another investigators (39) showed that there are approximately the same size as the enzyme from erythrocytes (61). .

There are two enzymes which carry out ADA activity , called ADA1 and ADA2 . ADA1 , a 40KD monomeric protein with a 200 KD, non catalytic combining protein is responsible for 90% of ADA . ADA2 is some what larger at 110 KD, appears to play a general adenosine deamination role in serum . Current evidence suggests that erythrocyte ADA and the catalytically active subunit of the higher M.W forms are product of the same genes (40) . ADA is a ubiquitous enzyme which appears to be particularly impotant in the development of thymocytes (64). .

Indeed the physiological roles of ADA can be seen in connection with adenosine whose concentration can be modulated by enzymatic action of ADA . Adenosine is both a metabolic precursor for nucleic acids (intra cellular adenosine) and a significant signaling molecule involved in regulation of various physiological processes (65). .

The physiological functions of adenosine are thought to be linked to its localized release (extracellular AD).Extracellular

AD triggers the changes within the cells through its interaction with adenosine receptors (62). .

The responses to adenosine include coronary vasodilation, reduction in heart rate and contractile force, inhibition of platelet aggregation , mast cell-degranulation, inactivation of eosinophil migration , renal vasoconstriction, regulation of ion channel activity , membrance potential , and neurotransmitt and hormone release (66). .

Adenosine levels may normally be controlled by the relative activities of the adenosine producing enzyme (ecto-5'-nucleotidase) and adenosine degrading enzyme (Adenosine deaminase) (62). So, any elevation in adenosine in serum is triggered with elevation of ecto-5-nucleotidase activity and decrease in ADA activity . .

1.2.7.3. Clinical Significance of ADA

In the kidney , adenosine is involved in the regulation of renal haemodynamies , tubular re-absorption of fluids and solutes , and in rennin release (65). .

Clinical investigations have shown an increase in urinary adenosine excretion following drug induced nephrotoxicity

acute renal failure , or acute renal allograft rejection . This increasing in adenosine in urine during renal failure may be attributed to the defficiency in ADA level (67).

Moreover , it has been observed that there is low molecular weight protein present in the urine of patients with kidney disorders . This glycoprotein is called adenosine deaminase binding protein . Adenosine binding protein is shed from the brush border of the proximal tubule in kidney damage , particularly in cases of renal failure (68). Release of adenosine deaminase binding protein into the urine appears to reflect the severity of the insult to the nephron. .

There is no study concerning on detection the ADA levels in patients with renal failure . However , some studies are carried out on ADA levels in cases other than renal failure. .

For instance , ADA levels are studied in patients with renal cell carcinoma and it has been found that there is decrease in ADA levels if it is compared to control group, while ADA activity has increased in children with nephrotic syndrom where urea levels in those patients are normal (67) .

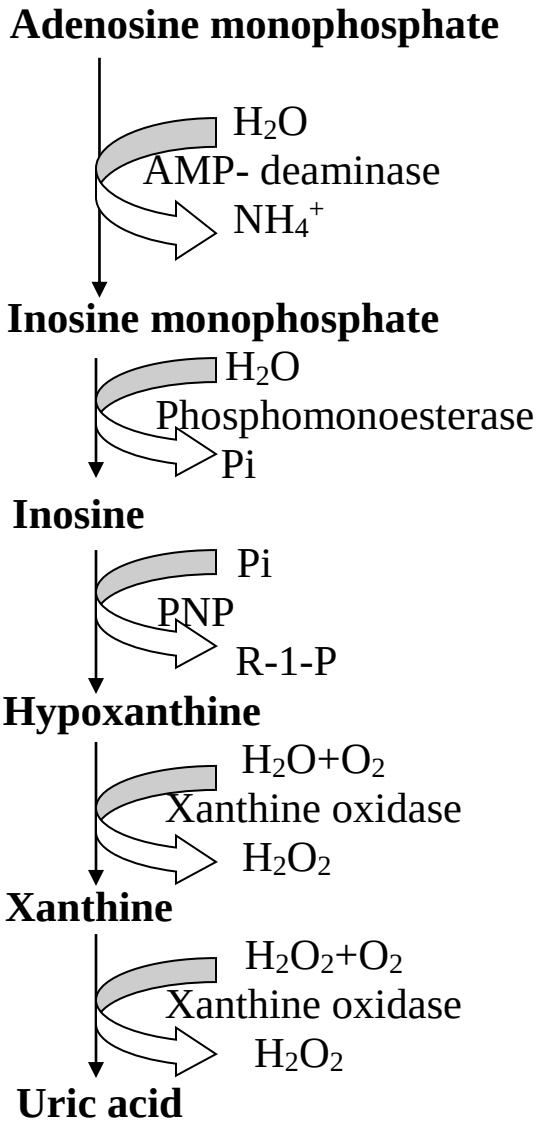


Fig.(1.3.) Degradation of Purine nucleotides (Adenosine monophosphate) (40)

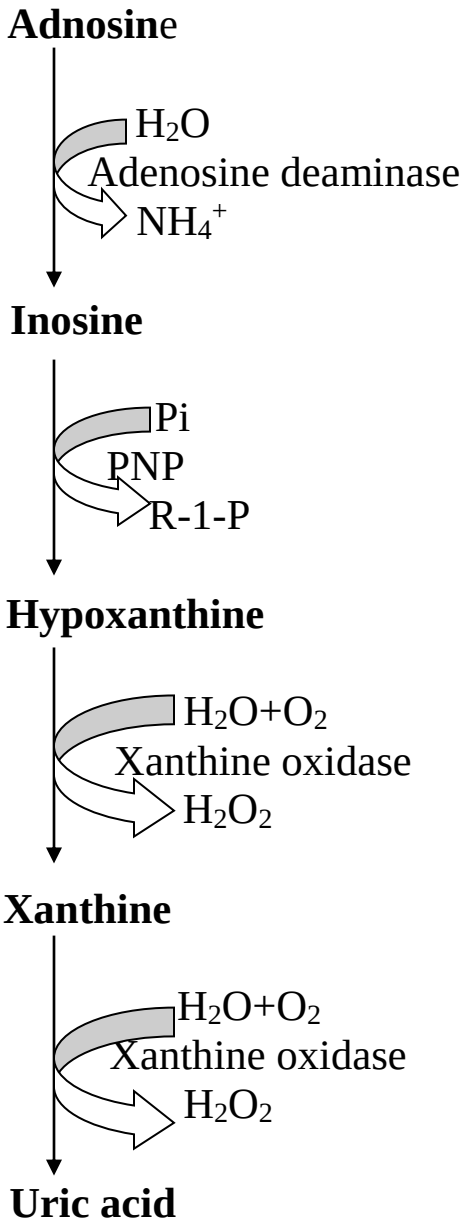


Fig. (1.4.) Degradation of Purine nucleotides

(Adenosine) (40)

1.2.8. Trace elements and renal failure

In uremic patients , the most important factors for trace elements concentration are the degree of renal failure and the modality of renal replacement therapy . In renal failure , some trace elements tend to increase , others tend to decrease . However the data of different studies are not entirely consistent (28). .

In healthy individuals , normal functioning kidney eliminate trace elements from the body . However , in uremia , declining kidney function leads to an accumulation of potentially nephrotoxic trace elements which may contribute to deterioration of renal function (36) . .

1.2.8.1. Copper (Cu)

Total body copper is about 100 mg . It is seen in muscles, liver , bone marrow , brain , kidney , heart and in hair . A major function of copper is as a component of enzymes involved in redox reactions , with many involving reactions with oxygen . These metalloenzymes include ceruloplasmin , cytochrom c oxidase , superoxide dismutase , tyrosinase , and ascorbate oxidase (69). .

Copper is essential for the formation of hemoglobin. Excess copper intake may lead to toxic manifestations . Chronic toxicity is manifested as diarrhea and blue-green discoloration of saliva . Copper poisoning may result in hemolysis , hemoglobinuria , proteinuria and renal failure (70). .

Copper deficiency is manifested as anemia , RBC count is reduced and the deficiency will result in microcytic hypochromic anemia. .

Many pathophysiologic states produce hypercupremia generally by means of a corresponding elevation of ceroloplasmin , which accounts for >90% of total plasma copper (71). .

In renal failure , copper levels differ from one study to another . Some investigators mention that copper levels have decreased in the serum of patients with acute renal failure,(28,72) but others (75,71) have found that copper levels remain normal in patients with renal failure and indicate that there is no significant difference in copper concentrations in the sera of the patients with renal failure and healthy individuals.

1.2.8.2. Iron

Iron is an essential component of hemoglobin which allows it to bind reversibly with oxygen in the lung and release oxygen to the tissue. .

The distribution of body iron stores shows the importance of iron to red cell production . Normally about 70% of iron is found in the circulating erythrocytes . Approximately 20% of iron is stored as ferritin , primarily in the liver (49) . Smaller amounts of iron are coupled with enzymes , myoglobins and other proteins . Iron is absorbed from the duodenum and proximal jejunum and is stored as ferritin , so , serum ferritin provides one measure of iron stores , and a low ferritin is a reliable indicator of iron deficiency. Availability of iron is a key for optimal erythropoiesis (36). .

Anemia is seen in patients with renal failure , this anemia has been attributed to reduced red cell survival , reduced red cell production , or to a combination of both mechanisms ((49) . .

The erythropoietin production by the kidneys , but probably the biochemical disturbances of severe uraemia also influence bone marrow activity (74) . It has been proved that

decreased erythropoietin mass is the primary underlying cause of anemia in chronic renal failure (38). .

Iron may be available at minimal normal levels in patients with chronic renal failure . However , iron deficiency anemia also occurs as a result of unavoidable dialyzed blood loss , clotted dialysis membranes , and frequent blood sampling (49).

Recent studies (75,76) have proved that in uremic or chronic renal failure patients or on haemodialysis program , free iron or non-transferrine bound iron does exist and may induce damage to biomolecules , thereby enhancing the disease process. .

Indeed , iron may decrease in patients with chronic failure especially those on haemodialysis or even on peritonesl dialysis and erythropoietin is also needed for treatment of anemia and if there is no response to treatment , the patients should be supplemented with iron (74). .

Aim of the study

This study aims to investigate the changes in some purine metabolic enzymes activities in the sera of patients with renal failure , such as adenosine deaminase activity and xanthine oxidase activities in addition to the main parameters associated with renal failure which include urea , creatinine , total protein and albumine . .

CHAPTER TWO

MATERIALS AND METHODS

2.1. Materials

2.1.1. Patients

In this study , 107 patients (51 male and 56 female) suffering from renal failure who were admitted to Mirjan Teaching Hospital unit of artificial kidney – were included.

Also , 20 healthy persons were included and distributed accordingly . Blood samples were obtained and the sera of them were subjected for testing. .

2.1.2.Chemicals

Table (2 – 1) the chemicals materials used throughout the study :

N0	Chemical Compound	Manufacture
1.	Sodium dihydrogen phosphate	Ltd,Jeddah 21484 Kingdom of Saudi
2.	Ammonium sulphate anhydrous	BDH AnalaR Poole , BH 151 TP England
3.	Adenosine	SCHUCHARDT MUNCHEN, Germany
4.	Sodium dihydrogen phosphate	BDH AnalaR BH 15 ITD , 301285D , England
5.	Phenol	Fluka Chemikab 02870 , USA
6.	Sodium nitroprusside	CHEM-Supply 4326410005598 GILLMAN,South Australia
7.	Sodium hydroxide	Fisher Scientific LE115RG,UK
8.	Sodium hypochloride	Fisher Scientific LE115RG,UK
9.	Xanthine	SCHUCHARDT MUNCHEN, GERMANY
10.	Trichloroacetic acid(TCA)	GAINLAND chemical company,CLWYD,UK
11.	Thiobarbutric acid (TBA)	BDH AnalaR BH 15 ITD,301285D,England
12.	4-Chlorophenol	Fluka Biochemica 24004023,USA
13.	Lipoprotein Lipase	Sandoz Co.
14.	Glycerol Kinase	SPIREACT , Spain
15.	Glycerol-3-P-oxidase	BDH , England
16.	Peroxidase	Flika chemika-Switzerland
17.	4- Aminophenazine	AFMA-DISPO , Jordan
18.	Cholesterol oxidase	BDH , England
19.	Chlesterol esterase	Merk – Darmstadt

N0	Chemical Compound	Manufacture
20.	Cholesterol	Analar Poole , England
21.	Disodium phenylphosphate	Fluka Biochemica ,USA
22.	Carbonate-bicarbonate	SIGMA-ALDRICH CHEMIE GmbH, Germany
23.	Puffer-PH-10	Merck Darmstdt, Germany
24.	Sodium merthiolate	BDH AnalaR Bh 15, ENGLAND
25.	Phenol	Fisher Scientific LE115RG ,UK
26.	4-Amino antipyrine	Fluka Biochemika 6042,Switzerland
27.	Sodium arsenate	CHEM-Supply,South Australia
29.	Double distilled water	Laboratory Distillator
30.	Distilled water	Laboratory Distillator
31.	Acetate buffer	Analar Poole , England
32.	Ascorbic acid	SIGMA-ALDRICH CHEMIE,GmbH,Germany
34.	Hydroxylamine	Merck Darmstdt , Germany
35.	Sodium acetate	Fluka Chemika , USA
36.	Albumin	GAINLAND chemical , UK
37.	Sodium potassium tartrate	Fluka Biochemica , USA
38.	Sodium iodide	BDH Analar Poole , England
39.	Potassium iodide	Fluka Chemika 02870 , USA
40.	Copper (II) sulphate	SIGMA-ALDRICH , Germany
41.	Picric acid	BDH AnalaR Poole,BH ,England
42.	Creatinine	Merk- Darmstadt
43.	Sodium salicylate	AFMA-DISPO , Jordan
44.	EDTA(Ethylen diamine Tetraacetic acid	SPIREACT , Spain
45.	Urease , Urea	Sandoz Co.

2.1.3. Instruments

Table (2 – 2) the equipments and apparatuses used in the study:

No.	Apparatus	Company
1.	Balancer	Sartorius AG GOTTINGEN BL 2105 , Germany
2.	Centrifuge	Griffin and George BS 4402-D , Britain
3.	Refrigerator	Concord , Lebanon
4.	Centrifuge (hematocrit)	CENTRIFUGA – MILANO, MOD , 4223. 1984 , Made in ITALY .
5.	Vortex(Electronic)	VIBROFIX , VF-I JANKE and KUNKEL IKA-Labortechnik , Germany
6.	Filter papers	What man 42 mm pore size ,9m diameter , England .
7.	Water bath	Schutzart DIN 40050-IP 20 Memmert GmbH, Schwabach FRG , Germany
8.	Incubator	Fisher Scientific , model 5370 .CAT . 11- 690 – 5380 , made in U.S.A.
9.	Magnetic stirrer with hot plate	Classico , India .
10.	Micropipettes – automatic (0.1,1) ml	SIAMED , Germany
11.	pH meter	(HANNA) HI – 9321 , Portugal
12.	Spectrophotometer (Digital ultraviolet and Visible)	Spectronic 21, MILTON ROY COMPANY , Bouch and Lamp , USA
13.	Water distiller	Ogawa Seiki , Japan

2.2. Methods

2.2.1. Determination of Xanthine Oxidase activity(77)

Principle

Xanthine oxidase activity (the oxidase form) was determined by the method of Ackerman and Bril (50) in sera of control subjects and patients with renal failure. This method depends on the enzymatic oxidation of xanthine which is followed spectrophotometrically by measuring uric acid formation at (293) nm. .

Xanthine + H_2O_2 + O_2 ------ ------ Urate + H_2O

Reagents:-

I-Phosphate Buffer (0.I M : pH 8.2). .

Solution 1-A weight of (27.8) g of NaH_2PO4 was dissolved in (800) ml distilled water , then diluted to (1000)ml.

Solution 2-A weight of (53.65) g of Na_2HPO_4 was dissolved in the same way .Volume of (5.3) ml of NaH_2PO_4 was added to (94.7) ml of Na_2HPO_4 and the pH was adjusted to (8.2) , then diluted to (200) ml.

II- Substrate solution (Xanthine solution I x 10^{-4} M) . Aweight of (15.2) mg of xanthine was dissolved in (10)ml of NaOH solution (0.2) M by vigorous shaking , and then (0. 5) ml of this solution was diluted to (50) ml with phosphate buffer. .

Procedure :-

Volume of (0.1) ml of sample was added to (3) ml of substrate solution and the rate of increase of absorbency at (293) nm was recorded against water blank. .

Calculations:-

The activity was calculated by using the following equation :

$$\text{Activity (U / L)} = \frac{\triangle \text{ A /min} \times V_t}{\varepsilon \times Vs} \times 1000$$

Where :

V_t = Total Volume = 3.1 ml

Vs = Sample Volume = 0.1 ml

ε = Molar extinction coefficient for xanthine= 9.6 cm^2 / µmole

2.2.2. Determination of Adenosine deaminase Activity.(78)

Principle

The adenosine deaminase assay is based on the enzymatic deamination of adenosine to inosine , which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP) . .

Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl – N-(2 – hydroxyl –3–sulfopropyl) -3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate guinone dye which is monitored kinetically. .

Reagents :-

All solutions were prepared with double distilled , ammonia free water . Ammonia was removed from distilled water by the addition of a little H_2SO_4 and $KMnO_4$, distillation was carried out using simple glass distiller apparatus. .

1-Phosphate buffer (0.05 M ; pH 6.5) : as prepared in (4.5) .

2- Buffered adenosine solution (adenosine 0.021 phosphate buffer ,(0.05 M ; pH 6.5): .

A weight of (0.05) g of adenosine was dissolved in phosphate buffer , then the volume was made up to (10) ml with the same buffer. .

3- Ammonium sulphate stock solution (0.1015 M) .

A weight of (1.982) g of anhydrous ammonium sulphate was dissolved in ammonia – Free water , mixed thoroughly and made up to (1000) ml. .

4- Ammonium sulphate standard solution (75 mM) volume of (0.5) ml of solution (3) was diluted to (100) ml with solution (1) .

5- Phenol nitroprusside solution (phenol 0.106 M , sodium nitroprusside 0.00017 M). .

Weights of (10) g of phenol and (0.05) g of sodium nitroprusside were dissolved in (500) ml boiled distilled water , then diluted to (1000) ml. .

6- Alkaline hypochloride solution (NaOCl 0.011 M ; NaOH, 0.125M): :

Volume (125) ml of NaOH (1N) was mixed with (16.4) ml Clorox (Contains (5%) W/V NaOCl) , then diluted to (1000) ml with boiled distilled water , solutions

(1,2,3,4) were stored in dark bottles at (4) Cº where they were stable for at least (2) months . Adenosine buffer solution was prepared freshly , solution (5) should be discarded if it becomes brown. .

Assay System Assay system was carried out in doublicate according to the following (78) :

Pipette Successively in test tubes	Reagent blank (ml)	Standard solution (ml)	Adenosine blank(ml)	Sample blank (ml)	Sample (ml)
Phosphate buffer (1)	0.1	---	---	1.0	---
Buffer adenosine solution (2)	---	---	1.0	---	1.0
Ammonium sulphate standard solution (4)	---	1.0	---	---	---
Sample (serum or tissue homogenate)	---	---	---	0.05	0.05
Ammonium- free water	0.05	0.05	0.05	---	---

Each tube was mixed and stoppered with parafilm and incubated for (10) min in a (37) Cº water bath , then each tube,

(3) ml of solution (5) and solution (6) were added with vortex , further incubation for (30) min in a (37) C^o water bath carried out , then the absorbencies were read against water at (628) nm. .

Calculation :-

The enzyme activity was calculated according to the following equation :-

$$\text{Activity (U/L)} = \frac{A - B}{C} \times \frac{1}{t} \times \frac{1}{Vs} \times \frac{V_1}{Vt} \times 1000$$

Where :

A= A sample – A sample blank

B= A adenosine blank – A reagent blank

C= A standard – A reagent blank

t= Incubation time Vt = Total Volume

Vs = Sample Volume V1= Incubation Volume

For the assay , the values were as following :

$$(U/L) = \frac{A-B}{C} \times \frac{1}{60} \times \frac{1}{0.050} \times \frac{1.05}{7.05} \times 1000$$

$$(U/L) = \frac{A-B}{C} \times 50$$

2.2.3. Determation of serum Malondialdehyde (MDA).(79)

Principle:

The principle of the following method was based on the spectrophotometric measurement of the color, occurred during the reaction with thiobarbutric acid (TBA) with MDA ,Fig.(2-1)

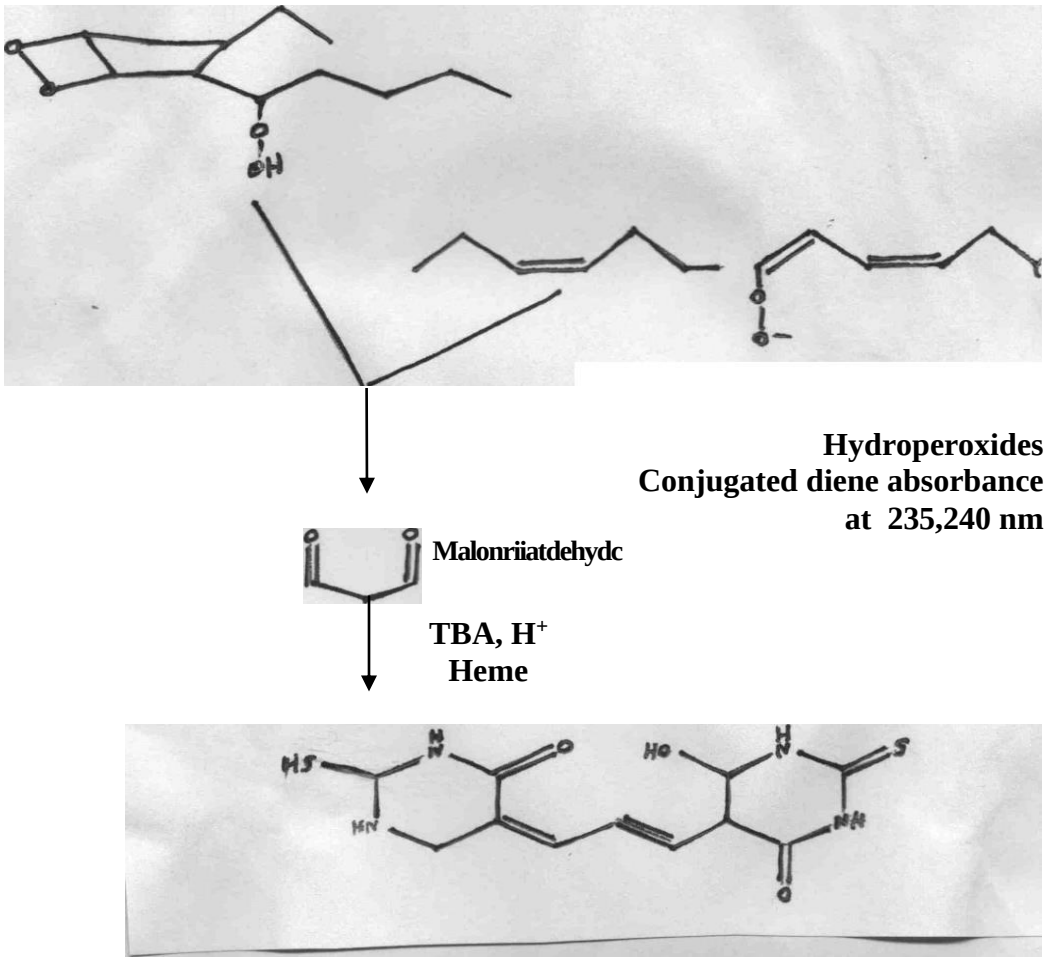


Fig (2-1) : Schematic diagram for assessment lipid peroxidation via determination the byproduct ; malondialdehyde (79)

Reagents

1- 17.5% Trichloroacetic acid (TCA)

2- 0.6% thiobarbutric acid (TBA)

3- 70% Trichloroacetic acid

Procedure :

Two set of tubes were prepared as following (79) :

Reagent	Sample	Blank
Serum	150 µL	-----
TCA(17.5%)	1 ml	1 ml
TBA(0.6%)	1 ml	1 ml
All tubes were mixed well by vortex, incubated it in boiling water bath for (15) minutes , then allowed to cool .		
TCA(70%)	1	1

The mixture was let to stand at room temperature for 20 minutes and centrifuged at 450 xg for 15 minutes and the supernatant was taken out to read the absorbance of sample at 532 nm by using spectrophotometer instrument . .

Calculation

$$\text{The concentration of MDA} = \frac{\text{A of sample}}{L \times \varepsilon} \times D = \mu mol/L$$

Where :

L= Light path (1 cm)

ε = Extinction coefficient ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$)=156000

D= Dilution factor =6.66

$$D = \frac{1\text{ ml (volume used in reference)}}{0.15\text{ ml (volume of the sample)}} = 6.66$$

2.2.4. Determination of Triglycerides

Principle

The triglycerides are enzymatically hydrolyzed to glycerol according to the following series of reaction. .

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-P + } O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone-P + } H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4- chlorophenol} \xrightarrow{\text{POD}} 4H_2O_2 + \text{Quinonimine}$$

Reagents

1-Buffer Reagent (1)

Pipes buffer pH2.5 (50) mmol/1 and 4-chlorophenol (2) mmol/1

2- Enzyme Reagent (2)**:**

Lipoprotein Lipase (150 000 U/L) .

Glycerol Kinase (500 U/L).

Glycerol 3-p-oxidase (4000 U/L).

Peroxidase (440 U/L).

4-Aminophenazone (0.7 mmol /L)

ATP (0.3 mmol / L)

3- Reagent (3) : 200 mg /dL

4- Standard : (2.26 mmol / L)

The contents of one vial (R2) are dissolved , with one bottle buffer reagent (R1) .

This working reagent is stable for 6 weeks at 2.8 C^o , and for 5 days at 25 C^o

Procedure :

	Reagent blank	Standard	Sample
Working reagent	1.0 ml	1.0 ml	1.0 ml
Standard	--------	10 µL	---------
Sample	---------		10 µL

Mix , and then incubate for 5 min at 37 Cº or 10 min at 25Cº, read absorbance of sample and standard against the reagent blank . The color is stable for 30 min. .

Calculation :-

$$\text{Triglycerides Constration} = \frac{\text{A . Sample}}{\text{A. standard}} \times n$$

n = 200 mg / dL n = 2.2 mmol / L

200×0.0114 = mmol

Normal value

***Female* :** 40 – 140 mg/dL ; 0.45 – 1.58 mmol/L

Male: 60 – 165 mg/dL ; 0.677 – 1.86 mmol/L

2.2.5. determination of Cholesterol (Biolabo-France)

Method

The method of cholesterol determination was enzymatic colorimetric assay using kits.

Principle:

The cholesterol is present in the serum originat a coloured complex according to the following reaction :

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{ChE}} \text{Cholesterol} + \text{free fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{COxase}} \text{Cholesten 3ene4} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{PAP} \longrightarrow \text{Quinoneimine (pink)} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in sample.

Reagents Composition

Contents		Initial concentration Of solution
Reagent1 (R1)	Buffer	
	Phosphate buffer	100 mmol
	Chloro-4-phenol	5 mmol
	Sodium Chloride	23 mmol/litter
	Triton	1.5 mmol/litter
Reagent2 (R2)	Enzymes	
	CHE (cholesterol esterase)	> 170 IU / L
	CHOD (cholesterol Oxidase)	> 100 IU / L
	POD (Peroxidase)	> 1200 IU / L
	4-Amino – antipyrine(4-AA)	0.25 mmol / L
Reagent3 (R3)	Standard	
	Standard cholesterol	200 mg/dl (5.17 mmol/L)

Reagent preparation	Add promptly the content of vial R2 (enzymes),into vial R1(Buffer).Mix gently unit complete dissolution (approximately 2 min) .

CHOD : Cholesterol Oxidase
CHE : Cholesterol esterase
4-AA : 4- Amino Antipyrine
POD : Peroxidase

Procedure

Three sets of tubes marked blank, standard and sample were prepared as follows :
Let stand reagent and specimens at room temperature.

Pipette into well identified test tubes	***Blank***	***Standard***	***Sample***
Reagent	1 ml	1 ml	1 ml
Demineralised Water	10 µL	----------	----------
Standard	--------	10 µL	--------
Specimen	--------	---------	10 µL
Mix and stand for 5 minutes at 37 Cº or 10 minutes at room temperature . Record absorbances at 500 nm against reagent blank . The color is stable for 1 hour .			

Calculation

Calculate the result as follows :

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (standard)}} \times \text{Standard Concentration}$$

Standard Conc. = 200 mg/dL (5.17 mmol/L)

200×0.0259 = mmol

Normal Value = 150 – 250 mg /dL or < 5.17 mmol/L

2.2.6. determination of alkaline phosphatase alkaline (BioMerieux-France)

Principle

Colorimetric determination of alkaline phosphatase activity according to the following reaction :

$$\text{Phenyl phosphate} \xrightarrow[\text{pH-10}]{\text{Alkaline phosphatase}} \text{phenol + phosphate}$$

The liberated phenol is measured in the presence of 4-aminoantipyrine and potassium ferriccyanide . .

The presence of sodium arsenate in the reagent stops the enzymatic reaction. .

Reagent 1 Substrate buffer	Disodium phenylphosphate carbonate-bicarbonate Buffer pH 10 Sodium merthiolate	5 mmol /l 50 mmol /l 0.1 g / l
Reagent 2 Standard	Phenol	equal to 20 kind and king 4
Reagent 3 Blocking reagent	4-aminoantipyrine Sodium arsenate	60 mmol/l 75 g/l
Reagent 4 Color reagent	Potasium ferric	150 mmol / l

This reagent contains a mercury derivative . Disposal in sewers or natural environments is forbidden .

Procedure

Wave length 510 nm (Hg 492)

Zero adjustment Reagent blank

Set up the following tubes :

	Serum Sample	Serum blank	Standard	Reagent blank
Reagent 1	2 ml	2 ml	2 ml	2 ml
Incubate for 5 minutes at 37 Cº				
Serum	50 µl	-----	-----	-----
Reagent 2	-----	-----	50 µl	-----
Incubate for exactly (15) minutes at 37 Cº				
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well or preferably vortex				
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	-----	50 µl	-----	-----
Distiled water	-----	-----	-----	50 µl
Mix let stand to (10) minutes in the dark measure				
Thr color intensity is stable 45 min				

Normal value :

Children: 10-20 kind and king U/100ml (71-142U/L)

Adults : 3-13 kind and king U/100ml (21-92 U/L)

Calculation:

$$\text{Calculation} = \frac{\text{Abs(serum sample)} - \text{Abs(serum blank)}}{\text{Abs Standard}} \times n$$

Kind and King U/100ml : n= 20 if U/100 ml

n= 142 if U/ L

2.2.7. Determination of Urea (Crescent- Saudi Arabia)

Principle

Urease catalyses the conversion of urea to ammonia . In a modified Berthlot reaction , the ammonium ions react with a mixture of salicylate , hypochloric , and nitroprusside to yield a blue- green dye (Indo phenol) . The intensity of this dye is proportional to the concentration of urea in the sample .

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} + \text{Sod. Hypochloric} \xrightarrow{\text{nitro prusside}} \text{2,2-dicarboxy indophenol(green complex)}$$

Reagent Composition

Contents		**Initial Concentration of solution**
Reagent R1	Phosphate buffer	120 mmol / L
	Sodium Salicylate	60 mmol / L
	Sodium nitro prusside	5 mmol / L
	EDTA	1 mmol /L
	Urease	5 K U / L
Reagent R2	Phosphate buffer	120 mmol / L
	Sodium hydroxide	400 mmol / L
	Sodium hypo chloride	12 mmol / L
Reagent R3	Urea Standard	80 mg/dl or mmol/L
Reagent Preparation : The reagents and standard are ready for use .		

Assay:-

Wave length : Hg 578 nm Optical path : 1 cm

Temperature : 25 C^o or 37 C^o

Measurment : Against reagent blank

Procedure

Pipette into cuvettes	***Micro***			***Macro***		
	Blank	***Standard***	***Sample***	***Blank***	***Standard***	***Sample***
Test Sample (ml)	-----	-----	0.01	-----	-----	0.025
Standard (ml)	-----	0.01	-----	-----	0.025	-----
Distilled water(ml)	0.01	-----	-----	0.025	-----	-----
Reagent 1 (ml)	1.0	1.0	1.0	2.5	2.5	2.5
Mix and incubate for (5) minutes at 25 C^{o} or (3) minutes at 37 C^{o} .						
Reagent 2 ml	1.0	1.0	1.0	2.5	2.5	2.5
Mix and incubate for (5) minutes at 37 C^{o} . Measure the absorbance of the sample (As) and standard (A std) against the reagent blank .						

Calculation

$$\text{Urea(mg/dl)} = \frac{\text{Asample}}{\text{A standard}} \times 50 \text{ (concentration of standard)}$$

Urea (g/ 24 h urine) = mg/dL x volume of 24 hours urine

To convert mg / dL to mmol / L . divide by 6.01 .
Normal Value for Urea = 10 – 50 mg / dL or 1.66 – 8.3 mmol/l

2.2.8. Determination of Creatinine (Linear Chemicals – Spain)

Principle

The procedure is based upon a modification of the original picrate reaction (Jaffe) . Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex . The formation rate of the complex measured through the increase of absorbance in a Prefixed interval of time is proportional to the concentration of creatinine in the sample(Linear chemicals-Spain). pH>12 .

Creatinine + Picric acid $\xrightarrow[37\ ^oC]{}$ Red addition complex

Reagent Composition

R	Contents	Initial Concentration of Solution
R1	Picric acid	Picric acid (40) mmol / L Potassium ferricyanide (40) mmol / L
R2	Alkalin buffer	Borate / phosphate buffer (300) mmol / L. PH (12.7)
CAL	Creatinine Standard	Creatinine(2) mg / dl (177 mmol/L).

Reagent Preparation

Working reagent . Mix (1) volume of R1 and 1 volume of R2 stable for (1) week or (5) hours at room temperature (25) C^o, when stored in dark bottle . .

Procedure

1-Preincubate working reagent . Sample and standard to reaction temperature (37 C^o) .

2- Sat the photometer to (10) absorbance with distilled water

3- Pipette into a cuvette .

Working reagent	1.0 ml
Sample or standard	100 mL

4- Mix and gently insert cuvette into the temperature – controlled instrument and start stopwatch .

5- Record absorbance at 500 nm after (30) seconds (A1) and after (90) seconds (A2) of the sample or standard addition. .

Materials Required

-Cuvettes with 1 cm pathlength .
Constant temperature incubator set at 37 C^o .

Calculation

$$\frac{(A2 - A1)\ \text{Sample}}{(A2 - A1)\ \text{standard}} \times \text{standard conc.} = \text{mg / dL creatinine}$$

Standard conc. = 2 mg / dL
mg / dL × 88.4 = μmol/L

Normal value
Male : 0.7 – 1.2 mg/dL (62 – 108 μmol/L)
Female : 0.5 - 0.9 mg/dL (44 – 80 μmol/L)

2.2.9. Determination of Total Protein (Spinreact – Spain)

Principle

Proteins give an intensive violet – blue complex with copper salts in an alkaline medium . Iodide is included as an antioxidant . .

The intensity of the color formed is proportional to the total protein concentration in the sample . .

Reagents

R	Sodium potassium tartrate Sodium iodide	15 mmol / L 100 mmol / L
Biuret	Potassium iodide Copper (II) Sulphate	5 mmol / L 19 mmol / L
T Protein Cal	Bovine albumin primary standard 7g / dl	

Preparation

The reagents are ready to be used .

Procedure

1-Assay conditions :

Wavelength	540 (530-550) nm
Cuvette	1 cm light bath
Temperature	37 C^o

2- Adjust the instrument to zero with distilled water .

3- Pipette into a cuvette .

	Blank	***Standard***	***Sample***
R (ml)	1.0	1.0	1.0
Standard (µL)	------	25	------
Sample (µL)	------	------	25

4- Mix and incubate (5) min at 37 C^{o} or (10) min at room temperature .

5- Read the absorbance (A) of the samples and standard , against the blank . The color is stable for at least (30) min .

Calculations

$$\frac{(A)\ \text{sample}}{(A)\ \text{standard}} \times 7\ (\text{standard conc.}) = \text{g / dL of total protein}$$

Normal value : in adults 6.6-8.3 g/dL in Newborn 5.2-9.1 g/dL

2.2.10. Determination of Albumin (Spinreact-Spain)

Principle

Albumin in the presence of bromcresol green at a slightly acid pH , produces a color change of the indicator from yellow-green to green-blue . The indensity of the color

formed is proportional to the albumin concentration in the sample(Spinreact-Spain). .

Reagents

R	Bromcresol green pH 4.2 0.12 m mol/ L
Albumin Cal	Albumin aqueous primary standard 5 g / dl

Preparation

Reagent and Calibrator are ready to use .

Procedure

1- Assay conditions :

Wavelength 630 nm (600- 650)

Cuvette 1 cm light path

Temperature 25 C^{o}

2- Adjust the instrument to zero with distilled water .

3- Pipette into a cuvette .

	Blank	***Standard***	***Sample***
R (ml)	1.0	1.0	1.0
Standard (ml)	------	5	------
Sample (ml)	------	------	5

4- Mix and incubate for (10) min at room temperature (25) C^o .

5- Read the absorbance (A) of the samples and standard , against the blank .

The color is stable (1) hour at room temperature .

Calculations

$$\frac{\text{(A) sample}}{\text{(A) standard}} \times 5 \text{ (standard conc .)} = \text{g / dL albumin}$$

Conversion factor : g / dL x 144.9 = mmol / L

Normal value : 3.5 – 5 g/dL

2.2.11. Determination of Iron (Linear chemicals – Spain)

Principle

The Fe bound to serum ferritine once dissociated in a week – acid medium by Teepol and guanidium chloride , is reduced by hydroxylamine to Fe , forming the ferrous ion a colored complex with Ferrozine proportional to the concentration of iron present in the sample. .

$$\text{Transferrine} - Fe^{+3} \xrightarrow[\text{Teepol}]{\text{PH} > 5} \text{Apotransferrine} + Fe^{+3}$$

$$Fe^{+3} + \text{hydroxylamine} \longrightarrow Fe^{+2}$$

$$Fe^{+2} + \text{Ferrozine} \longrightarrow Fe(\text{Ferrozine})_3^{+2} \text{ (complex)}$$

Reagent Composition

R1 = Buffer / Reductant . Guanidine chloride (1.0) mmol / L
Hydroxylamine (0.6) mol / L , acetate buffer 400 m mol / L
pH 4.0 , Teepol

R2 = Chromogen , Ferrozine 40 mmol / L , sodium acetate 400 mmol/L

R3= Iron standard , Ferric ion 100 µg / dl (17.9 µmol / L)

Reagent Preparation

Working reagent : Mix (4) volume of R1+1 volume of R2 stable (6) months at(4) C^o . .

Discard the mixture if the blank presents an absorbance above (0.050) at 560 nm against distilled water. .

Procedure

1- Bring reagents and samples to room temperature .

2- Pipette into labeled test tubes :

Tubes	***Reagent blank***	***Sample blank***	***Sample***	***Standard***
Distilled Water	200 µL	------	------	------
Sample	------	200 µL	200 µL	------
Standard	------	------	------	200 µL
R1	------	1.0 ml	------	------
Working reagent	1.0 ml	------	1.0 ml	1.0 ml

3- Mix and let the tubes stand (5) minutes at room temperature .

4- Read the absorbance (A) of the sample blank at 560 nm against distilled water ..

5- Read the absorbance (A) of the samples and the standard at 560 nm against the reagent blank .

Calculations

$$\frac{\text{A sample} - \text{A sample blank}}{\text{A standard}} \times 100(\text{Conc.standard}) = \mu g/dL \text{ iron}$$

Normal value : Male 60-175 µg/dL (10.7-31.3 µmol/L

Female 50-170 µg/dL (9.0-30.4 µmol/L)

Samples with concentrations higher than (1000) mg / dL should be diluted 1:2 with saline and assayed again . Multiply the results by 2 . .

μg / dl x 0.179 = μmol / L

2.2.12. Determination of Copper (Randox-UK)

Principle

Copper deficiency is characterized by growth failure and hypochromic – microcytic anaemia; acute copper toxicity can cause gastroenteritis and acute renal failure . .

Reagent Composition

Contents	**Initial Concentrations**
1- Buffer	
Acetate Buffer	0.2 mmol pH 4.7
Non reactive stabilizers	
2- Chromogen	

Acetate Buffer	0.2 mol / L pH 4.7
3- Reagent	
Ascorbic acid	
4- Standard	31.5mmol/L (200mg/dl)

Preparation of Reagent

1- Buffer :

Supplied ready for use. Stable up to expiry date when stored at + 4 Cº.

2- Chromogen :

Supplied ready for use. Stable up to expiry date when stored at + 4 Cº.

3- Reagent :

Dissolve the contents of 1 vial of reagent 3 with 20 ml of buffer 1 .

Ensure that contents are completely dissolved . Stable for 2 week at + 4 Cº

4- Standard :

Supplied ready for use. Stable up to expiry date when stored at + 4 Cº.

Procedure

Wavelength 580 nm (570 – 590 nm)

Temperature 37 C°

Pathlength 1 cm

Measurme Against Reagent blank

Pipette into cuvette : -

	Reagent blank	***Standard***	***Sample***
Double Distilled H2O	120 µl	------	------
Sample Supernatant	------	------	120 µl
Standard Supernatant	------	120 µl	------
Reagent 3	1000 µl	1000 µl	1000 µl
Mix and allow to stand for (60) seconds at 37 C° .Read intial absorbance (A1) of sample and standard against the reagent blank .			
Chromogen	250 µl	250 µl	250 µl
Mix , incubate for (5) minutes at 37 C° and read final absorbance (A2) against reagent blank .			

Normal value : Male 11 - 24 µmol/L (70 - 150 µg/dL)
Female 12.6 – 24.4 µmol/L (80 – 155 µg/dL)

Calculation

$\triangle A = A2 - A1$

$$\text{Concentration} = \frac{\triangle \text{ A sample}}{\triangle \text{ A standard}} \times \text{Conc. of standard}$$

Conc. of standard(31.5)µmol / L (200 µg /dL)

CHAPTER THREE

RESULTS

AND

DISCUSSION

3.1. Distribution of renal failure cases according to age.

In this study , 107 uremic patients (51 males and 56 females) suffering from renal failure who admitted to Merjan Teaching Hospital in Hilla province were included. Blood samples were obtained from those pre dialyzed patients compared with samples obtained from healthy individuals as a control group(20 samples). .

It was found that the disease was prevalent among the age groups ranging from 40 – 70 years old , where the highest distribution of this disease was found to be in the age groups (61 – 70)years old (Fig. 3-1). .

These results were in agreement with those observed by other investigators (2 , 80) who indicated that renal failure occurrence increases in elderly patients especially those suffering from chronic diseases such as hypertention and diabetes mellitus . .

However , the results obtained in this study also had pointed out that this disease was distributed among patients below 40 years old and this would confirm that renal failure might involve all age groups who had predisposing factors that made them liable to such diseases. .

Fig.(3-1) Distribution of renal failure patients according to their ages

3.2. Study of some biochemical indicators of renal failure.

3.2.1. Urea and Creatinine Concentration

Some biochemical parameters associated with renal failure (urea and creatinine) were studied (as shown in table (3-1) .

Table (3-1) : Mean and standard deviation of urea and creatinine concentration in patients with renal failure and control group

Tests	*Mean ± SD*		*P value*
	Patients	*Control*	
Urea (mmol/L)	24.558 ± 7.716	4.70 ± 1.320	0.001
Creatinine (mmol/L)	463.5 ± 228.3	57.4 ± 8.02	0.001

P< 0.01 Significant S.D Standard Deviation

P> 0.05 not significant

It was observed that all patients with renal failure had hyperuraemia and increase in serum creatinine level , with a mean value 24.55 and 463.5 respectively . The results of urea and creatinine in patients with renal failure were significant ($P<0.01$) when they were compared to control group.

Some researches had pointed out that urea and creatinine were the main indicators for renal failure at both types (acute and chronic) (64), but others indicated that at all stages renal insufficiency , the serum creatinine was a much more reliable indicator of renal function than blood urea because blood urea is far more likely to be affected by dietary and physiological conditions not related to renal function. .

Moreover , the results of urea and creatinine among both sexes in all age groups were not significant ($P> 0.05$) .These results were similar to those obtained by some investigators who pointed out that the mean values of urea and creatinine in patients with renal failure was not significant if the results of both indicators were distributed among patients ages and gender (81) . .

3.2.2. Total Protein , Albumin and globulin Concentration

Total protein, serum albumin and globulin concentration were tested in the sera of patients with renal failure. It was found that all patients understudy had low concentrations of total protein and low levels of serum albumin with a mean values 5.82 and 3.03 respectively . .

The results of total protein and albumin were not significant (P > 0.05) when compared to control group, as shown in Table (3-2) . Also, the globulin concentration was found to have no significanc when compared with control group.

Table (3-2) : Mean and standard deviation of total protein , albumin and globulin concentration in patients with renal failure and control group

Tests	*Mean ± SD*		*P value*
	Patients	*Control*	
Total protein (g/ dL)	***5.826 ± 0.88***	***7.335 ± 0.53***	***0.234***
Albumin (g / dL)	**3.035 ± 2.40**	**4.705 ± 0.49**	**0.584**
Globulin (g / dL)	**3.007 ± 0.90**	**2.725 ± 0.26**	**0.02**

P < 0.05 significant ***P > 0.05 not significant***

It was seen that the loss of protein and albumin in patients with renal failure might have occured as a result of increased transcapillary eccape rate of protein (82). .

Also , if proteinuria and microalbuminuria were defected in the urine of uremic patients ,they should be accompanied by decreasing in serum protein and albumin (81).

Proteinuria occurs as a result of glomerular capillary hypertention and damage to the permeability barrier in the glomerulus (24). .

The results of low total protein concentration and low albumin levels were similar to those results obtained by other studies (11,83),who found that there was no significant difference when protein and albumin were reduced in their levels in the sera of patients with renal failure . Many findings had suggested that a marked total protein depletion existed in chronic uremia , and that dietary treatment was not responsible for such a depleted state , instead , the depletion of protein stored observed in the steady phase of chronic uremia might have been originated by exaggeratedly increased catabolism in the early phase of renal failure , not compensated by a proportional increase of the synthetic rate , due to both the state of uremic intoxication and the reduced dietary protein intake during the early phase (82) . .

3.3. Adenosin deaminase activity :

Few studies had previously pointed out findings about the level of adenosine deaminase enzyme in uremic patients especially those suffering from renal failure. .

In this study , it was seen that adenosine deaminase levels decreased in all patients (n=107) with renal failure (with the value 14.30 U/L respectively) . This result was highly significant in renal failure cases when compared with the control group ($p<0.01$), as shown in Table (3-3) .

Table (3-3) : Mean and standard deviation of adenosine deaminase in patients with renal failure and control group .

Test	Mean ± SD		P values
	Patients	Control	
Adenosine Deaminase (ADA) U/L	14.30 ± 10.47	63.80 ± 22.98	0.001

ADA was expressed in the cytosol of all cells as deficiency of ADA results in accumulation of adenosine.This

would result in the excretion of some amount of adenosine in the urine (68). .

The presence of adenosine and adenosine deaminase binding protein at high levels in the urine of patients with renal failure might reflect the low levels of ADA activity in such patients (84). .

Regarding the sex of the patients , it was found that the mean value of ADA in male patients (n= 51) and female patients (n= 57) had four fold decrease when it is compared to enzyme level in control group. .

The mean values of ADA in male patients showed no significant difference in comparison to enzyme level in female patients ($P > 0.05$) , as shown in Table (3-4). .

Table (3-4) : Mean and standard deviation of adenosine deaminase activity in patients with renal failure according to their sexes.

Sex	Case	Number	Mean ± SD of ADA U/L	P value
Male	Patient	51	14.410 ± 9.739	0.219
Female	Patient	56	14.207 ± 11.183	

P > 0.05 not Significant

P < 0.05 Significant

These results can be related to the effect of renal failure at the same degree in male and female patients without any differences among them . .

3.4. Xanthine Oxidase activity

Xanthine oxidase activity is one of the most important enzyme in nucleotide metabolism . The enzyme activity was investigated in all patients of renal failure and in the control group. It was found that the mean value of enzyme activity in the sera of patients was (4453.9 U/L) and in the control group was (4669.45 U/L) . According to the results above , there was no significant difference between the enzyme activity of patients and in the control group (P>0.05) , as shown in Table (3-5). .

Table (3-5) : Mean and standard deviation of xanthine oxidase in patients with renal failure and control group .

Test	Mean ± SD		P. Value
	Patients	Control	
Xanthine Oxidase Activity U/L	4453.98 ±694.86	4669.45±811.80	0.260

P < 0.05 Significant

P > 0.05 not Significant

This mild decrease of xanthine oxidase in uremic patients may be attributed to the effect of high levels of urea in the sera of those patients which play the role of an inhibitor for this enzyme (57) . Also , when the enzyme activity is reduced , xanthine will accumulate in human tissues and then go out as an output in urine resulting xanthineurea (85,59). .

In addition , chronic renal failure was studied in animals and it showed a marked reduction in hepatic , renal , and enteric tissue xanthine oxidase activity and no significant change was seen when it was compared with healthy animals (86). .

Moreover , chronic renal failure was associated with oxidative stress where it was associated with depressed superoxide dismutase and elevated NADPH oxidase expression , which can be contributed to oxidative stress by increasing superoxide and at the same time xanthine oxidase activity was reduced in chronic renal failure which acted on xanthine oxide and the later was a major source of reactive oxygen system (87).

Regarding to sex , the mean value of xanthine oxidase mean activity in male patients was (4467.6) and in female patients was (4441.5) .There was no significant difference between the enzyme activity in male and female patients ($p > 0.05$), as shown in Table (3-6). .

Table (3-6) : Distribution of xanthine oxidase mean and standard deviation in patients with renal failure according to the sex .

Sex	Case	Number	Mean ± SD of XOD	P Value
Male	**Patient**	51	4467.67 ± 742.87	**0.362**
Female	**Patient**	56	4441.51 ± 876.16	

P < 0.05 Significant ***P > 0.05 not Significant***

These results were similar to those obtained by other researches (72,66) who showed that there was no differences in enzyme activity in male and female patients of renal failure .

3.5. Alkaline Phosphatase

Alkaline phosphatase was tested in this study to show whether kidney failure had an effect on this enzyme .It was observed that alkaline phosphatase had increased in patient above the normal values when compared to its values in healthy individuals. .

The mean value of alkaline phosphatase patients group was (94.22 U/L) whereas the mean value in control group was (29.17 U/L) . .

According to these results , there was a significant difference in alkaline values in renal failure patients when compared to the control group (P < 0.01), as shown in Table (3-7).

Table (3-7) : Mean and standard deviation of alkaline phosphatase in patients with renal failure and control group

Test	Mean ± SD		P Value
	Patients	Control	
Alkaline Phosphate (U/L)	94.22 ± 59.56	29.17 ± 8.50	0.01

P > 0.05 not Significant ***P < 0.01 Significant***

The increase of alkaline phosphatase may be attributed to the increase of ecto – 5 – nucleotidase the enzyme which involved in converting adenine into adenosine. .

Ecto – 5 – nucleotidase activity was present within alkaline phosphatase activity and it could be estimeted together by the same procedure as indicated by some studies (88,89). .

The presence of high activity in ecto – 5 – nucleotidase in the sera of patients with renal failure would result inturn in accumulation of adenosine in the serum and in some body

tissues and this accumulation may also be related to the decrease in the activity of adenosine deaminase which was diminished in patients with renal failure. .

As for the sex , the mean value of alkaline phosphatase was (97.70 U/L) in female patients and (90.31U/L) in male patients and it was found that there were no significant differences in the mean values of alkaline phosphatase between both sexes (P > 0.05), as shown in Table (3-8). .

Table (3-8) : Distribution of alkaline phosphatase mean and standard deviation in patients with renal failure according to patient sexes . .

Patient Sex	Number	Mean ± SD of Alp (U/L)	P Value
Male	51	90.31 ± 42.51	0.784
Female	56	97.79 ± 71.89	

P < 0.05 Significant

P > 0.05 not Significant

This result was similar to that obtained by other study which found that alkaline phosphatase values were similar in female and male patient with kidney failure (86) . .

3.6. Relationship between urea ,creatinine and relationship with nucleotide metabolic enzymes

Statistical analysis was performed to show whether there was relationship between the presence of urea and serum creatinine at high levels and the activities of xanthine oxidase and adenosine deaminase . The results obtained in this study reveal that there is no relationship between urea when presente at high concentrations and the decrease in xanthine oxidase ($r = - 0.001$; $P > 0.05$). .

However , urea acts as inhibitor for XOD , and it is suggested that urea acts by reversible attachment at the substrate binding site . So, its effect on XOD may have occurred by indirect mechanism (91). .

Thus, there was no direct relationship between urea and enzyme level in the sera of the patients .In addition to that , it was observed that high levels of creatinine had no relationship with the reduction of XOD levels in the sera of the patients. ($r = - 0.016$; $p > 0.05$). .

On the other hand , it was found that urea had no relationship with the decrease occurred in adenosine deaminase activity ($r = - 0.092$; $p>0.05$). Additionlly, creatinine elevation in renal failure patients had no relationship with the reduction in

the adenosine deaminase activity($r = -0.117$; $p > 0.05$). (Although urea plays a role as an inhibitor to xanthine oxidase (53) and adenosine deaminase (91) but its effect on the enzymes activities has little importance since there is increase in the xanthine in the sera of the patients (86) or adenosine (60) respectively. .

However , the main cause that results in decreasing such enzymes comes from haemodialysis procedure which can effect chiefly on adenosine deaminase activity but not at the same degree on xanthine oxidase activity(92) . Some studies (93,84) have showed that the high reduction in adenosine deaminase activity (4.5 folds decrease if compared to control) had stemmed from the effect of haemodialysis on the transcription of the gene encoding this enzyme , leading to decreas of ADA and increase of the concentration of adenosine . It has been seen that a deficiency in ADA activity causes moderate to complete lack of immune function .Therefore , most patients with renal failure suffer from weakening in immuno systems especially those on dialysis process (94). .

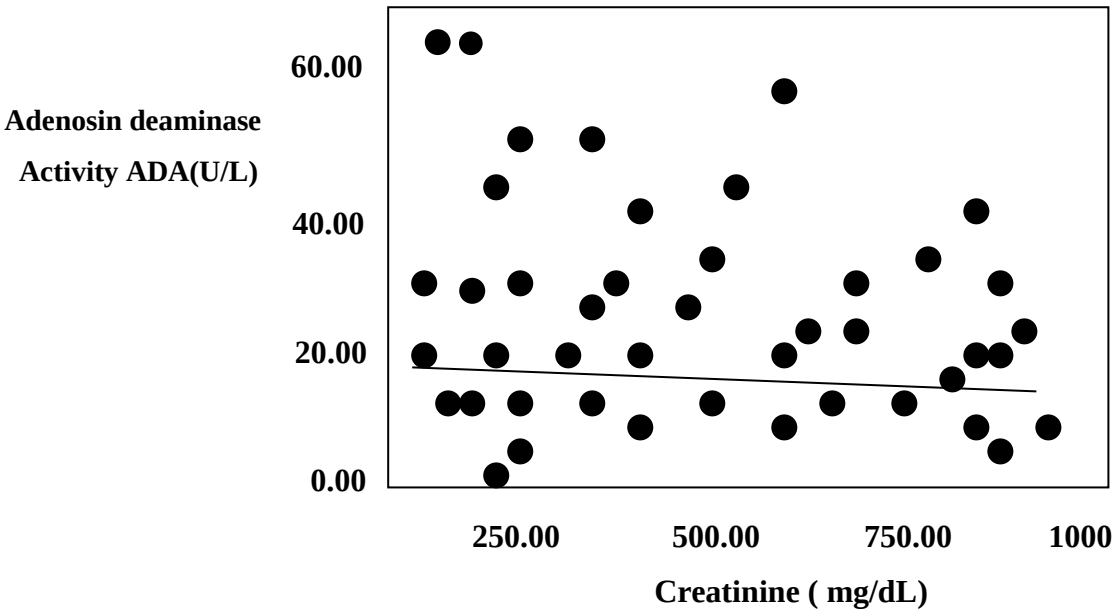


Fig.(3.2.) Relationship between creatinine and adenosine deaminase activity

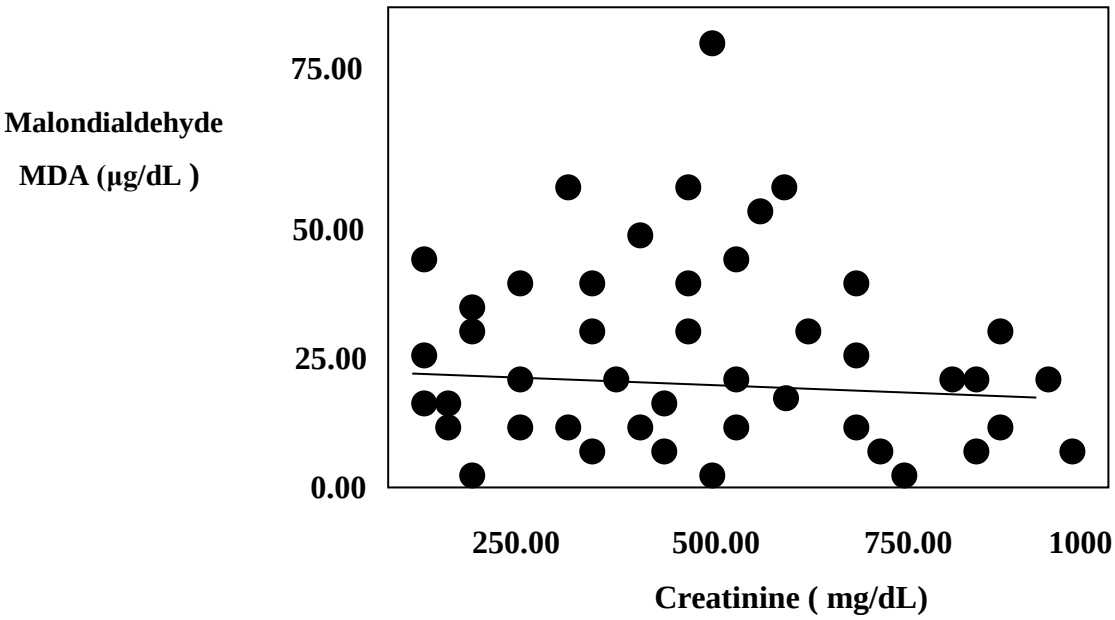
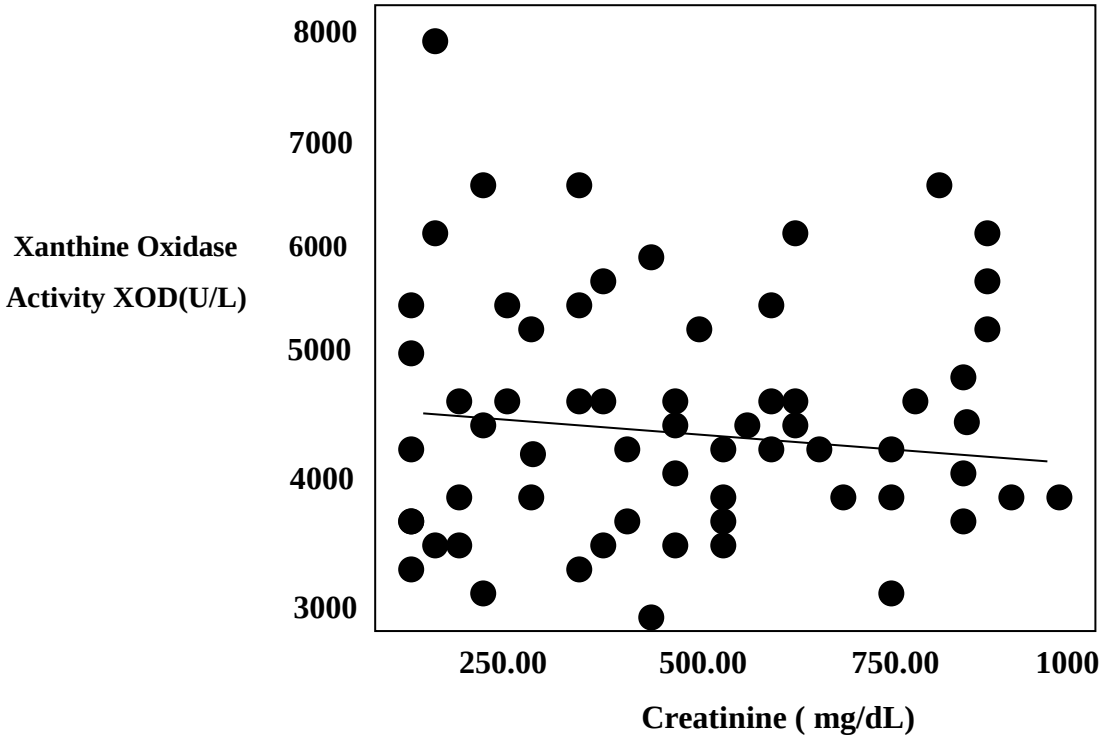


Fig.(3.3.) Relationship between creatinine and malondialdehyde



Fg.(3.4.) Relationship between creatinine and xanthine Oxidase activity

3.7. Cholesterol , triglyceride and Lipid Peroxidation in renal failure :

In this study , cholesterol , triglycerides and lipid peroxidation were also estimated . Lipid peroxidation was expressed by estimation of malondialdehyde (MDA) in the serum of patients with renal failure . .

It was seen that cholesterol decreased in all patients with a mean value (3.32 mmol / L) whereas the control group was with a mean value (4.36 mmol / L) and the results were not significant (p > 0.05) as shown in Table (3-9).

Table (3-9) : Mean and standard deviation of MDA , cholesterol and triglyceride in patients with renal failure and control group .

Test	Mean ± SD		P value
	Patient	Control	
MDA Malondialdehyde µmol / L	15.570 ± 12.34	5.810 ± 0.95	0.001
Cholesterol mmol / L	3.320 ± 1.22	4.365 ± 0.82	0.001
Triglyceride mmol / L	1.924 ± 1.05	1.030 ± 0.17	0.011

Many studies had shown that cholesterol either become less or remained at minimal normal values and they pointed out that renal failure had an effect on the enzymes associated with biosynthesis of cholesterol such as hydroxyl – 3 – methyl glutaryl – CoA reductase (HMG – CoA reductase) which was rate – limiting enzyme for cholesterol biosynthesis (95,47) .The expression and activity of this enzyme could be modified and reduced by proteinurea associated with renal failure. Morever, cholesterol – 7 - & - hydroxylase was seen to increase in patients with renal failure and this enzyme had played a role in cholesterol catabolism (46) . .

Triglyceride , on the other hand , was estimated and it was observed that triglyceride had increased above the normal levels when the mean value of triglyceride was (1.93 mmol / L). This value was high when compared to the healthy individuals where the mean value of triglyceride was (1.030 mol/L) . .

Hypertriglyceridemia was a common feature of chronic renal failure. Potential causes include increased synthesis and/or diminished clearance of TG from circulation that will result in increased levels of triglyceride (48,95) . .

Many studies had shown that hypertriglyceridemia in renal failure patients could occur as a result of deficiency of some lipases enzymes such hepatic lipases (96) which

catalyzes the hydrolysis and removal of triglyceride content of HDL and it was seen that chronic renal failure resulted in profouned hepatic lipase deficiency in humans (43) . Also, lipoprotein lipase deficiency was associated with increasing the level of triglycerides in the serum of patient with renal failure (96). .

On the other hand , lipid peroxidation was detected in the sera of the patients and it was seen that MDA level was high in all patients where the mean value was (15.57) and this result was higher than those reported in normal healthy persons (Mean value 5.81). .

These results were similar to those obtained by other studies (52,97) which showed that hypocholesteremia, hypertriglyceridemia and high elevation of MDA had been seen in patients with chronic renal failure. .

There was an evidence that lipid peroxidation was important in the pathogenesis of renal failure and data had shown that the heme center of myoglobin can initiate lipid peroxidation and renal injury without invoking release of free iron (95). .

It was reported that renal failure, especially chronic type, was associated with oxidative stress which promotes production of reactive carbonyl compounds and lipoperoxidas leading to the

accumulation of advanced glycation and lipoxidation and products (98). .

As regards the sex of the patients , it was noticed that malonyldialdehyde (MDA) levels were not significantly different between male and female patients (p > 0.05), as shown in Table (3-10). .

Table (3-10) : Mean and standard deviation of MDA in patients with renal failure and control group according to sex

Sex	Mean ± SD of MDA µmol / L		P value
	Patient	Control	
Male	14.47 ± 10.00	5.98 ± 1.08	0.751
Female	16.57 ± 14.15	5.65 ± 0.82	

P > 0.05 not significant

The results obtained above were similar to those obtained by other studies (32 , 62) which had showed that lipid peroxidation in male and female patients with renal failure increased , but there were no significant differences for such increase between them Moreover , in this study ,

the relationship between urea and creatinine with lipid peroxidation was investigated .It was seen that there was no relation between elevation of urea and malonyldialdehyde in the sera of the patients. (r = - 0.93 ; p > 0.05). .

Some studies confirmed that patients with renal failure who were subjected to dialysis especially peritoneal dialysis would be exposed to loss of some proteins , enzymes and chemical compound in addition to the increase of some compounds that made the patients at particular risk such as hypertriglyceridemia and high lipid peroxidation (97) . .

3 .8. Study of some trace elements in uremic patients :

3.8.1 Iron

Iron was investigated in the sera of (49) uremic patients of the subjects of this study. It was observed that iron decreased in (33) patients and was in normal values in other patients (n = 16) in which the mean value of the first group (33 patients) was (49.87 µg /dL)and in the normal group (88.16 µg /dL), as shown in Table (3-11). .

Table (3-11) : Mean and standard deviation of iron in renal failure patients

Group	Mean ± SD of Iron µg/L	P Values
First group n = 33	49.87 ± 12.91	0.001
Second group n = 16	88.16 ± 24.01	

According to the results shown in Table (3-11) ; there are significant differences between the two groups ($P < 0.01$). It had been proved that in ureamia or chronic renal failure patients or on hemodialysis program free iron or non – transferring bound iron would exist and there was no completed depletion of iron in such patients (76) .

Some studies had pointed out that if iron deficiency occurred it would happen mostly in those patients with hemodialysis (75,99).

Besides , packed cell volume (PCV) was estimated in all uremic patients (107) and it was found that all patients with renal failure had decreased PCV when compared to the control group where the mean value of the patients was 22.79 % and 43.0% for control group, as shown in Table (3-12).

Table (3-12) : Mean and standard deviation of PCV for patients with renal failure and control group

Test	Mean ± SD		P Value
	Patient	Control	
PCV (%)	22.79 ± 5.48	43.00 ± 5.25	0.001

P > 0.05 not Significant P < 0.05 Significant

The differences between PCV in the patients and in the control group were significant (P > 0.05). .

According to the results above , the patients were anemic and needed further management to treat the anemia . This anaemia was attributed to reduced red cell survival, to reduced red cell production , or to a combination of both (99) . The reduction in erythropoiesis may be attributable to lack of erythropoietin production by the kidney (100). .

On the other hand , the relationship between Fe values and PCV was studied . It was seen that although there was high decrease in PCV values for all the patients but the relationship between iron values and PCV was significant .(r = 0.035 ; p > 0.05) .

Erythropoietin is mostly administrated for uremic patients to treat anaemia and it will induce the formation of RBCs to elevation PCV levels, however some patients need blood transfusion to treat anemia. .

3.8.2. Copper (Cu)

Copper was estimated in (44) patients of renal failure and the results were compared with the control group (20 healthy individuals) . It was found that the mean value of copper in the patients was 101.64 and in control group was (133.35 µg/dL) ,as shown in Table (3-13). .

Table (3-13) : Mean and standard deviation of copper in patients and control group. .

Test	Mean ± SD		P Value
	Patient	Control	
Copper (µg/dL)	101.64 ± 37.60	133.35 ± 32.13	0.082

P > 0.05 not Significant P < 0.05 Significant

The data from different studies concerned with copper were not entirely consistent .Copper was an essential component of enzymes and other proteins and was critical for numerous reactions necessary for life. .

However , some studies showed that copper reduced in acute renal failure but it might elevate or fall within normal levels in chronic renal failure (71,73). .

Other studies showed that copper did not decrease in pre-dialysis patients, but through hemodialysis , or peritoneal dialysis there was a loss of copper and other trace elements (101). .

Some other studies showed that uremia may impair the hepatic metabolism of copper in a manner not reversible by dialysis (102) . Additionelly , in this study it was found that when copper was estimated in female and male patients , there was no significant difference ($p>0.05$) in the mean values of copper in all patients under investigation ,as shown in Table (3- 14). .

Table (3-14) : Mean and standard deviation of copper in patients according to their sex

Patients Sex	Mean ± SD of Copper µg/dL	P .Value
Male (n=22)	99.09 ± 35.76	0.448
Female (n=22)	104.19 ± 40.03	

P > 0.05 not Significant P < 0.05 Significant

According to all data obtained in this study, it can be noticed that renal failure may involve all patients whether male or female at the same manner and all will be subjected to the same mode of management and treatment . .

CONCLUSIONS

&

RECOMMENDATIONS

CONCLUSIONS

From this study the following findings can be concluded :

1. Renal failure is involved in all age groups .

2. Renal failure causes elevation in blood urea ,serum creatinine.

3. Renal failure causes decreases in total protein and albumin whereas there is elevation in globulin concentration .

4. There is a bit little decreases in xanthine oxidase levels in all patients with renal failure .

5. Adenosine deaminase also decreases at high level when compared to control group .

6. Lipid peroxidation and serum triglyceride also increase but serum cholesterol falls below the normal .

7. There is no relationship between creatine elevation and nucleotide metabolic enzymes reduction .

8. Copper and iron have different behavior in renal failure . They may increase or decrease or remain within a normal level according to the status of renal failure as well as the therapeutic processes .

Recommendations

1- Further studies are needed to study xanthine oxidase and adenosine deaminase isozymes levels in the sera of uremic patients .

2- Further research work on new techniques to evaluate the stage and type of renal failure is essential .

3- Study the effect of dialysis on nucleotide metabolic enzymes in patients with renal failure.

4- Investigation on other trace elements and their relationship to dialysis .

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الخلاصة

تضمنت هذه الدراسة , (107) مريض (51 من الذكور و 56 من الاناث) يعانون من عجز الكلية والذين براجعون مستشفى مرجان التخصصي للطب الباطني . كما شملت (20) شخص من الاصحاء كمجموعة سيطرة .

فقد وجد ان مرض عجز الكلية كان سائدا بدرجة كبيرة بين المرضى للاعمار بين (40 – 70) سنة تم التحري عن بعض المؤشرات البايوكيمياوية لمرضى عجز الكلوي ومقارنة ذلك بالأصحاء (مجموغة السيطرة) وقد وجد ان تركيز اليوريا والكرياتين قد سجلا معدلات معنوية عليه عند جميع المرضى كما لوحظ ان تركيز كل من البروتين والالبومين كانا واطئين في حين هناك زيادة في الكلوبيولين كذلك درست فعالية انزيم (ادينوسين دي امينيز) في مصول المرضى وقد لوحظ ان فعالية هذا الانزيم قد تضاءلت بدرجة كبيرة وبشكل معنوي في حين لاتوجد هناك فروقات معنوية من دراسة فعاليته لدى المرضى موزعة حسب الجنس .

اضافة الى ذلك , تم دراسة فعالية انزيم زانثين اوكسيديز في مصول مرضى العجز الكلوي وقد وجد ان فعالية الانزيم قد تضاءلت بدرجة غير معنوية كما وجد ان ليس هناك اية فروقات عند قياس فعالية الانزيم لدى الاناث والذكور من مرض العجز الكلوي .

لوحظ ايضا زيادة واضحة في فعالية انزيم الكلاين فوسفيت الذي قد يعود الى الزيادة الحاصلة في انزيم 5– اكتو نيوكليوتايد في حالات العجز الكلوي .

وعند دراسة مستوى بعض الليبيدات في مصول المرضى فقد لوحظ ان هناك زيادة كبيرة في ليبيد بيروكسيديس من خلال الزيادة الحاصلة في تركيز مادة مالون داي الديهايد, كما لوحظ ان هناك نقصان في تركيز الكولسترول وزيادة واضحة في تركيز ثلاثي الكليسيرايد .

الخلاصة

كما تم تقدير مستوى كل من الحديد والنحاس في 49 عينة من المرضى وقد لوحظ ان الحديد يقل في (33) مريض بينما (16) مريض بقت مستويات الحديد ضمن الحد الطبيعي . كما لوحظ ان تركيز النحاس تكون عند المرضى اقل من تركيزه في مجموعة السيطرة .

دراسة بعض الانزيمات الايضية للبيورينات لمرضى الفشل الكلوي

رسالة مقدمة إلى
كلية الطب – جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في
علم الكيمياء الحياتية السريرية

من قبل
سوسن حسن كاظم
بكالوريوس علوم كيمياء – الجامعة المستنصرية

ذي الحجة 1428 هـ **كانون الأول 2008 م**