The Effect of Chronic Renal Failure on Hepatic Functions in Sudanese Patients

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قال الله تعالى :

(وإذا مرضت فهو يشفين)

صدق الله العظيم

الاية (80) – سورة الشعراء

Dedication

To MY FAMILY

BEDOUR

ABSTRACT

There is abundant evidence that animal studies in chronic renal failure show a major down regulation of hepatic cytochrome P450 metabolism and hepatic functions are reduced; however, direct evidence in humans is lacking.

This study *is carried out in the period from October 2003 to October 2004 and* the purpose of it is to outline the effect of chronic renal failure on human hepatic functions. Liver functions in Sudanese patients with chronic renal failure were studied. Study subjects were 45 patients (11 females and 34 males) with chronic renal failure under regular heamodialysis (2 times per week) and 20 healthy persons (control). Blood samples were collected from the patients (pre-dialysis) and the controls in order to measure serum albumin, total protein, serum bilirubin levels as well as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase activities (ALP).

Chronic renal failure patients exhibited a decrease in serum albumin level ($p<0.05$) while total protein level and alkaline phosphatase activity were increased ($p<0.05$). Chronic renal failure did not alter AST and ALT activities. Serum bilirubin levels were unaffected.

From this study it is concluded that chronic renal failure has no clear effect upon the liver functions in humans and that it may be attributed to the positive effect of dialysis removal of toxins outside the body.

الخلاصة

هناك أدلة واضحة أثبتت أن الفشل الكلوى المزمن فى الحيوانات يؤدى الى خلل فى وظائف الكبد وانخفاض فى مستوى استقلاب cytocrome P 450 الكبدى بينما لا تتوفر مثل هذه الأدلة فى الانسان.

أجريت هذه الدراسة فى الفترة من اكتوبر 2003 الى اكتوبر 2004 وكان الهدف منها معرفة تأثير الفشل الكلوي المزمن على وظائف الكبد فى الانسان، واشتملت هذه الدراسة على 45 مريضا سودانيا يعانون من مرض الفشل الكلوي المزمن تحت الغسيل الدموى المنتظم (مرتين فى الاسبوع) من بينهم 11 اناث و 34 ذكور و 20 شخصا صحيحا للمقارنة.

أخذت عينات الدم من كل المرضى قبل الغسيل الدموى و كذلك عينات من الأصحاء و ذلك لقياس مستوى ال *Albumin* , *كامل البروتين* ال total protein و البيليروبين Bilirubin وكذلك قياس نشاط انزيمات الكبد ال(Transaminases (GOT,GPT و Alkaline. (ALP) phosphatase

و أوضحت الدراسة أن هناك انخفاض فى مستوى الAlbumin فى المرضى عند مقارنته بالأصحاء كما أن هنالك ارتفاع فى مستوى كامل البروتين Total protein.

ايضا هناك زيادة فى نشاط انزيم Alkaline phosphatase (ALP) بينما لا يوجد تأثير على مستوى ال Bilirubin و نشاط انزيم(Transaminases (GOT,GPT.

نخلص من هذه الدراسة إلى أن الفشل الكلوى المزمن تأثيره غير واضح على وظائف الكبد فى الانسان و ربما يعزى ذلك الى الأثر الأيجابى للغسيل فى ازالة السموم من الجسم.

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LIST OF ABBREVIATIONS

ADH	**Antiduretic hormone**
Alb	**Albumin**
ALP	**Alkaline phosphatase**
ALT	**Alanine transaminase**
ARF	**Acute renal failure**
AST	**Aspartate transaminase**
ATN	**Acute tubular necrosis**
CCD	**Cortical collecting duct**
CMOAT	**Canalicular multispecific organic anion transporter**
CRF	**Chronic renal failure**
DCT	**Distal convoluted tubule**
enPCR	**Equilibrated normalized protein catabolic rate**
FFA	**Free fatty acid**
GBM	**Glomerular basement membrane**
GFR	**Glomerular filtration rate**
HBSAg	**Hepatitis B surface antigen**
HCV	**Hepatitis C virus**
HDL	**High-density lipoproteins**
ICT	**Initial collecting tubule**

IDL	**Low-density lipoproteins**
IL	**Interlukine**
LCAT	**Lecithin cholestrol acyltransferase**
LDH	**Lactate dehydrogenase**
MDH	**Malate dehydrogenase**
MRP 2	**Multidrug-resistance protein 2**
NAD	**Nicotinamide adenine dinucleotide (oxidized)**
NADH	**Nicotinamide adenine dinucleotide (reduced)**
NAT-2	**N-acety transferase -2**
OD	**Opical density**
P450	**Cytochrome P 450**
PD	**Potential difference**
PTH	**Parathyroid hormone**
RBF	**Renal blood flow**
SnGFR	**Single nephron glomerular filtration rate**
TAL	**Thick ascending limb**
TNF	**Tumor necrosis factor**
Tm	**Transport maximum.**
TP	**Total protein**
VLDL	**Very-low-density lipoproteins**

1-1 General introduction

Renal failure has been recognized as a major health problem occurring in almost all population of the world. In Sudan, it has been disclosed from hospital records that the number of patients admitted to hospital suffering kidney diseases is increasing month after month and year after year.

Once renal failure occurs, requires immediate management such as dialysis and even then prognosis is often not good unless transplantation is done.

The chronic renal failure has a multiple complication that affects some organs in the body. One of them is the liver.

The liver has a central metabolic role in the body. It is an important metabolic factory for plasma proteins, blood glucose and lipid. It is also a major site for detoxification and excretion of drugs, hemoglobin metabolites and ammonium ion. Its anatomic position as filter of splanchnic blood flow makes it a critical determinant of the pharmacodynamics of drugs and crucial for the detoxification of absorbed metabolic poisons from the colon. These roles become especially evident when the liver dose not do it's job.

Liver involvement with a variety of viral disease is a frequent finding in patients with end stage renal diseases on regular haemodialysis. Such patients are at increased risk for infection with hepatitis B virus (HBV).

Non renal clearance and the distribution volume of drugs are altered by chronic renal failure via changes in hepatic clearance, plasma proteins binding and tissue bindings.

Chronic renal failure can significantly affect the disposition of both low and high hepatic extraction drugs which are cleared predominantly by the liver. Hepatic clearance of low-extraction drugs is limited by intrinsic hepatic clearance and plasma protein binding. A reduction in intrinsic hepatic clearance will lead to reduced systemic clearance and produce an increase in steady-state plasma levels for both free and total drugs concentration of low-extraction drugs. Intrinsic hepatic clearance produced by down-regulation of hepatic cytochrome P450.

This study was performed to determine the effect of chronic renal failure on liver functions and whether the liver is affected by the toxicants that are clear by the kidneys. That by assessment certain enzymes activity, bilirubin, total proteins and albumin levels in patients with chronic renal failure compared with persons without renal insufficiency.

It is reasoned that such an understanding was essential to learning the relation between renal disease and hepatic functions**.**

Objectives:

The aim of this study is to determine the effect of chronic renal failure on liver functions and whether the liver is affected by the toxicants that are produced from the kidneys.

1- To measure blood urea and creatinine.

2- To assess the levels of electrolytes.

3-To measure the levels of serum bilirubin, total protein and albumin.

4- To measure the activity of certain enzymes.

5- To study the effects on sex and age on the level of these parameters.

6- To study the impact of family history and post history on these parameters.

1-2 The kidney:

1 -2-1 kidney Structure:

The kidneys are vital organs that perform a variety of important functions in the body. The human kidneys are paired; bean-shaped organs located in the retroperitoneal space. The right organ usually is more caudal, whereas the left organ tends to be slightly larger. Each adult human kidney weighs about 115 to 170 gram, measures approximately 11x 6 x 2.5 cm, and is surrounded by a tough, fibroblastic capsule. The kidney has a complex multicellular composition. The cut surface of a bisected kidney reveals a darker inner region, the medulla and a pale outer region approximately 1 cm in thickness, the cortex. The human kidney has a multi-papillary configuration in which the medulla is divided into 8 to 18 striated conical masses called pyramids. Each human kidney contains about 0.8 to 1.2 $\times$ 10^6 nephrons, the functional units of the kidney. A nephron is a complex apparatus that consists of the glomerulus or renal corpuscle, the proximal tubule, the Henle's loop and the distal tubule. The connecting tubule, transitional segment, joins the nephron to the collecting duct system. Although not anatomically precise, the term nephron is commonly used to also include the entire collecting duct (Bennett,et al, 2000; Bishop, 1995). Figure (1) shows the anatomy of the kidney and the principal part of the nephron (Guyton, et al, 2001; Kumar, et al, 2003)

1-2-1-1 The glomerulus (the renal corpuscle):

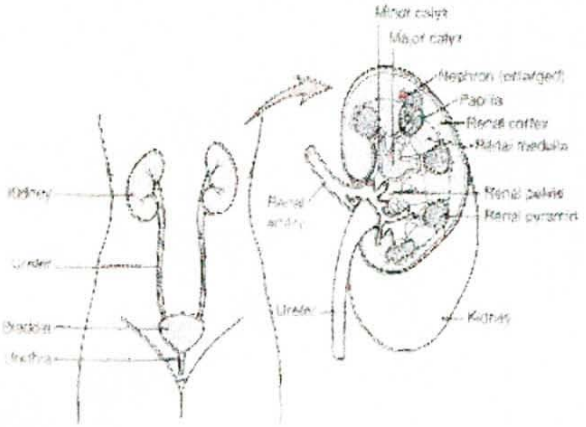
The glomerulus includes the glomerular tuft and the Bowman's capsule. The glomerular tuft contains three specialized cells, a basement membrane and a supporting framework, the mesangium. A fourth cell type, the parietal epithelial cells, lines Bowman's capsule. Each glomerulus is supplied by an afferent arteriole carrying the blood in and an efferent arteriole carrying the blood out.

Bowman's space, also called urinary space, represents the area between the visceral epithelial cells (specialized cells) and the parietal epithelial layer lining Bowman's capsule. A filtration barrier is formed between the blood and the urinary space by the peripheral glomerular basement membrane (GBM) and the overlying visceral epithelial cells. In humans, the area of the filtration surface per glomerulus is approximately 0.136 sq mm.

The GBM is a hydrated gel containing cross-linked molecules that form a complex, type IV and V collagen, iaminin, heparin sulfate proteoglycans and nidogen or entactin as well as other components. The polyanionic character of the heparin sulfate proteoglycans is largely responsible for the net negative charge of the GBM.

1-2-1-2 The proximal convoluted tubule:

The proximal convoluted tubule is located in the cortical labyrinth and the straight portion of the cortex. The cells contain a well-developed endocytic-lysosomal apparatus that has an important role in the absorption and degradation of macromolecules such as albumin from the glomerular filtrate. The localization of Na^+, K^+-ATPase (the sodium pump) to the basolateral membranes explains the



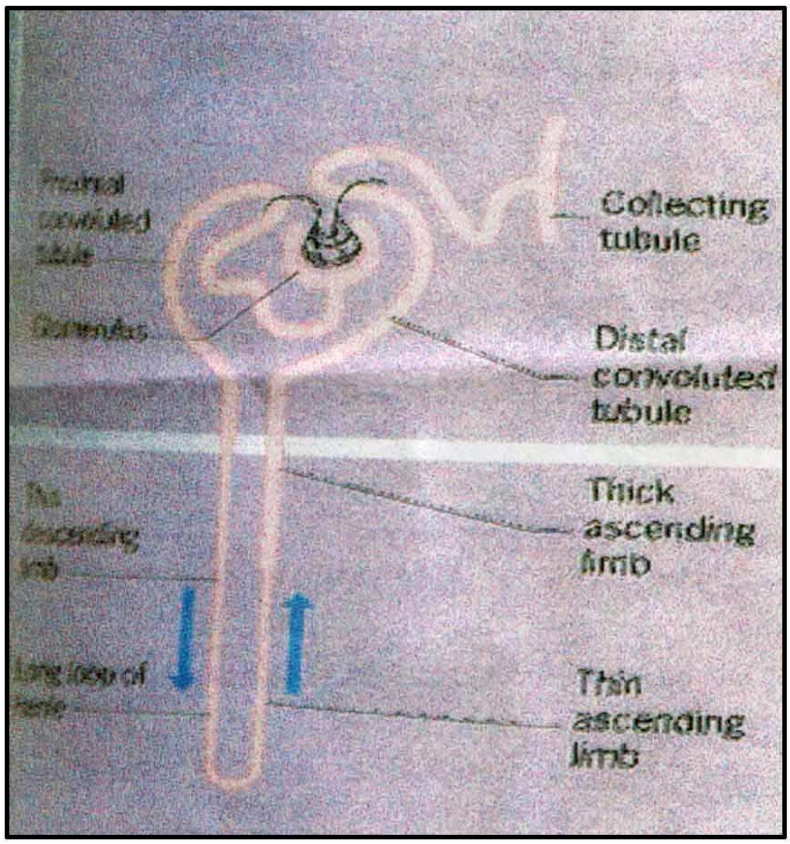


Figure (1): Shows general organization of the kidneys and the uniary System and principal part of the nephron (Guyton etal , 2001;Kumar etal, 2003)

active transport of sodium characteristic of this tubule segment.

1-2-1-3 The Henle's loop:

There is an abrupt transition from the terminal proximal tubule to the descending thin limb of Henle's loop at the junction between the outer and inner stripes of the out medulla. Short-looped nephrons have a short descending thin limb that continues into the thick ascending limb near the bend in the loop. Long looped nephrons have a long descending thin limb that enter the inner medulla, forms are lined with a low lying simple epithelium. The sending limb located in both the medulla and the cortex (Bennett et al, 2000; Bishop, 1995).

1-2-1- 4 The distal tubule:

The distal tubule includes two morphologically distinct segments: the thick ascending limb (TAL) of Henle's loop and the distal convoluted tubule (DCT). The TAL traverses the outer medulla upward in to the cortex near its glomerulus origin to end just beyond the macula densa. Thus the TAL can be divided into a medullary and a cortical segment.

The TAL is composed of cubical cells with extensive basolateral plasma membrane. The DCT represent the terminal part of the distal tubule and begins at a variable distance beyond the macula densa. The cells of the DCT resemble those of the TAL.

The connecting tubule or connecting segment joins the DCT with the collecting duct system. Representing a transitional segment in the human kidney, the connecting tubule composed of four specific cell types resulting from an intermixing of cells from adjacent DCT

and the initial collecting tubule (ICT). The most characteristic cell type is the connecting tubule cell, which is intermediate in appearance between the DCT cell and the principal cell of the collecting duct.

1-2-1-5 The collecting duct:

The collecting duct is begins in the cortex and descends through the medulla to the tip of the papilla. It can be divided into cortical and medullary segments. There is remarkable cellular heterogeneity along the collecting duct.

The cortical collecting duct (CCD) can be subdivided into the initial collecting tubule (ICT) and medullary ray-portion. The CCD is composed of both principal cells and intercalated cells. The principal cells, which represent a pproximately two thirds of the total cells population, have a light-staining cytoplasm and relatively few organelles but prominent in folding of the basal plasma membrane. The intercalated or (dark) cells comprise approximately one third of the cells in the CCD (Bennett et al, 2000; Bishop 1995).

1-2-2 Kidney physiology:

The complex multicellular composition of the kidney reflects the complicated nature of it functional properties. This organ is responsible for maintaining both the volume and ionic composition of the body fluids: excreting fixed metabolic waste products such as urea, creatinine and uric acid and eliminating exogenous drugs and toxins. The kidney is a major endocrine organ, because it produces rennin, erythropoietin, 1,2 dihydroxy cholecalciferol, prostaglandins and kinins. It also serves as a target organ for many hormones. The kidney also catabolizes small-molecular-weight proteins and is

responsible for host of metabolic functions (ammoniagensis and gluconeogensis).

In a 70 kg person, a renal blood flow (RBF) amount is one fourth to one fifth of the resting cardiac output or 1.2 L/min. The renal cortex receives 85 to 90% of this flow compared with 10% for the outer medulla and 1 to 2% for the inner medulla including the papilla.

RBF and glomerular filtration rate (GFR) remain relatively constant over a wide range of perfusion pressure, a process that is termed autoregulation. An intrinsic property of smooth muscle cells in the renal vasculature permits instantaneous alterations in the tone of the vessel wall to maintain RBF and GFR constant over a pressure range of 80 to 180 mmHg.

There are a host of hormonal and neural factors that can alter RBF. The three basic renal processes are glomerular filtration, tubular reabsorption and tubular secretion (Bennett et al, 2000; Bishop, 1995).

1-2-2-1 Glomerular function:

The glomeruli act as "filters" and the fluid which passes from the blood in the glomerular capillaries into Bowman's capsule is of the same composition of protein-free plasma. The effective filtration pressure which forces fluid through the filters is the result of:

1- The blood pressure in the glomerular capillaries.

2- The opposing osmotic pressure of plasma proteins.

The glomerular is the first part of the nephron to receive incoming blood and functions to filter this blood. Each substance such as urea, creatinine, amino acids, glucose and electrolytes except cells and large molecules continues into the proximal convoluted

tubule. In a 70 kg person, the kidney forms approximately 180 L of glomerular rate each day through a process termed ultrafiltration. This represents the initial step in urine formation. The driving force to move fluid from the glomerular capillaries across the glomerular capillary wall to the urinary space (Bowman's space) is derived from the hydraulic pressure that is generated by the pumping action of the heart. Each glomerulus has a filtration rate (single nephron glomerular filtration rate) (Sn GFR) of 60nL/min. The GFR is the volume of blood filtered per-minute. The rate of filtration is proportional to the net ultrafiltration pressure that is present across the glomerular capillary wall and is determinate by the balance of hydraulic and oncotic pressure that are operative between the glomerular capillary lumen and Bowman's space. Several factors facilitate filtration. One is the unusually high pressure in the glomerular capillaries, which is due to their position between two arterioles. Other factor is the GBM in both size-selective and charge - selective barrier to the passage of macromolecules. The size-selective properties of the GBM allow molecules such as inulin, with radius of about 1.4 nm, to pass freely from the capillary lumen to the urinary space. As the radius macromolecules increases above 2.0nm, their passage is restricted across the GBM, and molecules with a radius greater that 4.2nm are completely restricted. Thus their fractional clearance approaches zero under normal circumstances. In addition to size, the charge of a molecule can greatly affect its ability to cross the glomerular capillary wall.

The juxtaglomerular apparatus is believed to be responsible for tubuloglomerular feedback, in which the composition of tubular fluid

delivered to the macula densa changes the filtration rate of the associated glomerulus, presumably by altering rennin secretion, which ultimately regulates glomerular homodynamic (Chatterjea, et al, 1994).

1-2-2-2 Tubular function:

1-2-2-2-1 Mechanisms of tubular reabsorption and secretion:

Small protein and some peptide hormones are reabsorbed in the proximal tubules by endocytosis other substances are secreted or reabsorbed in the tubules by passive or facilitated diffusion down chemical or electrical gradients or actively transported against such gradients. Movement is by way of ion channels, exchangers, co-transported against and pumps. Renal active transport systems have a maximal rate, or transport maximum (Tm), at which they can transport a particular solute. Thus, the amount of a particular solute transported is proportionate to the amount present up to the Tm for the solute, but higher concentration, the transport mechanism is saturated and there is no appreciable increment in the amount transported.

However, the Tm for some systems are high, and it is difficult to saturate them (Gonog, 1997).

1-2-3 Renal disease:

Many diseases affect renal function. In some several different functions are affected, whereas in others there is selective impairment of glomerular function or of one or more tubular functions. The term glomerulonephritis encompasses group of renal diseases, which are characterized by pathological changes in the glomeruli with an immunological basis, such as immune complex deposition.

Glomerulonephritis may present in many ways as acute nephritic syndrome with heamaturia, hypertension and oedemma, and as acute or chronic renal failure.

Many disorders primarily affect renal tubular function but most are rare. Their metabolic and clinical consequences range from the trivial, such as renal glucosuria, to the severe cystinuria. Most types of renal disease cause destruction of complete nephrons. This is particularly true for the chronic renal failure.

Clinical investigations are mainly of value in detecting the presence of renal disease, by its effects on normal function, and in assessing its progress. They are of less value in detecting the cause of the disease.

Glomerular function tests are essential steps in initial assessment of renal function. Tubular function tests are less convenient, since their performance usually requires conditions in which the tubules are stressed by artificial loading (Whitby et al, 1998; Marshall 1997).

1-2-3-1 Renal failure:

Renal failure means failure of renal excretory function owing to a variable extent by failure of erythropoietin production, vitamin D hydroxylation, regulation of acid-base balance and blood pressure.

Renal failure results in reduced excretion of nitrogenous waste products of which urea is the most commonly measured.

A raised serum urea concentration (uremia) may conveniently be present in an individual patient.

1-2-3-1-1 Acute renal failure (ARF):

Means abrupt deterioration in parenchyma renal function which is usually, but not invariably, reversible over a period of days or weeks.

In clinical practice, such deterioration in renal function is sufficiently severe to result in uremia. Oliguria is usually, but not invariable a feature. Clinical features are determined by the underlying condition and by the rapidly developing uremia if the patient survives, renal function usually returns to normal or near normal (Kumar et al, 2003; Macsween et al, 1995).

The most commonly acute uraemia due to renal Parenchymal is acute renal tubular necrosis (Kumar et al, 2003).

1-2-3-1-2 Chronic renal failure:

Chronic renal failure (CRF) implies long standing and usually progressive, impairment in renal function. Chronic Renal failure is the major cause of death from renal disease. The healthy kidney has a large reserve of function and while the biochemical evidence of renal insufficiency will occur earlier it is only when around 75% of renal function has bean lost that the symptoms of CRF make themselves manifest.

1-2-3-1-2-1 Causes of chronic renal failure:

Chronic renal failure may be caused by any condition which destroys the normal structure and function of the kidney, the etiology of chronic renal failure are: congenital and inherited disease such as glomerular disease, that chronic renal failure due to atherosclerosis renal vascular disease is more common in the elderly than in the young (Kumar et al, 2003; Edwards et al, 1995).

1-2-3-1-2-2 Clinical features of CRF:

The predominant symptom of chronic renal failure is tiredness, due mainly to accompanying anaemia. Urinary symptoms may include polyuria resulting from the osmotic diuresis. This in turn may cause polydipsia and thirst. Dyspnoea is common particularly in the older patient, due again to the anaemia and often also to heart failure. Hypertension may be a primary cause of chronic renal failure but much more often it is a sequel to the renal disease. Anoxia, nausea and vomiting are features of more advanced ureamia while neuromuscular feature include myopathy, peripheral neuropathy and encephalopathy with convulsions. Renal osteody strophy feature include muscle weakness, bone pain and hyperparathyroidism. There are endocrine abnormalities

(i) Anaemia:

Anaemia is present in the great majority of patients with chronic renal failure. Several factors have been implicated:

erythropoietin deficiency, bone marrow toxins in renal failure and fibrosis secondary to hyperparathyroidism, haemostatic deficiency-iron-vitamin B_{12} and folate, increased red cell destruction and abnormal red cell membrane causing increased osmotic fragility, increased blood loss occult gastrointestinal bleeding, blood sampling and blood loss during heamodialysis or because of platelet dysfunction.

(ii) Bone disease renal osteodystrophy:

The term renal osteodystrophy embrances the various form of bone disease which may develop alone or in combination in chronic renal failure hyperparathyroidism bone disease. Many patients with chronic renal failure are found histologically to have a mixed bone disease that is a combination of hyperparathyroidism (Kumar et al, 2003; Edwards et al, 1995).

(iii) Skin disease:

Pruritus (itching) is common in severe renal failure and is usually attributed in the main to retention of nitrogenous waste products of protein catabolism. Other causes of itching include: hypercalcaemia, hyperphosphataemia, and hyperparathyroidism and iron deficiency.

Chronic renal failure may also cause pseudoprophyria, results from decrease in hepatic uroporphyrinogen decarboxylase combined with decreased clearance of prophyrins in the urine by dialysis.

(iv) Endocrine function:

A number of hormonal abnormalities may be present. In female amenorrhoea is common due to absence of normal cyclical changes in female sex hormones, decreased serum testosterone levels,

complex abnormalities of growth hormone secretion and action, abnormal thyroid hormone levels, partly because of altered protein binding (Kumar et al, 2003; Edwards et al, 1995).

(v) Cardiovascular disorders:

Hypertension develops in approximately 80% of patient with chronic renal failure. It must be controlled, as it causes further vascular damage, thus increasing renal failure. Atherosclerosis is common due to abnormalities of lipid and carbohydrate metabolism and may be accelerated by hypertension.

(vi) Infection:

Both cellular and humeral immunity are impaired and thus there is increased susceptibility to infection. Urinary tract infections are common and must be treated, as they lead to further destruction of functioning renal tissue (Edwards et al, 1995).

1-2-3-1-2-3 Investigations of CRF:

There are some investigations by which diagnosis the chronic renal failure, urine, serum biochemistry, haematology, immunology, microbiology, radiological investigation and renal biopsy.

(A) Urine:

1-Urinalysis: Haematuria may indicate glomerulonephritis but other source my be considered, proteinuria, glycosuria with normal blood glucose.

2-Urine microscopy: Eosinophiluria is strongly suggestive of allergic tubulointerstitial; granular casts are formed from abnormal in the tubular lumen and indicate active renal disease and red cell casts are highly suggestive of glomulonephritis.

3-Urine biochemistry**:**

24 hour creatinine clearance is useful in assessing the severity of renal failure, urine osmolality is a measure of concentrating ability and urine electrophersis is necessary for the detection of light chains, which can be present without detectable serum proportion.

(B) Blood:

1-Serum biochemistry:

Urea, creatinine and electrolytes sodium and potassium, while the blood urea level will reflect the degree of renal failure it is also affected by dietary protein and rate of tissue break down and the serum ceratinine is more accurate guide to renal function. Other nitrogenous products whose blood levels are elevated include uric acid. Serum sodium is often normal but may be low due to decreased tubular capacity to conserve sodium. The same applies to potassium although in very advanced cases the blood level may become high hence the glomerular filtration rate falls to very low levels , or in association with oliguria or severe acidosis and is characterized by a lowering of the serum pH , pCo_2 and bicarbonate. The serum phosphate rises due to the decrease in phosphate filtered and this is usually associated with a fall in serum calcium (Kumar et al, 2003; Macsween et al, 1995).

1-2-3-1-2- 4 Management:

The management of chronic renal failure falls into**:**

1- The underlying cause of renal disease should be treated aggressively where possible and both family history and drug history are important.

2- Blood pressure control **:**

Blood pressure should be reduced to 130/80 mm/h or lower. Adequate control may require combination of drugs together doses of diuretics to correct sodium and water retention.

3- Hyperkalaemia:

It often responds to dietary restriction of the potentional should be stopped (Kumar et al, 2003; Edwards et al, 1995).

4- Calcium and phosphate **:**

Hypocalcaemia and hyperphosphataemia should be treated aggressively, preferably with regular measurements of serum parathyroid hormone to assess how effectively parathyroidism being suppressed.

5- Dietary restrictions **:**

In advanced renal disease, reduction of protein intake lessens the amount of nitrogenous waste products generated and this may delay the onset of symptomatic restricted in sodium and potassium.

6- Fluid intake **:**

Fluid depletion and overload should be avoided. Dilutional hyponatraemia may occur if water intake exceeds the capacity to excrete the water load. Conversely, the large majority of patients with moderate chronic renal impairment do not need to restrict fluid intake (Kumar et al, 2003; Edwards ea t l, 1995).

7- Drug therapy **:**

This should be minimized in patients with chronic impairment. Tetracyclines should be avoided in view of their antianabolic effect and tendency to worsen uraemia. Drugs excreted by the kidneys should be avoided.

8- Anaemia **:**

The anaemia of erythropoietin deficiency can be treated with synthetic human erythropoietin, starting at a close of 25-50 u/kg three times a week; subcutaneous administration is more effective than intravenous. Blood pressure and hemoglobin connection and reticulocycte count are measured every two weeks.

9- Replacement of renal function:

The excretory function of the kidney can be partially replaced by dialysis. The best results are obtained by an integrated approach to management, using the most peritoneal dialysis or transplantation for the patient depending on the clinical circumstances present.

(A) Haemodialysis:

In haemodialysis, blood from the patient is pumped through an array of semiperneable membranes which bring the blood into close contact with dialysate, flowing counter current to the blood. The plasma biochemistry changes towards that of the dialysate owing to diffusion of molecules down their concentration gradients (Kumar et al, 2003; Edwards et al, 1995).

The dialysis machine comprises a series of blood pumps, with pressure motions and blood leak detectors. Blood flow during dialysis is usually 200- 300 ml per minute and the dialysate flow usually 500 ml per minute. The efficiency of dialysis in achieving biochemical change depends on blood and dialysate flow and the surface area of dialysis membrane (Kumar et al, 2003; Edwards et al, 1995).

The introduction of regular intermittent haemodialysis has prolonged the lives of many patients with chronic renal failure. Haemodialysis should be started when despite adequate medical

treatment, and preferable the patient has developed serious consequences of uraemia.

(B) Renal Transplantation:

This offers the possibility of restoring normal kidney function and thereby correcting the many metabolic abnormalities (kumar et al, 2003; Edwards et al, 1995).

1-3 The liver:

1- 2-1 liver Structure:

The liver is the largest internal organ in the body and situated in the right hypochondrium. Functionally, it is divided into right and left lobes by the middle hepatic vein. The right lobe is larger and contains the caudate and quadrate lobes.The liver is further subdivided into a total of eight segments by divisions of the right, middle and left hepatic veins. Figure (2a) shows the segmental anatomy of the liver (Kumar et al, 2003).The blood supply to the liver constitutes 25% of the resting cardiac output and is via two main vessels: The hepatic artery supplies25% of the total blood flow. Auto regulation of blood flow by the hepatic artery ensures a constant total liver blood flow. And the portal vein drains most of the gastrointestinal tract and the spleen. It supplies 75% of the blood flow. The normal portal pressure is 5-8 mmHg; flow increases after meals. Both vessels enter the liver via the hilum (portal hepatic).The blood from these vessels is distributed to the segments and passes into the sinusoids via the portal tracts. Blood leaves the sinusoids, entering branches of the hepatic vein which join in to three main branches before entering the inferior vena cava.

The caudate lobe is an autonomous segment as it receives an independent blood supply from the portal vein and hepatic artery, and its hepatic vein drains directly into the inferior vena cava. Lymph, formed mainly in the perisinusoidal space, is collected in lymphatics which are present in the portal tracts. These small lymphatics enter larger vessels which eventually drain into the hepatic ducts (Kumar etal, 2003).

The functional unit of the liver is an acinus. This consists of parenchyma supplied by the smallest portal tracts containing portal vein radicals, hepatic arterioles and bile ductless .The hepatocytes near this triode (zone I) are well supplied with oxygenated blood and are more resistant to damage than the cells near the terminal hepatic (central) veins (zone 3). (Figure 2b) shows the diagram of an acinus (Kumar,et al, 2003).The sinusoids lack a basement membrane and are loosely surrounded by specialist fenestrated endothelial cells and Kupffer cells (phagocytic cells). Sinusoids are separated by plates of liver cells (hepatocytes). The sub-endothelial space that lies between

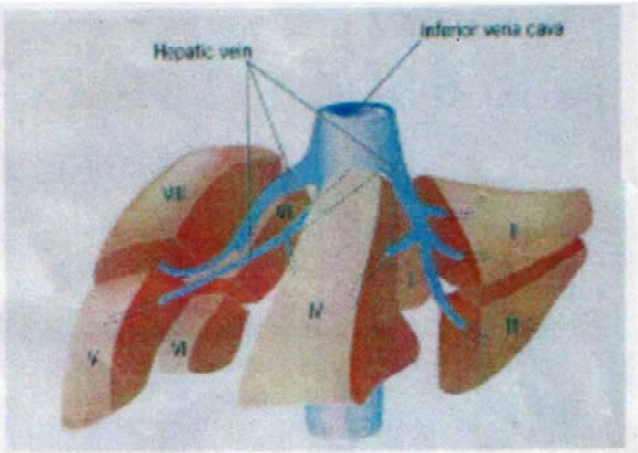


Figure (2a): segemental anatomy of the liver showing the eight hepatic segments.I caudate labe,II-IM the left hemiliver, V-VIII the right hemiliver(Kumar, 2003).

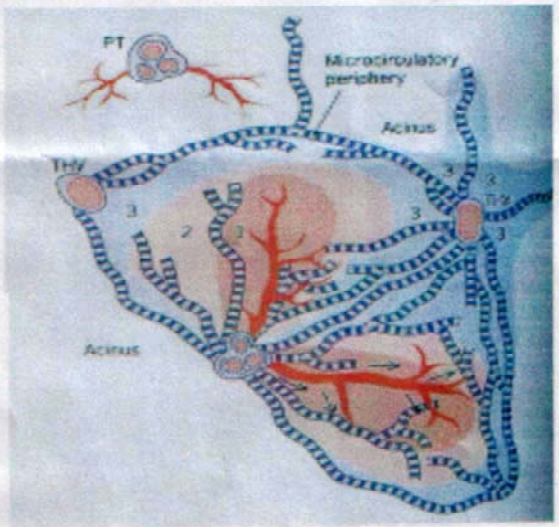


Figure (2b): Diagram of an acinus.Zones 1,2 and 3 represent areas supplied by blood with zone 1 being oxygeneated.Zone 3 is supplied by blood remote from afferent vessels and is in the microcirculatory periphery of the acinus. THV terminal hepatic venule, PT portal triad(Kumar, 2003).

the sinusoids and hepatocytes is the space of Disse, which contains a matrix of basement constituents and stellate cells.

Stellate cells store retinoids in their resting state and contain the intermediate filament, desmin. When activated they are contractile and probably regulate sinusoidal blood flow endothelin and nitric oxide play a major role in modulating stellate cell contractility. Stellate cells, after activation, produce collagen types I, III and IV (Kumar et al, 2003).

1-3-2 liver Functions:

The liver has important metabolic functions.

1-3-2-1 Protein metabolism:

(a)Synthesis:

The liver is site of synthesis of all circulating proteins apart from α-globulins, which are produced in the reticulo-endothelial system. The liver receives amino acids from the intestine and muscles and, by controlling the rate of gluconeogesis and transamination regulates levels in plasma. Plasma contains 60-80 g/L of protein, mainly albumin, globulin and fibrinogen.

Reduced synthesis of albumin over prolonged period produces hypoalbuminaemia which is seen in chronic liver disease and malnutrition. Hypoalbuminaemia is also found in hyper-metabolic State and in diseases where there is an excessive loss (e.g. nephritic syndrome).

Transport or carrier proteins such as transferrin, caeruloplasmin, acute phase proteins and other proteins (e.g. α_1- antitrypsin and α-fetoprotein) are also produced in the liver.

The liver also syntheses all factors involved in coagulation (a part from factor VIII) that are, fibrinogen, pro-thrombin, factor V, VII, IX and XIII, protein C and S and anti-thrombin as well as the component of the complement system.

(*b) Degradation *(*Nitrogen excretion*):

Amino acids are degraded by transamination and oxidative deamination to produce ammonia, which is then converted to urea and excreted by the kidneys. This is a major pathway for the elimination of nitrogenous waste. Failure of this process occurs in severe liver disease (Kumar et al, 2003).

1-3-2-2 Carbohdyrate metabolism:

Glucose homeostasis and maintenance of the blood sugar is a major function of the liver. It stores approximately 80 g of glycogen. In the immediate fasting state, blood glucose is maintained either by glucose released from the break down of glycogen (glycogenolysis) or by newly synthesized glucose (gluconeogensis) from lactate, pyruvate, amino acids from muscle (mainly alanine and glutamine) and glycerol from lipolysis of fat stores. In prolonged starvation, ketone bodies and fatty acids are used as alternative sources of fuel and the body tissues adapt to a lower glucose requirement (Kumar et al, 2003).

1-3-2-3 Lipid metabolisms:

Fats are insoluble in water and are transported in the plasma as protein-lipid complex (lipoproteins).

The liver has a major role in the metabolism of lipoproteins. It syntheses very low-density lipoproteins (VLDLs) and high-density lipoproteins (HDLs). HDLs are substrate for lecithin cholesterol

acyltransferase (LCAT) which catalyses the conversion of free cholesterol to cholesterolester. Hepatic lipase removes triglyceride from intermediate density lipoproteins (IDLs) to produce low-density lipoproteins (LDLs) which are degraded by the liver after uptake by specific cell-surface receptors.

Triglycerides are mainly of dietary origin but are also formed in the liver from circulating free fatty acids (FFA) and glycerol and incorporated into VLDLs. Oxidation or denovo synthesis of FFA occurs in the liver, depending on the availability of dietary fat (Kumar et al, 2003).

Cholesterol may be of dietary origin but most is synthesized from acetyl-CoA mainly in the liver, intestine, adrenal cortex and skin. It occurs either as free cholesterol, or is esterified with fatty acids, this reaction catalyzed by LCAT. This enzyme is reduced in severe liver disease, increasing the ratio of free cholesterol to ester, which alters membrane structure. One result of this is red cell abnormalities seen in chronic liver disease. Phospholipids are synthesized in the liver (Kumar et al, 2003).

1-3-2-4 Formation of bile secretion:

Bile consists of water, electrolytes, bile acids, cholesterol, phospholipids and conjugated bilirubin. Two processes are involved in bile secretion across the canalicular membrane of the hepatocyte- a bile salt-dependent and a bile salt-independent process, each contributing about 230 ml per day. The remainder of the bile (about 150 ml daily) is produced by the epithelial cells of the bile duct (Kumar et al, 2003).

Bile formation requires firstly the uptake of bile acids and other organic and inorganic ions across the basalateral (sinusoidal) membrane by multiple transport proteins, this process driven by Na^+ - K^+ ATPase in the basolateral membrane. Intercellular transport a cross the hepatocyte is partly through microtubules and partly by cytosol transport proteins. The canalicular membrane contains additional transporters, mainly ATPase-dependent, which carries molecules into the biliary canaliculi against a concentration gradient. The canalicular multispecific organic anion transporter (CMOAT) also known as multi drug-resistance protein 2 (MRP2) mediates transport of a broad range of compounds including bilirubin diglucuronide, glucuronidated and sulphated bile acids and other organic anions. Na^+ and water follow the passes of bile salts in to the biliary canaliculus by diffusion across the tight junction between hepatocytes (a bite salt-dependent process). In the bile salt-independent process water flow is due to other osmotically active solutes such as glutathione and bicarbonate.

The average total bile flow is approximately 600 ml per day. An adequate bile flow is dependent on bile salts being returned to the liver by the enterohepatic circulation (Kumar et al, 2003).

Bile acids are synthesized in hepatocytes from cholesterol. The two primary bile acids, cholic acid and chenodeoxycholic acid are conjugated with glycine or taurine and this process increases their solubility. Intestinal bacteria convert these acids into secondary bile acids, deoxycholic and lithocholic.

Bile acids act as detergents, their main function is lipid solubilization. In aqueous solution they aggregate to form micelles.

2-2-2-5 Hormone and drug inactivation:

The liver catabolizes hormones such as insulin, glycogen, estrogens, growth hormone, glucocorticoids and parathyroid hormone. It is also the prime target organ for many hormones (e.g. insulin). It is major site of the metabolism of drugs and alcohol. Fat soluble drugs are converted to water-soluble substances that facilitate their excretion in the bile or urine (Kumar et al, 2003).

1-3-2-6 Immunological function:

The reticuloendothelial system of the liver contains many immunological active cells. The liver act as a sieve for the bacterial and other antigens carried to it via the portal tract from the gastrointestinal tract. These antigens are degraded by Kupffer cells, which are macrophages attached to the endothelium. Kupffer cells have specific membrane receptors for ligands and are activated by several factors such as infection (Kumar et al, 2003).

The antigens are degraded with the production of antibody, as there is very little lymphoid tissue.

The reticulo endothelial system is also thought to play a role in tissue repair, T and B lymphocyte interaction and cytotoxic activity in disease processes. Following stimulation by, for example, an endotoxin, the Kupffer cells release IL-6, IL-8 and TNF-α and play a key role in producing parenchyymal damage. These cytokines stimulate the sinusoidal cells, stellate cells and natural killer cells to release proinflammatory cytokines. The stimulated hepatocytes

themselves express adhesion molecules and release IL-8, which is a potent neutrophil chemo attractant (Kumar et al, 2003).

1-3-3 Biochemical test for liver disease:

The mechanisms underlying hepatic diseases can be divided into three main groups; these often co-exist, but one usually predominates in any reflecting hepatocellular damage condition. Liver-cell damage is typified release of enzymes from damaged hepatocytes, which causes a rise in their plasma activities, cholestasis is typified by retention of conjugated bilirubin and alkaline phosphates (ALP) and a considerably reduced mass of functioning cell is typified by a reduction in prothrombin and albumin synthesis, which cause a prolonged prothrombin time and hypo albuminaemia (Zilva et al, 1991).

1-3-3-1 Investigative tests can be divided into:

1-Blood tests:

(a) Liver function tests:

i-Total protein.

ii- Serum albumin.

iii- Bilirubin.

iv- Serum aspartate and alanine aminotransferase.

v- Serum alkaline phosphatase,αglutamyltransferase.

vi- Prothrombin time.

(b) Viral markers.

(c) Additional blood investigation haematological, biochemical and immunological**.**

2**-** Urine tests: for bilirubin and urobilinogen

3- Imaging techniques**:** to define gross anatomy.

4- Liver biopsy for histology.

Most routine liver function tests sent to the laboratory processed by an automated multichannel analyzer to produce serum levels of bilirubin, aminotransferases, alkaline phosphatase and total proteins (Kumar et al, 2003).

Liver function tests

(1) Serum albumin:

Albumin is simple protein. The molecular weight is 65000 migrate faster in electrophoresis and precipitate last in salting out (Danish, 1997).

It is synthesized in the liver about 10-12 g/day and has a half life of 16-24 days (kumar et al, 2003).

About 60 percent of albumin in the extra cellular fluid is in the large intestinal compartment. However, the concentration of albumin in the smaller plasma compartment is much higher because of the relative impermeability of the blood vessel wall. This concentration gradient is important in maintaining plasma volume (Danish, 1997).

Its main functions are first to maintain the intravascular oncotic pressure and second to transport water-insoluble substances such as bilirubin, hormones, fatty acid and drugs.

Serum albumin is a marker of synthetic function and is a valuable guide to the severity of chronic liver disease. As falling serum albumin in the liver disease is a bad prognostic sign, in acute liver disease initial albumin levels may be normal.

(2) Bilirubin:

Bilirubin is produced mainly from the breakdown of mature red blood cells in the Kupffer cells of the liver in the reticuloendothelial

system; 15% of bilirubin comes from the catabolism of other haem-containing proteins, such as myoglobin, cytochromes and catalyst.

Normally, 250-300 mg of bilirubin is produced daily. The iron and globins are removed from the haem and this are reused. Biliverdin is formed form the haem and this is reduced to form bilirubin. The bilirubin produced is unconjugated and water-insoluble, and is transported to the liver attached to albumin. Bilirubin dissociates from albumin and is taken up by the hepatic cell membrane and transported to the endoplasmic reticulum by cytoplasmic proteins, where it is conjugated with glucuronic acid and excreted into the bile, the microsomal enzyme, uridine diphosphoglucurosyl transferase, catalyses the reaction. This conjugated bilirubin is water-soluble and is actively secreted into the bile canaliculi and excreted into the intestine with the bile. It is not absorbed from the small intestine because of large molecular size. In the terminal ileum, bacterial enzymes hydrolyse the molecule, releasing free bilirubin, which is then reduced to urobilinogen. Some of this is excreted in the stools as stercobilinogen. The remainder is absorbed by the terminal ileum, passes to the liver via the enterohepatic circulation and is re-excreted into the bile. Urobilinogen bound to albumin enters the circulation and is excreted in the urine via the kidneys (Danish, 1997).

In the serum, bilirubin is normally almost all unconjugated. When hepatic excretion of conjugated bilirubin is impaired a small amount of conjugated bilirubin is found strongly bound to serum albumin, and leads to hyperbilirubinaemia for a short time after cholestasis. In liver disease, increase of serum bilirubin is usually

accompanied by other abnormalities in liver biochemistry. Determination of whether the bilirubin is conjugated or unconjugated is only necessary in congenital disorder of bilirubin metabolism or to exclude haemolysis (Danish, 1997).

3- Total proteins:

Total protein estimation is of limited clinical value. Acute changes in concentration, like those of proteins, reflect both the protein and the fluid content of the vascular compartment. Acute changes are more likely to be due to loss from, or by the vascular compartment of protein-free fluid than of protein. Only marked changes of the major constituents, albumin and immunoglobulins, are likely to alter total protein, levels significantly.

Low total protein levels may be due to: Dilution, if blood is taken near the site of an intravenous infusion, hypoalbuminaemia and profound immunoglobulin deficiency.

Raised total protein levels may be due to: Loss of protein (free fluid), which may lead to increase in one or more immunoglobulins.

Total protein levels may be misleading and may be normal in the face of quite marked changes in the constituent proteins, for example; fall in plasma albumin concentration may roughly be balanced by arise in immunoglobulin levels, most individual proteins, other than albumin, make a relatively small contribution to total protein, quite a large percentage change in the concentration of one of them may not cause a detectable change in total protein concentration (Zilva et al, 1991).

The globulin fraction consists of many proteins that can be separated on electrophoresis. A raised globulin fraction, seen in liver

disease, is usually due to increased circulating immunoglobulins and is polyclonal. (Bennett et al, 2000).

4- Transaminases:

The aspartate (AST; GOT) and alanine (ALT; GPT) Transaminase are most commonly plasma enzymes measured as indicators of liver cell damage. Arise in plasma transminases activities is a sensitive indicator of damage to cytoplasmic or mitochondrial membranes even if there is no detectable impairment of function; the hepatic synthetic and secretary capacities are large; only severe and usually prolonged liver disease demonstrably impairs prothrombin and albumin synthesis (Zilva et al, 1991).

AST is primarily a mitochondrial enzyme (80%); (20% in cytoplasm) and it is also present in heart, muscle, kidney and brain, high levels are seen in hepatic necrosis, myocardial infraction, muscle injury and congestive cardiac failure.

ALT is a cytosol enzyme, more specific to liver so that arise only occurs with liver disease (Kumar et al, 2003).

5- Alkaline phosphatase (ALP):

Alkaline phosphatase is present in the canalicular and sinusoidal membranes of the liver, but is also present in many other tissues, such as bone, intestine and placenta. Its origin can be determined by electrophoretic separation of isoenzymes or bone-specific monoclonal antibodies. Alternatively, if there is also an abnormality of, for example, the γ glutamy l transpeptidase, the ALP can be presumed to come from the liver. Serum ALP is raised in cholestasis from any cause, whether intrahepatic or extra hepatic disease. The synthesis of ALP is increased in cholestatic jaundice; level may be four to six

times the normal limit. Raised levels also occur in conditions with infiltration of the liver and in cirrhosis, frequently in the absence of jaundice (Kumar et al, 2003).

Alteration in liver function is not typically present in patients with uraemic syndrome ,but varying degrees of liver dysfunction were observed in animals with experimental uraemia and to lesser degree in patients with chronic renal failure . There was study investigated the carbohydrate , lipid ,and proteins metabolism in liver with uraemia , particularly attention is given to the role of cystoslic calcium (Ca^{++}) regulation and calcium signal transduction in hepatocytes in chronic renal failure . It is proposed that the parathyroid hormone (PTH) mediated increase in the calcium ions of hepatocytes in chronic renal failure is the major signal for the down regulation of hepatic receptors for hepatic lipase , vasopressin ,and angiotensin II.

It is possible that the mRNA or other hormone receptors and various proteins of the liver cells are affected similarly by the elevated basal levels of (Ca^{++}) in CRF (Omar et al, 1995).

Metabolic changes due to chronic renal failure were studied in isolated rats liver cells. To study gluconeogenesis, ureagenesis, oxygen consumption as well as cytosolic and mitochondrial adenine nucleotide content .CRF rat hepatocytes exhibited a 25% to 45% decrease in gluconeogenesis and ureagenesis while endogenous rates unaffected. CRF did not alter ketone body production from oleate or octanoate. Thus, this study of hepatocytes intermediary metabolism during CRF showed an alteration of only gluconeogenesis and ureagenesis pathway (Kaysen et al, 2004).

Pharmacokinetics studies conducted in patients with CRF demonstrated that the non-renal clearance of drugs is reduced. Although the mechanism of by which this occurs is unclear , several studies shown that CRF affects metabolism of drugs by inhibiting key enzymatic systems in the liver , intestine and kidney . The down regulation of selected isoforms of the hepatic cytochrome P450 activity in CRF appears to be the accumulation of circulating factors which can modulate cytochrome P450 activity (Picette et al, 2003).

The cytochrome P450 oxidative enzyme system, located primarily in the liver and intestine , is responsible for metabolism and detoxification of numerous endogenous and exogenous substances .

In experimental models of renal failure, both hepatic function and cytochrome P450 enzyme content are reduced, however direct evidence in humans is lacking .An association between renal disease and reduced hepatic metabolism has been observed in animal's models ,in which significant correlation have been found between indices of renal function (blood urea nitrogen and serum creatinine levels)and hepatic function (amino levulinic acid syntheses, amino pyrine ,N.demethylase and cytochrome P450) in animal models.

This finding suggests that hepatic function (and drug metabolism) may be altered in renal failure and supports the hypothesis that accumulation of ureamic toxins ,such as organic ions and phenol conjugates may inhibit hepatic drugs metabolism by either reducing cytochrome P450 enzyme content or inhibiting uptake of drugs in hepatocytes (Dreisbach et al, 2003) .

There is abundant evidence that animal studies in chronic renal failure have shown a major down regulation (40 – 85 %) of hepatic cytochrome P 450 metabolism involving specific isozyme. Phase II reactions such as acetylation and glucourenidation are also involved with some isoenzymes showing induction and others inhibition .Hepatic enzymes exhibiting genetic polymorphisms as N-acetyltransferase -2 (NAT-2)which is responsible for the rapid and slow acetylator phenotypes ,have been shown to be inhibited by chronic renal failure and reversed by transplantation. There is some evidence pointing to the possibility of inhibitory factors circulating in the serum in chronic renal failure patients which may be dialyzable .One mechanism of the reduced non-renal clearance appears to down-regulation of protein expression of specific hepatic cytochrome P450 isozymes .This review includes all significant animal and clinical studies using the search terms “chronic renal failure “,”cytochrome P450 “ and “liver metabolism “ over the past 10 years. Citations were derived from MEDLINE (1990 –2001) including relevant articles dating back to 1969 (Kliem et al, 1998).

Chronic renal failure is associated with a decrease in liver cytochrome P450, the mechanism remains poorly understood.

One study investigated the effects of the serum of rats with chronic renal failure on liver P450. In normal hepatocytes incubated for 24 hours with serum from rats with chronic renal failure, total P450 level, protein expression and mRNA levels of several P450 isoforms were decreased by more than 35% compared to serum from control animals . In conclusion uraemic serum contains mediator(s)

that down regulate the cytochrome P450 of normal hepatocytes secondary to reduced gene expression (Smogorzewski et al, 2003).

The chronic renal failure has effect on the disposition highly hepatically metabolized drugs that by changes in plasma proteins binding and on hepatic metabolism (Dowling et al, 2003).

Pantoprazole is a selective inhibitor of the gastric H^+/K^+ - ATPase with low potential to interact with the cytochrome P450 enzyme system. Since Pantoprazole is metabolized in the liver to metabolites which are mainly cleared by the renal route , this is a study that is to investigate its pharmacokinetics in patients with end stage renal failure undergoing regular haemodialysis. Pantoprazole was well tolerated. In particular these were no clinically relevant changes in blood count or liver enzymes (Cano et al, 1995).

Patients with end stage renal diseases on regular haemodialysis were screened for antibodies against hepatitis C virus (HCV) and hepatitis B surface antigen (HBSAg).All patients were examined for liver functions and serum proteins electrophoretic pattern. Some patients were negative for both anti (HCV) and (HBSAg) Group I some patients were positive only for anti (HCV) Group II , and other patients were positive for anti (HCV) and (HBSAg) Group III , No significant correlation was noted between the elevation of liver enzymes and the groups studied, but a significant increase in total proteins and gamma globulin levels were absorbed in group II and III in comparison with group I. Serum albumin levels were significantly decreased (Yuan et al, 2000).

The concentration of albumin in serum is maintained by the rate of synthesis, catabolism and distribution between vascular and extra

vascular compartment .Albumin synthesis is suppressed when there is inflammation or inadequate protein intake. A decreased serum albumin of > 0.3 g/dl that persist for a period of 6 weeks is associated with a decrease in albumin synthesis .This response is associated with evidence of activation of acute phase response (inflammation) , but not with change in equilibrated normalized catabolic rate (enPCR).In well dialyzed patients , inflammation is the principle cause of a decrease in serum albumin while protein intake plays an insignificant role .The decrease in serum albumin concentration was resulting from reduced albumin synthesis and not a result of stable shift of albumin from the vascular to the extra vascular pool , nor was it a consequence of dilution resulting from plasma volume expansion .As pointed out recently , the diagnosis of malnutrition as a sole cause of a low serum albumin in haemodialysis patients would only rarely be appropriate (Guevinc et al. 2002).

2- MATERIALS AND METHODS

2-1 Study design:

This study was conducted in Khartoum State during the period from October 2003 to October 2004. The study aimed to assess liver functions in specimens obtained from patients with chronic renal failure, using chemical analysis.

2-2 Materials:

2-2-1 Study population:

Fourty five patients suffering from chronic renal failure and undergoing haemodyalsis for duration of 60 months participated in this study. The patients included 11 females and 34 males of average ages between 15 years to 80 years.

All patients in this study who were asked to give blood specimens were briefed and consented for the purpose of the study. The etiology of CRF was hypertension (N=17), diabetes mellitus (N=5), kidney disease N= (10) and unknown N= (13) where N = number of the patients.

Twenty healthy persons were also selected to participate in this study to serve as a control group. All patients and control subjects were seen at the kidney haemodialysis unit, Khartoum Teaching Hospital, in which all clinical examination and assessment were done by physicians. Samples from patients and controls were taken after an informed consent.

2-2-2 Sample collection and processing:

After wiping the skin with local antiseptic (70% ethanol), 5.0 ml of venous blood was taken with minimum stasis using sterile disposable syringes. Pre-analysis samples were then transferred to plain containers and allowed for 30-60 minutes for clot retraction, and then samples were centrifuged at 100g for 10 minutes. The sera were transferred by

pasture pipettes into glass containers and stored at 20 °C following methods of Vareley (Vareley, 1991).

2-3 Methods:

The clinical assessment and chemical analysis of all parameters except bilirubin and electrolytes were done using a universal system called Vita Lab Selector, attached to a computer controlling the analyzer unit of the machine, collecting raw data and providing the user interface. Details of the system can be found as appendix. Bilirubin was measured by a manual procedure.

2-3-1 Procedure:

Two ml of each of the prepared serum samples of patients and controls were entered in the Vita Lab Selector for estimation of albumin, total protein, alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP**),** urea and creatnine. The samples were put in the tubes of Vita Lab Selector after being numbered. At the same time the calibrator tube was prepared by mixing reagent one with reagent two. An amount of two ml were then taken. Also two ml of distilled water was put in a tube as a blank. The 45 samples of patients were done in 5 patches and the samples from control subjects were analyzed in 2 patches. The patch analyses took 100 minutes and the Vita Lab Selector gave the results automatically.

The estimation of bilirubin was done manually by preparing two sets of tubes, one set was used for blank control samples and the second set for experimental samples. Then reagent one, reagent two and the samples were added. Contents of each tube were mixed well and the optical density was determined using a spectrophotometer.

Quantitative estimation of electrolytes was done by using a flame photometer. The blank was prepared by pippetting 5ml of distilled water into a tube. For other samples 5ml of distilled water was pipette

in clear tube and 0.05 ml of the standard solution was added to it. In addition 5 ml of distilled water were mixed with o.o5 ml of each of the serum samples. Then the serum would be read against the specific filter by the flame photometer.

2-3-2 Estimation of Bilirubin:

Principle: Sulfanilic acid was expected to react with sodium nitrite to form diazotized sulfanilic acid. In the presence of dimethylsulfoxide, total bilirubin would react with diazotized sulfanilic acid to form azobilirubin. In the absence of dimethyl sulfoxide only the direct bilirubin would react to give azobilirubin. The Reagents of bilirubin were composed of two reagents, one was sulfanilic acid 28.9 mmol/L and Hydrochloric acid 165 mmol/L, (dimethy sulfoxide 7mmol/L in total bilirubin) and the other one was Sodium nitrite 43mmol/L. The calibrator used was BIEN- 4050.

Procedure:

	Sample blank	Sample
Reagent (1)	1.5 ml	1.5ml
Reagent (2)	-	50 ml
Sample	100ml	100 ml

After 5 minute incubation, contents were mixed again and the optical density (OD) at wavelength range of 555nm (530-580) was determined by the spectrophotometer. The spectrophotometer cuvette allowed 1 cm light path and the working temperature was 37ºC. The (OD) was proportionate with the colour intensity which was pink.

Calculation:

$$\text{With calibrator} = \frac{\text{OD sample} - \text{OD sample blank}}{\text{OD calibrator} - \text{OD calibrator blank}} \times n$$

With factor (OD sample) – (OD sample blank) XF

n = Calibrator concentration.

2-3-3 Estimation of Albumin (Alb):

Principle: Serum albumin in the presence of bromcresol green at a slightly acid pH produced a colour change of the indicator from yellow-green to green-blue. The reagents of albumin detection were composed of succinate buffer pH 4.20 (75 mmol/L), bromocresol green (0.14 g/L) and brij 35 (7ml/L).The calibrator was ELICAL, a lyophilized calibration serum (36.39g/L).

Procedure: The mode of this reaction was end point reaction and the unit was g/L. In the mono made normal volume (360Ml) and rerun volume (2Ml) were taken from the reagent as follows:

$$\frac{\text{OD sample}}{\text{OD calibrator}} \times n$$

n = Calibrator concentration.

2-3-4 Estimation of total proteins: (TP)

Principle: Serum proteins were expected to form a coloured complex in the presence of copper salt in alkaline solution (Biuret reactions). The total proteins reagents were composed of potassium iodide (6 mmol/L), potassium sodium tartrate (21 mmol/L), copper sulfate (6 mmol/L) and sodium hydroxide (58 mmol/L). The calibrator was ELICAL (54.1 g/L).

Procedure: The mode of this reaction was end point reaction and the unit was g/L. In the mono made normal volume (300 ml) and rerun volume (301 ml) were taken from the reagent bottle and then added to normal volume (3 ml) and rerun volume (2 ml) of the sample. After that samples were incubated in the Vita Lab Selector for about 11.5

minutes. The optical density at wavelength 620 nm was read and results were calculated as follows:

$$\frac{\text{OD sample}}{\text{OD calibrator}} \times n$$

n = concentration of calibrator

2-3-5 Estimation of Alanine amino transferase (ALT) (*GPT*):

Principle: Kinetic determination of alanine aminotransferase (ALT) was based upon IFCC recommendation:

L-Alanine + α - ketoglutarate $\xrightarrow{ALT}$ pyruvate + L-Glutamatic

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-lactate+ NAD^+

LDH = Lactate dehydrogenase.

The ALT reagents were composed of two reagents one was Tris buffer pH 7.5(110 mmol/L), L-Alanine (600 mmol/L) and LDH (≥ 1500 U/L) and the other one was α -ketoglutarate (16 mmol/L) NADH (0.24 mmol/L), the calibrator was (96.8 U/L 37ºC). Ten and volumes of reagent I were mixed with one volume of reagent 2. The reagents were stable 5 days at 20-25ºC or for 4 weeks at 2-8 ºC.

Procedure: The mode of this reaction was kinetic and the unit was U/L. The working temperature was 37ºC. In this reaction, normal volume (250 ml) and rerun volume (262 ml) were taken from the reagent bottle and then added to normal volume (25 ml) and rerun volume (13 ml) of the sample. After that the Vita Lab Selector read the optical density at wavelength 340 nm and the result calculated as follows:

Activity (U/L) = Δ OD/ min x 1746

2-3-6 Estimation of Aspartate aminotransferase (AST,GOT):

Principle: Kinetic determination of the aspartate aminotransferase (AST) based upon IFCC recommendation:

L- aspartate + α -Ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate.

Oxaloacetate + NADH+H^+ $\xrightarrow{MDH}$ L-Malate + NAD^+

MDH = Malate dehydrogenase.

The AST reagents were composed of two reagents; one was Tris buffer, pH 7.888 mmol/L, L-Aspartate 260 mmol/L, LDH≤ 1500 U/L and MDH ≥ 900 U/L. The second one was α- ketoglutarate 12 mmol/L and NADH 0.24 mmol/L. The calibrator was 106 U/L 37ºC. Ten volumes of reagent 1 were dissolved in 1 volume of reagent 2. The reagent was stable 5 days at 20-25º C or 4 weeks at 2-8 ºC.

Procedure: The mode of this reaction was kinetic and the unit was U/L. The working temperature was 37ºC. In this reaction, normal volume (250 ml) and rerun volume (262 ml) were taken from the reagent bottle and then added to normal volume (25 ml) and rerun volume (13 ml) of the sample. After that the Vita Lab Selector read the optical density at wavelength 340 nm and calculated the result as follows:

Activity (U/L) = Δ OD/ min x 1746.

2-3-7 Estimation of Alkaline phosphatase (ALP):

Principle: Kinetic determination of the alkaline phosphatase was based upon DGKC and SCE recommendations:

P -Nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ P- Nitrophenol + Inorganic phosphate.

The reagents of ALP were composed of, diethanol amine buffer PHID.21.25 mol/L and magnesium chloride 0.625 mmol/L. four volumes of reagent 1 were mixed with one volume of reagent 2. The

reagent was stable 24 hours at 20-25 ºC (avoiding direct exposure to light), or 7 days at 2-8 ºC (avoiding direct exposure to light).

Procedure: The mode of this reaction was kinetic and the unit was U\L. The working temperature was 37 ºC. In this reaction, normal volume (250 ml) and rerun volume (252 ml) were taken from the reagent bottle and then added to normal volume (5 ml) and rerun volume (3 ml) of the sample. After that the Vita Lab Selector read the optical density at wavelength 405 nm and the result was calculated as follows:

Activity (U/L) = Δ OD/ min x 3424.

2-3-8 Estimation of serum urea:

Principle: Enzymatic determination was performed according to the following reactions:

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH3 + CO_2$$

$$2NH_4^+ + 2\,\alpha\text{ - Ketoglutarate} + 2\,\text{NADH} \xrightarrow{\text{GDH}} \text{L Glutamate} + 2NAD^+ 2H_2O$$

(GDH = Glutamate dehyrogenase)

The urea reagents were composed of, Tris buffer, pH7.6 (100m mol/L), ADP (0.7 mmol/L), α- ketoglutarate (9 mmol/L), urease (≥ 6500 U/L), GDH≥ 1 100 U/L and NADH (320 mmol/L). The calibrator was ELICAL (1,050 g/L). The working reagents were stable for 5 days at 20 - 25 °C or for one month at 2 - 8 °C.

Procedure: The mode of this reaction was two points reaction and the unit was mg/dl. The samples were incubated by the Vita Lab Selector 24,103 minutes and read the optical density at wavelength 340 nm and calculated the results as the follows:

$$\frac{\text{OD sample}}{\text{OD calibrator}} \quad \text{x n}$$

n = concentration of calibrator.

2-3-9 Estimation of serum creatinine:

Principle: The rate of formation of a colour complex between creatinine and alkaline picrate was measured. The effect of interfering substances was reduced using the kinetic procedure.

The reagents for detecting creatinine were composed of reagent one, picric acid (8.73 mmoI/L) and reagent two, sodium hydroxide (300 mmol/L) and disodium phosphate (25m mol/L). The calibrator was ELICAL (34.7 mg/L). The working reagent was prepared by mixing one volume of the reagent I with one volume of reagent 2 and stable for a month at 20 - 25 °C (contact with air was avoided).

Procedure: The mode of this reaction was two points reaction and the unit was mg/dl. The Vita Lab Selector incubation was for 24,103 minutes and the optical density read at wavelength 505 nm and results calculated as the follows:

$$\frac{\text{OD sample}}{\text{OD calibrator}} \times n$$

n= calibrator concentration.

2-3-10 Estimation of electrolytes:

Principle: electrolytes were known to move either to cathode (-ve) charge or to anode (+ve) charge when supplied to electrical field and would carry either a negative or positive charge.

The most common electrolytes were K^+ and Na^+ and they were measured by flame photometer, Na^+ a major extra -cellular cation and K^+ a major intracellular cation.

Procedure: The selected K^+ filter was (766 - 770 nm), the electrolyte button was switched on, gas was supplied, the flame was ignited and the distilled water was sucked with a pump that was used to adjust the blank as zero and wash the machine. The flame was adjusted until blue a concentration shape was formed, the standard **(**STD) was

sucked low at first and then high. After that distilled water was sucked for zero again. Then the specimens were sucked and read. The flame photometer calculated the results as: T/S x concentration of STD.

The Na^+ and K^+ STD reagents were prepared by using**,** either Na^+ chloride or K^+ chloride. k^+ CL (mwt 74.5) was dissolved in one liter of distilled water.

3-4 Analysis:

Data collected was subjected to student's t test and analysis of variation (ANOVA) computer programs.

3- RESULTS

This study aimed at studying the liver functions and their correlation with chronic renal failure of patients and the impact of different levels of the studied parameters on subjects under healthy conditions.

Fourty five patients suffering from chronic renal failure disease participated in this study; they included 34 males and 11 females between the ages of 15 to 80 years. Another twenty healthy subjects were also selected to participate in this study in which they served as a control group. Results obtained are presented in tables and figures.

Table (I) represented the means and standard deviation with minimum and maximum levels of urea, creatinine, K^+ and Na^+, in chronic renal failure patients.

Table (II) shows the means, standard deviation with minimum and maximum levels of total protein, albumin, transaminases, alkaline phosphatase and bilirubin.

Table (III) and figures (3-9) illustrate the levels of serum total protein, albumin, transaminases, alkaline phosphatase and bilirubin in healthy subjects as well as in chronic renal diseased patients, we can see from the table and figures that transaminase and bilirubin levels in both control and diseased subjects are nearly the same in which no significant difference in the level between the two study groups was seen. Transaminases being GOT 21.91$\pm$ 2.12 and 9.05 $\pm$ 2.89, GPT 11.32 $\pm$ 1.01, and 9.05 $\pm$ 2.89, total bilirubin 0.72$\pm$ 0.181 and 0.69 $\pm$ 0.13 and direct bilirubin 0.16 $\pm$ 0.09 and 0.20$\pm$ 0.05 in patients and control group respectively, total protein ,albumin and alkaline

phosphatase levels have remarkable significant differences between study group with a total protein 9.30± 1.00 and 8.09±0.41 , albumin 2.50 ± 0.37 and 2.94 ± 0.92 alkaline phosphatase 265.64 ± 4.23 and 161.05 ± 2.05 in patients and control group respectively.

As far the level of all of serum albumin, total protein, transaminases, alkaline phosphatase and bilirubin in males and females in diseased group under the study are concerned, Table (IV) and figures (10), (11) and (12) illustrated that there are no differences between total protein, albumin, and alkaline phosphatase levels in both males and females in CRF patients. In both sexes levels are nearly the same.

Table (V) and figures (13) and (14) indicated no remarkable difference in albumin and total protein levels in patients from different age groups. However, alkaline phosphatase, as shown in table (V) and figure (15) are remarkably affected by age. The levels of alkaline phosphatase is lower in the age group (30-40) compared to the age group of (45-74), however, at older ages (69-80) the levels are increased. The levels of alkaline phosphatase are affected by age in varying degrees, results indicating that patients at younger ages have higher levels than the patients of middle age.

Table (VI) illustrates the means ± standard deviation of albumin, total protein and alkaline phosphatase of chronic renal failure patients according to family post history of renal and non renal causes; figures (16), (17) and (18) show the percentages of the three parameters.

Table (VII) illustrates the means ± standard deviation of albumin, total protein and alkaline phosphatase of chronic renal failure patients according to family of renal and non renal causes; figures (19), (20) and (21) show the percentages.

Table (VIII) represents the source of variation when using ANOVA test for the two study groups.

Table (I):
Average means of serum in patients with minimum and maximum levels.

Variable	S.Urea	S.Creatinine	$S.K^+$	$S.Na^+$
N	45	45	45	45
Mean	146.69	9.50	5.09	103.74
Std. Deviation	3.91	2.65	1.28	1.22
Minimum	63.00	3.50	2.40	105.00
Maximum	254.00	14.00	8.50	169.00

S= serum
Std= standard

Table (II):
Average means of serum in patients with minimum and maximum levels.

Variable	Total protien	ALb	GOT	GPT	ALP	Total bilirubin	Direct Bilrubin
N	45	45	45	45	45	45	45
Mean	9.34	2.50	21.62	13.20	265.64	1.05	0.16
Std. Deviation	1.00	0.38	2.12	1.01	4.23	0.18	0.09
Minimum	5.80	2.00	4.00	3.00	87.00	0.40	0.10
Maximum	11.30	3.60	127.00	54.00	992.00	1.00	60.50

Table (III):

Shows comparison between the means of serum total protein (g/dl), serum albumin (g/dl), transaminasee (GOT, GPT) U/L, alkaline phosphatase U/L and bilirubin (mg/dl) in patients with chronic renal failure and the control group. The table gives the mean ± standard deviation.

Variables	Grouping	N	Mean± Std.D	P
Serum total protein	Patient Control	45 20	9.34 ± 1.00 8.09 ± 0.417	< 0.05
Serum albumin	Patient Control	45 20	2.50 ± 0.38 2.95 ± .93	< 0.05
Serum GOT	Patient Control	45 20	21.63 ± 2.12 21.73 ± 2.41	> 0.05
Serum GPT	Patient Control	45 20	11.20±1.01 9.05±2.8946	> 0.05
Serum ALP	Patient Control	45 20	265.64±4.23 161.05±2.05	< 0.05
Serum total bilirubin	Patient Control	45 20	0.72± 0.18 0.69± 0.13	> 0.05
Serum direct bilirubin	Patient Control	45 20	0.16 ± 0.09 0.20± 0.05	> 0.05

P= Probability.

N=number of cases.

t test was used for comparison.

Table (IV):

Shows comparison between serum total protein (g/dl), serum albumin (g/dl) and alkaline phosphatase (U/L) in males and females patients with chronic renal failure. The table gives means ± standard deviation. Probability (P) and N=number of cases.

Variables	sex	N	Mean± Std.D	P
Total protein	Male	34	9.26± 1.10	>0.05
	Female	11	9.57± 0.59	
Albumin	Male	34	2.49± 0.28	>0.05
	Female	11	2.64± 0.43	
Alkaline phosphatase	Male	34	254.44± 2.11	>0.05
	Female	11	285.36± 1.91	

t test was used for the comparison.

Table (V):

Shows comparison between serum total protein (g/dl), serum albumin (g/dl) and serum alkaline phosphatase (U/L), in deferent grouped ages. The table gives mean± standard deviation. N=number of cases S = serum.

Grouped age	**N**	**S.Total Protein**	**S.Albumin**	**S.Alkaline phosphatase**
15-29	9	9.32±0.86	2.46±3.05	322.44± 0.03
30-44	11	9.09±1.23	2.65±1.12	189.18± 0.03
45-59	17	9.28±0.96	2.54±2.05	293.18± 0.03
60-74	7	9.71±0.87	2.44±1.70	212.71± 0.03
75-89	1	10.60±0.00	2.00±0.00	334±0.00

t test was used for the comparison.

Table (VI):

Shows comparison between serum total protein (g/dl), serum albumin (g/dl) and serum alkaline phosphatase (U/L), in family history of patients with chronic renal failure. The table gives mean ± standard deviation.

Variable	**Family history**	**N**	**Mean ± Std.D**
Albumin	Renal	23	2.60± 0.29
	Non renal	22	2.44 ± 0.35
ALP	Renal	23	320.78±2.55
	Non renal	22	200.55±1.10
protein	Renal	23	9.48±1.01
	Non renal	22	9.19±0.98

t test was used for the comparison.

Table (VII):

Shows comparison between serum total protein (g/dl), serum albumin (g/dl) and serum alkaline phosphatase (U/L), in post history of patients with chronic renal failure. The table gives mean ± standard deviation.

Variable	**Post history**	**N**	**Mean ± Std.D**
Albumin	Renal	9	2.53±0.25
	Non renal	36	2.52±1.99
ALP	Renal	9	221.67±1.99
	Non renal	36	272.08±2.08
Total protein	Renal	9	9.20±1.47
	Non renal	36	9.38±0.87

t test was used for the comparison.

Table (VIII):
Source of variation by using ANOVA test under the two studies groups

Variable	Source of variation	Sum of Squares	Df	Mean of Square	Sig.	F
Total Protein	Between Groups	20.80	1	20.80	0.00	24.09
	Within Groups	54.41	63	0.86		
	Total	75.21	64			
Alb	Between Groups	2.79	1	2.79	0.00	8.97
	Within Groups	19.61	63	.31		
	Total	22.40	64			
GOT	Between Groups	0.31	1	.31	0.98	0.00
	Within Groups	309.71	63	491.61		
	Total	309.71	64			
GPT	Between Groups	259.26	1	259.26	0.07	3.47
	Within Groups	470.25	63	74.64		
	Total	496.17	64			

ALP	Between Groups	155.49	1	155.49	0.03	5.25
	Within Groups	186.75	63	29643.06		
	Total	202.30	64			
Total Bilirubin	Between Groups	1.95	1	1.94	0.41	0.70
	Within Groups	1.76	63	2.80		
	Total	1.78	64			
Direct Bilrubin	Between Groups	28.05	1	28.05	0.48	0.50
	Within Groups	355.04	63	56.36		
	Total	357.84	64			

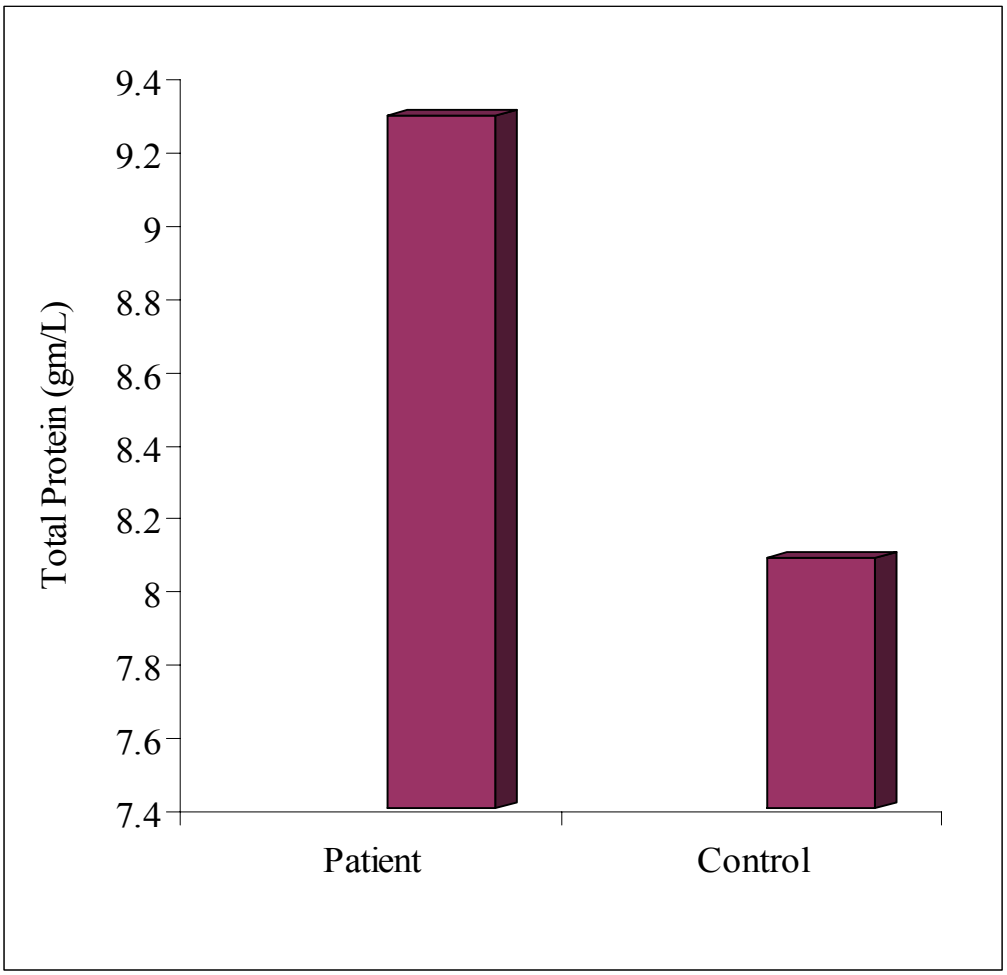


Figure (3): Comparison between serum total protein means (gm/dl) in patients with chronic renal failure (CRF) and control subject.

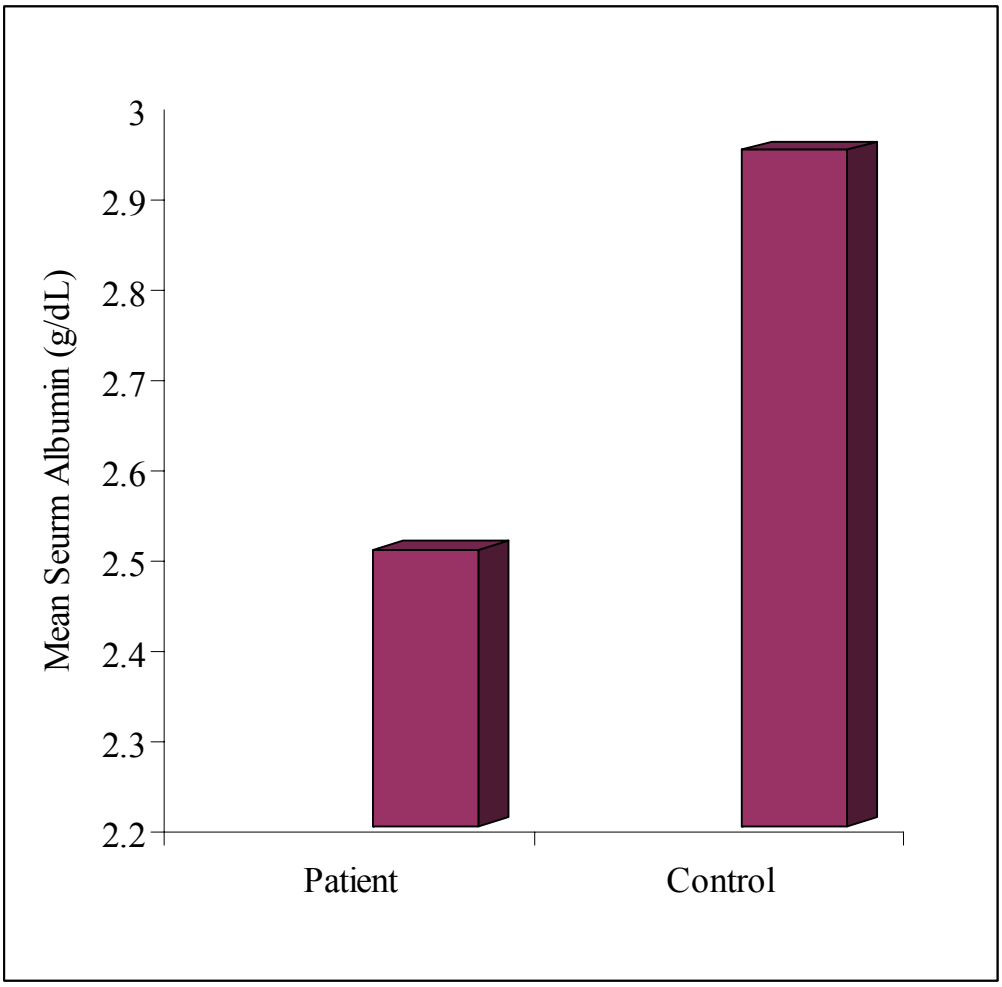


Figure.(4): Comparison between serum albumin means (gm/dl) in patients with chronic renal failure (CRF).

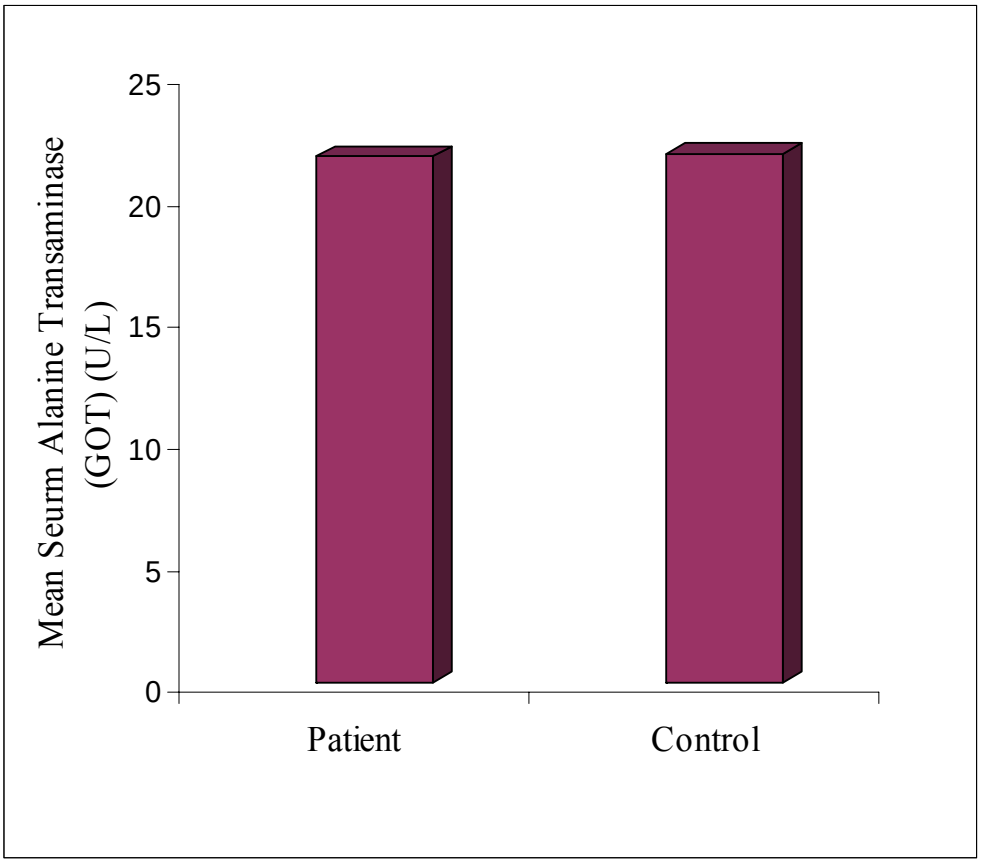


Figure (5): Comparison between serum alanine transaminase (GOT) means (U/L) in patients with chronic renal failure (CRF) and control subject.

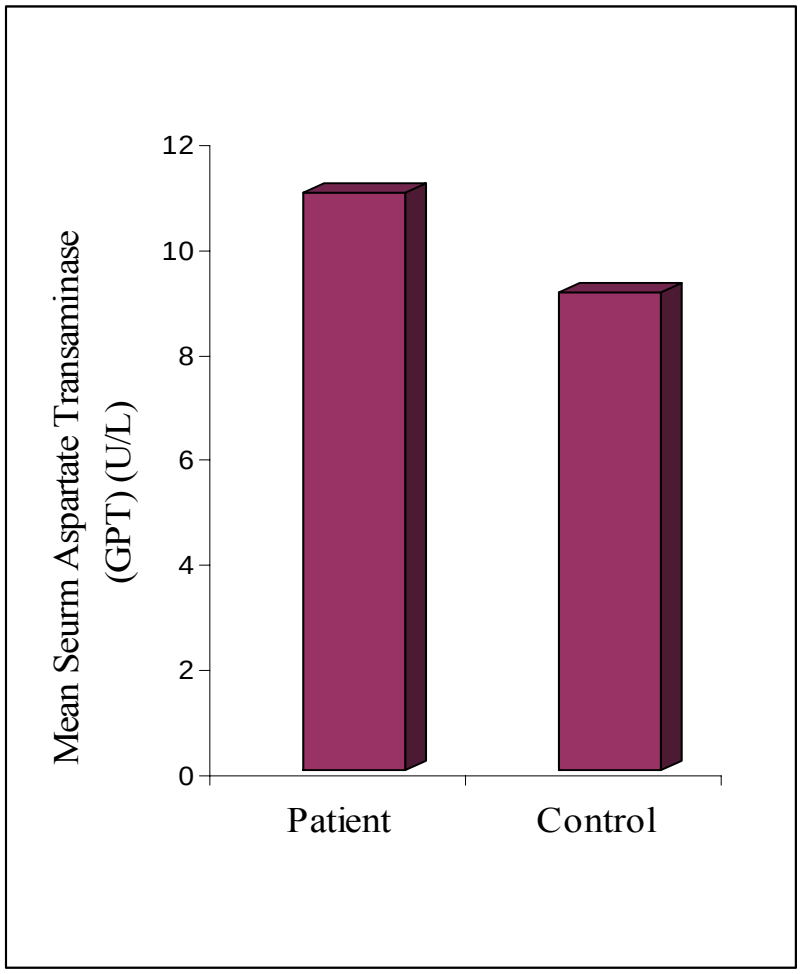


Figure (6): Comparison between serum spartate transaminase (GPT) means (U/L) in patients with chronic renal failure (CRF) and control subject.

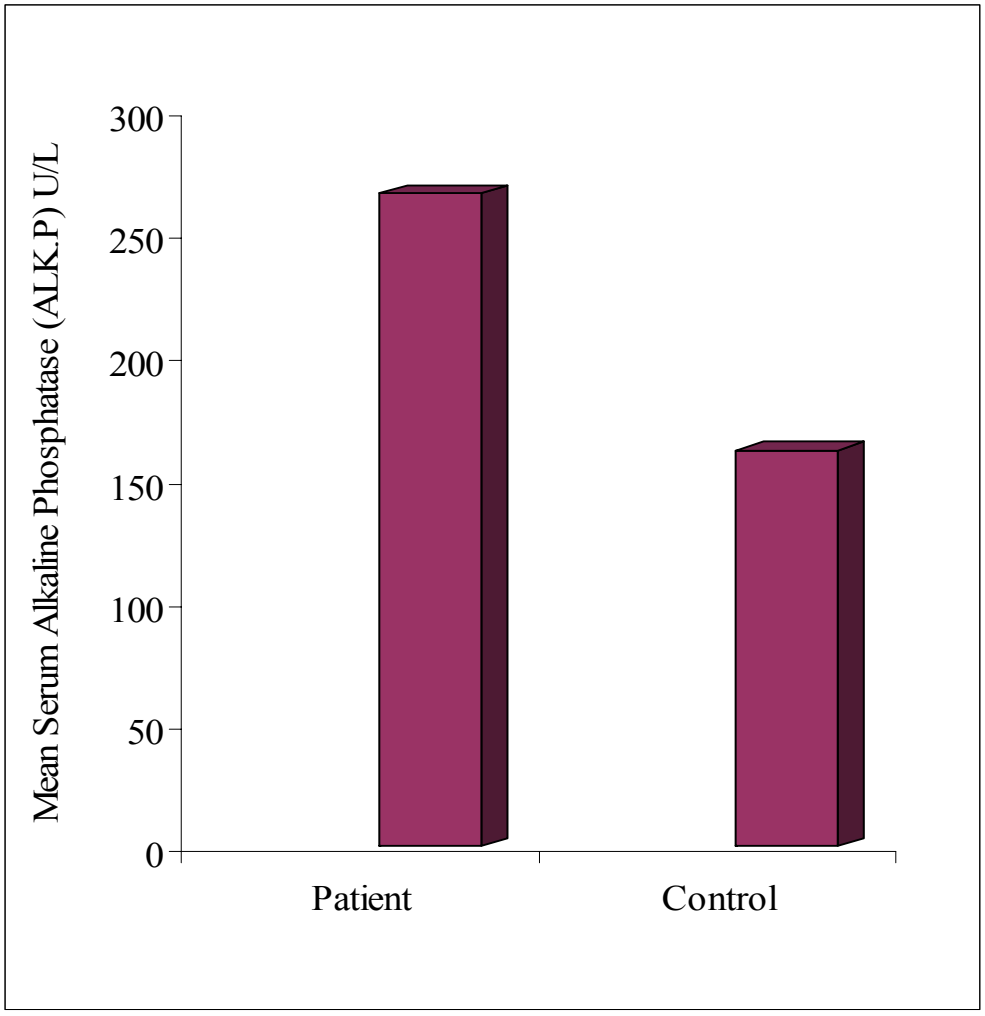


Figure (7): Comparison between serum alkaline phosphatase means (U/L)in patients with chronic renal failure (CRF) and control subject.

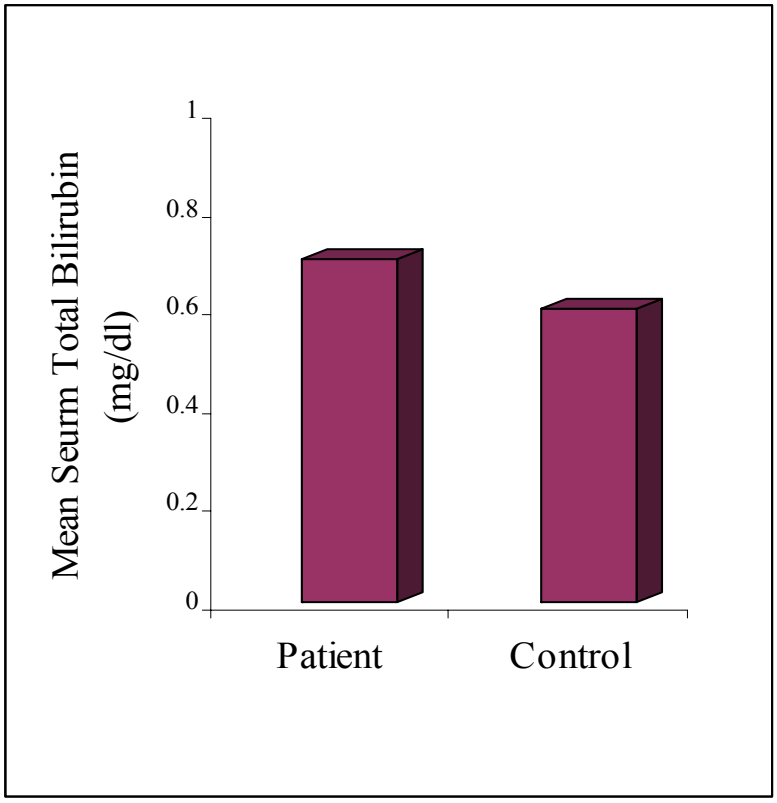


Figure (8): Comparison between serum total bilirubin means (mg/dl) in patients with chronic renal failure (CRF) and control subject.

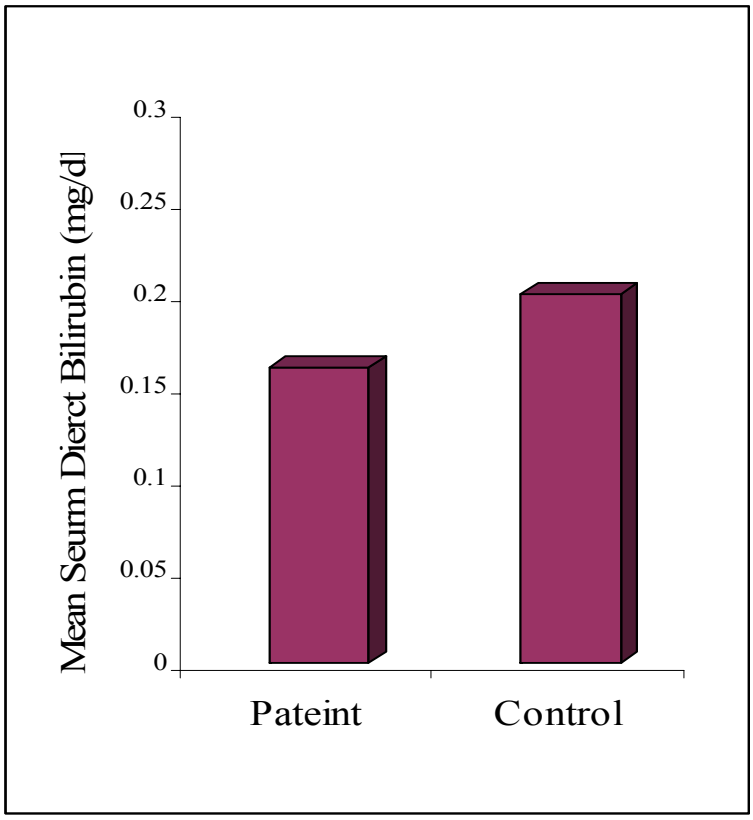
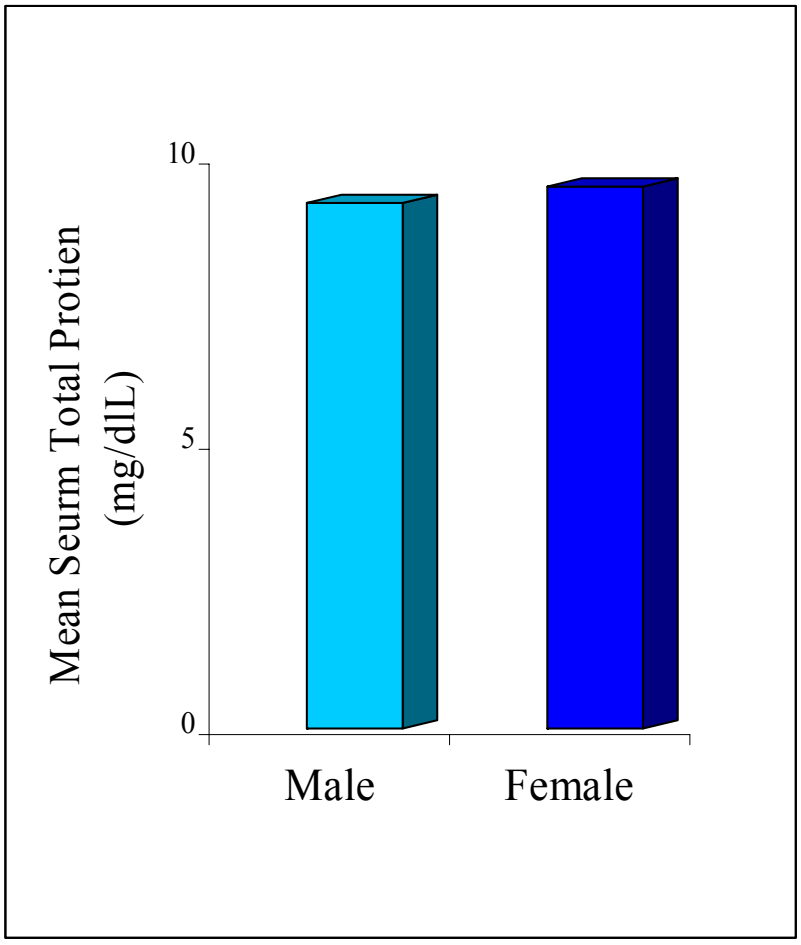
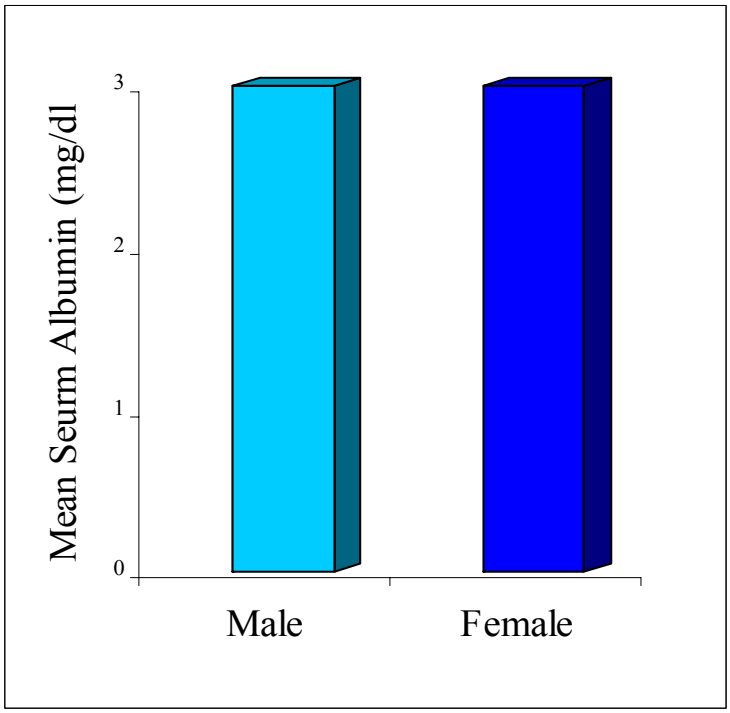


Figure (9): Comparison between serum direct bilirubin means (mg/dl) in patients with chronic renal failure (CRF) and control subject.



Figure(10): Serum total protein means (gm/dl) in male and female patients with chronic renal failure (CRF).



Figure(11): Serum albumin means (gm/dl) in male and female patients with chronic renal failure (CRF).

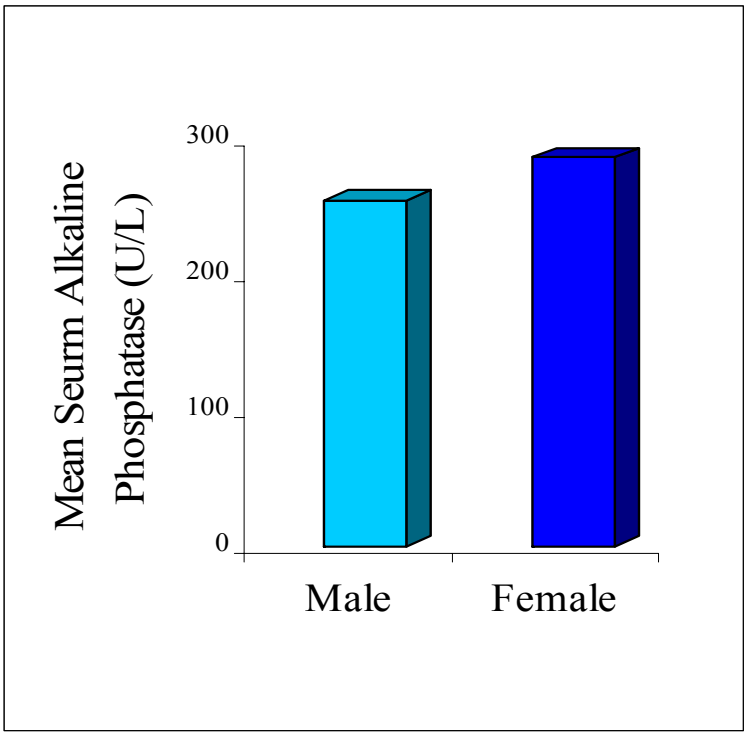


Figure (12): Serum alkaline phosphatase means (U/L) in male and female patients with chronic renal failure (CRF).

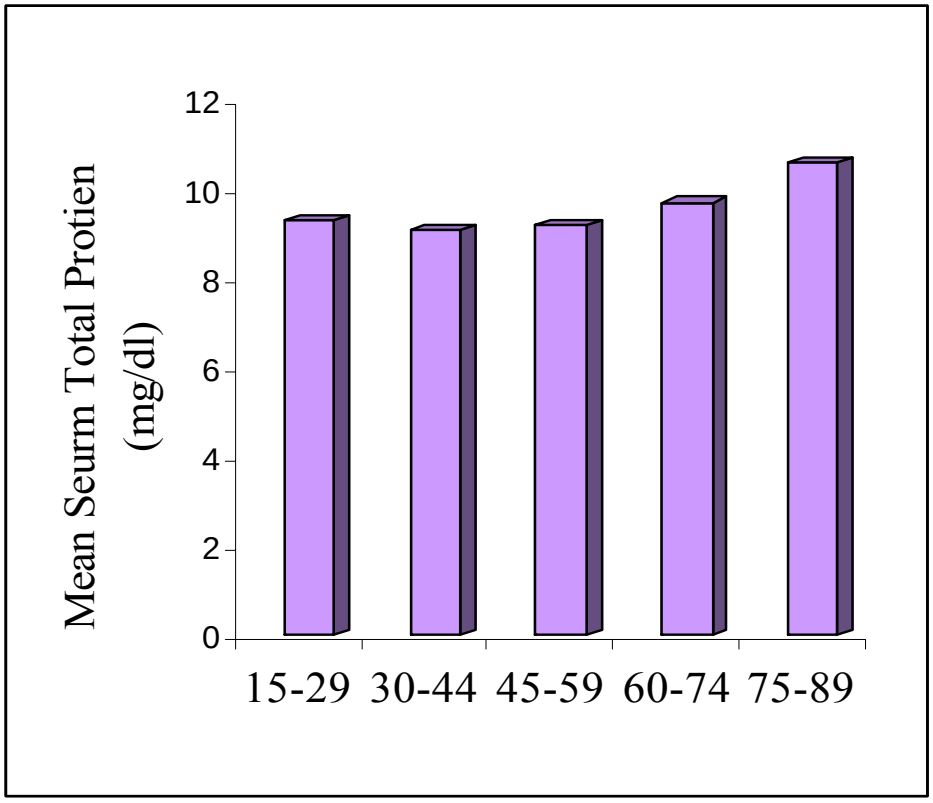


Figure (13): Comparison between serum total protein means (gm/dl) in different grouped ages of patients with chronic renal failure.

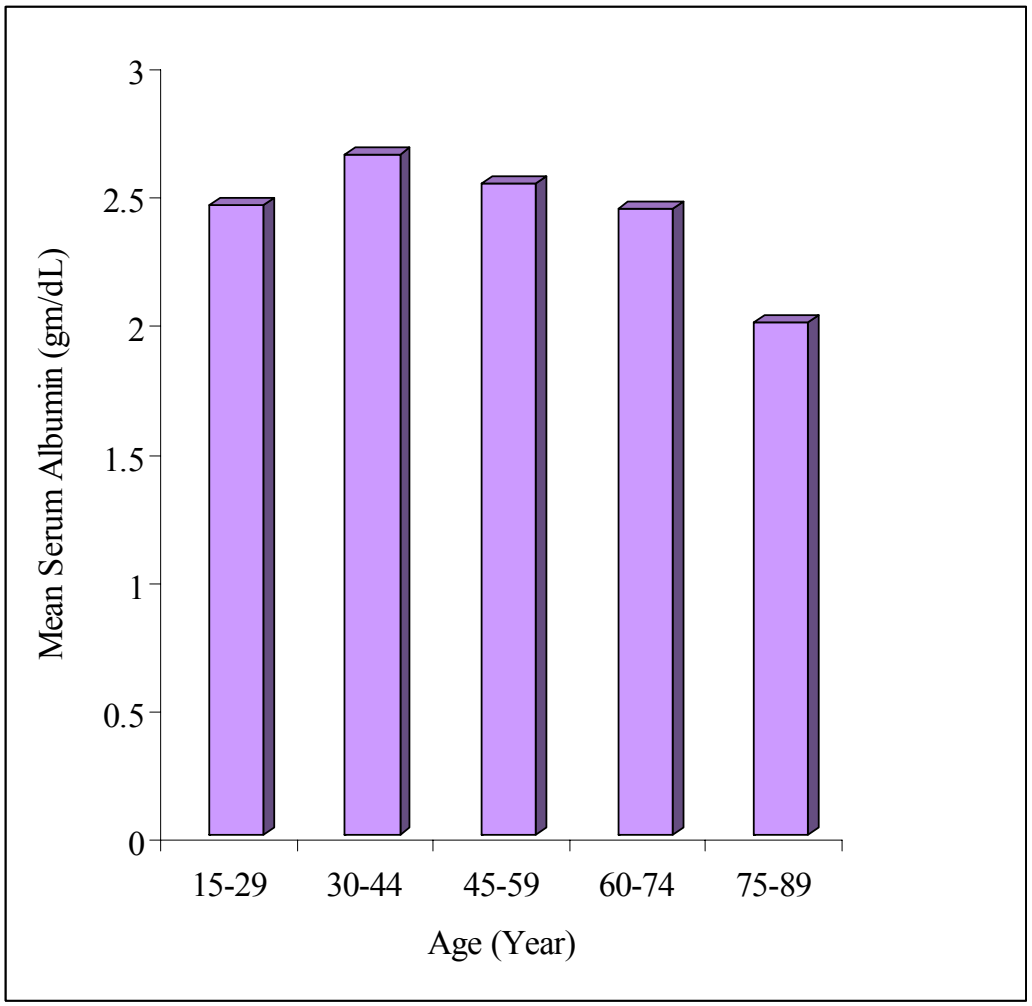


Figure (14): Comparison between serum albumin means (gm/dl) in different grouped ages of patients with chronic renal failure

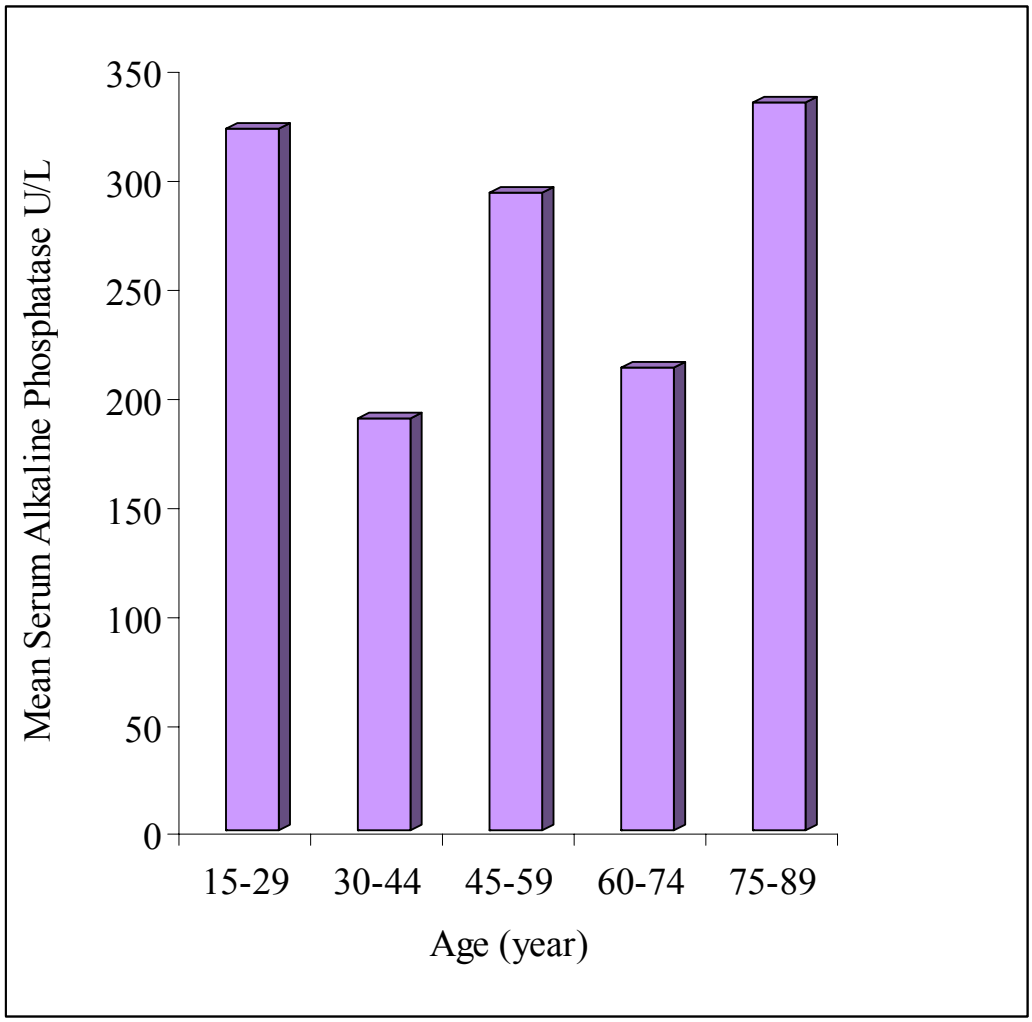


Figure (15): Comparison between serum alkaline phosphatase means (U/L) in different grouped ages of patients with chronic renal failure.

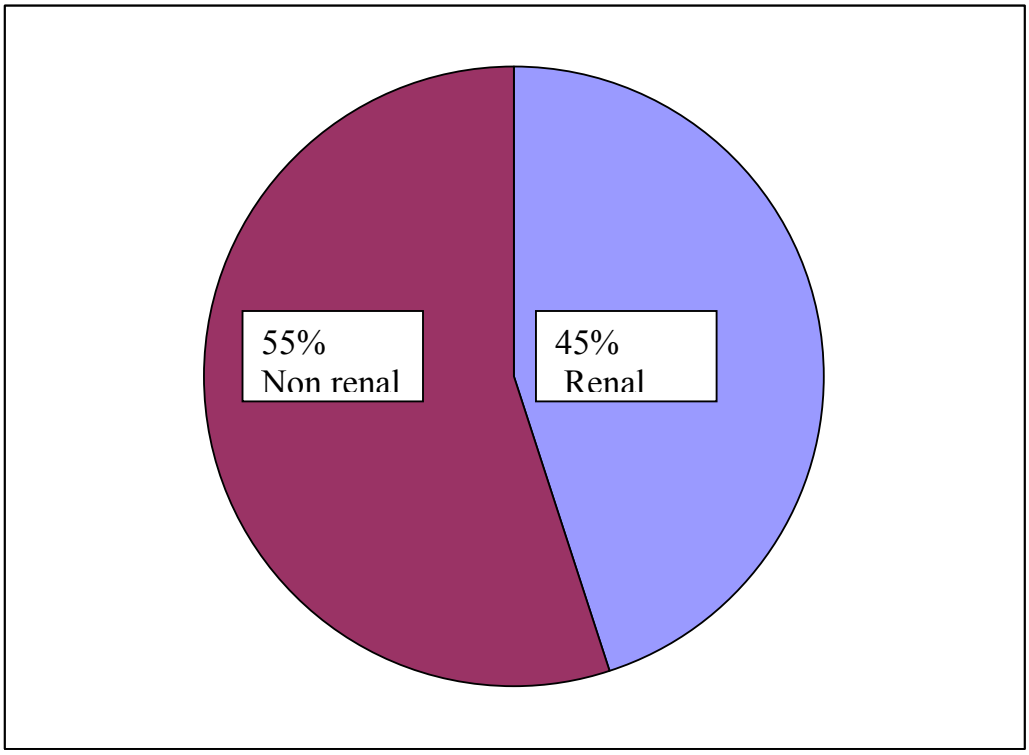


Figure (16): Comparison between percentage (%) of serum alkaline phosphatase means by using post history in patients with chronic renal failure (CRF).

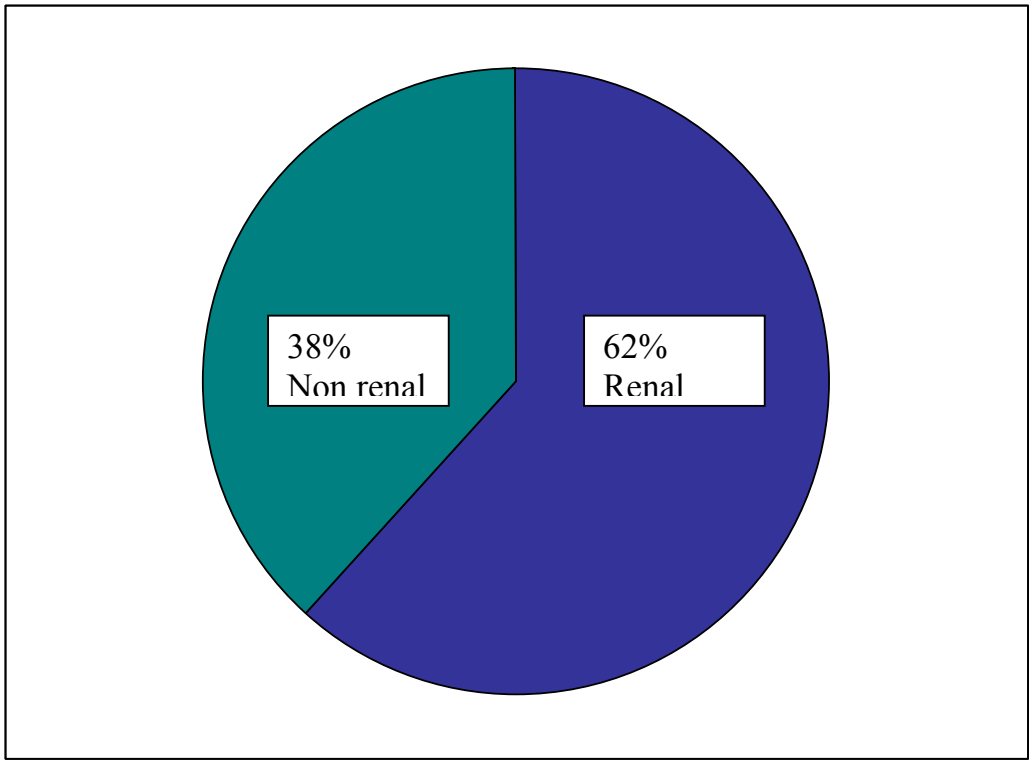


Figure.(17): Comparison between percentage (%) of serum alkaline phosphatase means by using family history in patients with chronic renal failure (CRF).

4- Discussion

In the last years the numbers of patients suffering renal failure disease are increasing month after month. Renal dialysis although not a cure is used as a mean to sustain life to kidney failure patients.

Although studies in animal models have found a favorable effect of chronic renal failure on the hepatic functions reduction, the result of trials in human are less clear. This study is the first to our knowledge to assess prospectively the association between chronic renal failure and subsequent decline in the hepatic functions in the Sudanese population. In our study chronic renal failure patients before heamodialysis have showed the same level of bilirubin and transaminases activity as in control group, this indicates that chronic renal failure disease which was treated with regular dialysis has no effect on these parameters, that no increase or decrease was observed.

On the other hand, albumin, total protein levels and alkaline phosphatase activity showed remarkable significant differences.

This result persisted after adjustment for demographic and clinical information, including sex, age, post history and family history.

Although the liver plays an important role in protein metabolism, changes in serum proteins are not specific for the liver damage. However detection of change to the various function of plasma protein may be of some value.

The combination of an absolute low value for serum albumin, total protein levels or either of these alone is typical of liver damage. Only marked changes of major constituents, such as albumin and immunoglobulins, are likely to alter total protein concentrations significantly. The finding showed that, albumin concentrations were

found to be low in patients with chronic renal failure disease. And decrease of about (71.4) of albumin level under the value of the control subjects was seen in chronic renal failure patients this may indicate that people suffering chronic renal failure have abnormal lowering albumin concentrations.

In our study total protein concentrations was much higher in patients with chronic renal failure disease. These indicate that they are increased in immunoglobulin, because albumin is low.

The study has show that the age and sex have no effect in level of albumin and total protein in patients with chronic renal failure.

Alkaline phosphatase is one of enzymes that have been used to measure obstructive process in hepatobilirary system. It has isoenzymes that are widely distributed throughout the body. By measuring the total alkaline phosphatase activity it is impossible to a certain the origin of ALP activity. Increase in the alkaline phosphatase activity in liver disease is usually in response to cholestasis, which may be extra hepatic or intra hepatic. The magnitude of increases of alkaline phosphatase due to increases in osteoblastic activity or other pathological non hepatic disorders is usually more than three times the normal serum level. Values indicating cholostasis are usually well above the level associated with non hepatic disorders.

Our study has reported that the alkaline phosphatase showed remarkable significant increase in all patients, it represents less than three times the normal serum level. So these results indicate that cholestasis was excluded.

The study showed that the sex has no effect on the alkaline phosphatase level in the chronic renal failure patients but the age showed variable differences. On the other hand, urea, creatinine and

electrolytes are frequently estimated to show the glomerular filtration rate.

The study showed that they have no effect of post and family history on the total protein and albumin levels and alkaline phosphatase activity in patients with chronic renal failure.

5-CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The liver is the central organ in the body that has important metabolic and synthetic functions, beside the major role in the detoxification of some substances.

There is a abundant evidences that liver functions are reduced in the chronic renal failure experimental animals but in human is less clear.

In this study we concluded that the bilirubin level and transaminases activities results were normal and not affected by chronic renal failure disease. Our result extend the alkaline phosphatase activity findings were significantly increased but the level of increase was lesser three times than normal level of alkaline phosphatase, this result raise the possibility that cholestasis was excluded.

In addition to that the albumin level was decreased this indicate that the level of albumin synthesis was reduced; at the same time results from the present study provide that total protein was increased and this would be due to the increase in immunoglobulins.

Hence the association between the decrease of albumin and increase of total protein indicate that there was slight damage in the liver.

Recommendations:

1- More studies investigating the effect of chronic renal failure on liver functions and the effect of dialysis are needed.

2- Electrophoreses to ALP is important that to know the ALP isoenzymes to determination the origin which come from.

3- Increase the centers of dialysis; because the dialysis is a means is sustain life to the kidney failure patients.

4- More work on Sudanese chronic renal failure patients is needed to perform more information about the areas where there is increased incidence of the disease.

5- Conduction of health education programs about the risk factors, mode of transmission and protective measure that can be used to prevent the spread of the disease.

6- More information about the disease and the importance of compliance should be provided to chronic renal failure patients at the time of diagnosis and initiation of therapy.

7- Organization of community members to participate in the control of chronic renal failure disease at community level.

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APPENDIX

UNIVERSITY OF KHARTOUM
Faculty of Veterinary Medicine
Department of Biochemistry
Questionnaire
The effect of chronic renal failure on hepatic functions in Sudanese patients

I- <u>Personal information</u>:

1. Name ……

2. Age ……

3. Sex ……

4. Duration of the diseases ……..……..

5. Residence ……..……

6. Occupation ……

II- <u>Family history of disease</u>:

1. Hypertension…….

2. Diabetes mellitus ……

3. Kidney diseases…….……

4. Liver diseases …….……

III- <u>Past history</u>:

1. Hypertension ……

2. Diabetes mellitus ……

3. Malaria ……

4. Kidney diseases …….……

IV- <u>Investigation</u>:

1. Serum urea (mg/dl) ……...……

2. Serum creatinine (mg/dl) ……..……..

3. Serum electrolyte (mg/dl) ……..…….

4. Serum albumin (g/dl) ……..……...……

5. Serum bilirubin (mg/dl) ……..……...……

6. Serum total protein (g/dl) ……..…….

7. Serum aspartate amino transferase (U/L) …….....

8. Serum alanine amino transferase (U/L) …….

9. Serum alkaline phosphatase (U/L) ……..

Instruments

Vita lab selector:

System presentation:

The vita lab analyzer is a universal system with a price performance relation optimized for small and medium throughput the main unit of the system is the analyzer where all liquid handling and measurements take place. A separate computer controls the analyzer unit, collects raw data and provides the user interface. A cooling unit permits the system to operate in such a way that it ensures the precision of all on board parameters.

Analyzer unit:

The freely programmable analyzer can be used for in vitro diagnosis of serum, plasma, CSF and urine, with a maximum through put of 180 photometric tests per hour in the mono mode or 133 tests per hour in the Dual mode. Up to 26 parameters can be determined selectively for one patient maple. Thirty two parameters can be loaded on to the respective positions of the reagent rotor.

A total of 60 tests and 15 controls can be programmed per programmable reagent disc. Up to 3 controls can be assigned to each test.

The required reagent is pipetted by the reagent arm. Volumes between 110Ml and 399Ml are possible for reagent I. Reagent 2 may be pipetted with volumes between 10 and 180 Ml (Scriptor. Vita lab selector).

Including the sample material, volumes between 220Ml and 400Ml are allowed. The reagent arm is equipped with a heating element to warm up the cooled reagent prior to use. The reagent needle is rinsed inside and outside after each pippetting process. Subsequently to pippetting of the sample to the prepared reagent, liquids are mixed with the sample

needle. The sample needle is also rinsed inside and outside after each pippetting process. After pippetting of reagent 2, a new mixing process is performed, using the reagent needle. The needle is then rinsed.

Fifty one patient samples can be positioned in one loading operation as soon as all test for a sample are completed, the sample can be removed from the sample rotor and replaced by a new sample. The system automatically provides a printout of result and evaluations.

During testing unused positions on the reagent rotor must be covered with the supplied caps to ensure optimum cooling and avoid water of energy. The caps also prevent evaporation of the reagents.

Incubation and measurement are carried out on the reaction or cuvettes (path length 7mm, minimum volume 220 Ml) are available, incubation is performing at 37°C. Maximum incubation time is 11.5 minutes.

Computer control:

An external computer provides the user interface to the analyzer unit. The operation software is windows based in order to reduce the work expenditure to a minimum. Test and sample data can be entered via the keyboard or a barcode reader connected to the computer (Scriptor. Vita lab selector).

All test results are saved on the external computer and can be printed on a printer to his computer.

Entering sample data and test requests:

The request samples mean contains all functions required for test requests and entry or assignment of patient samples, with a minimum of complexity and time you can manually enter sample and patient specific data and request the designated tests. Recorded samples can later be edited or deleted. If needed, the system prints a work list.

Define the type of sample in this field. After pressing enter or clicking on the arrow, a drop- down list opens with the choices available.

Select the type of sample needed:

normal patient sample, state emergency sample, pediatrics sample of children and teenagers are labeled pediatric, control requests labeled with control are recognized by the system as controls, calibrator a request labeled labled calibrator is used to calibrate tests and blank. One position on the inner ring of the rotor research for the reagent blank measurement.

In accordance with the programming made in the TEST PROGRAMMING menu, the screens display a variable number of tests (Scriptor. Vita lab selector).The incoming results of each sample and test can be checked and if necessary validated in the EVALUATE SAMPLE menu. In the graphic mode the results of a dichromatic end point test, run in the DUAL MODE, is displayed. Six points are displayed by the system.

Quality control:

The quality control menu provides an over view of all measured control data. You can select one of the up to three after selection of control and corresponding test, you can check the test in the graphic mode, e.g. for stability, no graphic representation is displayed for cut off tests, test results can be switched directly to PROGRAM CONTROL to change assignment of control and to quality control which can even be called up during a measuremen

Calibration: Elical

Principle:

Elical, a calibrator for automated system, is used for the calibration of clinical chemistry analysts.

Product description**:** Elical is a lyophilized calibration serum.

Resents (1) Freeze dried based on human serum.

Resents (2) Solvent

Preparation:

Carefully open the reagent 1 a voiding the loss of freeze dried powdered and pipette exactly 3 ml of solvent (Reagent 2) (Scriptor. Vita lab selector).

Carefully close the bottle and dissolve the contents completely by occasional gentle swirling with 30 minutes a voiding the formation of foam.

Stability of reagent:

Prior to reconstitution, when stored at 2.8ºC and protected from light, the reagents 1 and 2 are stable until the expiry date stated on the label.

After reconstituting stability of components in reconstituted calibrator: 4 hours at 25ºC, 2 days at 4ºC and 1 month at -20ºC (only freeze once)

Values:

The calibration values were determined under strictly standardized conditions. The concentrations of the components are lot- specific.

Calibration curve:

Can be done if three or more standards (maximum 9) are used for calibration procedure. By means of this calibration curve the instrument will calculate a concentration from absorbance results. The values of the standards must be programmed in the program calibrator screen.

Calculated results:

The results calculated test are not performed on the analyzer but are derived from applying a calculated test formula to the results of the tests that are actually performed on the analyzer. Up to 10 formulas can be defined calculated results are not used for quality control (Scriptor. Vita lab selector).

Flame photometer:

One of the most important instroments to estimate electrolyte. It has many types and the most important is flame emission photometry (Flame photometry). It is used to estimate electrolyte in solution.

General principle:

After preparation of solution from serum, the solution is sprayed as fine droplets into the flame. The metal emits radiation or light, the color of flame is changed. By characterizing of metal emission light, the light wavelength corresponding to element being analyzed is selected by filter or spectrophotometer. The light is allowed to fall in to photo cell convert light current and detector (to measure current) and this light is proportional to concentration of element being analyzed (Kaplan, etal, 199).

Reference values:

Reference value of: Total bilirubin < 1.0 mg/dl.

Reference value of: direct bilirubin < 0.3 mg/dl.

Factor total bilirubin 21.6 mg/dl.

Factor direct bilirubin 16.8 mg/dl.

Reference value of albumin: 3.8 – 5.4 g/dl.

Reference value of total protein: 6.2 – 8.0 g/dl.

Reference value of ALT: at 37ºC up to 49 U/L.

Reference value of AST: at 37ºC up to 46 U/L.

Reference values of ALP: at 37ºC Children 180-370 U/L, Adults 100-290 U/L.

Reference values of urea 15-50 mg/dl 0.15 - 050 g/L, 2.50 -8.33 mmol/L.

Reference values of creatinine: 6 -13 mg/dl 53- 115 mmol/L.

Normal serum Na^+ = 136-149 mmol/L.

Normal serum K^+ = (3.8 - 5.0) mmol/L.

Low K^+ STD = 5.0 mmol/L.

High K^+ STD = 7.0 mmol/L.

Low Na^+ STD = 140 mmol/L.

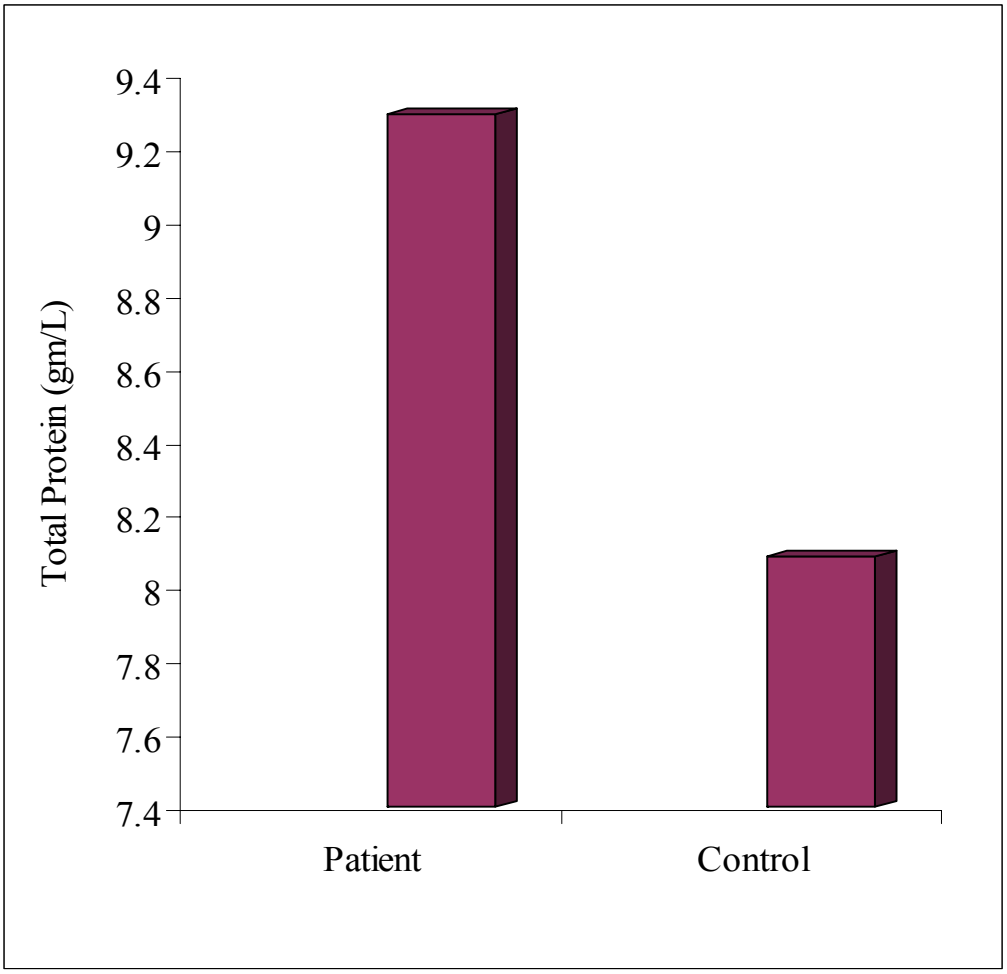


Figure (3): Comparison between serum total protein means (gm/dl) in patients with chronic renal failure (CRF) and control subject.

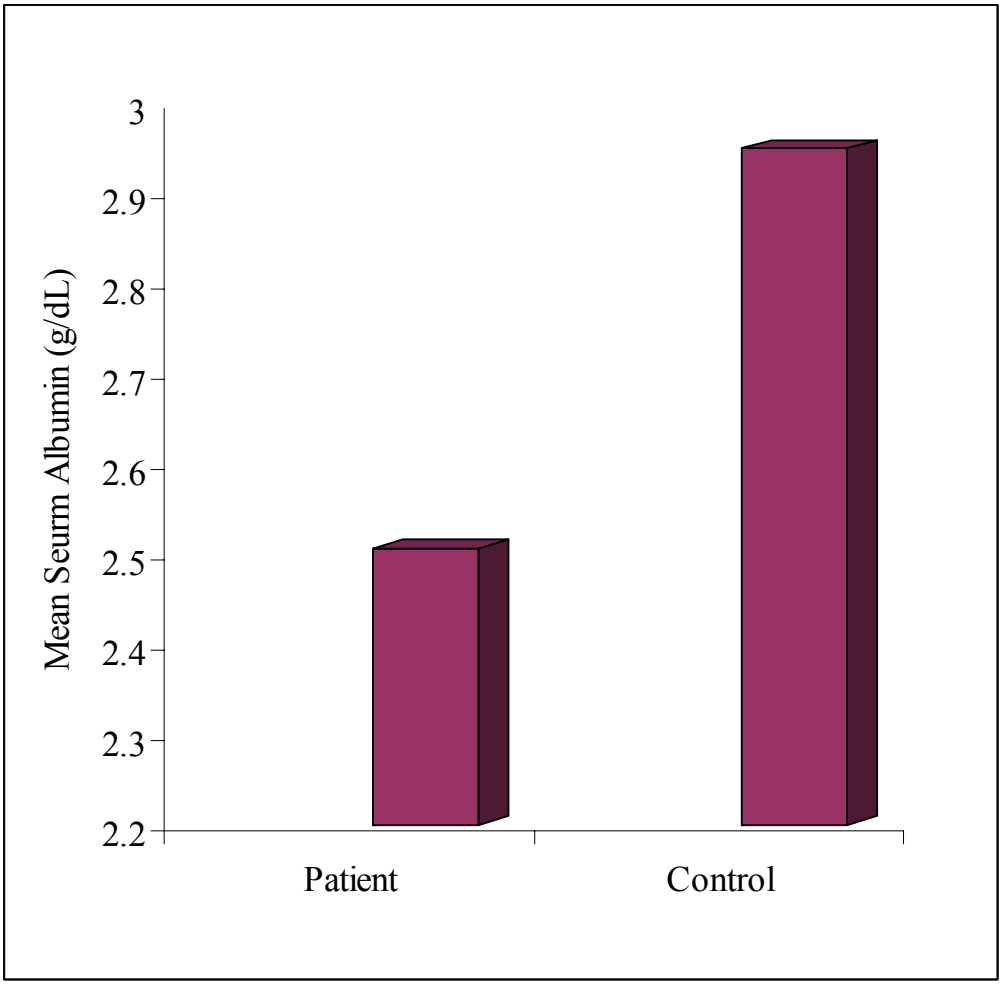


Figure.(4): Comparison between serum albumin means (gm/dl) in patients with chronic renal failure (CRF).

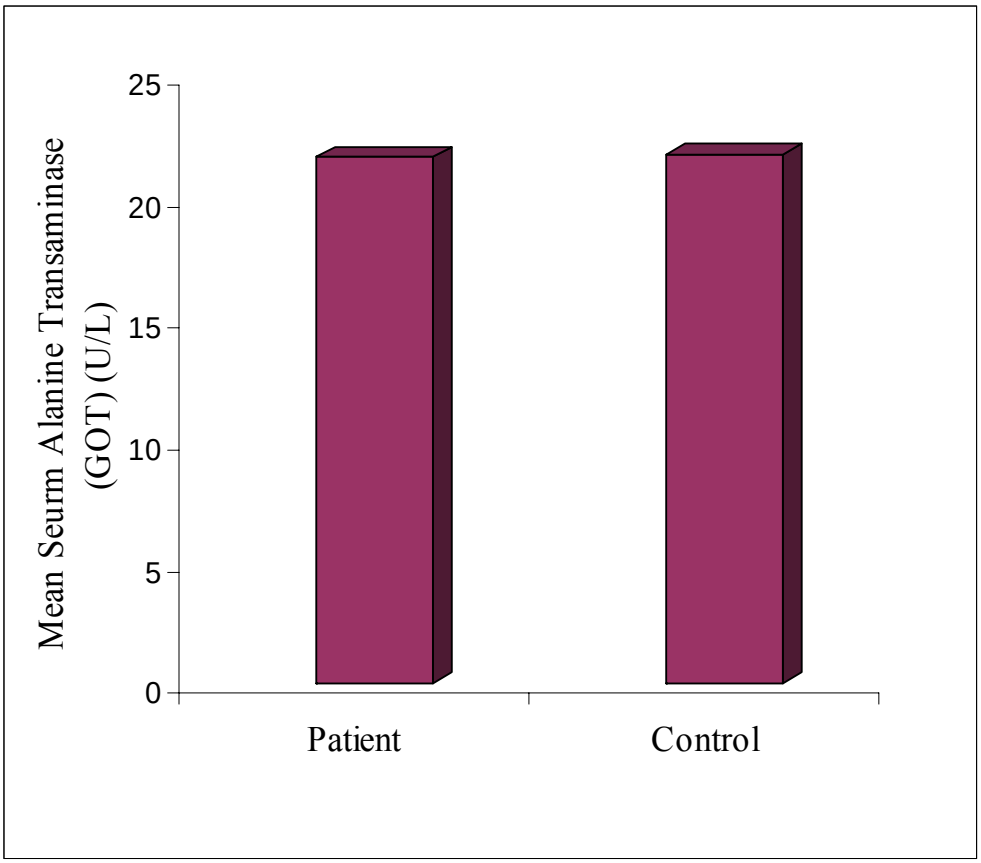


Figure (5): Comparison between serum alanine transaminase (GOT) means (U/L) in patients with chronic renal failure (CRF) and control subject.

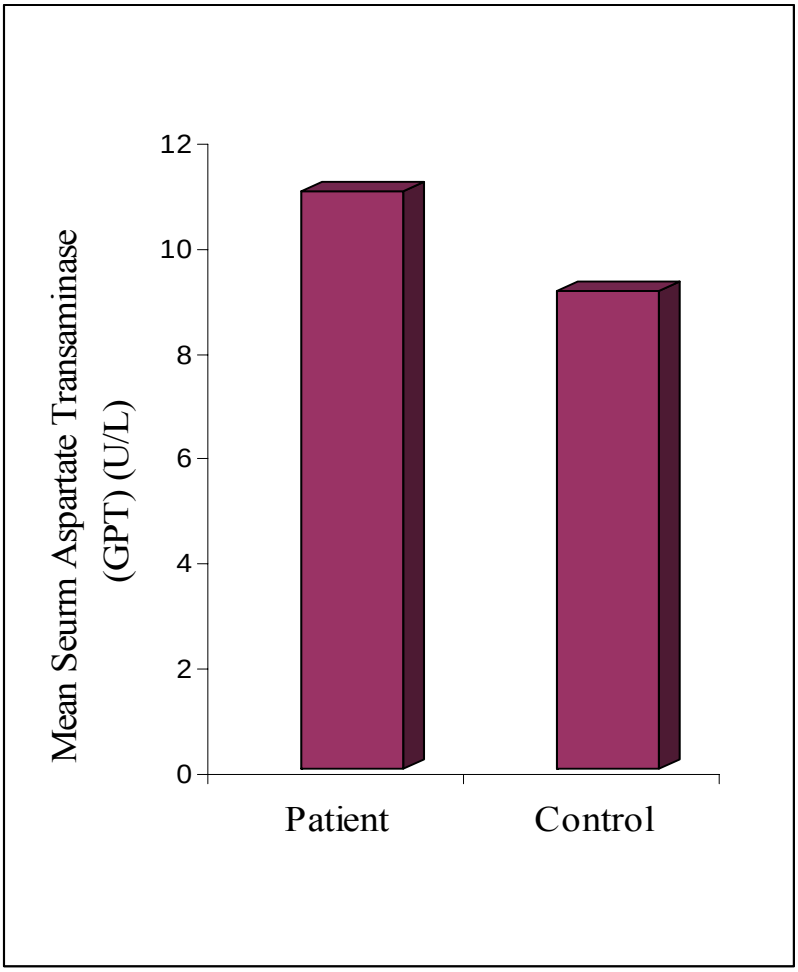


Figure (6): Comparison between serum spartate transaminase (GPT) means (U/L) in patients with chronic renal failure (CRF) and control subject.

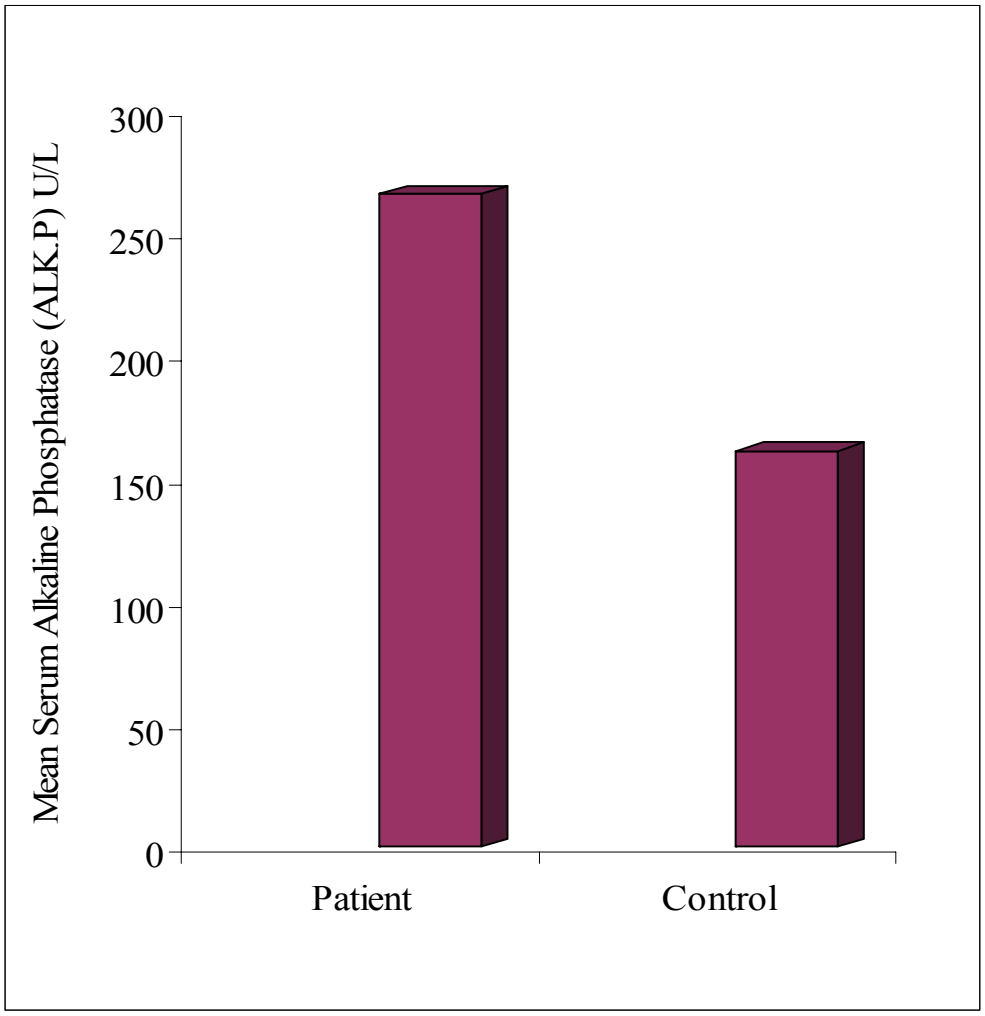


Figure (7): Comparison between serum alkaline phosphatase means (U/L)in patients with chronic renal failure (CRF) and control subject.

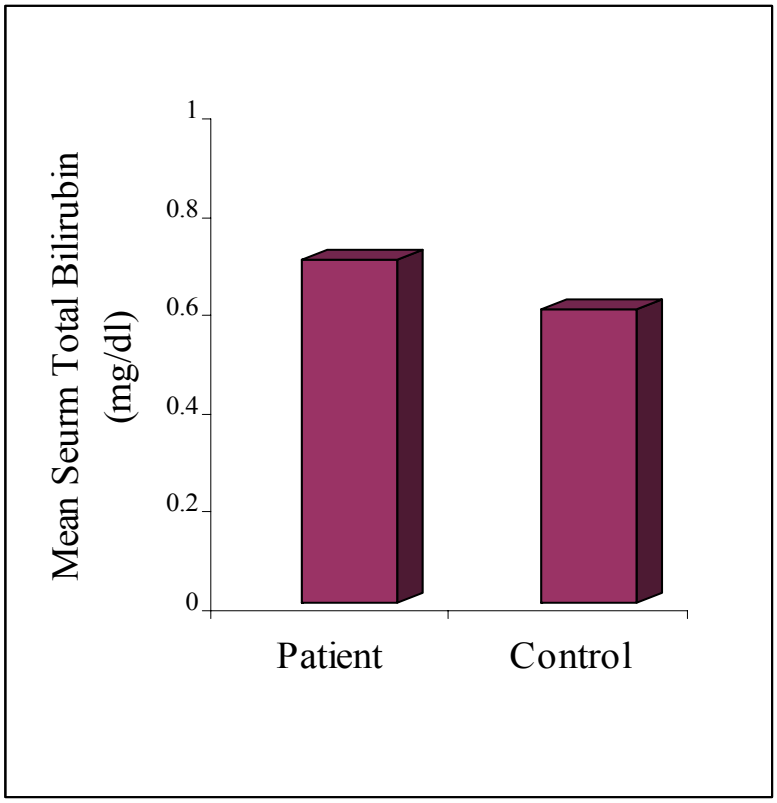


Figure (8): Comparison between serum total bilirubin means (mg/dl) in patients with chronic renal failure (CRF) and control subject.

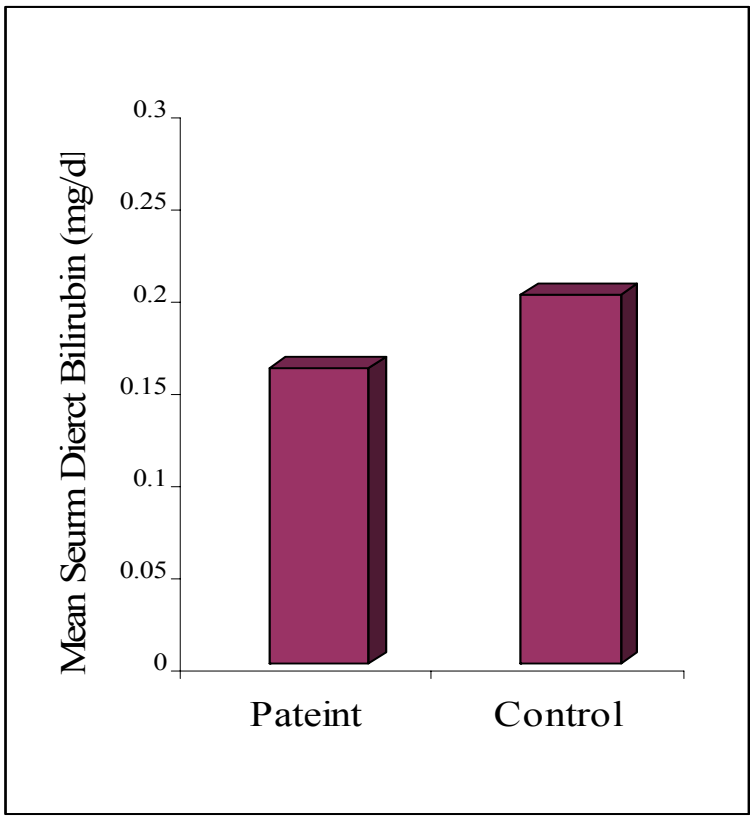
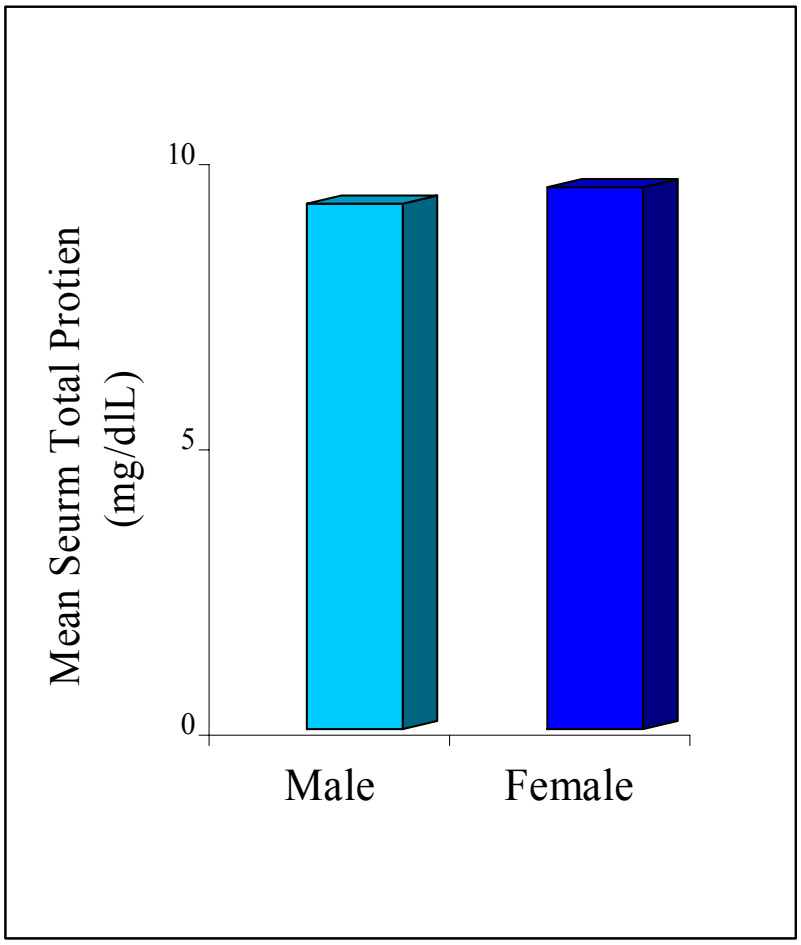
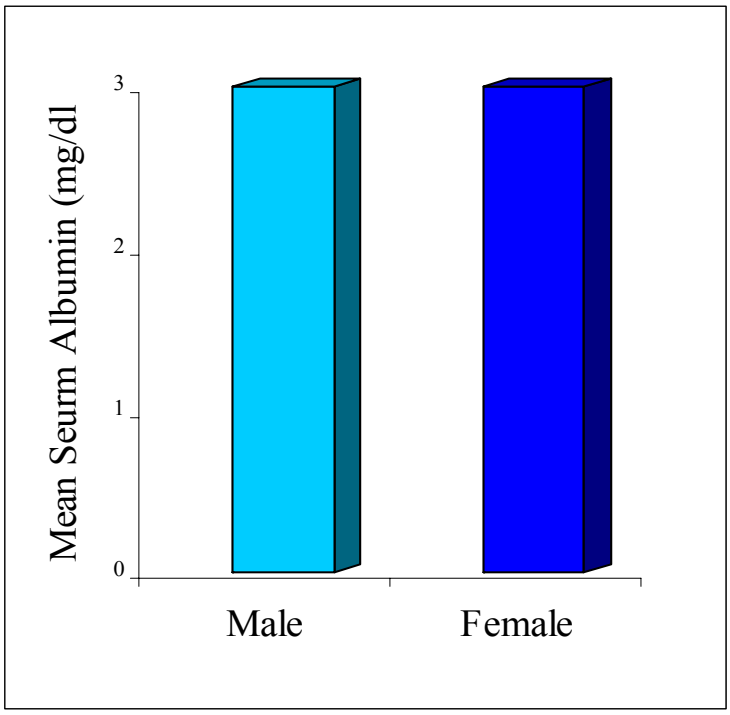


Figure (9): Comparison between serum direct bilirubin means (mg/dl) in patients with chronic renal failure (CRF) and control subject.



Figure(10): Serum total protein means (gm/dl) in male and female patients with chronic renal failure (CRF).



Figure(11): Serum albumin means (gm/dl) in male and female patients with chronic renal failure (CRF).

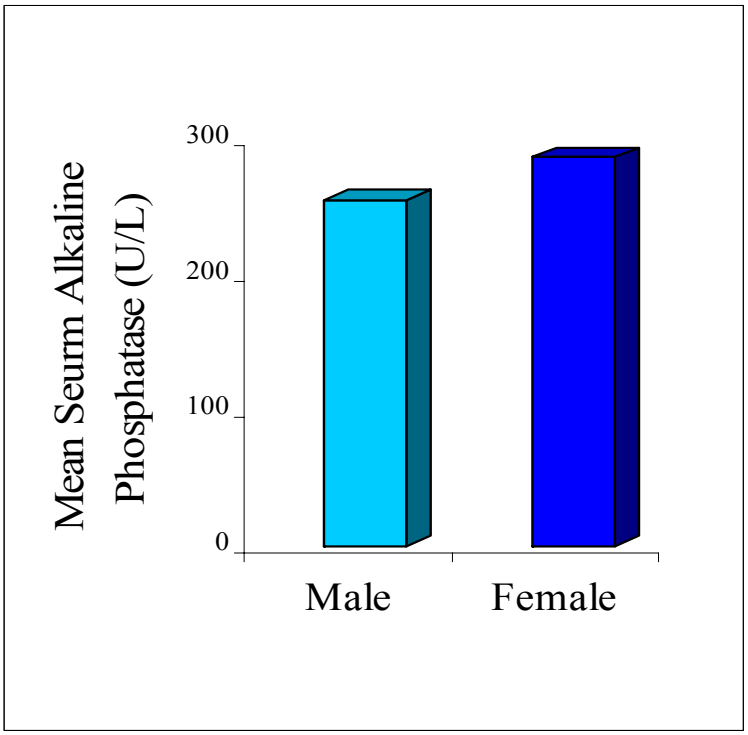


Figure (12): Serum alkaline phosphatase means (U/L) in male and female patients with chronic renal failure (CRF).

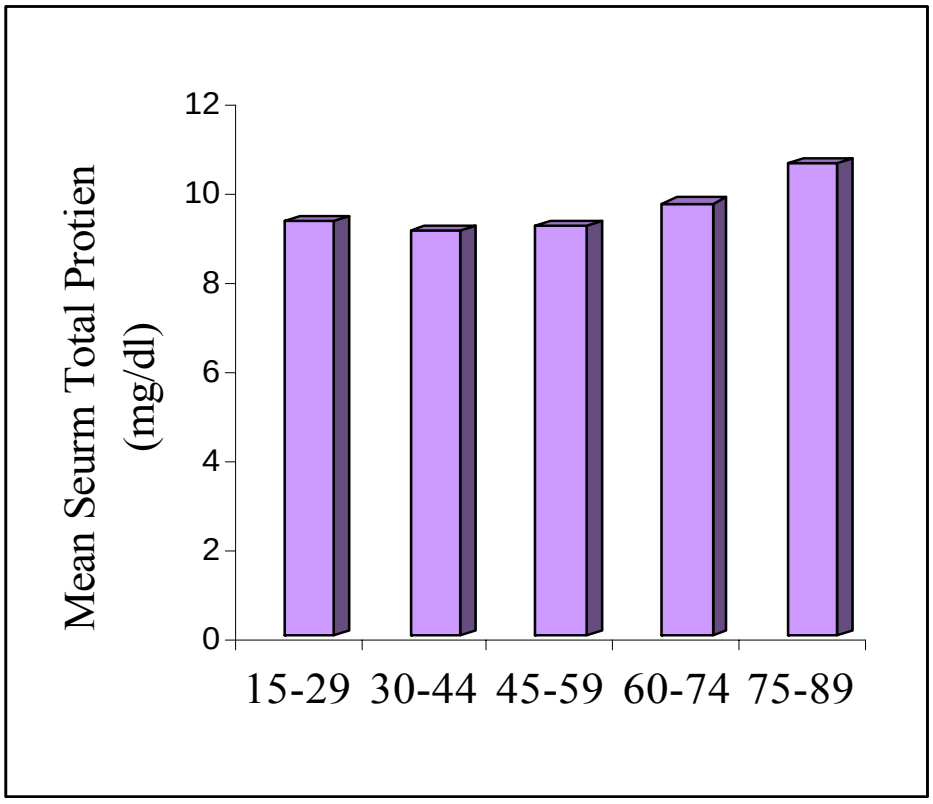


Figure (13): Comparison between serum total protein means (gm/dl) in different grouped ages of patients with chronic renal failure.

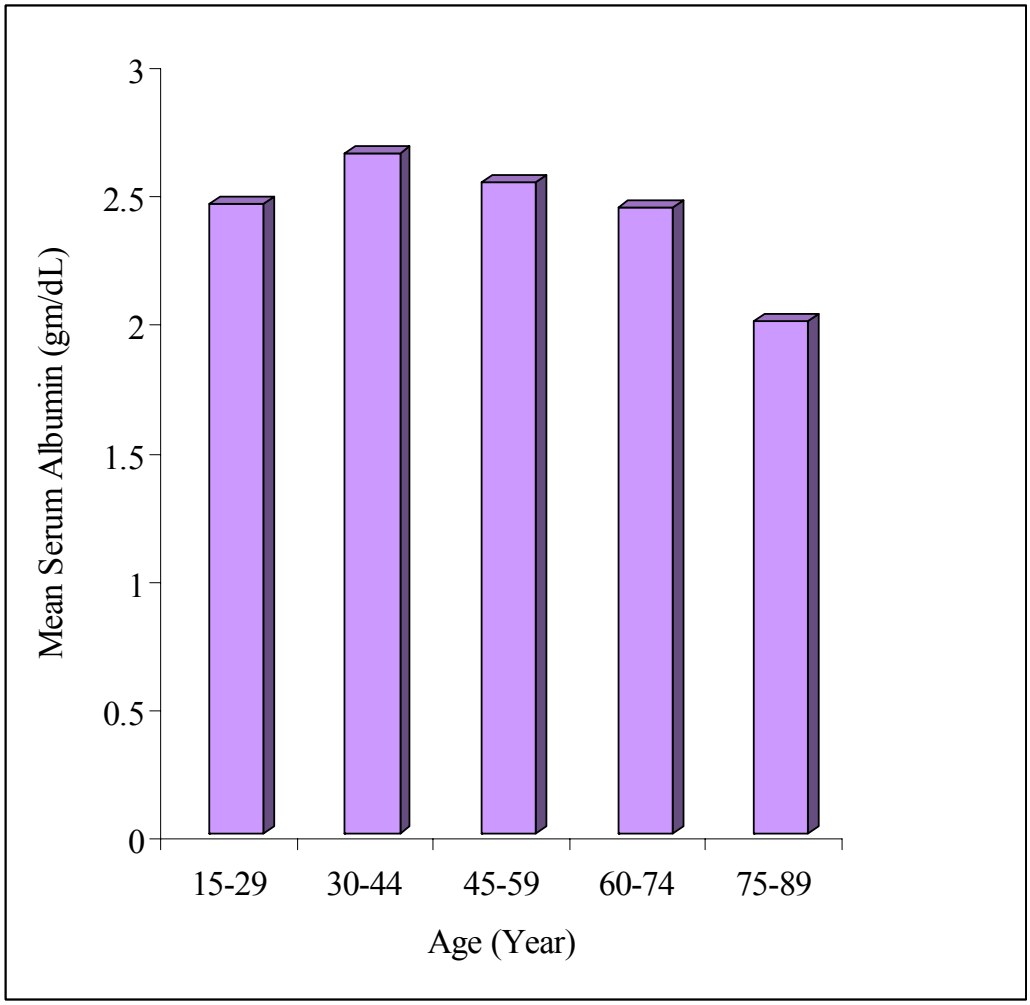


Figure (14): Comparison between serum albumin means (gm/dl) in different grouped ages of patients with chronic renal failure

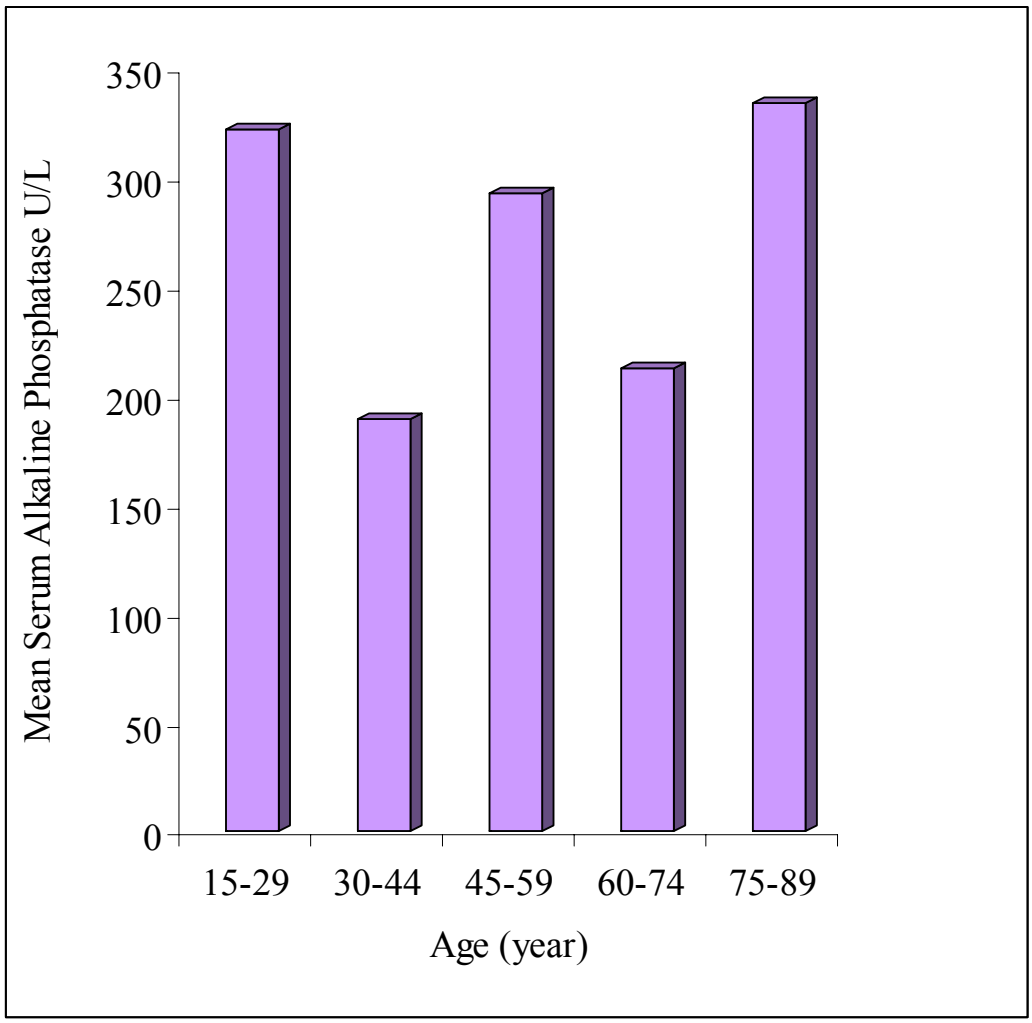


Figure (15): Comparison between serum alkaline phosphatase means (U/L) in different grouped ages of patients with chronic renal failure.

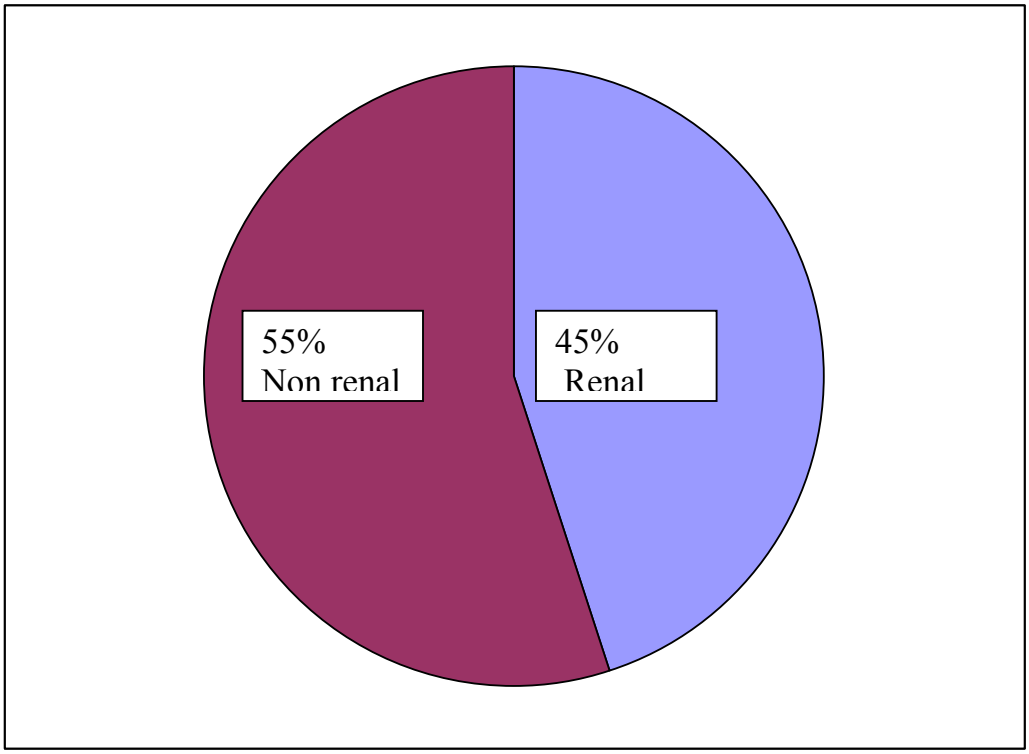


Figure (16): Comparison between percentage (%) of serum alkaline phosphatase means by using post history in patients with chronic renal failure (CRF).

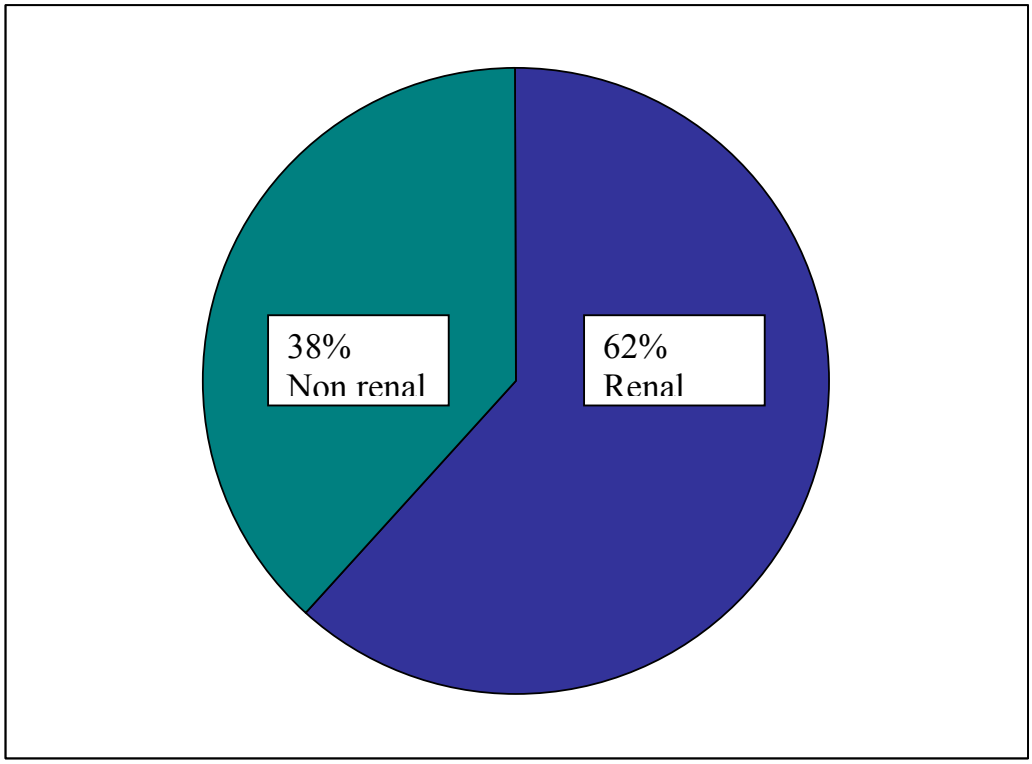


Figure.(17): Comparison between percentage (%) of serum alkaline phosphatase means by using family history in patients with chronic renal failure (CRF).

4- Discussion

In the last years the numbers of patients suffering renal failure disease are increasing month after month. Renal dialysis although not a cure is used as a mean to sustain life to kidney failure patients.

Although studies in animal models have found a favorable effect of chronic renal failure on the hepatic functions reduction, the result of trials in human are less clear. This study is the first to our knowledge to assess prospectively the association between chronic renal failure and subsequent decline in the hepatic functions in the Sudanese population. In our study chronic renal failure patients before heamodialysis have showed the same level of bilirubin and transaminases activity as in control group, this indicates that chronic renal failure disease which was treated with regular dialysis has no effect on these parameters, that no increase or decrease was observed.

On the other hand, albumin, total protein levels and alkaline phosphatase activity showed remarkable significant differences.

This result persisted after adjustment for demographic and clinical information, including sex, age, post history and family history.

Although the liver plays an important role in protein metabolism, changes in serum proteins are not specific for the liver damage. However detection of change to the various function of plasma protein may be of some value.

The combination of an absolute low value for serum albumin, total protein levels or either of these alone is typical of liver damage. Only marked changes of major constituents, such as albumin and

immunoglobulins, are likely to alter total protein concentrations significantly. The finding showed that, albumin concentrations were found to be low in patients with chronic renal failure disease. And decrease of about (71.4) of albumin level under the value of the control subjects was seen in chronic renal failure patients this may indicate that people suffering chronic renal failure have abnormal lowering albumin concentrations.

In our study total protein concentrations was much higher in patients with chronic renal failure disease. These indicate that they are increased in immunoglobulin, because albumin is low.

The study has show that the age and sex have no effect in level of albumin and total protein in patients with chronic renal failure.

Alkaline phosphatase is one of enzymes that have been used to measure obstructive process in hepatobilirary system. It has isoenzymes that are widely distributed throughout the body. By measuring the total alkaline phosphatase activity it is impossible to a certain the origin of ALP activity. Increase in the alkaline phosphatase activity in liver disease is usually in response to cholestasis, which may be extra hepatic or intra hepatic. The magnitude of increases of alkaline phosphatase due to increases in osteoblastic activity or other pathological non hepatic disorders is usually more than three times the normal serum level. Values indicating cholostasis are usually well above the level associated with non hepatic disorders.

Our study has reported that the alkaline phosphatase showed remarkable significant increase in all patients, it represents less than three times the normal serum level. So these results indicate that cholestasis was excluded.

The study showed that the sex has no effect on the alkaline phosphatase level in the chronic renal failure patients but the age showed variable differences. On the other hand, urea, creatinine and electrolytes are frequently estimated to show the glomerular filtration rate.

The study showed that they have no effect of post and family history on the total protein and albumin levels and alkaline phosphatase activity in patients with chronic renal failure.

5-CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The liver is the central organ in the body that has important metabolic and synthetic functions, beside the major role in the detoxification of some substances.

There is a abundant evidences that liver functions are reduced in the chronic renal failure experimental animals but in human is less clear.

In this study we concluded that the bilirubin level and transaminases activities results were normal and not affected by chronic renal failure disease. Our result extend the alkaline phosphatase activity findings were significantly increased but the level of increase was lesser three times than normal level of alkaline phosphatase, this result raise the possibility that cholestasis was excluded.

In addition to that the albumin level was decreased this indicate that the level of albumin synthesis was reduced; at the same time results from the present study provide that total protein was increased and this would be due to the increase in immunoglobulins.

Hence the association between the decrease of albumin and increase of total protein indicate that there was slight damage in the liver.

Recommendations:

8- More studies investigating the effect of chronic renal failure on liver functions and the effect of dialysis are needed.

9- Electrophoreses to ALP is important that to know the ALP isoenzymes to determination the origin which come from.

10- Increase the centers of dialysis; because the dialysis is a means is sustain life to the kidney failure patients.

11- More work on Sudanese chronic renal failure patients is needed to perform more information about the areas where there is increased incidence of the disease.

12- Conduction of health education programs about the risk factors, mode of transmission and protective measure that can be used to prevent the spread of the disease.

13- More information about the disease and the importance of compliance should be provided to chronic renal failure patients at the time of diagnosis and initiation of therapy.

14- Organization of community members to participate in the control of chronic renal failure disease at community level.

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Fifty one patient samples can be positioned in one loading operation as soon as all test for a sample are completed, the sample can be removed from the sample rotor and replaced by a new sample. The system automatically provides a printout of result and evaluations.

During testing unused positions on the reagent rotor must be covered with the supplied caps to ensure optimum cooling and avoid water of energy. The caps also prevent evaporation of the reagents.

Incubation and measurement are carried out on the reaction or cuvettes (path length 7mm, minimum volume 220 Ml) are available, incubation is performing at 37°C. Maximum incubation time is 11.5 minutes.

Computer control:

An external computer provides the user interface to the analyzer unit. The operation software is windows based in order to reduce the work expenditure to a minimum. Test and sample data can be entered via the keyboard or a barcode reader connected to the computer (Scriptor. Vita lab selector).

All test results are saved on the external computer and can be printed on a printer to his computer.

Entering sample data and test requests:

The request samples mean contains all functions required for test requests and entry or assignment of patient samples, with a minimum of complexity and time you can manually enter sample and patient specific data and request the designated tests. Recorded samples can later be edited or deleted. If needed, the system prints a work list.

Define the type of sample in this field. After pressing enter or clicking on the arrow, a drop- down list opens with the choices available.

Select the type of sample needed:

normal patient sample, state emergency sample, pediatrics sample of children and teenagers are labeled pediatric, control requests labeled with control are recognized by the system as controls, calibrator a request labeled labled calibrator is used to calibrate tests and blank. One position on the inner ring of the rotor research for the reagent blank measurement.

In accordance with the programming made in the TEST PROGRAMMING menu, the screens display a variable number of tests (Scriptor. Vita lab selector).The incoming results of each sample and test can be checked and if necessary validated in the EVALUATE SAMPLE menu. In the graphic mode the results of a dichromatic end point test, run in the DUAL MODE, is displayed. Six points are displayed by the system.

Quality control:

The quality control menu provides an over view of all measured control data. You can select one of the up to three after selection of control and corresponding test, you can check the test in the graphic mode, e.g. for stability, no graphic representation is displayed for cut off tests, test results can be switched directly to PROGRAM CONTROL to change assignment of control and to quality control which can even be called up during a measuremen

Calibration: Elical

Principle:

Elical, a calibrator for automated system, is used for the calibration of clinical chemistry analysts.

Product description**:** Elical is a lyophilized calibration serum.

Resents (1) Freeze dried based on human serum.

Resents (2) Solvent

Preparation:

Carefully open the reagent 1 a voiding the loss of freeze dried powdered and pipette exactly 3 ml of solvent (Reagent 2) (Scriptor. Vita lab selector).

Carefully close the bottle and dissolve the contents completely by occasional gentle swirling with 30 minutes a voiding the formation of foam.

Stability of reagent:

Prior to reconstitution, when stored at 2.8ºC and protected from light, the reagents 1 and 2 are stable until the expiry date stated on the label.

After reconstituting stability of components in reconstituted calibrator: 4 hours at 25ºC, 2 days at 4ºC and 1 month at -20ºC (only freeze once)

Values:

The calibration values were determined under strictly standardized conditions. The concentrations of the components are lot- specific.

Calibration curve:

Can be done if three or more standards (maximum 9) are used for calibration procedure. By means of this calibration curve the instrument will calculate a concentration from absorbance results. The values of the standards must be programmed in the program calibrator screen.

Calculated results:

The results calculated test are not performed on the analyzer but are derived from applying a calculated test formula to the results of the tests that are actually performed on the analyzer. Up to 10 formulas can be defined calculated results are not used for quality control (Scriptor. Vita lab selector).

Flame photometer:

One of the most important instroments to estimate electrolyte. It has many types and the most important is flame emission photometry (Flame photometry). It is used to estimate electrolyte in solution.

General principle:

After preparation of solution from serum, the solution is sprayed as fine droplets into the flame. The metal emits radiation or light, the color of flame is changed. By characterizing of metal emission light, the light wavelength corresponding to element being analyzed is selected by filter or spectrophotometer. The light is allowed to fall in to photo cell convert light current and detector (to measure current) and this light is proportional to concentration of element being analyzed (Kaplan, etal, 199).

Reference values:

Reference value of: Total bilirubin < 1.0 mg/dl.

Reference value of: direct bilirubin < 0.3 mg/dl.

Factor total bilirubin 21.6 mg/dl.

Factor direct bilirubin 16.8 mg/dl.

Reference value of albumin: 3.8 – 5.4 g/dl.

Reference value of total protein: 6.2 – 8.0 g/dl.

Reference value of ALT: at 37ºC up to 49 U/L.

Reference value of AST: at 37ºC up to 46 U/L.

Reference values of ALP: at 37ºC Children 180-370 U/L, Adults 100-290 U/L.

Reference values of urea 15-50 mg/dl 0.15 - 050 g/L, 2.50 -8.33 mmol/L.

Reference values of creatinine: 6 -13 mg/dl 53- 115 mmol/L.

Normal serum Na^+ = 136-149 mmol/L.

Normal serum K^+ = (3.8 - 5.0) mmol/L.

Low K^+ STD = 5.0 mmol/L.

High K^+ STD = 7.0 mmol/L.

Low Na^+ STD = 140 mmol/L.

The Vitalab Selector