STUDY OF SERUM ALBUMIN, THYROID AND LIPID PROFILE IN CHRONIC KIDNEY DISEASE – A CROSS SECTIONAL STUDY

BY

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DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH,

TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE
IN
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UNDER THE GUIDANCE OF

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APRIL 2013

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Date: *Signature of the Candidate*

Place:Kolar

DR G GANESH

DEDICATED WITH REVERENCE

TO

MY FAMILY

FOR THEIR SELFLESSNESS AND INSPIRATION THAT MOTIVATES ME IN ALL MY ENDAVEOURS

LIST OF ABBREVIATIONS

CKD - Chronic Kidney Disease

CVD – Cardio Vascular Disease

ESRD – End Stage Renal Disease

CRF – Chronic Renal Failure

GFR – Glomerular Filteration Rate

eGFR – estimated Glomerular Filteration Rate

TRH – Thyrotropin Releasing Hormone

TSH – Thyroid Stimulating Hormone

TH – Thyroid Hormone

TT3 – Total Tri-iodothyronine

TT4 – Total Tetra-iodothyronine

fT3 – free Tri-iodothyronine

rT3 – reverse Tri-iodothyronine

TBG – Thyroid Binding Globulin

LDH – Lactate Dehydrogenase

CPK – Creatine Phospho Kinase

AST – Aspartate Transaminase

TNF-α – Tumor Necrosis Factor – α

IL-1 – Interleukin – 1

NTI – Non Thyroid Illness

HD – Hemodialysis

VLDL – Very Low Density Lipoprotein

LDL – Low Density Lipoprotein

IDL – Intermediate Density Lipoprotein

HDL – High Density Lipoprotein

TG – Triglycerides

CE – Cholesteryl Esters

LPL – Lipoprotein Lipase

LCAT – Lecithin Cholesterol Acyl Transferase

ACAT – Acyl CoA Cholesterol Acyl Transferase

mRNA – messenger Ribonucleic Acid

ABSTRACT:

BACKGROUND:

Chronic kidney disease (CKD) is a debilitating condition associated with high cardiovascular morbidity and mortality. Other than traditional risk factors such as diabetes mellitus, hypertension, dyslipidemia and old age which is prevalent in CKD, there are some non-traditional associated risk factors that is involved in the pathogenesis of cardiovascular disease in CKD such as hypoalbuminemia and hypothyroidism.

OBJECTIVES:

To measure serum albumin, serum thyroid and lipid profile in CKD patients and healthy controls. To compare the levels of above parameters in the different stages of CKD.

MATERIALS AND METHODS:

Total number of 80 subjects of which 40 were CKD patients and 40 were healthy controls were selected. Serum was collected to estimate albumin, thyroid and lipid profile. eGFR was calculated using MDRD (Modification of Diet in Renal Disease) formula to stage the CKD patients.

RESULTS:

There was a reduced levels of serum albumin in CKD patients compared to controls. CKD patients had reduced levels of TT3 and TT4 compared to controls. CKD patients had increased levels of TSH compared to controls. TT4 levels were significantly reduced in stage 4 compared to stage 3 of CKD. There were reduced

levels of HDL in CKD patients compared to controls.

CONCLUSION:

There was hypoalbuminemia due to inflammation and malnutrition in CKD patients. Hypothyroidism observed in CKD patients was due to the alteration in protein binding of TT4 and decreased activity of deiodinase. Decreased HDL level was seen in cases due to the abnormalities in enzyme activity of HDL metabolism. These alterations may increase the risk of cardiovascular morbidity in CKD patients

Key words: Chronic kidney disease, Cardiovascular disease, Dyslipidemia, Hypoalbuminemia, Hypothyroidism.

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INTRODUCTION:

Chronic kidney disease (CKD) caused by different etiologies such as diabetic kidney disease, hypertension, non-diabetic kidney diseases like nephrotic and nephritic syndrome, cystic kidney disease and tubulo-interstitial disease are the emerging non-communicable disease in both rural and urban part of India.

These chronic kidney diseases pose a greater need for the control of the primary diseases causing this chronic kidney disease leading on to end stage renal disease (ESRD). This ESRD causes a lot of financial burden for the patients when they reach the stage for the need for renal replacement therapies[1].

In the developing countries, the awareness and burden of chronic renal failure (CRF) on society has been highlightened during last decade. In India, incidence of CRF is not well documented because of lack of national registry and data regarding its incidence. It has been estimated that the prevalence of CRF in India may be up to 785 people per million population[2].

CRF is accompanied by anemia, malnutrition, impaired metabolism of lipids, carbohydrates and proteins & defective utilization of energy. With advancing renal disease, clear disturbances in electrolytes and endocrine functions takes place.

Cardiovascular disease (CVD) is the most common cause of morbidity and mortality in patients with chronic kidney disease (CKD). Although traditional risk factors such as diabetes mellitus, hypertension, dyslipidemia and old age are prevalent in CKD, they may not be sufficient by themselves to account for the high prevalence of CVD in them. Hence the study of non-traditional associated risk factors that is involved in the pathogenesis of CVD in CKD patients becomes necessary[3].

Some of these factors contributing to the pre-mature development of CVD are thyroid disorder like hypothyroidism[4] and generalised causes like hypoalbuminemia[5].

CKD affects both hypothalamus–pituitary–thyroid axis and TH peripheral metabolism leading to hypothyroidism mainly sub-clinical hypothyroidism. Uremia influences thyroid gland functions by a variety of mechanism one of which is disturbing the circadian rhythm and glycosylation of TSH. The features caused by affection of thyroid in CKD is typical of illness called "non-thyroid illness" [6].

This hypothyroidism caused by CKD in turn causes hyperlipidemia. CKD by itself by various mechanisms causes abnormal lipid metabolism leading on to hyperlipidemias.

Albumin being a negative acute phase reactant, it is said that it can predict chronic inflammation and clinical events. Hypoalbuminemia occurring due to chronic malnutrition and increased catabolism by cytokines in CKD, is a known marker of cardiovascular morbidity and mortality in CKD[5].

All these above said factors like hypothyroidism, dyslipidemia and hypoalbuminemia becomes the important causes for increase in cardiovascular morbidity and mortality in CKD patients.

Early assessment of these factors at different stages of CKD may help in decreasing the complications of CVD in CKD[3]. Studies with reference to these parameters in our population are limited and varied. Studies assessing the thyroid levels at different stages of CKD in India are scarce. When these parameters assessed together may give greater insight in to the risk of cardiovascular complication in CKD patients.

Hence this study was undertaken to measure the levels of serum albumin, serum thyroid hormones and serum lipid levels to know the degree of hypoalbuminemia, hypothyroidism and dyslipidemia at different stages of CKD in the rural population of Kolar.

AIMS AND OBJECTIVES

AIMS & OBJECTIVES OF THE STUDY:

1. To measure serum albumin in chronic kidney disease cases and controls.
2. To measure serum thyroid profile in chronic kidney disease cases and controls.
3. To measure serum lipid profile in chronic kidney disease cases and controls.
4. To compare the above parameters in different stages of CKD.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

INTRODUCTION:

CHRONIC RENAL FAILURE:

HISTORY

Raffer, Smith and Dawson showed that the early inhabitants of Nile Valley had atrophied kidneys. Charaka explained the different varieties of prameha or urinary affections (2^{nd} century); Hippocrates has given a detailed description of renal diseases. He diagnosed certain affections of the kidney by urine examination.

Boerhaave and Rouelle le Cadet suggested the presence of unknown "soapy" substance in urine. Between 1797 and 1808, A Fourcroy and N. Vauquelin isolated and crystallized it and called it as 'urea'. They suggested that the principle function of the kidney is to "denitrogenize" the body by excreting urea in urine.

In 1821, J.L. Prevost and J.B. Dumas reported that following a bilateral nephrectomy in many different animal species, a significant rise in the concentration of blood urea occurred uniformly.

In 1827, F. Wohler was able to synthesize urea, the first organic substance belonging to the animal kingdom to be produced in the laboratory.

Henri Dutrochet in 1827, described his investigations in the phenomenon of osmosis in vegetables and animals. Thomas Graham in 1850 used a semi-permeable membrane in vitro and was able to separate large molecules from small molecules. The concept of dialysis was born and the seed idea for the artificial kidney had been sown.

J. Bostock, G.H. Barlow and O.W. Rees proposed that urea might be a

retained product in kidney disease.

In 1839 Christison shed light on the matter by considering two categories of clinical findings noted in patients with the end stage of "Bright's disease". He described the modern concept of the "Uremic syndrome".

In 1847, Piorry coined the word "Uremia". In 1851, E.T. Frerich's focused more on the Uramische Intoxication and its clinical signs than on what happened to the kidney itself. He described the clinical uremic syndrome and dared to accept a toxic mechanism as its etiology.

In 1856, J. Picard, developed a reproducible and sensitive method for measurement of blood urea. Later Recklinghausen proved that the renal failure was a condition accompanied by rise in blood urea concentration.

Several years later, Claude Bernard suggested that uremic toxicity was due to ammonium carbonate absorbed from the gut.

An association between lipids and kidney disease was first noted by Virchow, who described 'fatty degeneration' of the renal epithelium in Bright's disease in 1860[7].

DEFINITION:

Chronic Renal Failure (CRF) / chronic kidney disease (CKD) is the state which results from a permanent and usually progressive reduction in renal function, in a sufficient degree to have adverse consequence on other systems[8].

The need for the insight into the burden of CRF has been stressed upon since last decade. It's been estimated that 785 people per million populations will be suffering from CRF in India[2].

CRF is accompanied by anemia, malnutrition, impaired metabolism of lipids, carbohydrates and proteins & defective utilization of energy. With advancing renal disease, clear disturbances in electrolytes and endocrine functions takes place.

Renal function deterioration may be described in successive stages as follows:

1. **Stage of Decreased Renal Reserve:**

The earliest stage common to all forms of CRF is a loss of renal reserve when kidney function is entirely normal, glomerular filtration rate (GFR) can be augmented by 20-30% in response to the stimulus of a protein challenge. During the earliest stage of loss of renal reserve, basal GFR may be normal or even elevated (hyper filtration), but the expected further rise in response to a protein challenge is attenuated. At this stage, there are no symptoms or prominent biochemical alterations. Proteinuria and hypertension may or may not be present. Diminished creatinine clearance is the only observed change which is below the normal but above 50ml/min.

2. **Stage of Moderate Renal Insufficiency:**

In this stage creatinine clearance is below 50ml/min, nocturia, mild anemia, loss of energy, decreasing appetite are usual. At the first and second stages, inter current clinical stress may compromise renal functions still further, inducing signs and symptoms of overt uremia. Such intercurrent clinical conditions include infection (urinary, respiratory, GIT), poorly controlled hypertension, hyper or hypovolemia & drug or radio contrast nephrotoxicity among others.

3. **Stage of Serve Renal Insufficiency (Frank Renal Failure)**

Here, the creatinine clearance is about 10-15 ml/min. Serum creatinine ranges between 5.5-7mg/dl. Symptomatic stage, anemia becomes severe, metabolic acidosis sets in, hypocalcaemia, hypochloremia and hyponatremia occurs; hyperkalemia is uncommon.

4. Stage of Uremia

This is the stage when patient is symptomatic with symptoms referral to all major organs systems. Renal function is 5-10% of the normal. Serum creatinine is 8mg/dl and above. Hypocalcaemia is frequent, anemia, bleeding and bone disease may coexist.

5. End Stage Renal Disease:

End stage renal disease occurs when GFR falls below 5-10% of normal (< 3 ml/min/1.73m^2). Continued survival without renal replacement therapy becomes impossible[9].

The K/DOQI definition and classification are accepted with clarifications. CRF is defined as kidney damage or glomerular filtration rate < 60 ml/min/1.73 m^2 for 3 months or more irrespective of cause[10].

CLASSIFICATION OF CHRONIC RENAL FAILURE (CRF) [10]:

Stage	Description	GFR ml/min/ 1.73m^2	Related terms	Classification by treatment
1	Kidney damage with normal or decreased GFR	≥ 90	Albuminuria, proteinuria, hematuria	T if kidney transplant recipient
2	Kidney damage with mild decreased GFR	60-89	Albuminuria, proteinuria, hematuria	
3	Moderate decreased GFR	30-59	Chronic renal insufficiency, Early renal insufficiency	
4	Severe decreased GFR	15-29	Chronic renal insufficiency, Late renal insufficiency, Pre-ESRD	
5	Kidney failure	<15 (or dialysis)	Renal failure, uremia, ESRD	D if dialysis (hemodialysis, peritoneal dialysis)

ESRD – End stage renal disease; GFR – Glomerular filtration rate

TABLE 1: CLASSIFICATION OF CRF

CAUSES OF CHRONIC RENAL FAILURE[11]:

- Diabetic glomerulosclerosis
- Hypertensive nephrosclerosis
- Glomerular diseases
  - Glomerulonephritis
  - Amyloidosis light chain disease
  - Systemic lupus erythematosus
  - Wegener's granulomatosis
- Tubular diseases
  - Reflux nephropathy (chronic pyelonephritis)
  - Analgesic nephropathy
  - Obstructive nephropathy (stones, benign prostatic hyperplasia)
  - Myeloma kidney
- Vascular diseases
  - Scleroderma
  - Ischaemic nephropathy
  - Atheroembolic renal disease
  - Vasculitis
- Cystic diseases
  - Autosomal dominant polycystic kidney disease
  - Medullary cystic kidney disease

PATHOGENESIS:

Approximately one million nephrons are present in each kidney, each contributing to the total GFR. Regardless of the etiology of renal injury, with progressive destruction of nephrons, the kidney has an ability to maintain GFR by hyperfiltration and compensatory hypertrophy of the remaining healthy nephrons. This nephron adaptability allows for continued normal clearance of plasma solutes such as urea and creatinine. Their levels are increased in plasma only after total GFR has decreased to 50%, when renal reserve has been exhausted. The hyperfiltration and hypertrophy of residual nephron although beneficial, it is known to cause progressive renal dysfunction. This occurs because of increased glomerular capillary pressure which damages the capillaries leading to focal and segmental glomerulosclerosis and eventually to global glomerulosclerosis[12].

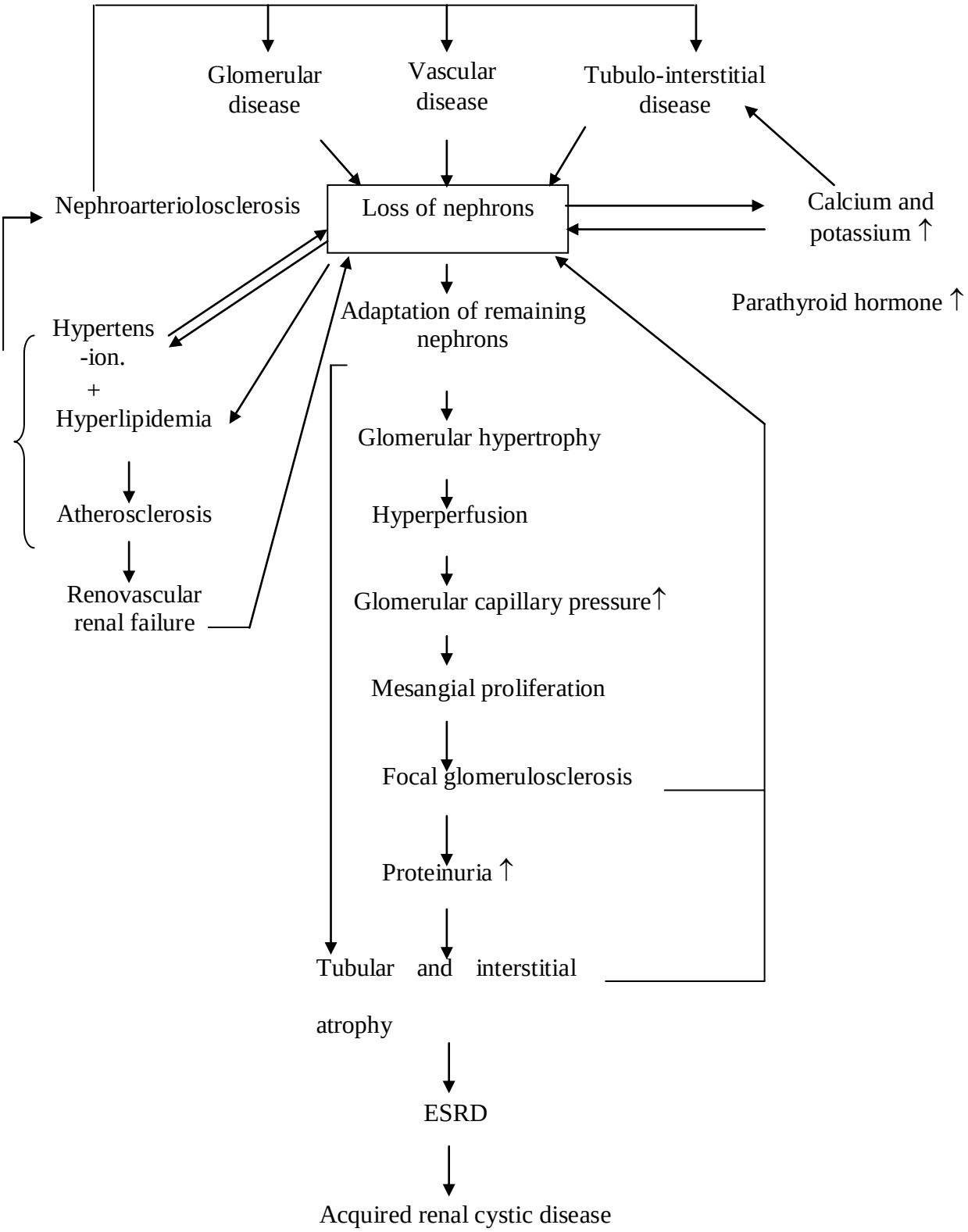


FIG 1: PATHOGENESIS OF CHRONIC RENAL FAILURE[11]

CLINICAL FEATURES OF CHRONIC RENAL FAILURE[11]:

- **Early stage**
  - Hypertension
  - Proteinuria, elevated blood urea or serum creatinine
  - Nephrotic syndrome
  - Gross hematuria
- **Late stage** (GFR <15ml/min, Blood urea > 60mg/dl)
  - Cardiac failure
  - Anemia
  - Serositis
  - Confusion, coma
  - Vomiting
  - Peripheral neuropathy
  - Hyperkalemia
  - Metabolic acidosis

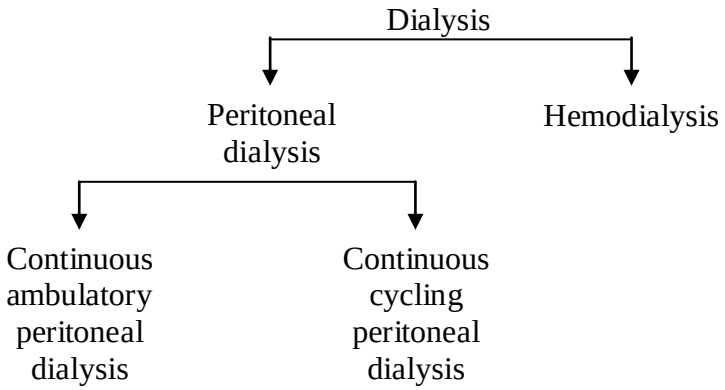
INDICATIONS FOR DIALYSIS[8]:

When conservative treatment fails to sustain quality of life in chronic renal failure, renal replacement therapy is indicated.

Absolute indications for dialysis include

- Hyperkalemia
- Fluid over load
- Severe hypertension
- Pericarditis
- Encephalopathy
- Neuropathy

TYPES OF DIALYSIS[13]:



Peritoneal dialysis:

Continuous ambulatory peritoneal dialysis: This simple process can be performed even in small hospitals. Two liters of sterile peritoneal dialysis solution are instilled into the peritoneal cavity. Exchange of water and solutes takes place across the semi-permeable peritoneal membrane between the solution and the blood flowing through the peritoneal surface.

The fluid is drained by gravitational force after about 20-30 minutes of dwell time. 24 to 36 such exchanges are done. Clearance of water and solutes can be improved by using hypertonic dialysis solutions and adding vasodilator substances in fluid. This modality of treatment is being used in about 10% patients with end stage renal disease in India as they stay far away from major dialysis units.

Continuous cycling peritoneal dialysis: This is similar to intermittent peritoneal dialysis but patient uses a cycler to carry out exchanges. However these techniques require additional machines hence the cost of treatment is more.

Hemodialysis:

Hemodialysis involves single pass of blood and dialysis solution (dialysate) across a semi-permeable membrane. The solutes move across the membrane by diffusion while water moves by ultrafiltration to reach a state of equilibrium.

The regular dialysis process lasts for about 4 hours and to be repeated three times a week. At least 12 hours of dialysis per week is necessary to get acceptable long term survival in patients with ESRD.

CRF is associated with premature atherosclerosis and increased cardiovascular morbidity and mortality.

THYROID:

HISTORY[14]:

After the initial description of the thyroid by Galen in his "DEVOCE", significant milestones related to thyroid and its related diseases are given below in brief.

Vesalius gave a detailed description of the thyroid. Wharton named the organ as thyroid i.e., oblong shield. King described the internal secretory functions of the thyroid. In 1835 Grave correlated symptoms of hyperthyroidism with thyroid gland. In 1874 Gull first described clinical syndrome of hypothyroidism. In 1896 Baumann described the association of the iodine with the working of the thyroid. In 1926 Harriton and Bayer described the chemical structure of the thyroxine.

ANATOMY OF THYROID GLAND[15]:

In the adult normal thyroid gland weighs about 20-25gms. It is the largest endocrine gland in man. Two large lateral lobes are connected in the midline by a broad isthmus from which on occasion a pyramid lobe may protrude superiorly.

Embryology:

The thyroid gland develops as tubular invagination of the endoderm from the root of the tongue called Foramen caecum about the third week of embryonic life. This endodermal proliferation invaginates and grows downwards to form a diverticulum, the caudal end of which gives rise to median isthmus and two lateral lobes of the thyroid. The ultimobranchial bodies developing from the fourth pharyngeal pouch on each side amalgamates with the development lateral lobes of the thyroid gland and contributes to the parafollicular or C cells of thyroid.

Histology:

The functioning unit is the lobule, which contains 20 - 40 variably sized follicles that are lined by regular cuboid cells. Delicate fibrous tissue stroma and small collections of lymphocytes separate these follicles. Dispersed within the follicles are the calcitonin secreting C cells. The resting follicles contain colloid in which thyroglobulin is stored. In active follicle, with absorption of colloid, cells become more columnar.

Arterial Supply:

The arterial supply consists of the superior thyroid arteries arising from the external carotids and inferior thyroid arteries arising from the subclavian artery.

Venous Drainage:

The venous drainage consists of the superior thyroid veins and the middle thyroid veins draining into the internal jugular vein and inferior thyroid veins draining into brachiocephalic vein.

Lymphatic Drainage:

There is an extensive lymphatic network within the gland. The subcapsular plexus drains principally to the juxtathyroid nodes i.e., pretracheal and paratracheal nodes and nodes on the superior and inferior thyroid veins and then to the deep cervical and mediastinal group of nodes.

PHYSIOLOGY:

Thyroid secretes two significant hormones thyroxine (T_4) and triidothyronine (T_3), Iodide a substrate for thyroid hormone synthesis, also plays an auto regulatory role in the metabolism of the thyroid gland.

IODINE METABOLISM:

A typical daily dietary intake of iodide is about 500µg. Iodide is almost completely absorbed in the gastrointestinal tract, where it enters the inorganic iodide pool in the extracellular fluid. In the presence of normal renal function inorganic iodide in the extracellular fluid is rapidly cleared, with a half-life of about 2 hrs. The only two significant pathways of iodide clearance from the extracellular fluid are the kidneys and the thyroid. When the dietary intake of iodide increases, the fractional uptake of extracellular fluid iodine by the thyroid decreases and the proportionate urinary excretion of iodide increases. The iodine content of the hormonal pool is

about 600µg. Cellular uptake of thyroid hormones from this pool is approximately 75µg of iodine per day in an euthyroid individual. Of this 75 µg of thyroid hormone iodine, about 60µg reenters the extracellular fluid iodide pool following intracellular enzymatic de-iodination of the thyroid hormones. The remainder is conjugated in the liver and excreted in the bile and subsequently in the stools.

SYNTHESIS OF THYROID HORMONES:

The thyroid hormone biosynthesis can be considered in the various stages.

1. Active transport of iodide into the gland.
2. Oxidation of iodine to active iodine by thyroperoxidase using NADPH.
3. Iodination of tyrosyl residues within thyroglobulin to yield hormonally inactive iodotyrosines.
4. Coupling of iodotyrosines to form the hormonally active iodothyronines namely tri-iodothyronine (T_3) and tetra-iodothyronine or thyroxine (T_4).
5. Storage of thyroglobulin as colloid in acini with eight T4 residues per molecule.
6. Utilisation by taking up thyroglobulin into the cell by pinocytosis.
7. Hydrolysis of thyroglobulin occurs to release T4 by specific proteases.
8. T4 is released into blood stream, T3 is produced by deiodination at 5′ position by deiodinase.
9. Mono-iodotyrosine and di-iodotyrosine which are not utilised are deiodinised and salvaged for re-utilisation.

REGULATION OF HORMONE SYNTHESIS:

Thyroid hormone synthesis and secretion are regulated by thyrotropin and intrathyroidal autoregulatory mechanism. Thyrotropin is the major modulator of

thyroid activity. Thyrotropin a glycoprotein with a molecular weight of 28000 kD is secreted by thyrotrophs of anterior pituitary. It consists of alpha and beta chains. Alpha chain is identical to the alpha chain of gonadotrophins. TSH specificity is determined by beta chain. TSH secretion is modulated by thyroid hormones in a negative feedback mechanism. TSH stimulates thyroid hypertrophy and hyperplasia, accelerates all intermediary metabolisms in the thyroid. TSH enhances synthesis of thyroglobulin and stimulates synthesis and secretion of thyroid hormones. The actions of TSH are mediated by the binding of TSH to specific receptors on the surface of follicular cells and activation of the plasma membrane enzyme adenylate cyclase. The second messenger cAMP initiates all the responses that characterize the actions of TSH.

Regulation of TSH is affected by two opposing influences at the thyrotrophic cell. Thyrotropin Releasing Hormone (TRH) a peptide of hypothalamic origin stimulates secretion and synthesis of TSH whereas T_3 inhibits TSH secretion and antagonize the action of TRH. The homeostatic control of TSH is mediated via negative feedback control of thyroid hormone the "threshold" thermostat for which is set by TRH.

Intra-thyroidal Regulation:

In some manner glandular iodine content causes reciprocal changes in thyroid iodide transport activity and regulates growth, amino acid uptake and nucleic acid synthesis. These influences are evident in the absence of TSH, hence termed auto regulation. Their most important role is to modify the response to TSH.

HORMONE TRANSPORT AND METABOLISM:

Thyroid hormones in the blood are almost entirely bound to plasma proteins. They are bound to a globulin named thyroid binding globulin (TBG), thyroxine binding prealbumin and albumin. Affinity of thyroxine is maximum for thyroid binding globulin and hence TBG is the major determinant of binding. Affinity for T_3 and T_4 are slightly different because T_3 is not bound significantly by thyroxine binding prealbumin and binds to TBG less firmly than T_4. The levels of free T_3 are 8 to 10 times more than T_4. As only the free hormone is available to tissues, metabolic state correlates closely with the concentration of free thyroxine.

Thyroid hormones undergo metabolism mainly through sequential removal of iodine atoms (mono deiodination). Deiodination accounts for 70% of T_3 / T_4 disposal. In case of T_4, 5 mono deiodination yields T_3 and 30% of T_4 is converted to T_3 and virtually all metabolic actions of T_4 can be ascribed to T_3. Normally extra glandular T_3 accounts for 80% of total T_3. 40% of T_4 disposal is accounted by its conversion to reverse T_3 which has no metabolic function. The second major pathway of T_3/T_4 metabolism is conjugation with glucuronide in the livers which are de-iodinated in the liver and excreted in the bile. 20% of T_4 disposal occurs via fecal loss.

ACTIONS OF THYROID HORMONE:

1. Effects on Fetal Development

Thyroid hormones are critically important in fetal development particularly of the neural and skeletal systems. Thus, intrauterine hypothyroidism leads to cretinism.

2. Effects on Oxygen Consumption and Heat Production

Thyroid hormones increase O_2 consumption in all tissues except the brain, spleen, and testes. Compared to the effects of TSH on thyroid hormone secretion, most thyroid hormone actions on peripheral tissue are induced relatively slowly over a period of hours or days.

3. Cardiovascular Effects

Thyroid hormones have marked chronotropic and inotropic effects on the heart.

4. Sympathetic Effects

Many thyroid hormone effects, particularly on the cardiovascular system, are similar to those induced by catecholamines. Thyroid hormones increase the number of catecholamine receptors in heart muscle cells. Post receptor actions of catecholamines are also amplified by thyroid hormones.

5. Hematopoietic Effects

Thyroid hormones increases erythropoiesis and also increases 2,3 bisphosophoglycerate concentrations in erythrocytes.

6. Endocrine Effects

Thyroid hormones generally increase the metabolism and clearance of various hormones and pharmacologic agents.

THYROID FUNCTION TESTS:

Thyroid function tests can be classified as:

1. Bio-Chemical studies:

- Those that measure concentration of hormones or biologically inactive products secreted by the thyroid - Serum total T_4 & T3 and Free T_4 & T3 concentration, serum Thyroglobulin.
- Those that test effects of thyroid hormones on peripheral tissues.

  1. Serum Lipids: Serum Cholesterol, Triglycerides and LDH levels are increased in primary hypothyroidism.

  2. Serum Enzymes: The levels of CPK (MM), LDH, AST are increased in hypothyroidism, decreased in hyperthyroidism.
- Those that evaluate hypothalamic-pituitary-thyroid axis - measurement of TSH, TRH stimulation test, T3 suppression test.

2. Thyroid Imaging Studies:

- Radioactive Iodine (I^{123}) uptake radionuclide scan.

- Technicium 99mm Pertechnate scan

- Ultrasound

3. Invasive Studies:

- Aspiration Cytology: Fine Needle Aspiration Cytology (FNAC)

4. Immunological Tests:

- Thyroid auto antibodies

- Thyroperoxidase antibodies (TPO Ab)

- Antithyroglobulin (Tg Ab)

HYPOTHYROIDISM:

DEFINITION:

Deficiency of thyroid hormones results in hypothyroidism. It is called primary when caused due to pathology of thyroid gland and secondary, when the pituitary or the hypothalamus is the cause. It can affect people of both sexes and all ages. The term myxoedema denotes severe hypothyroidism in which there is accumulation of hydrophilic mucopolysaccharides in the ground substance of the dermis and other tissues leading to thickening and doughy indurations of skin.

ETIOLOGY:

1. **Primary causes:** Iodine deficiency states, surgical or radio ablation of thyroid as a treatment for Grave's disease, Idiopathic, or it may coexist in diabetes mellitus, CRF, autoimmune diseases like SLE, Rheumatoid arthritis, etc.
2. **Secondary causes:** It is caused by pituitary or hypothalamic disorders like tumors, hemorrhages, granulomatous (tuberculosis, histiocytosis) and auto immune (lymphocytic hypophysitis) disease.

CLINICAL FEATURES:

Diminished sweating, slow movement, hoarseness of voice, delayed ankle reflex, paraesthesia, coarse skin, dry skin, periorbital puffiness, constipation, cold intolerance, impairment of hearing, weight increase.

DIAGNOSIS:

Measurement of TSH, T3 and T4 – primary hypothyroidism has high TSH and Low T3 and T4 and secondary hypothyroidism has low TSH, T3 and T4.

TREATMENT:

Thyroxine as Levothyroxine sodium is the therapy of choice.

HYPERTHYROIDISM:

The term hyperthyroidism is reserved for disorders that result from over production of hormone by the thyroid gland itself, of which graves disease is the most common.

ETIOLOGY:

1. **Primary causes:** Graves' disease, Toxic multinodular goiter, Toxic adenoma, Iodine - induced .
2. **Secondary causes:** Trophoblastic tumor, increased TSH secretion due to pituitary cause

CLINICAL MANIFESTATIONS:

- They depend on the severity and the duration of the disease, the age of the patient, presence or absence of extra thyroidal manifestations and the specific disorder producing the thyrotoxicosis.
- Symptoms are tremors, excessive sweating, oncholysis, exophthalmos, restlessness, anxiety, syncope, tachycardia, signs of heart failure, dyspnoea, diarrhoea, menstrual irregularities, anemia.

DIAGNOSIS:

Measurement of TSH, T3 and T4

TREATMENT:

Medical treatment using anti thyroid drugs, radio ablation or surgical removal of thyroid.

EFFECTS OF THYROID HORMONES ON RENAL DEVELOPMENT[16]:

Thyroid hormones influence protein synthesis and cell growth. Thyroid hormone status affects the functional renal mass which is measured as the kidney to body mass ratio. Hypothyroidism reduces this ratio and hyperthyroidism increases the ratio. Thyroid hormones influence the neonatal renal function. Perinatal thyroid hormone status affects the mitochondrial energy metabolism enzymes in the cells of proximal convoluted tubules (PCT). There is an increase activity of the Na-P cotransporter, Na-H exchanger and Na/K ATPase in the PCT.

EFFECTS OF THYROID HORMONE ON RENAL PHYSIOLOGY[16]:

Thyroid function influences water and electrolyte balance on different compartments of the body. The kidney also plays a role on the regulation of metabolism and elimination of TH and is an important target organ for TH actions. The decrease in the activity of TH is accompanied by an inability to excrete an oral water overload. This effect is not due to an incomplete suppression of vasopressin production, or a decrease in the reabsorptive ability in the dilutor segment of the kidney tubule, but rather to a reduction in the glomerular filtration rate (GFR).

TH have a hold upon tubular transport of sodium, via their actions on the sodium–potassium ATP pump (Na/K ATPase) and on the potassium permeability in the membrane of proximal tubules. In fact, tubular reabsorption of Na per gram of kidney tissue in rats was the lowest in thyroidectomized rats than in controls and was accompanied by a similar reduction of the specific activity of the Na-K ATPase pump. On the contrary, that activity increased when the reabsorption of Na increased in euthyroid rats treated with tri-iodothyronine (T3). As it occurs with Na, the reduction of TH activity at kidney level is accompanied by a decrease in the absorption of calcium at tubular level without affecting magnesium.

TH stimulate renin release by the juxta-glomerular cells through a mechanism independent of the ouabain - sensitive sodium pump and protein synthesis and influence kidney angiotensinase activity. T3 is also involved in sulfate homeostasis through the regulation of kidney sodium-sulfate cotransporter, NaS-1, a protein entailed in the control of serum sulfate levels. Finally, different studies in animals have shown that TH act on the regulation of kidney dopaminergic system.

EFFECTS OF HYPOTHYROIDISM ON KIDNEY[16]:

- The renal blood flow (RBF) is reduced by decreased cardiac output by negative chronotropic and ionotropic effects.
- Increased peripheral vascular resistance and intra-renal vasoconstriction
- Reduced renal response to vasodilators
- Reduced expression of renal vasodilators like VGEF (vascular endothelial growth factor) and IGF-1(insulin like growth factor)

- GFR is reversibly reduced due to decreased sensitivity to beta adrenergic stimulus, decreased renin release and impaired RAAS activity.
- Reduced proximal tubular absorption of sodium, chloride and water.
- Reduced tubular transport capacity with decrease in Na-K ATPase activity.
- Impaired urine concentrating ability of kidney
- Increased free water retention due to increase in vasopressin sensitivity of collecting ducts.

EFFECTS OF HYPERTHYROIDISM ON KIDNEYS[17,18]:

- Intra glomerular hypertension and causing hyperfiltration
- Causes proteinuria which predisposes to direct renal injury
- Increased mitochondrial energy metabolism and down regulation of superoxide dismutase increases free radical generation and leading to kidney injury
- Contributes to anemia in CKD patients

EFFECT OF CHRONIC KIDNEY DISEASE ON THYROID[6,16]:

1. CKD affects both hypothalamus–pituitary–thyroid axis and TH peripheral metabolism. Uremic patients have an increased thyroid volume compared with subjects with normal renal function and a higher prevalence of goiter, mainly in women.

2. Serum TSH concentrations are usually normal or elevated in CKD, but its response to its releasing hormone (TRH) is generally low. These findings suggest the presence of intra-thyroidal and pituitary disturbances associated with uremia. Also, both TSH circadian rhythm and TSH glycosylation are altered in CKD. The latter may compromise TSH bioactivity.

3. Free and total T3 and T4 concentrations are usually normal or low in patients with CKD.

4. The reduction in T3 levels (low T3 syndrome) is the most frequently observed thyroid alteration in these patients. This reduction in T3 concentrations has been linked to:

    - Chronic metabolic acidosis and chronic protein malnutrition which affects iodothyronine deiodination and protein binding of T3, decreasing the peripheral synthesis of T3 from T4.

    - Tumor necrosis factor-α (TNF- α) and interleukin – 1 inhibit expression of type 1 5′ deiodinase which is responsible for peripheral conversion of T4 to T3

    - Impaired renal handling of iodine increases serum iodine levels, causing prolonged Wolf-Chaikoff effect.

5. Low T4 level also is seen in CKD. This is called sick euthyroid state in CKD which is called "Non Thyroid Illness (NTI)". But compared to other NTI , CKD patients show no increase in rT3

6. In CKD patients, the ESRD is characterized by the absence of total rT3 rising, a typical feature in other patients with non-thyroidal disease. Despite the fact

that the total rT3 clearance in CKD patients is diminished, there is a redistribution of rT3 from the vascular to the extravascular space and an increase in rT3 cellular uptake. However, free rT3 concentrations are high due to a reduction in its renal clearance.

7. CKD is associated with a higher prevalence of primary hypothyroidism, both overt and subclinical, but not with hyperthyroidism. In fact, the prevalence of primary hypothyroidism, mainly in the subclinical form, increases as GFR decreases. A recent study has shown a prevalence of subclinical hypothyroidism of 7% in patients with estimated GFR < 90 ml/min per 1.73 m2 that increased to 17.9% in subjects with GFR < 60 ml/min per 1.73 m2. The prevalence of hypothyroidism is higher in women and is associated with an increased frequency of high titers of anti-thyroid antibodies.

8. A greater prevalence of non-autoimmune primary hypothyroidism has been reported in patients with advanced diabetic nephropathy under conservative treatment in comparison with non-diabetic patients with nephropathy.

9. The prevalence of hyperthyroidism in CKD is similar to that found in general population in areas. Moreover, hyperthyroidism has been considered as one of the many causes of anemia resistant to recombinant human erythropoietin (rh-EPO) in CKD patients on HD with an adequate response to antithyroid treatment.

PLASMA LIPOPROTEINS[19]:

Blood contains triglycerides, cholesterol and cholesterol esters at concentrations that far exceed their solubility in water. Blood lipids are kept in solution or at least thoroughly dispersed in the circulation by virtue of being incorporated into macromolecular structures called lipoproteins.

STRUCTURE OF LIPOPROTEINS:

The lipoproteins are spherical particles in which the most hydrophobic lipids such as cholesterol esters and triacylglycerols are located in the core of the structure, sequestered away from water, whereas free (non-esterified) cholesterol, phospholipids

and proteins are arrayed on the surface. All plasma lipoproteins contain one or more of surface proteins called apoproteins. It is mainly the amphipathic phospholipids and proteins that keep highly insoluble lipids like cholesterol and triacylglycerols in solution.

FUNCTIONS OF LIPOPROTEINS[19]:

1) The plasma lipoproteins are the vehicles by which cholesterol, cholesterol esters and triacylglycerols are transported from one tissue to another in the body.

2) The plasma lipoproteins facilitate lipid metabolism by acting as substrate for lipid metabolising enzymes in blood.

3) The apolipoproteins on the surface of the particles also serve as structural components, ligands for cell receptors and cofactors for enzymes involved in lipoprotein metabolism.

CLASSIFICATION OF LIPOPROTEINS[20]:

Lipoproteins are mainly classified into five groups according to their density on ultracentrifugation and their mobility on agarose gel electrophoresis.

- Chylomicrons
- Very Low Density Lipoproteins (VLDL)
- Low Density Lipoproteins (LDL)
- Intermediate Density Lipoproteins (IDL)
- High Density Lipoproteins (HDL)

Variable	Chylomicron	VLDL	IDL	LDL	HDL	Lp (a)
Density (g/ml)	< 0.95	0.95-1.006	1.006-1.019	1.019-1.063	1.063-1.210	1.040-1.130
Electrophoretic mobility	Origin	Pre-beta	Between pre-beta and beta	Beta	Alpha	Pre-beta
Molecular weight (Da)	$0.4\text{-}30x10^9$	$5\text{-}10x10^6$	$3.9\text{-}4.8x10^6$	$2.75x\ 10^6$	$1.8\text{-}3.6x10^5$	$2.9\text{-}3.7x10^6$
Diameter (nm)	> 70	26-70	22-24	19-23	4-10	26-30
Lipid-lipo protein ratio	99:1	90:10	85:15	80:20	50:50	75:26 -64:36
Major lipids	Exogenous triglycerides	Endo-genous TG	Endo-genous TG and Cholesteryl esters (CE)	CE	Phospho-lipids (PL)	CE PL
Major proteins	A-I B-48 C-I C-II C-III	B-100 C-I C-II C-III E	B-100 E	B-100	A-I A-II	(a) B-100

TABLE 2 – CHARACTERISTICS OF HUMAN LIPOPROTEINS

LIPOPROTEIN METABOLISM:

CHYLOMICRONS:

Structure: Chylomicrons contain mainly triglyceride along with cholesterol, small amounts of phospholipids, and specific apoproteins (apo B-48, A-I, A-II, C-I, C-II, C-III with small amounts of apo B and E-II, E-III, E-IV). The neutral lipids triglycerides and cholesteryl esters are partially surrounded by an outer shell of phospholipids, free cholesterol and protein. Under fasting conditions chylomicrons are generally absent in the blood of healthy persons. The presence of chylomicrons makes the serum appear turbid or milky[21].

Metabolism:

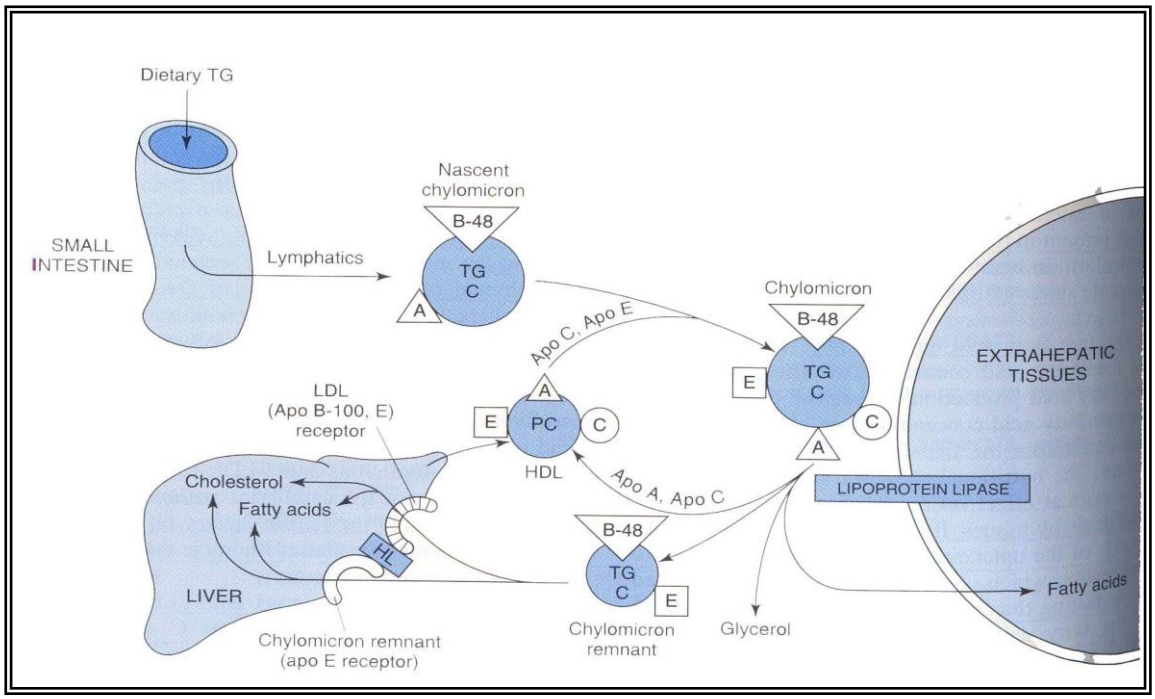


FIG 3: Metabolic fate of chylomicrons[22] (A: apolipoprotein A; B-48: apolipoprotein B-48; C: apolipoproteinC; E: apolipoprotein E; HDLc: High Density Lipoprotein; TG: triacylglycerol; C: cholesterol and cholesteryl ester; P: phospholipids, HL: Hepatic Lipase).

Chylomicrons are the vehicle for the transport of dietary lipids in the plasma. These are synthesized within the enterocytes from packing of fat droplets containing (Triglycerides, cholesterol ester and phospholipids) with a number of apolipoproteins

including apo B-48, apo A-I, apo A-II and apo A- IV. The nascent chylomicrons are then released into the circulation via the lymphatic system. The nascent chylomicrons acquire apo E, apo C and additional cholesterol from HDL-2 in exchange for apo A-I, apo A-II and phospholipids in circulation. This transaction with HDL is necessary for subsequent lipolysis of chylomicrons by lipoprotein lipase (LPL) because apo E is necessary for chylomicron binding to endothelial surface and apo C-II is required for activation of LPL. In the perfusing muscle and adipose tissues, mature chylomicrons form a transient binding to the endothelial surface via their constituent apo E leading to the activation of endothelium bound LPL by apo C-II. Triglyceride content of chylomicrons is hydrolyzed by LPL with release of free fatty acid. These free fatty acids are taken up by myocytes for energy production or by adipocytes for energy storage. The remaining free fatty acids are carried to distant sites by albumin and various lipoproteins. Chylomicron remnants thus formed returns the borrowed apo C and apo E to HDL before their eventual removal by the liver and other tissues through LDL receptor. The remnants are immediately internalized by receptor mediated endocytosis and degraded in hepatic lysosomes[21,23].

VERY LOW DENSITY LIPOPROTEIN (VLDL):

Structure: VLDL contains 52% triglyceride, 18% phospholipids, 22% cholesterol and about 8% protein. Cholesterol and cholesteryl esters occur in a ratio of about 1:1 by weight. The major phospholipids being sphingomyelin and phosphotidylcholine. The larger the size of a VLDL particle greater the proportion of triglycerides and apoC and smaller the portion of phospholipids, apo B and other apoproteins. Apo B is present constantly in all VLDL fractions. Apo B-100 accounts for approximately 30% to 35% with apo C-I, apo C-II and C-III making up over 50% of the apoprotein content in the VLDL.

Apo E-II, E-III and E-IV and varying quantities of other apoproteins (A-I, A-II, B-48) may also be present[21].

Metabolism:

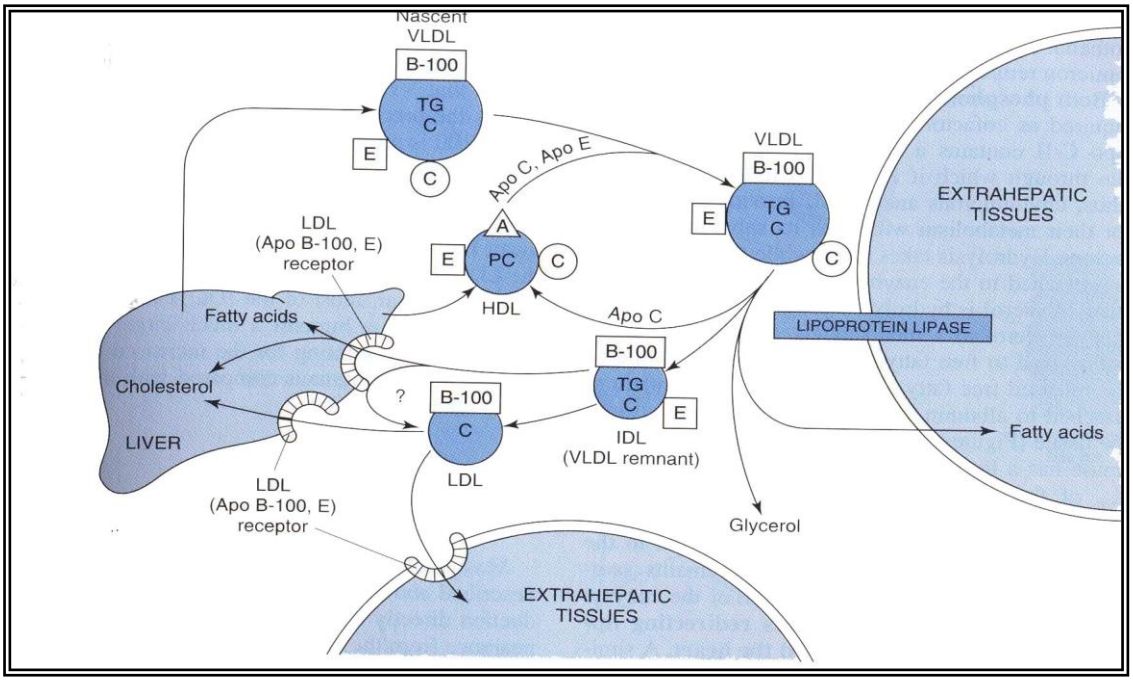


FIG 4: Metabolic fate of Very Low Density Lipoproteins (VLDL)[22] and production of Low Density Lipoprotein (LDL).
A: Apolipoprotein A; B-100: apolipoprotein B-100; C: apolipoprotein C; E: apolipoprotein E; HDL: High Density Lipoprotein; TG: Triacylglycerol; IDL: Intermediate Density Lipoprotein; C: Cholesterol and Cholesteryl ester, P: phospholipids.

After the postprandial rise in chylomicron-TG, a secondary rise in triglyceride levels occurs 4-6 hours after a meal. This represents predominantly hepatic VLDL-TG synthesized from glucose and chylomicron-TG not hydrolyzed in peripheral tissues.

VLDL particles are synthesized by the liver and serve as the vehicle for delivery of endogenous lipids to the peripheral tissues. Nascent VLDL is formed within the hepatocytes from the fusion of apolipoproteins like apo B-100, apo E, apo A-I and apo A-II with triglycerides rich lipid droplet. In the hepatocytes, the enzymes such as acyl-CoA diacylglycerol acyltransferase (DGAT) and acyl CoA cholesterol acyl transferase (ACAT) serve as the sources for triglycerides and

cholesterol ester respectively. The fatty acids and cholesterol supplies are derived from a combination of de novo synthesis and uptake from the circulating blood.

When nascent VLDL reaches circulation, acquires apo C and apo E from HDL-2 in exchange for apo A-I, apo A-II and phospholipids. When VLDL reaches the capillary beds of muscle and adipose tissue it binds to the endothelial surface via apo E. This leads to activation of LPL which hydrolyzes the triglyceride content of VLDL with release of two free fatty acids. These fatty acids are utilized by adjacent myocytes or adipose tissue for energy production or storage respectively. Remaining fatty acids transported to liver and other tissue by binding to albumin and lipoproteins. Lipolysis of VLDL by LPL results in a 70% reduction in their triglyceride content and detachment and release of a remnant particle commonly known as IDL[21,23].

LOW DENSITY LIPOPROTEIN (LDL):

Structure: LDL contains by weight 80% lipid and 20% protein. LDL is smaller (21 to 25nm) and is of higher hydrated density (1.006 to 1.63g/ml) than VLDL and chylomicrons. About 50% of LDL lipid is cholesterol. LDL constitutes 40% to 50% of the plasma lipoprotein mass in humans. Apo B-100 is the major apoprotein of normal LDL and represents 90% to 95% of the total plasma apo B-100. Based on the floatation density LDL is frequently separated into two classes. LDL_1 (or intermediate density lipoprotein IDL) and LDL_2. IDL (1.006 to 1.109 g/ml) is more lipid rich than LDL_2 (1.109 to 1.063g/ml) and probably represents an intermediate in VLDL catabolism. Thus a comparison of IDL with LDL_2 demonstrates the gradual disappearance of triglyceride and of apoproteins more characteristic of VLDL (apo C and apo E) and an enrichment with apo-B 100 and cholesterol ester[21].

Metabolism :

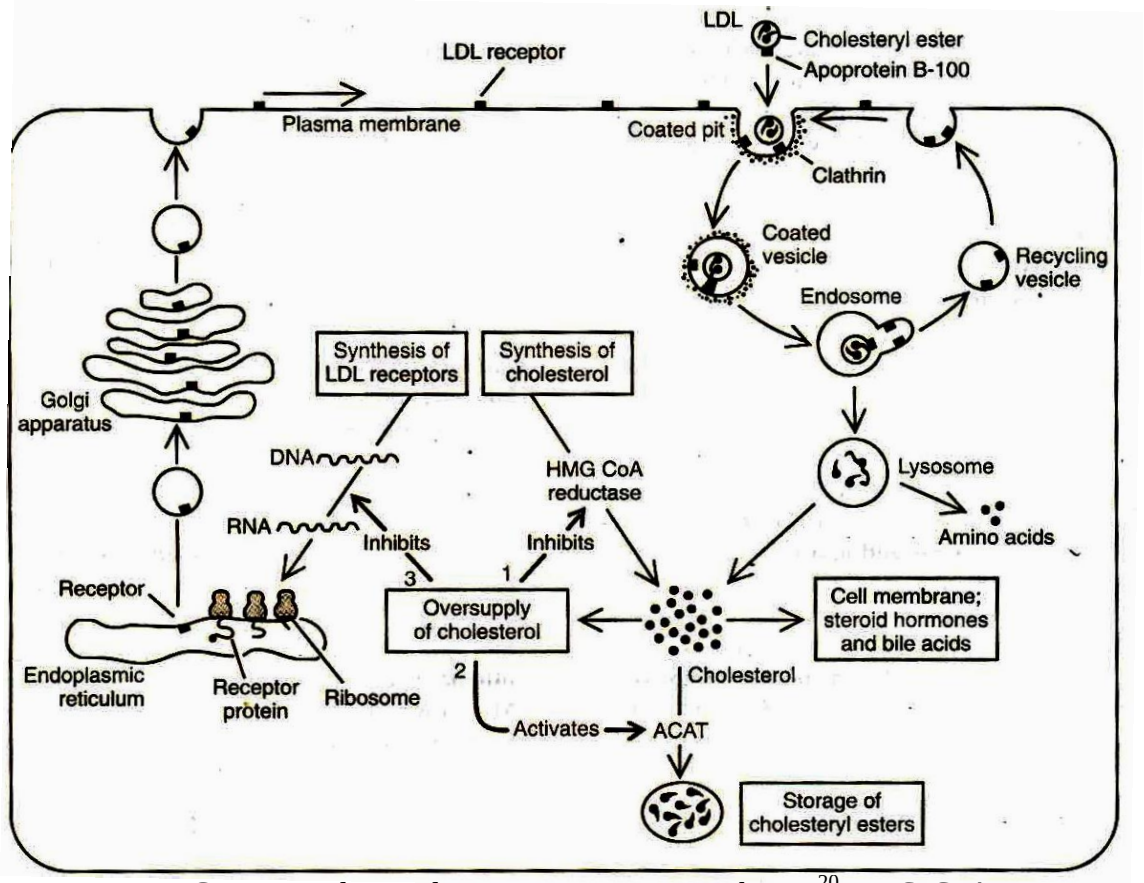


FIG 5 : Low density lipoprotein receptor pathway.[20] HMG-CoA: 3-hydroxy 3-methyl glutaryl coenzyme A; ACAT: acyl CoA cholesterol acyl transferase

LDL formation occurs during the catabolism of VLDL. Function of LDL is to deliver cholesterol to extrahepatic tissues where it is utilized, deposited or excreted. In the peripheral tissues LDL binds to high affinity receptors located in regions of the plasma membrane called coated pits. These pits invaginate into the cell and pinch off to form endocytic vesicle and later fuses with lysosome to form endosome. Because of the acidic milieu of the endosome, LDL dissociates from the receptor which returns to the cell surface for reuse whereas LDL migrates with the lysosome. In the lysosome, apo B-100 is degraded to small peptides and amino acids. Cholesterol esters are also hydrolyzed which is utilized for the synthesis of cell membranes, steroid hormones in adrenal glands and bile acids in hepatocytes.

Cells have the ability to regulate their cholesterol content. Excess cholesterol activates the enzyme ACAT leading to intracellular cholesteryl ester storage. The inhibition of HMG-CoA reductase and suppression of the LDL receptor may occur through the interaction of the cholesterol derivative, hydroxyl cholesterol with the regulatory portions of the respective genes.

LDL is sometimes degraded by less efficient mechanisms at high plasma levels to achieve significant rates of removal. One of these mechanisms includes degradation by macrophages of the reticulo-endothelial system. When overloaded with cholesteryl esters, these macrophages get converted into foam cells which are classic components of atherogenic plaques. In humans, estimates of the proportion of plasma LDL degraded by the LDL receptor system range from 33% to 66%. The remainder is degraded by the scavenger cell system and perhaps by other mechanisms not yet elucidated[19,23].

INTERMEDIATE DENSITY LIPOPROTEIN (IDL)[21]:

IDL is a transient particle (22 to 28nm), which are produced during VLDL catabolism. These are usually present in very low concentrations in plasma from fasting persons. In the plasma LCAT (Lecithin cholesterol Acyltransferase), esterifies the excess HDL free cholesterol with fatty acid derived from the carbon-2 position of lecithin, the major phospholipid of plasma. With the help of a plasma cholesteryl ester transfer protein (CETP), the newly synthesized cholesteryl ester is transferred back to IDL particles from HDL. This results in the replacement of most of the original TG core of VLDL with cholesteryl ester. In the circulation IDL particles undergo a further conversion where most of the remaining TG are removed and all the apoproteins except apo B are lost. The resultant particle is LDL which contains almost pure

cholesteryl ester in the core and apo B at the surface.

HIGH DENSITY LIPOPROTEIN[21]:

Structure: The HDL macromolecular complex contains approximately 50% protein and 50% lipid. HDL is the smallest of the lipoproteins (9 to 12nm) and with the highest density (1.063 to 1.21gm/ml). HDL contains high phospholipids, major species being phosphotidyl choline (also known as lecithin) which accounts for 70 to 80% of total phospholipid. It has an important functional role as a reactant in plasma cholesterol esterification which is catalyzed by the enzyme LCAT.

HDL may be further sub-fractionated by differential ultracentrifugation into HDL_2 (with a density of 1.063 to 1.110g/ml) and HDL_3 (1.110 to 1.21g/ml).

Metabolism:

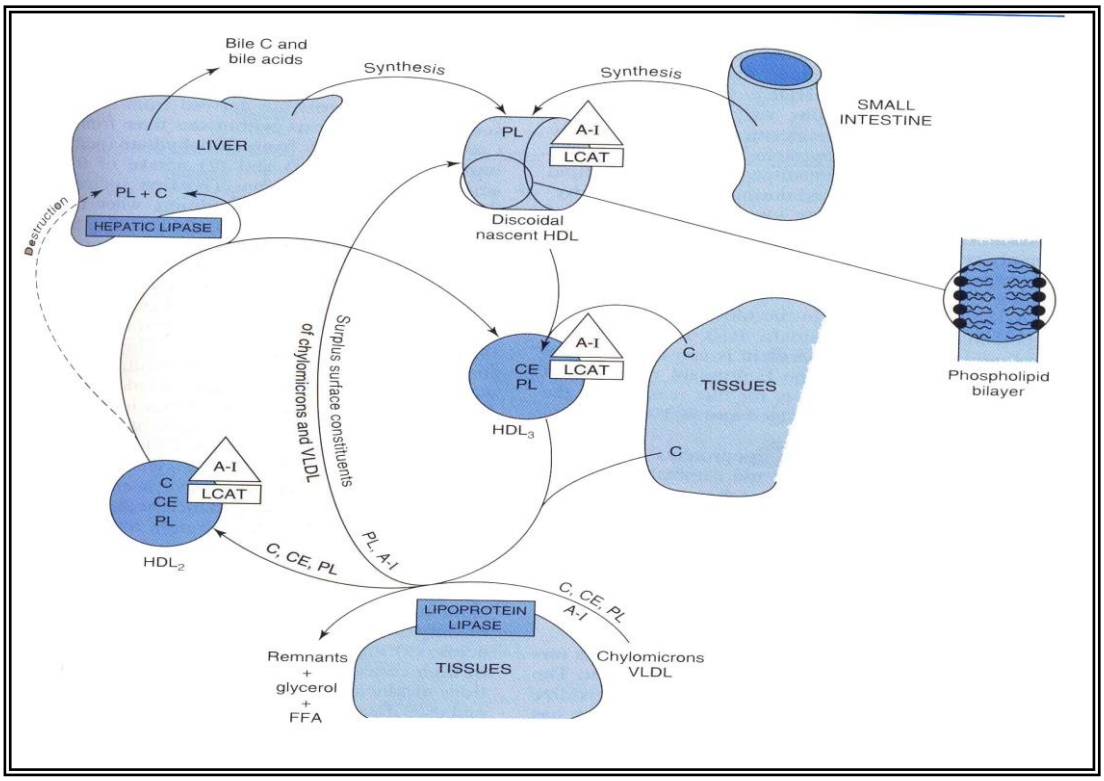


FIG 6: Metabolism of high density Lipoprotein (HDL) in reverse cholesterol transport[22] (LCAT: Lecithin cholesterol acyltransferase, LPL: Lipoprotein Lipase, C: Cholesterol, CE: Cholesteryl ester, PL: Phospholipid, A-I: apolipoprotein A-I). The figure illustrates the role of the three enzymes hepatic lipase, LCAT and lipoprotein lipase in HDL cycle for the transport of cholesterol from the tissues to the liver (Reverse cholesterol transport).

Intestinal mucosal cells and hepatocytes synthesize nascent HDL molecule by incorporating lipids and apolipoproteins. During the synthetic process, phospholipid and free cholesterol after combining with specific apoproteins form disc like structures that undergo extensive compositional and structural modifications after secretion. The most important of these modifications is the esterification of free cholesterol to form cholesteryl ester by an enzymatic reaction catalyzed by LCAT.

In humans this is the major source of plasma cholesteryl ester. Persons with LCAT deficiency have an accumulation of these cholesteryl ester deficient particles in plasma. This finding possibly indicates that the expansion of the disc like structures to form spheres characteristic of normal plasma HDL. Cholesteryl ester thus formed may be transferred to VLDL during catabolism.

The apoproteins of nascent HDL is modified along with change in lipid content. Nascent HDL mainly contains apo E unlike the plasma HDL, which contains mainly apo A with minor contributions by apo C and apo E. The functional significance of this modification is not completely understood, but apo A-I is an activator of LCAT. In addition HDL participates in the regulation of triglyceride catabolism and cholesteryl ester formation by providing the respective cofactors, apo C-II for activation and apo C-III for inhibition of lipoprotein lipase activity.

Also normal HDL may balance LDL transport by mediated cholesterol removal from peripheral sites to degradative and excretory sites. This role of HDL in reverse cholesterol transport may be the basis for the protection afforded by HDL against cardiovascular disease.

MECHANISMS OF CRF INDUCED DYSLIPIDEMIA:

Number of studies has been done to know the features and mechanisms of CRF induced dyslipidemia. Recent studies were conducted to elucidate the molecular mechanisms of CRF induced dyslipidemia[23].

I. Abnormalities in Triglyceride metabolism[24]: Hypertriglyceridemia is the most common lipid abnormality which occurs in 50% to 75% of patients with chronic renal insufficiency.

The mechanisms responsible for the hypertriglyceridemia include:

- Reduced catabolism of the triglyceride rich lipoprotein is the predominant defect.
- Decrease in the activity and deficiency of lipoprotein lipase, hepatic lipase or both these enzymes. The reason for the decrease in enzyme activity is unclear. Possibilities include the presence of a circulating inhibitor of lipolytic enzymes in the uremic serum leads to deficiency of enzymes LPL and hepatic lipase. The changes in apoprotein concentrations leads to decreased activity of LPL and hepatic lipase.

II. Abnormalities in cholesterol metabolism[23]: Plasma total cholesterol is usually normal or elevated in patients with CRF. Renal insufficiency along with heavy proteinuria leads to post transcriptional upregulation of HMG-CoA reductase which is a rate limiting enzyme in cholesterol synthesis and decrease in LDL receptors, which plays an important role in the genesis of hypercholesterolemia.

III. Abnormalities in HDL Metabolism[23]: CRF is consistently associated with reduced plasma HDL cholesterol concentration. It is primarily due to CRF induced dysregulation of several important proteins like,

a) LCAT: Plasma LCAT activity is diminished due to reduction in hepatic production and inhibition by unknown uremic toxin leading to increased plasma free cholesterol and marked decrease in esterified cholesterol.

b) CETP: CETP mediates transfer of cholesterol ester from HDL to IDL in exchange for triglycerides. Plasma CETP levels are increased in CRF resulting in reduction in HDL cholesterol ester and elevation of HDL triglycerides.

c) HEPATIC LIPASE: CRF results in pronounced hepatic lipase deficiency. Thus hepatic lipase can potentially contribute to increased HDL triglyceride content.

d) SRB-1 (Hepatic HDL receptor): Hepatic SRB-1 is the primary pathway for disposal of HDL-borne cholesterol ester and triglycerides. Heavy glomerular proteinuria has been shown to significantly reduce hepatic SRB-1 protein expression.

e) ACAT : ACAT is the main enzyme for intracellular esterification of cholesterol. CRF has been recently shown to increase hepatic ACAT-2 mRNA as well as total ACAT activity.

IV. Abnormalities in VLDL Metabolism:

CRF is associated with impaired clearance of VLDL and chylomicrons and accumulation of their atherogenic remnants. These abnormalities are primarily caused by dysregulation of LPL, hepatic lipase, VLDL receptor, hepatic ACAT and LRP (LDL receptor related protein) expressions/activities as well as impaired HDL metabolism.

V. Apolipoproteins in chronic renal failure:

Apo A-1 and apo A-II are significantly reduced in early renal insufficiency and more marked in advanced renal failure. Apo B levels are often normal in early renal insufficiency and variably altered in advanced renal insufficiency and those on

dialysis. These changes result in a reduced ratio of apo A to apo B, which is considered to represent an antiatherogenic index.

The most characteristic features of apolipoprotein profile are an early and marked elevation of apo C-III levels. There is decreased apo C-II to apo C-III ratio. Apo E concentrations are within normal range in predialytic patients, but tend to be moderately increased in hemodialysis patients[25].

LIPID ABNORMALITIES IN PERITONEAL AND HEMODIALYSIS PATIENTS:

Dyslipidemia in CRF is often related to the type of dialysis.

Hemodialysis:

Hemodialysis patients often have normal or near normal levels of total cholesterol and LDL-C. Approximately 20 to 40% of hemodialysis patients have been estimated to have elevated triglycerides and reduced HDL-C. In addition, hemodialysis patients have increased oxidized LDL levels and increased Lp (a).

Peritoneal dialysis:

Peritoneal dialysis seems to be associated with a relatively more atherogenic lipid profile than hemodialysis. About 20 to 40% of peritoneal dialysis patients have been shown to have elevated total cholesterol and LDL-C and 25 to 50% of patients have been reported to have elevated triglycerides and apo B and HDL-C. In addition, increased oxidized LDL levels and increased Lp (a) levels have been reported in peritoneal dialysis patients[26].

HYPERLIPIDEMIA AND PROGRESSION OF RENAL DISEASE:

Lipids play a causative role in progressive glomerular damage. Mesangial cells resemble modified smooth muscle cells and can accumulate lipid material. They show specific binding and uptake of LDL and proliferate in response to LDL. These mesangial cells have been shown to have receptors for oxidized LDL which is more toxic form and is thought to be involved in the production of atherosclerosis[27].

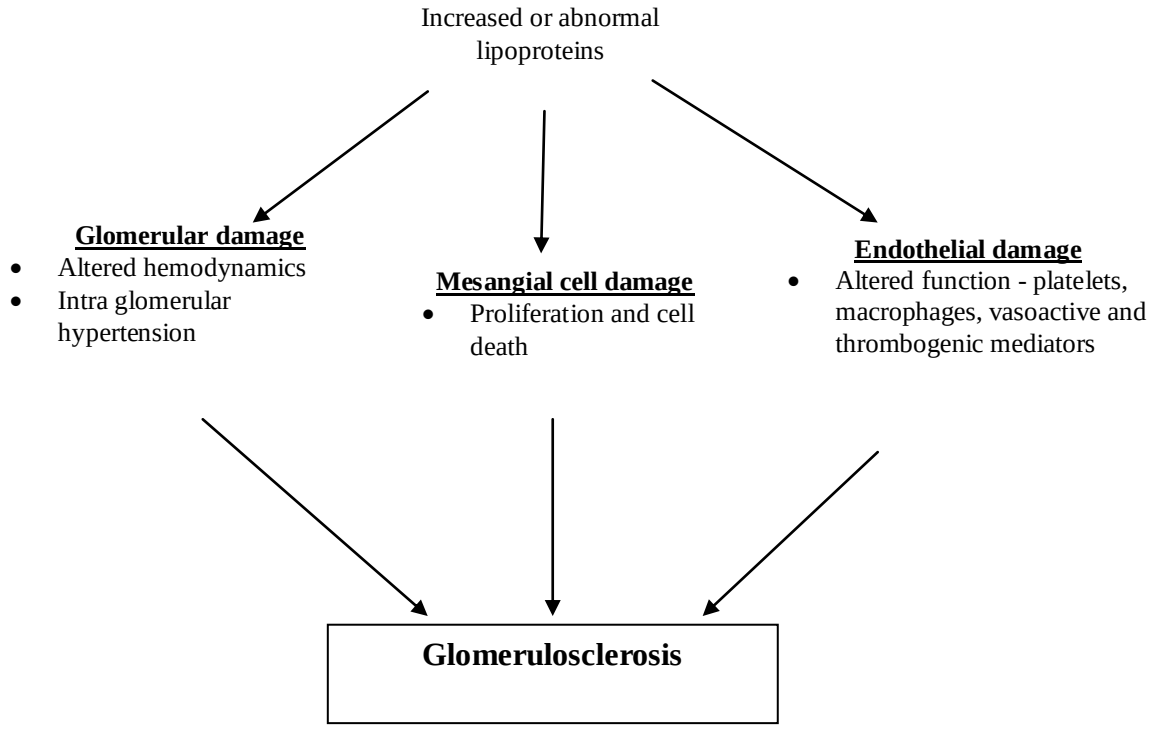


FIG 7 : POTENTIAL MECHANISM OF LIPID – INDUCED RENAL DAMAGE[27].

ALBUMIN[28]:

HISTORY:

The name albumin evolved from the more general term, albumen, the early German word for protein. The Greek physician Hippocrates noted in his Aphorisms that foamy urine, in all likelihood caused by the presence of albumin, indicates chronic kidney disease. The French physiologist, C. Denis, in 1840 performed the first recorded dialysis by placing blood serum in a sac of intestine immersed on water; he found that some of the protein precipitated as the salt was removed through the sac. Unlike the action of heat, this precipitation was reversible when small amounts of salt were added. The protein soluble in water without salt was called albumin and that which precipitated in little globules, globulin.

CHEMISTRY[29]:

The peptide sequence of human albumin was known before its cDNA sequence. The complete sequence was reported by Brown in Austin, Texas and Meloun in Prague. Albumin is the major constituent (60%) of plasma proteins with a concentration of 3.5-5.0 g/dl. Human albumin has a molecular weight of 69,000 daltons and consists of a single polypeptide chain of 585 amino acids with 17 disulfide bonds.

SYNTHESIS[29]:

Albumin is exclusively synthesized by the liver. For this reason, measurement of serum albumin concentration is conveniently used to assess liver function. Liver produces about 12g of albumin per day which represents 25% of total hepatic protein synthesis. Albumin has a half-life of 20 days.

FUNCTIONS OF ALBUMIN[29]:

1. **Osmotic function:** Due to its high concentration and low molecular weight, albumin contributes to 75-80 % of the total plasma osmotic pressure (25 mm Hg). Thus, albumin plays a predominant role in maintaining blood volume and body fluid distribution. Decrease in plasma albumin level results in a fall in osmotic pressure, leading to enhanced fluid retention in tissue spaces causing edema.

2. **Transport functions:** Plasma albumin binds to several biochemically important compounds and transports them in circulation. These include free fatty acids, bilirubin, steroid hormones, calcium and copper.

3. **Nutritive functions:** Albumin serves as a source of amino acids for tissue. protein synthesis to a limited extent, particularly in nutritional deprivation of amino acids.

4. **Buffering function:** Among the plasma proteins, albumin has the maximum buffering capacity.

EFFECTS OF CHRONIC KIDNEY DISEASE ON ALBUMIN LEVELS:

The renal glomeruli become slightly more permeable as renal disease progresses; this leakiness leads to slight increases in urinary albumin excretion. With decreasing glomerular filtration rate (GFR), kidney function diminishes and uremia results, with build up of toxic metabolites that affect liver function and ligand binding by the albumin molecule.

As the glomerular filtration rate falls and chronic renal failure sets in, proteins are no longer lost in large quantity in the urine, and albumin degradation in the proximal tubules subsides[30].

A buildup of secreted toxins depresses albumin synthesis by the liver and there is an increase in intravascular and extra vascular fluid volume, so the albumin level remains mildly subnormal. The responsible toxins is not identified but they act by destabilizing the albumin mRNA. Hence transcription is normal.

Toxic compounds affecting protein function, if not biosynthesis, have been widely sought in patients with renal failure. Because urea concentrations rise so markedly, carbamylation of amino groups by small amounts of cyanate produced from urea was first suspected. Aromatic compounds indoxyl and furanoic acids are highly active in depressing albumin-binding capacity for small anionic compounds. The likely culprit is a furan dicarboxylic acid, 3-carboxyl-4-methyl-5-propyl-2-furanpropanoic acid[31,32].

Cysteine is lost from disulphide bonds on therapy by hemodialysis causing increased formation of mercaptoalbumin after hemodialysis[33].

MARKERS OF CARDIOVASCULAR DAMAGE IN CKD:

- Decreased thyroid function is accompanied by reduced activity of HMG-CoA reductase, TC and LDL-C levels are increased in patients with overt hypothyroidism[34-38].
- This is due to the decreased LDL-receptor activity, resulting in decreased catabolism of LDL and IDL[39-41].
- Moreover, a decrease in LPL activity is found in overt hypothyroidism, decreasing the clearance of TG-rich lipoproteins[42].
- The above abnormalities of lipid metabolism associated with overt

hypothyroidism predispose to the development of atherosclerotic coronary artery disease (CAD)[43,44].

- Subjects with overt hypothyroidism also exhibit impaired endothelial function[45].

- As described earlier, dyslipidemia induced by chronic kidney disease will predispose the patients to increased risk of cardiovascular morbidity.

- Cardiovascular damage starts quite early in the time course of progressive CKD. CKD patients in pre-dialysis stage are at increased risk of CVD and its complications[46].

- There is association between CVD, malnutrition and inflammation in ESRD patients.

- Hypoalbuminemia, a marker of malnutrition and inflammation is a powerful predictor of mortality in patients with ESRD[47,48].

- In a study done in Spain, low albumin has been shown to predict morbidity and mortality in stage 3-5 CKD patients[49].

- Reason being appetite suppression and increased catabolism by inflammatory cytokines. Albumin being a scavenger of free radicals, hypoalbuminemia leads to decreased antioxidant activity favoring noxious effects on arterial wall[5,50].

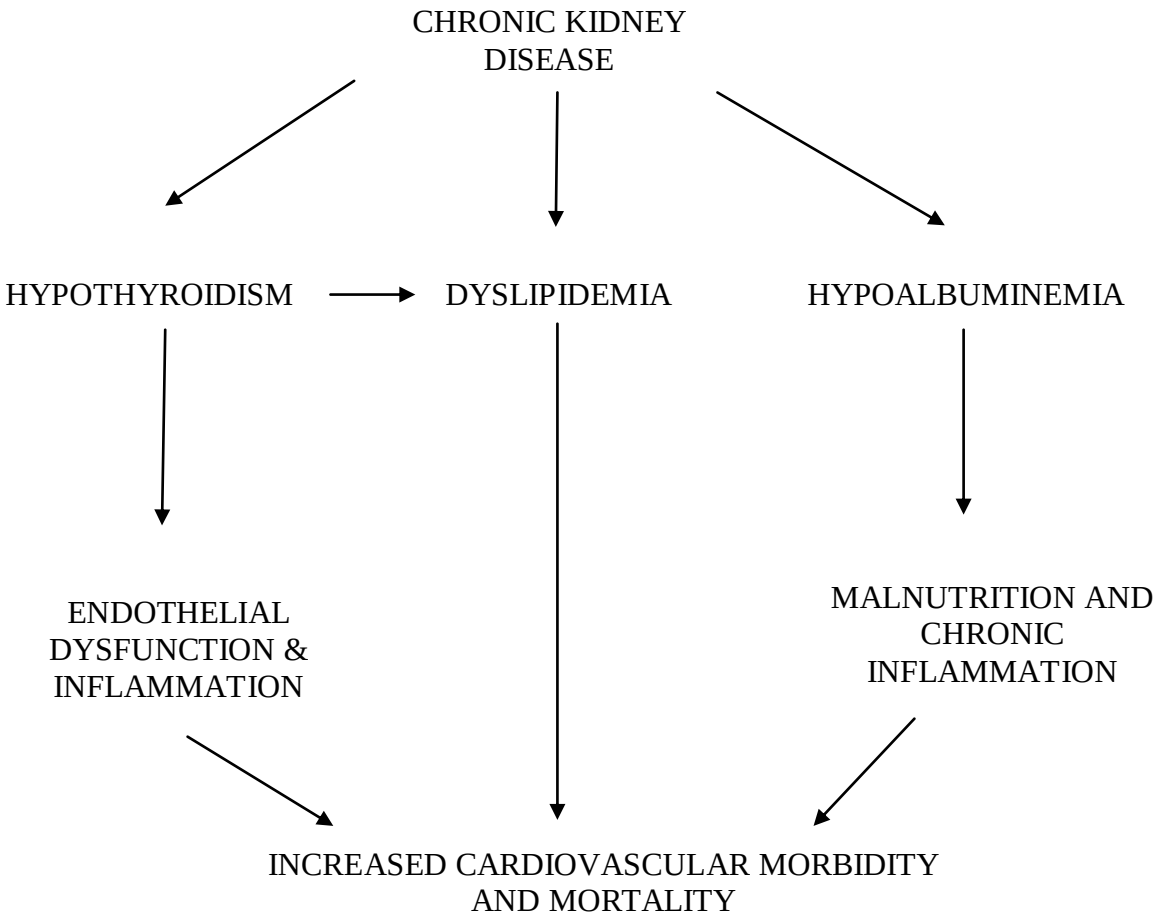


FIG 8: CVD INDUCED BY CKD

METHODOLOGY

MATERIAL & METHODS:

STUDY DESIGN: Cross sectional study

SOURCE OF DATA:

With the calculation taking Odd's ratio as 5 based on the mortality of CKD patients with CVD at 95% confidence interval and 80% power, sample size of 39 was determined. Hence the sample size is rounded off to 40 in each group.

STUDY GROUP: consist of 80 individuals.

Case group - 40 Cases of Chronic Kidney Disease.

Control group - 40 Normal individuals.

(I) **CASE GROUP**

Inclusion criteria:

1. 40 diagnosed cases of chronic kidney disease at different stages based on their eGFR (estimated glomerular filtration rate) from RL Jalappa Hospital and Research Center, Kolar.
2. Stage II, Stage III, Stage IV of chronic kidney disease
3. CKD due to causes like diabetes mellitus, hypertension, polycystic kidney disease, infections
4. Above the age of 18 years

Exclusion criteria:

1. Stage I and Stage V of chronic kidney disease
2. Acute renal failure patients
3. Diagnosed cases of CVD later developing CKD
4. Patients with hypoalbuminemia due to other causes like chronic liver diseases and malabsorption
5. Diagnosed cases of hypothyroidism/hyperthyroidism on treatment

6. Patients with altered lipid profile due to familial hypercholesterolemia

(II) **CONTROL GROUP**

1. Age and gender matched normal population, with no chronic kidney diseases.

2. Control group was screened for the serum urea and creatinine, if the values fell within the normal reference range, they were included as control.

(III) **METHOD OF COLLECTION OF DATA**:

1. After obtaining informed consent, 5ml of blood after 12 hours fasting from the study group and the control group was drawn under complete aseptic precautions.
2. Samples were collected before dialysis.

(IV) **PARAMETERS MEASURED**:

In the present study the following parameters were estimated by using the serum.

1. Blood glucose
2. Blood urea
3. Serum creatinine
4. Albumin
5. Thyroid stimulating hormone
6. Total T3
7. Total T4
8. Total cholesterol
9. Triglycerides
10. High density lipoprotein
11. Low density lipoprotein

- Lipid profile and albumin were analyzed by using semi-autoanalyser.

- Thyroid hormones were estimated using chemiluminescense method.
- eGFR was calculated using MDRD (Modification of Diet in Renal Disease) formula.

ESTIMATION OF BLOOD GLUCOSE:

METHOD: Glucose oxidase- peroxidase (GOD-POD) method[51,52].

PRINCIPLE:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts, under catalysis of peroxidase (POD), with phenol and 4-aminophenazone (4AAP) to form a red violet quinoneimine dye as indicator.

REAGENT:

- Enzyme reagent- Phosphate buffer 50 mmol/L, Phenol 15 mmol/L, 4AAP 2.5 mmol/L, GOD 18 KU/L, POD 2.5 KU/L
- Glucose standard- 100 mg/dl
- Preservative: Sodium azide (0.02%)

PROCEDURE:

	BLANK	**STANDARD**	**TEST**
SAMPLE	-	-	10µl
STANDARD(STD)	-	10µl	-
REAGENT	1000µl	1000µl	1000µl

Mix & incubate for 05min at 37^0 C or 15 min at room temperature (RT). Measure absorbance of sample (AT) and standard (AS) against reagent blank at 505 nm. The color is stable for 30 min at RT.

CALCULATION:

TOTAL GLUCOSE (mg/dl) = AT/AS X Concentration of standard (100 mg/dl)

LINEARITY: 400 mg/dl at 37^0 C

EXPECTED VALUES:

RANDOM BLOOD GLUCOSE: 75-140 mg/dl

FASTING BLOOD GLUCOSE: 75-126 mg/dl

POSTPRANDIAL BLOOD GLUCOSE: <140 mg/dl

ESTIMATION OF UREA:

METHOD: Glutamate dehydrogenase kinetic method (GLDH/KINETIC METHOD)[53].

PRINCIPLE:

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

$$NADH + NH_3 + \alpha\text{- Ketoglutarate} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD + H_2O$$

The rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD^+ is proportional to urea concentration in the sample

REAGENTS:

Reagent 1: Buffer- TRIS buffer 75mM/l, pH 7.55

Reagent 2: Enzymes

Urease - $\geq$ 30000 U/l

GLDH - $\geq$ 1000 U/l

Ketoglutarate - 9mM/l

NADH - 0.32 mM/l

ADP- 0.66 mM/l

Reagent 3: Standard, Urea- 50mg/dl

PREPARATION OF WORKING REAGENT: Reagent 1= 50 ml to reconstitute with one bottle of reagent 2.

PROCEDURE:

Wave length: 340 nm

Temperature: 30^0 C

	STANDARD	TEST
WORKING REAGENT	1ml	1ml
STANDARD	10µl	-
SAMPLE	-	10µl
Mix and record the change in absorbance of the test (ΔA_T) and standard (ΔA_S) between 30 seconds and 90 seconds.		

CALCULATION:

Urea concentration (mg/dl) = $\Delta A_T/\Delta A_S$ X 50 (Standard concentration)

NORMAL VALUES:

Serum urea: 15-45 mg/dl

Urine: 20 to 35 g/24 hrs

LINEARITY: 300 mg/dl. For sample values higher than 300 mg/dl, dilute the sample suitably with normal saline and repeat the assay. Apply proper dilution factor to calculate the final result.

ESTIMATION OF SERUM CREATININE:

METHOD: JAFFE'S KINETIC METHOD[54].

PRINCIPLE:

The rate of formation of a color complex between Creatinine and alkaline picrate is measured using Jaffe Kinetic method. The effect of interfering substances are reduced using the kinetic procedure.

REAGENTS:

Reagent 1: Picric acid

Reagent 2: Sodium hydroxide, Disodium phosphate

Reagent 3: Creatinine standard 2mg/dl

Storage at room temperature

Preparation of working reagent: Mix 1 volume of picric acid with 1 volume of sodium hydroxide.

PROCEDURE:

Wavelength: 492 nm (480-520)

Temperature: 30^0 C (27^0 C)

Read against distilled water

	STANDARD	**TEST**
WORKING REAGENT	1ml	1ml
STANDARD	100µl	-
SAMPLE	-	100µl

Mix and read the optical density (OD1) at 10 seconds after sample or standard addition. Exactly 2 min after first reading, take second reading (OD2), to obtain ΔOD= (OD2-OD1).

CALCULATION:

$$\frac{\Delta OD\ Sample}{\Delta OD\ Standard} \times n$$

mg/dl n=2
mg/L n= 20
µmol/L n=176.6

LINEARITY:

The method is linear up to a concentration of 25 mg/dl

REFERENCE VALUES:

Serum, Plasma: 0.6-1.3 mg/dl

Urine: 0.8-1.8 g/ 24 hrs

MODIFICATION OF DIET IN RENAL DISEASE EQUATION[55]:

Estimated GFR (eGFR) in ml/min/1.73 m^2 = 186 x $(P_{Cr})^{-1.154}$ x $(age)^{-0.203}$

Multiply by 0.742 for women

Multiply by 1.21 for African Americans

Age - in years

Serum creatinine - in milligrams per decilitre

ESTIMATION OF ALBUMIN:

METHOD: BROMOCRESOL GREEN (BCG) METHOD[56]:

PRINCIPLE:

BCG (3,3′,5,5′ – tetrabromo-m-cresol sulphophthalein) binds quantitatively and specifically with the albumin giving a green/blue colored compound whose color intensity is proportional to the albumin concentration in the sample.

REAGENTS:

SUCCINATE BUFFER – 60 mmol/L

BCG – 0.15 mmol/L

PROCEDURE:

	BLANK	**STANDARD**	**TEST**
REAGENT (µL)	1000	1500	1500
DISTILLED WATER (µL)	10	--	--
STANDARD (µL)	--	10	--
SAMPLE (µL)			10

Mix and incubate for 10 minutes at 37'C and measure OD at 630nm

CALCULATION:

Serum Albumin = (Absorbance of sample/Absorbance of standard) x Concentration of standard mg/dl

LINEARITY: 0.5 to 8 mg/dl.

EXPECTED VALUES: 3.5 -5.0 g/dl

ESTIMATION OF T3 & T4 [57,58]:

The principle & procedure for estimation of T3 & T4 are similar

PRINCIPLE:

The T3, T4 assay employs a competitive test principle with polyclonal antibodies specially directed against T3,T4. Endogenous T3,T4 released by the action of 8 anilino-1- naphthalene sulphonic Acid (ANS), competes with the added biotinylated T3,T4 derivate for the binding sites on the antibodies labeled with the ruthenium complex.

REAGENTS:

1. Streptavidin-coated microparticles contains 0.72mg/ml in preservative
2. Anti T3-Ab & Anti T4-Ab (separate for both) contains polyclonal Anti-T3 & Anti T4 antibody (sheep) labeled with ruthenium complex 75 ng/ml for T3 & 100ng/ml for T4; ANS 0.8 mg/ml for T3 & 1 mg/ml for T4; phosphate buffer 100 m mol/l, pH 7-4; preservative for both.
3. T3 or T4 - biotin: Contains biotinylated T3 3 ng/ml & biotinylated T4 20ng/ml, phosphate buffer 100 m.mol/l, pH 7-4; preservative.

PROCEDURE:

- Total duration of assay 18min.
- 1st incubation: 30 µl for T3 & 15 µl for T4 of sample and a T3 or T4 specific antibody labeled with a ruthenium complex; bound T3 and T4 will be released from the binding proteins in the sample by ANS.
- 2nd incubation: After addition of strepatavidin coated microparticles and biotinylated T3 or T4, the still free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The

entire complex becomes bound to the solid phase via interaction of biotin & streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured into the surface of the electrode.
- Unbound substances are then removed with pro-cell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by photomultiplier.
- Results are detected via calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

ESTIMATION OF TSH[58,59]:

PRINCIPLE (SANDWICH METHOD):

The TSH assay employs monoclonal antibody specifically directed against human TSH. The antibodies labeled with ruthenium complex consist of chimeric construct from human & mouse specific components. As a result, interfering effects due to HAMA (human anti-mouse antibodies) are largely eliminated.

REAGENTS:

- Streptavidin -coated microparticles Contains -0.7 mg/ml in preservative.
- Anti-TSHAb-biotin Contains biotinylated monoclonal anti-TSH-antibody (mouse) 2.0 mg/l; phosphate buffer 100 m.mol/l pH 7.2 in preservative.
- Anti-TSH-Ab contains-monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex1-2 mg/l; phosphate buffer100 m.mol/l pH 7.2 in preservative.

PROCEDURE:

Total duration of assay - 18minutes

- 1st incubation: 50 µl of sample, a biotinylated monoclonal TSH specific antibody and a monoclonal TSH specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interactions of biotin & streptavidin.
- The reaction mixture is aspirated in to the measuring cell where the micro particles are magnetically captured on to the surface of the electrode.

- Unbound substances are then removed with pro-cell
- Application of voltage to the electrode then induces chemiluminescent emission, which is measured by photomultiplier.
- Results are determined via calibration curve which is instrument specifically generated by 2 point calibration and a master curve provided via the reagent barcode.

ESTIMATION OF SERUM TRIGLYCERIDES

METHOD: Enzymatic method (GPO-PAP method) [20].

PRINCIPLE: Triglycerides are hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol is first phosphorylated to glycerol-3-phosphate by glycerol kinase and then oxidized by glycerol phosphate oxidase forming hydrogen peroxide and dihydroxy acetone phosphate. This hydrogen peroxide in the presence of peroxidase causes oxidative coupling of 4- chlorophenol and 4- aminoantipyrine to form red colored quinoneimine dye which is measured at 505 nm. The decrease in absorbance is directly proportional to the concentration of triglycerides.

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[Mg^{2+}]{\text{Glycerol kinase}} \text{Glycerol-3-phosphate+ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy acetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4 aminoantipyrine + 4 chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + H_2O$$

REAGENT: Triglycerides DES reagent

Active reagent	Concentration
ATP	2.5 mmol/L
Mg^{2+}	2.5 mmol/L
4 aminoantipyrine	0.8 mmol/L
3,5Dichloro-2-hydroxy benzene sulfonate	1 mmol/L
Peroxidase	>2000 U/L
Glycerol kinase	> 550 U/L
Lipoprotein lipase	> 3500 U/L
Buffer (pH 7.0 ± 0.1) at 20^0C	53 mmol/L

TRIGLYCERIDE STANDARD – 200 mg/dl (2.3 mmol/L)

PROCEDURE:

	BLANK	**STANDARD**	**TEST**
WORKING REAGENT	1000 µl	1000 µl	1000 µl
DISTILLED WATER	10 µl	-	-
STANDARD	-	10 µl	-
TEST (SERUM)	-	-	10 µl

Mixed and incubated for 10 minutes at 37^0C. Read the absorbance of standard and each test at 505 nm.

CALCULATIONS:

Triglycerides (mg/dl) = (OD of sample / OD of standard) x concentration of standard

= (OD of sample / OD of standard) x 200

= ---------- mg/dl

LINEARITY: The assay is linear upto 900 mg/dl

EXPECTED VALUES: According to National Cholesterol Education Programme (NECP)[60]

Normal: < 200mg/dl

Borderline: 200-400mg/dl

High: 400-1000mg/dl

ESTIMATION OF SERUM TOTAL CHOLESTEROL

METHOD: Enzymatic CHOD/PAP method[20].

PRINCIPLE: Cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and fatty acids. Cholesterol is oxidized by cholesterol oxidase forming hydrogen peroxide and cholest-4ene-3one. In presence of peroxidase, hydrogen peroxide formed brings about oxidative coupling of phenol and antipyrine to form red colored quinoneimine dye.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol ester} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4ene-3one} + H_2O_2$$

$$2H_2O_2 + \text{4- aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

REAGENTS:

- Phosphate buffer (pH 6.5 ± 0.1) – 68 mmol/L
- 4- amino antipyrine – 0.5 mmol/L
- Peroxidase - > 2000 IU/L
- Cholesterol esterase - > 200 IU/L
- Cholesterol Oxidase - > 150 IU/L
- Sodium phenolate – 20 mmol/L

CHOLESTEROL STANDARD: 200 mg/dl

PROCEDURE: Wavelength: 505 nm

Optical path: 1cm

Temperature: 37^0C

	BLANK	STANDARD	TEST
ENZYME REAGENT	100 µl	1000 µl	1000 µl
DISTILLED WATER	20 µl	--	--
STANDARD	--	20 µl	--
SAMPLE (SERUM)	--	--	20 µl

Mixed and incubated for 10 minutes at 37^0C and absorbance of standard and sample was measured at 505 nm against blank.

CALCULATIONS:

Total Cholesterol (mg/dl) = (OD of sample / OD of standard) x concentration of standard

= (OD of sample / OD of standard) x 200

= ---------- mg/dl

LINEARITY: up to cholesterol concentration of 600 mg/dL

EXPECTED VALUES: Recommendation of National Cholesterol Education Programme (NCEP)[60].

Desirable: < 200 mg/dl (< 5.2 mmol/L)

Borderline: 200 – 239 mg/dl (5.2 – 6.2 mmol/L)

High: ≥ 240mg/dl (≥ 6.2 mmol/L)

ESTIMATION OF SERUM HDL CHOLESTEROL

METHOD: Enzymatic CHOD-PAP method[20].

PRINCIPLE: Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to sample. Centrifugation leaves only HDL in the supernatant. The cholesterol content in it is determined enzymatically.

REAGENTS: Precipitating reagent

Phosphotungstic acid	2.4 mmol/L
Magnesium chloride	40 mmol/L

HDL CHOLESTEROL STANDARD – 25 mg/dl

PROCEDURE FOR PRECIPITATION:

PIPETTE	**VOLUMES**
Test (serum)	250 µl
Precipitating reagent	500 µl

Mixed well and allowed the reaction mixture to stand for 10 minutes at room temperature, centrifuge at 4000 rpm (1800xg) for 10 minutes to obtain a clear supernatant. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

PROCEDURE:

	BLANK	**STANDARD**	**TEST**
CHOLESTEROL WORKING REAGENT	1000 µl	1000 µl	1000 µl
DISTILLED WATER	50 µl	--	--
HDL STANDARD	--	50 µl	--
SUPERNATANT	--	--	50 µl

Mixed well, incubated for 10 minutes at 37^0C. Read the absorbance of the standard and each test at 505 nm against reagent blank.

CALCULATION:

HDL (mg/dl) = (OD of sample / OD of standard) x Concentration of standard x Dilution factor

= (OD of sample / OD of standard) x 25 x 3

= ---------- mg/dl

LINEARITY: The assay is linear upto 125 mg/dl or 3.23 mmol/L of HDL.

EXPECTED VALUES:

Positive coronary heart disease risk < 35 mg/dl

Negative coronary heart disease risk > 60 mg/dl

ESTIMATION OF SERUM LDL CHOLESTEROL

METHOD: Direct enzymatic CHOD-POD method[20].

PRINCIPLE: Direct determination of serum LDLc levels without the need for any pre treatment or centrifugation steps.

$$\text{Cholesterylester} \xrightarrow{\text{CHE}} \text{cholesterol + fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{TOOS} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} 2H_20 + O_2 + \text{Colored product}$$

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

REAGENTS:

R1	GOOD pH 7	50 mmol/L
	Cholesterol esterase	380 U/L
	Cholesterol oxidase	380 U/ L
	Catalase	400 U/ mL
	N-2hydroxy-sulfopropyl - 3,5dimethoxyaniline (TOOS)	0.45 mmol/L
R2	GOOD pH 7	50 mmol/L
	4 aminoantipyrine	1 mmol/L
	Peroxidase(POD)	1000µ/L
LDLc CAL	Standard lyophilized serum	

PROCEDURE:

	BLANK	**STANDARD**	**TEST**
R1 (ML)	375	375	375
STANDARD (ML)	--	5	--
SAMPLE (ML)	--	--	5
Mix and incubate for 5 min at 37^0C			
R2 (ML)	125	125	125

LDL CHOLESTEROL STANDARD – 75 mg/dl

Mixed well, incubated for 5 minutes at 37^0C. Read the absorbance of the standard and each test at 546 nm against reagent blank.

CALCULATION:

LDL (mg/dl) = (OD of sample / OD of standard) x concentration of standard

= (OD of sample / OD of standard) x 75

= ---------- mg/dl

LINEARITY: The assay is linearity is from 3.7 mg/dl to 1000 of LDL.

EXPECTED VALUES FOR LDL: According to National Cholesterol Education Programme (NECP) [60]

Desirable: < 130 mg/dl (< 3.36 mmol/L)

Border line: 130-160 mg/dl (3.36 – 4.13 mmol/L)

High risk: > 160 mg/dl (> 4.13 mmol/L)

STATISTICAL ANALYSIS

- The data collected was tabulated and analyzed using descriptive statistical tool.
- Mean and standard deviation was calculated for serum albumin, lipid profile and thyroid profile individually for cases and controls.
- This mean and standard deviation of cases and controls was compared using independent ‘t’ test.
- Serum albumin, lipid profile and thyroid profile was compared between the stage II,III and IV of chronic kidney disease patients using independent t test.
- p value of < 0.05 was taken statistically significant.

RESULTS

RESULTS OF THE STUDY:

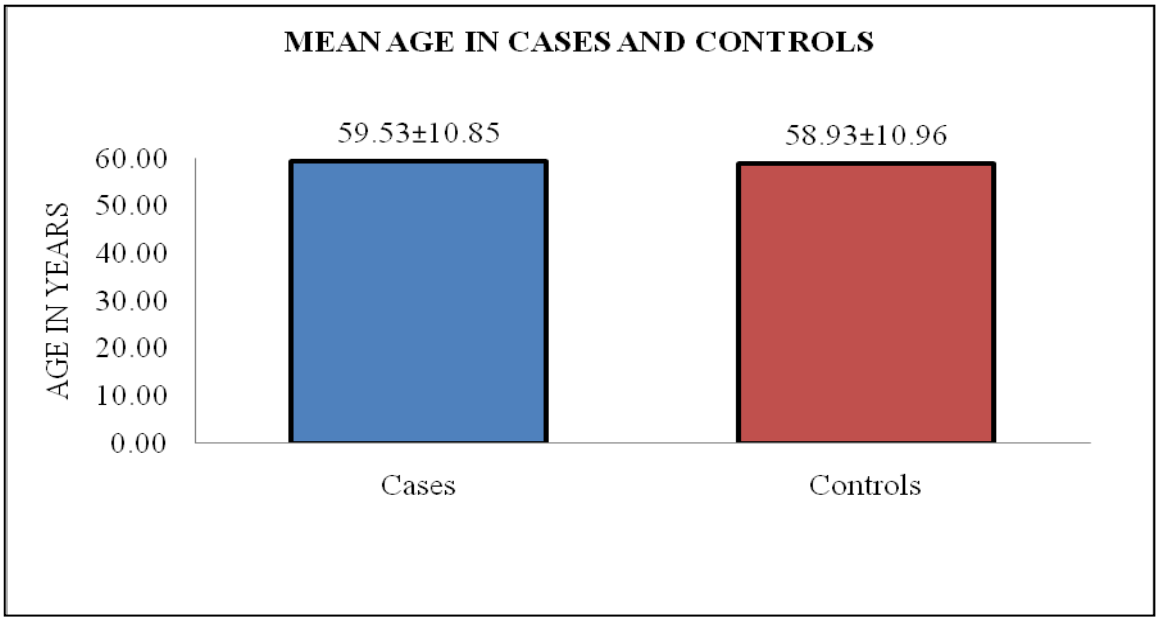
In the present study, 80 subjects were selected considering the inclusion and exclusion criteria stated in the methodology. Among them 40 were CKD cases and 40 were age and gender matched controls.

PRESENTATION OF DATA:

Master chart showing the blood glucose, blood urea, serum creatinine, eGFR, serum albumin, serum cholesterol, serum triglycerides, serum HDL, serum LDL, serum TSH, serum TT3 and serum TT4 levels with hospital number, age and gender of the subjects obtained during the study in annexure 1.

AGE DISTRIBUTION OF CASES AND CONTROLS:

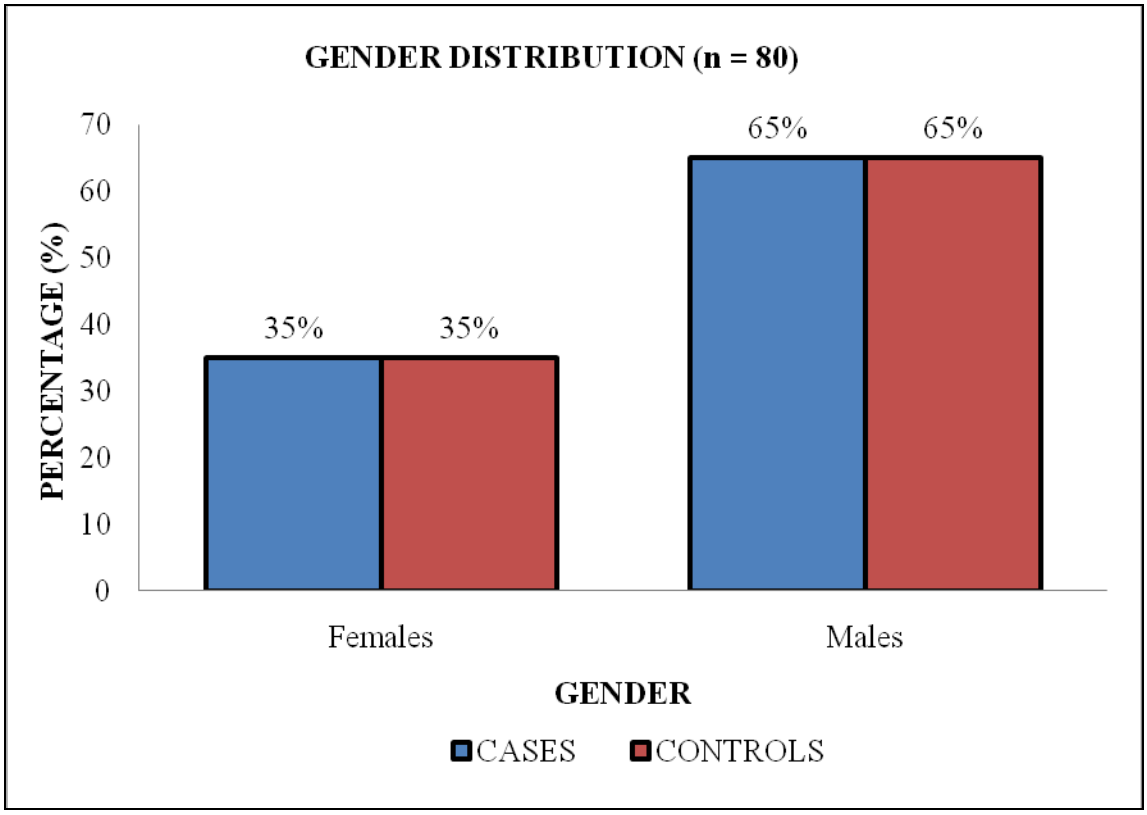
Mean age of the CKD cases was 59.83 ± 10.85 and control group was 58.93 ± 10.96 as shown in the graph 1.



GRAPH 1: MEAN AGE IN CASES AND CONTROLS.

GENDER DISTRIBUTION OF CASES AND CONTROLS:

The percentage of females in the cases and controls were 35% and the percentage of males were 65% as shown in the graph 2.



GRAPH 2: GENDER DISTRIBUTION

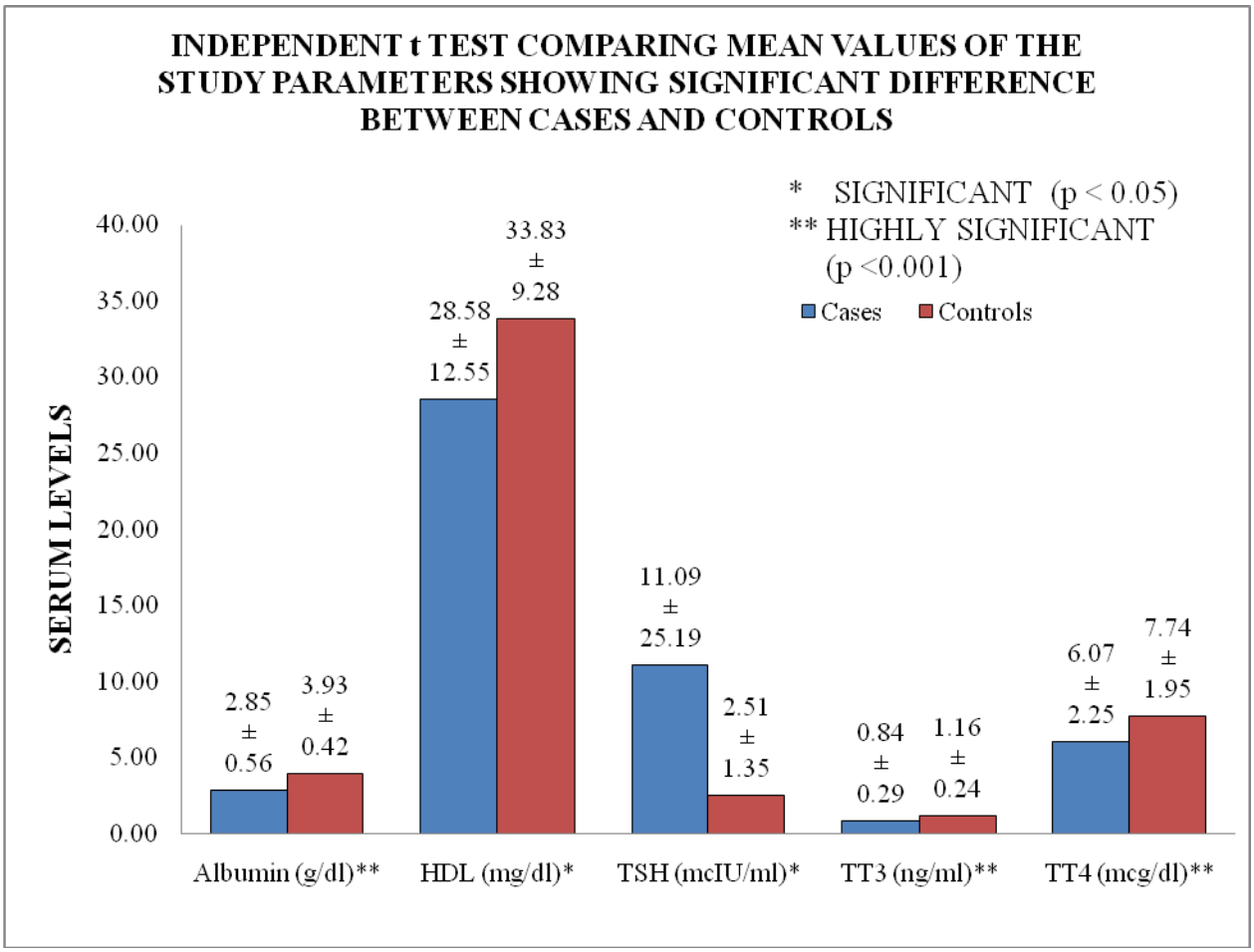
PARAMETERS	CASES	CONTROLS	t VALUE	p VALUE
Random Blood Sugar (mg/dl)	188.6 ± 91.25	107.75 ± 22.68	5.44	<0.001**
Blood Urea (mg/dl)	65.23 ± 24.51	30.60 ± 8.69	8.42	<0.001**
Serum Creatinine (mg/dl)	2.2 ± 0.51	0.82 ± 0.2	15.97	<0.001**
eGFR (ml/min/1.73m^2)	28.38 ± 8.10	94.08 ± 22.95	-17.07	<0.001**
Serum Albumin (g/dl)	2.85 ± 0.56	3.93 ± 0.42	-9.77	<0.001**
Serum Cholesterol (mg/dl)	148.25 ± 64.35	154 ± 36.6	-0.49	0.62
Serum Triglycerides (mg/dl)	171.25 ± 94.35	153.6 ± 55.45	1.02	0.31
Serum HDL (mg/dl)	28.58 ± 12.55	33.83 ± 9.28	-2.13	0.04*
Serum LDL (mg/dl)	85.50 ± 53.68	89.57 ± 30.03	-0.42	0.68
Serum TSH (mcIU/ml)	11.09 ± 25.19	2.51 ± 1.35	2.15	0.03*
Serum TT3 (ng/ml)	0.84 ± 0.29	1.16 ± 0.24	-5.31	<0.001**
Serum TT4 (mcg/dl)	6.07 ± 2.25	7.74 ± 1.95	-3.54	<0.001**

* SIGINIFICANT ** HIGHLY SIGNIFICANT

TABLE 3: INDEPENDENT t TEST COMPARING THE MEAN VALUES OF THE PARAMETERS BETWEEN THE CASES OF CKD AND CONTROLS

- The mean blood glucose levels were raised in cases (188.6 ± 91.25 mg/dl) compared to the controls (107.75 ± 22.68 mg/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 3.
- The mean blood urea levels were raised in cases (65.23 ± 24.51 mg/dl) compared to the controls (30.60 ± 8.69 mg/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 3.
- The mean serum creatinine levels were raised in cases (2.2 ± 0.51 mg/dl) compared to the controls (0.82 ± 0.2 mg/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 3.
- The mean eGFR values were reduced in cases (28.38 ± 8.10 ml/min/1.73m^2) compared to the controls (94.08 ± 22.95 ml/min/1.73m^2) which was highly significant statistically ($p < 0.001$) as shown in the table 3.
- The mean serum albumin levels were reduced in cases (2.85 ± 0.56 g/dl) compared to the controls (3.93 ± 0.42 g/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 3 and graph 3.
- The mean serum HDL levels were reduced in cases (28.58 ± 12.55 mg/dl) compared to the controls (33.83 ± 9.28 mg/dl) which was significant statistically ($p < 0.05$) as shown in the table 3 and graph 3.
- The mean serum TSH levels were raised in cases (11.09 ± 25.19 mcIU/ml) compared to the controls (2.51 ± 1.35 mcIU/ml) which was statistically significant ($p < 0.05$) as shown in the table 3 and graph 3.
- The mean serum TT3 levels were reduced in cases (0.84 ± 0.29 ng/ml) compared to the controls (1.16 ± 0.24 ng/ml) which was highly significant statistically ($p < 0.001$) as shown in the table 3 and graph 3.

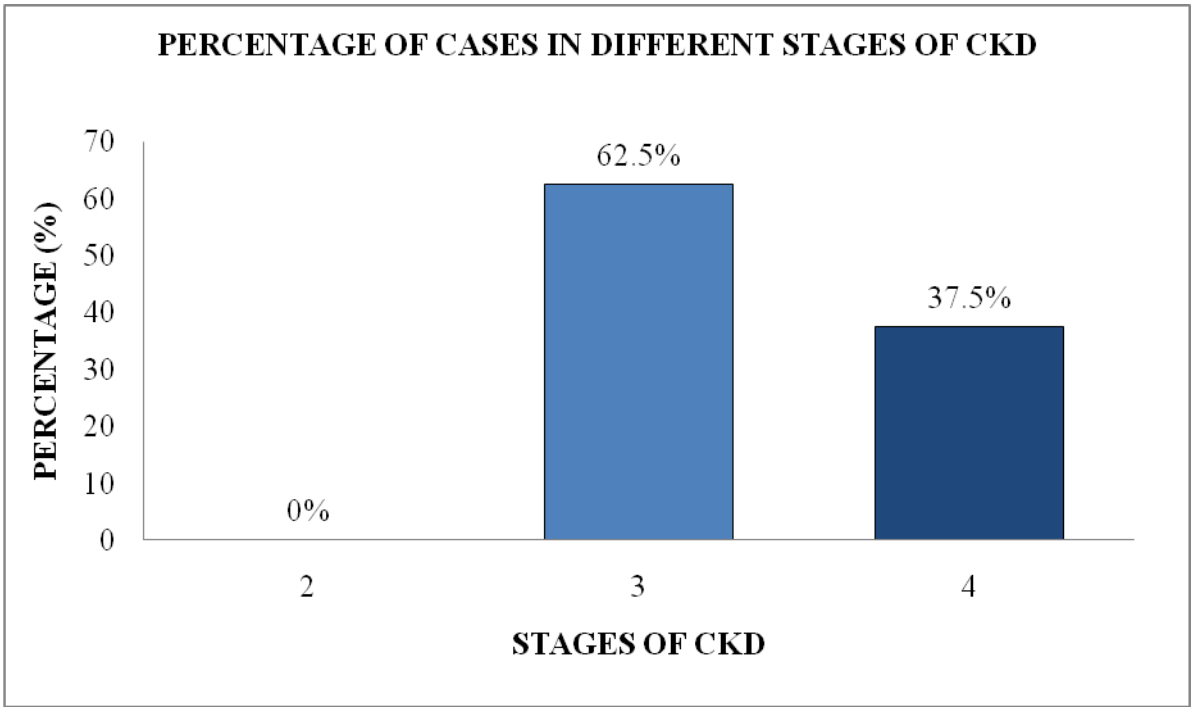
- The mean serum TT4 levels were reduced in cases (6.07 ± 2.25 mcg/dl) compared to the controls (7.74 ± 1.95 mcg/dl) which was highly significant statistically ($p < 0.001$) as shown in the table 3 and graph 3.
- There was no significant difference in the serum levels of cholesterol, triglycerides and LDL between the CKD cases and the controls as shown in the table 3.



GRAPH 3: INDEPENDENT t TEST BETWEEN CASES AND CONTROLS

PERCENTAGE OF CASES IN DIFFERENT STAGES OF CKD:

Out of 40 CKD cases 62.5% were in stage 3 and the rest 37.5% were in stage 4. There were no cases in stage 2 in the study group as shown in the graph 4.



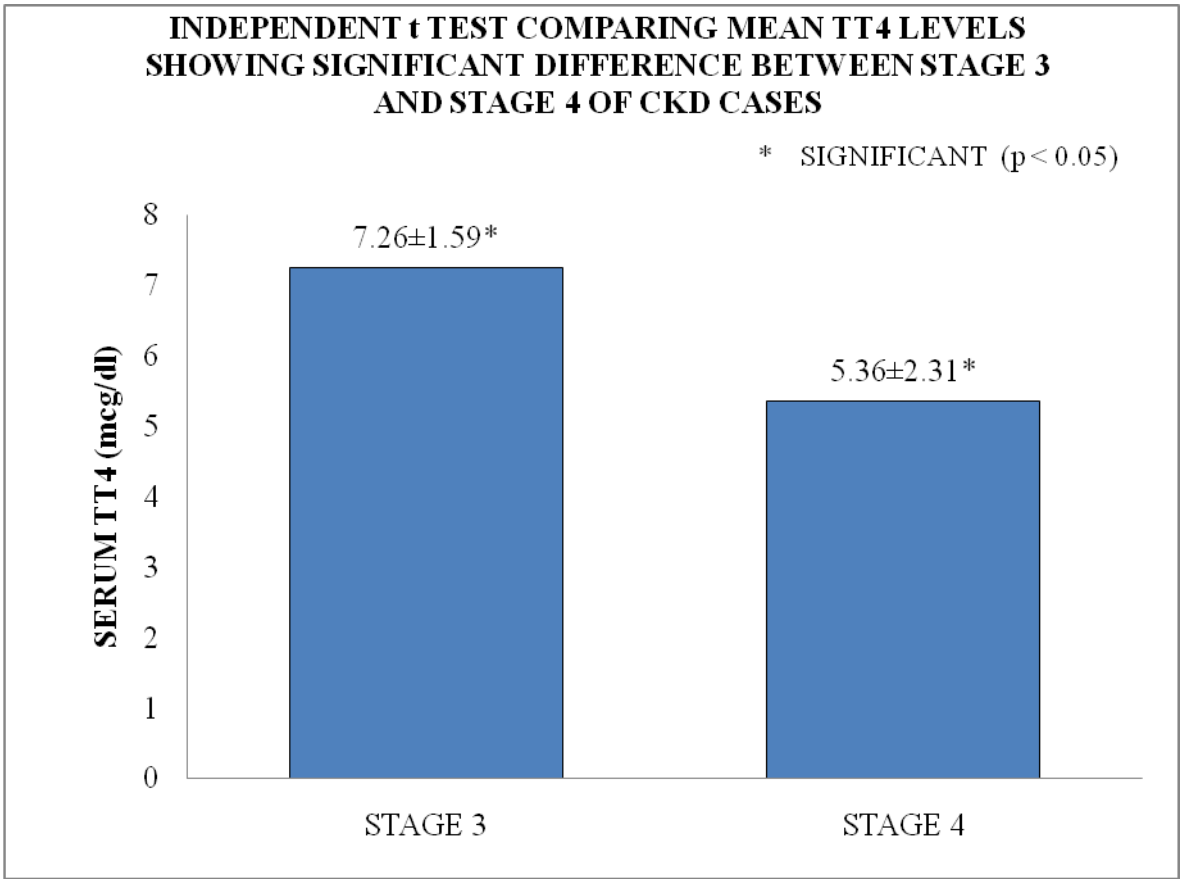
GRAPH 4: PERCENTAGE OF CASES IN DIFFERENT STAGES OF CKD

PARAMETERS	**STAGE 3**	**STAGE 4**	**t VALUE**	**p VALUE**
Serum Albumin (g/dl)	2.85 ± 0.44	2.85 ± 0.63	0.029	0.9
Serum Cholesterol (mg/dl)	142.33 ± 40.54	151.8 ±7 5.74	-0.446	0.6
Serum Triglycerides (mg/dl)	176.8 ± 87.68	167.92 ± 99.74	0.285	0.7
Serum HDL (mg/dl)	25.2 ± 8.58	30.6 ± 14.19	-1.331	0.1
Serum LDL (mg/dl)	81.8 ± 32.18	87.7 ± 63.75	-0.334	0.7
Serum TSH (mcIU/ml)	9.24 ± 25.17	12.19 ± 25.65	-0.335	0.7
Serum TT3 (ng/ml)	0.93 ± 0.32	0.79 ± 0.25	1.551	0.1
Serum TT4 (mcg/dl)	7.26 ± 1.59	5.36 ± 2.31	2.794	0.008*

* SIGNIFICANT

TABLE 4: INDEPENDENT t TEST COMPARING THE MEAN VALUES OF THE STUDY PARAMETERS BETWEEN DIFFERENT STAGES OF CKD

- The mean serum TT4 levels were significantly ($p < 0.05$) reduced in the stage 4 (5.36 ± 2.31 mcg/dl) compared to the stage 3 (7.26 ± 1.59 mcg/dl) of the CKD cases as shown in the table 4 and graph 5.
- There was no significant difference in the serum levels of albumin, cholesterol, triglycerides, HDL, LDL, TSH and TT3 between the stage 3 and stage 4 of the CKD cases as shown in the table 4.



GRAPH 5: INDEPENDENT t TEST BETWEEN STAGES OF CKD

DISCUSSION

DISCUSSION:

The present study was a cross sectional study done by selecting 80 subjects of which 40 were cases of CKD and 40 were age and sex matched normal healthy controls. The percentage of males in the cases and controls were 65% and females were 35%. Staging of CKD patients was done based on eGFR calculated using MDRD formula. Baseline parameters namely glucose, urea and creatinine were estimated in cases and controls. Measurement of the serum levels of albumin, lipid profile and thyroid profile in the cases was done and compared with the controls. Comparison of the above parameters between the stage 3 and stage 4 of chronic kidney disease was also done. The percentage of CKD cases in stage 3 was 67.5% and stage 4 was 32.5% respectively.

The levels of serum albumin were found to be significantly reduced in the cases when compared with the controls (graph 3). This is because, renal glomeruli become progressively permeable with advancing renal disease and this leakiness leads to increased urinary albumin excretion causing hypoalbuminemia in CKD patients[33]. The analysis did not show any significant difference in the levels of serum albumin between stage 3 and stage 4 of CKD.

Kaysen et al described that serum albumin levels have been shown to decrease in situation of volume overload, which is highly prevalent in CKD patients[61]. Acchiardo et al proposed that hypoalbuminemia is caused by malnutrition in CKD in a study involving 120 hemodialysis patients[62]. Hence serum albumin can be used as a marker of malnutrition in CKD patients[63].

However, study done by Heimburger and his co-workers in Sweden showed albumin levels do not differ significantly between well nourished and malnourished pre-dialysis patients suggesting albumin as a poor nutritional marker in CKD[64].

Study done by Kaysen and Schoenfeld showed that in Continuous Ambulatory Peritoneal Dialysis (CAPD) patients, there will be loss of albumin due to extracorporeal losses[65].

It is noted that inflammatory processes occuring due to increase in pro-inflammatory cytokines in CKD cases itself can lead to marked hypoalbuminemia by suppressing albumin synthesis[66,67,68]. These cytokines also causes muscle wasting by stimulating protein catabolism via ubiquitin-proteosome pathway, which may lead to hypoalbuminemia[69]. Interleukin-1, tumor necrosis factor-α and endotoxins may induce muscle catabolism by stimulating branched chain ketoacid dehydrogenase, which causes increased oxidation of branched chain amino acids[70].

Another mechanism which contributes is buildup of uremic toxins that depresses albumin synthesis by the liver and thus, there is an increase in intravascular and extra vascular fluid volume, due to which the albumin level remains mildly subnormal[31,32]. The present study also revealed these findings.

Hypoalbuminemia may be caused due to decrease in dietary intake due to decreased appetite in CKD patients[71]. Inflammation in combination with low protein intake plays a significant role in causing hypoalbuminemia in CKD patients[71,72,73].

In the present study there was no significant difference between the stage 3 and stage 4 albumin levels, because as the glomerular filtration rate falls and chronic renal failure sets in, proteins are no longer lost in large quantity in the urine, and albumin degradation in the proximal tubules subsides[30].

Hypoalbuminemia, a marker of malnutrition and inflammation is a powerful predictor of mortality in patients with ESRD[47,48]. Albumin being a scavenger of free radicals, hypoalbuminemia leads to decreased antioxidant activity favoring noxious effects on arterial wall[50].

In the present study, measurement of TSH, TT3 and TT4 showed a significant difference among the cases and controls (graph 3). Serum TSH was found to be increased significantly in CKD patients compared to the controls. Serum TT3 and TT4 was reduced significantly in CKD patients compared to the controls.

Study done by El-Hana and his co-workers, showed increases in TSH levels and decrease in TT3 levels in children with CKD. There was no significant decrease difference in TT4 levels in their study group[74].

In a population based study done by Asvold et al in Norway showed that high TSH levels were associated with higher prevalence of CKD[75]. Chonchol et al in Denver, USA, found that subclinical hypothyroidism is a common condition among persons suffering from CKD showing normal TT3 and TT4 levels and increased TSH levels[76].

Lo and his co-investigators found that there is elevated TSH in patients with CKD and their study did not show any significant difference in TT4 levels[77]. Targher et al found that 26 % of their study group with GFR < 60 ml/min/1.73m^2 had subclinical hypothyroidism[78]. The present study supports this finding of significant elevation of TSH in CKD cases, but this study also shows significant reduction in levels of both TT3 and TT4, which means that CKD patients exhibit overt hypothyroidism.

However, there are studies which showed normal or reduced TSH in CKD patients. Study done by Lims et al in adult patients showed normal TSH levels[79]. Also, in pediatric CKD patients there might be normal TSH[80,81].

There are studies, which have shown decrease in TSH levels. In a study done by Drabczyk et al, they have found low levels of TSH in CKD pateints[82].

Sang and his co-workers in Korea found that the number of people with CKD suffering from low T3 syndrome is increasing as the stage of the diseases progresses[83]. A similar observation was made in the present study, which showed significant decrease in TT3 levels in CKD cases compared to controls. Kaptein et al report in 1988 of 287 euthyroid patients with ESRD, 76% had low T3 irrespective of TSH levels[81]. Zoccali et al in their prospective study found that fT3 in patients with ESRD is frequently reduced and is also associated with inflammation and cardiovascular damage[84].

In a study done by Kayima and his co-investigators in Kenya on 52 CRF patients on conservative management, they found low serum TT4 and TT3 levels[85]. A low serum TT4 level was also found in adult CKD patients on conservative treatment in a study done by Hegedus and his co-workers[86]. Identical results have been obtained in children with CKD by Hershman et al and Hardy et al[87,88]. The present study supports these findings, including significantly low levels in stage 4 compared to stage 3. In addition, there are studies reporting normal T4 levels. Afrasiabi et al reported normal TT4 levels in CKD patients in children[89].

All these changes in thyroid hormone is seen because, of the disturbance in the hypothalamus–pituitary–thyroid axis due to uremia[90-93]. T3 tends to decrease due to the reduced deiodination by inhibition of 1 5′deiodinase causing decrease in peripheral conversion of T4 to T3[94]. Low T4 levels in CKD patients are due to impaired protein binding of T4[95].

In the present study mean levels of HDL is significantly reduced in CKD cases compared to controls. Many studies, such as the one conducted by Massy et al[96], Das et al[97] and Shoji et al[98] have also observed the same results. The reason for decreased concentration of HDL-C in CRF is not fully understood. It may be due to decreased activities of LPL, hepatic triglyceride lipase (HTGL), lecithin cholesterol acyl transferase (LCAT) and increased concentration of cholesterol ester transfer protein (CETP) and decreased apolipoprotein concentrations[23].

Triglycerides in CKD cases shows a tendency to increase, which may occur due to decreased catabolism rate due to the diminished activity of lipoprotein lipase caused by down regulation of gene coding for the enzyme and presence of lipase inhibitors[99,100]. But in this study, the difference between cases and controls were not statistically significant.

In the present study serum cholesterol and LDL levels were decreased in CKD cases compared to controls, but the differences were not statistically significant. All these changes in the thyroid hormones causing hypothyroidism in CKD as shown in the study by itself may contribute to dyslipidemia by causing decreased catabolism of LDL and decreased activity of lipoprotein lipase[41,42].

Increased oxidative stress caused due to hypoalbuminemia[47] and sub-endothelial inflammation caused by TNF-α and Interleukin-1 in hypothyroidism[101] added with CKD induced dyslipidemia increases the risk for cardiovascular complication in CKD patients.

CONCLUSION

CONCLUSION:

- CKD is a debilitating condition and cardiovascular complications is the main cause of morbidity and mortality in these patients
- Measurement of albumin and thyroid status combined with lipid profile gives an insight into the ongoing patho-physiological changes leading to risk of development of cardiovascular complications in CKD patients.
- Being a negative phase reactant, reduction in serum albumin levels indicates the inflammation and also reflects the malnutrition in CKD patients.
- Overt hypothyroidism was observed in CKD patients compared to controls with the levels of TT4 being reduced in stage 4 compared to stage 3.
- Amongst lipid profile, decreased HDL levels in the CKD patients might contribute in increasing the risk of cardiovascular complications
- Existence of hypothyroidism in CKD patients might contribute to an increase in the risk of cardiovascular complications by causing inflammation and dyslipidemia.
- Hence identifying these abnormalities early in the disease process may help the clinician to effectively manage the developments of cardiovascular complications in CKD patients at an earlier stage.

SUMMARY

SUMMARY:

This is a cross sectional study done in RLJH & RC, Tamaka, Kolar to estimate the levels of serum albumin, serum thyroid and lipid profile in CKD patients. 80 subjects were chosen of which 40 were diagnosed cases of CKD and 40 were age and sex matched healthy controls. CKD patients were staged based on eGFR calculated using MDRD formula. The CKD patients showed hypoalbuminemia, hypothyroidism and decreased HDL levels compared to the controls. The levels of TT4 were found to be reduced in stage 4 compared to stage 3.

These abnormalities may contribute as a non-traditional risk marker of CVD in CKD patients. Hence, early assessment of these parameters may help in decreasing the cardiovascular complications and effective management of CKD patients.

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ANNEXURES

ANNEXURE 1:

CASE HISTORY OF THE PATIENTS

Case No:

Name: Mr/Mrs OP No:

Age: IP No:

Gender: Ward:

Date: Occupation:

Weight:

Address:

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS:

PAST HISTORY:

Hypertension : yes/no if yes, duration:

Diabetes : yes/no if yes, duration:

Tuberculosis : yes/no if yes, duration:

Heart diseases : yes/no if yes, duration:

Liver diseases : yes/no if yes, duration:

Thyroid disorders : yes/no if yes, duration:

Drug ingestion : yes/no if yes, duration & details:

Acute renal failure: yes/no

Others :

FAMILY HISTORY:

Diabetes : yes/no if yes, duration:

Hypertension : yes/no if yes, duration:

Tuberculosis : yes/no if yes, duration:

Familial hypercholesterolemia: yes/no if yes, duration:

OCCUPATIONAL HISTORY:

PERSONAL HISTORY:

Economic status:

Diet: vegetarian / mixed

Smoking: yes/no if yes, duration:

Alcohol: yes/no if yes, duration:

MENSTRUAL HISTORY: Regular/irregular/not applicable.

GENERAL PHYSICAL EXAMINATION:

Built: normal / below normal / well built / obese

Nourishment: well / poor nourished

Oedema : Icterus :

Pallor: Clubbing:

Cyanosis: Lymphadenopathy:

Blood pressure: Pulse rate:

SYSTEMIC EXAMINATION:

CVS:

RS:

CNS:

PER ABDOMEN:

DIAGNOSIS:

INVESTIGATIONS:

URINE:

Albumin - Sugar –

BLOOD:

Hemoglobin: g/dl

Blood sugar: mg/dl

Serum Albumin: g/dl

RENAL FUCTION TEST:

Blood Urea: mg/dl

Serum Creatinine: mg/dl

eGFR: mL/min/1.73 m^2

LIPID PROFILE:

Total Cholesterol: mg/dl

Triglycerides: mg/dl

HDL cholesterol: mg/dl

LDL cholesterol: mg/dl

THYROID FUNCTION TEST:

TSH: IU/L

TT3: ng/dl

TT4: ng/dl

OTHERS:

Informed consent:

The details of the study have been explained to me in my own language. I confirm that I have understood the above study and had the opportunities to ask questions . I understand that my participation in the study is voluntary and that am free to withdraw at any time , without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose. I fully consent to participate in the above study.

Signature of the patient

ANNEXURE 2: MASTER CHART OF THE CKD CASES

S No.	HOSPITAL NO.	SEX	AGE	RBS	ALB	BU	CREAT	EGFR	CHL	TG	HDL	LDL	TSH	TT3	TT4
1	459696	M	60	198	2.1	57	3.1	16	117	157	39	47	4.73	0.53	4.36
2	678739	M	55	205	3.5	70	3.1	16	270	136	34	209	2.08	0.63	6.56
3	707300	M	50	140	2.8	57	1.8	32	106	86	21	68	0.94	0.94	7.16
4	710858	M	56	240	2.2	120	2.1	26	194	67	61	120	90.44	0.59	2.89
5	711305	M	62	133	3.7	53	2.1	25	161	206	21	99	0.8	0.63	6.16
6	684588	M	53	65	3.3	104	2	28	76	96	16	41	1.94	0.8	6.21
7	643314	M	58	158	3.4	44	2.6	20	93	77	31	46	10.1	0.93	5.94
8	783157	M	50	114	3.2	95	1.6	37	66	55	23	32	3.67	1.01	6.94
9	784711	M	58	223	2.6	99	1.5	38	169	131	7	136	0.11	0.49	6.12
10	787845	M	55	53	1.6	45	2	28	106	82	25	65	24.8	0.54	2.01
11	788170	M	70	229	2.1	107	2.9	16	124	74	34	75	2.15	0.7	2.01
12	739071	M	71	220	2.7	47	2.8	16	99	104	31	47	2.27	0.99	6.93
13	787527	M	65	87	1.9	49	2	26	174	176	45	94	3.04	0.58	4.55
14	789952	M	50	208	3.3	50	2	28	173	264	34	87	4.26	0.85	6.75
15	823069	M	54	144	2.8	70	2.5	29	122	203	25	56	13.7	0.8	6.97
16	827218	M	80	200	3.2	42	2	34	197	252	23	124	3.94	1.22	8.89
17	824446	M	60	210	2.7	58	2.4	29	127	165	25	69	0.45	1.39	5.57
18	826863	M	80	157	2.3	100	2.6	25	160	444	13	59	2.9	1	3.58
19	789652	M	60	220	3	78	2	36	126	136	28	71	100	0.58	7.28
20	578546	M	48	154	3.7	98	3.2	22	144	145	18	97	1.9	1.03	3.71

RBS – RANDOM BLOOD SUGAR, ALB- ALBUMIN, BU – BLOOD UREA, CREAT – CREATININE, eGFR – ESTIMATED GLOMERULAR FILTRATION, CHL –CHOLESTEROL, TG – TRIGLYCERIDES, HDL – HIGH DENSITY LIPOPROTEIN, LDL – LOW DENSITY LIPOPROTEIN, TSH – THYROID STIMULATING HORMONE, TT3 – TOTAL TRI-IODOTHYRONINE, TT4 – TOTAL TETRA-IODOTHYRONINE.

MASTER CHART OF THE CKD CASES

S No.	HOSPITAL NO.	SEX	AGE	RBS	ALB	BU	CREAT	EGFR	CHL	TG	HDL	LDL	TSH	TT3	TT4
21	825953	M	75	167	2.5	52	2.2	31	117	82	35	65	0.44	0.74	4.51
22	824806	M	62	130	2.2	109	2.1	34	88	124	17	46	3.56	0.77	5.24
23	826609	M	77	114	3.3	47	2.2	31	123	165	21	69	4.48	0.95	5.69
24	823127	M	65	145	2.9	54	2.4	29	88	263	12	23	4.09	0.95	7.02
25	780432	M	56	156	2.3	47	2	37	114	113	29	62	4.12	0.84	5.82
26	822507	M	48	205	3	54	2.9	25	139	122	56	59	1.9	0.98	7.42
27	787784	F	50	85	2	55	2.1	27	156	75	49	92	7.93	0.53	3.58
28	786494	F	60	214	3.2	42	1.1	55	185	162	29	123	5.49	0.99	10.6
29	710822	F	68	132	3.4	60	2	25	108	186	19	52	14.54	1.18	8.87
30	615727	F	65	446	3.8	30	2.2	23	447	424	25	337	100	0.24	0.53
31	711222	F	70	412	3.5	54	1.6	32	156	330	18	73	0.55	0.77	7.63
32	789250	F	48	457	2.2	48	1.5	41	197	175	39	123	0.884	1.84	8.61
33	686558	F	45	269	2.7	32	2	30	150	326	35	49	3.09	1.21	8.47
34	825093	F	65	341	2.7	36	1.4	40	155	248	19	87	2.17	0.82	8.03
35	748907	F	30	138	2.9	56	2.6	23	211	229	56	109	0.26	0.83	4.66
36	825193	F	80	125	3.4	109	1.4	38	186	267	34	99	5.12	0.81	7.9
37	627997	F	55	164	3.4	73	2	27	118	119	21	73	6.9	1.09	10.9
38	824683	F	46	185	3.1	82	2.5	22	114	57	30	73	1.76	0.77	7.2
39	825991	F	56	134	2.9	48	2.8	19	178	143	36	114	0.22	0.52	5.61
40	825966	F	65	167	2.5	78	2.7	19	96	184	9	50	1.73	0.66	4.07

RBS – RANDOM BLOOD SUGAR, ALB- ALBUMIN, BU – BLOOD UREA, CREAT – CREATININE, eGFR – ESTIMATED GLOMERULAR FILTRATION, CHL –CHOLESTEROL, TG – TRIGLYCERIDES, HDL – HIGH DENSITY LIPOPROTEIN, LDL – LOW DENSITY LIPOPROTEIN, TSH – THYROID STIMULATING HORMONE, TT3 – TOTAL TRI-IODOTHYRONINE, TT4 – TOTAL TETRA-IODOTHYRONINE.

MASTER CHART OF THE CONTROLS

S No	HOSPITAL NO	sex	Age	RBS	ALB	BU	CREAT	EGFR	CHL	TG	HDL	LDL	TSH	TT3	TT4
1	830350	M	65	135	3.5	34	0.8	98	192	253	29	113	5.53	1	5.29
2	829999	M	71	161	4.3	30	0.9	89	188	200	40	107	2.09	1.28	10.1
3	812451	M	50	85	4.6	26	0.9	91	135	120	35	76	0.24	0.86	6.86
4	830698	M	63	95	3.9	25	0.8	98	123	98	40	63	4.3	0.91	3.8
5	790118	M	86	110	3.6	38	1.1	75	178	227	35	97	1.01	1.36	9.22
6	830812	M	60	79	4.9	23	0.6	102	118	232	18	54	4.18	0.84	5.88
7	804361	M	69	145	4.7	42	1	80	111	150	30	51	2.14	0.87	6.68
8	831113	M	60	122	3.9	37	0.8	97	200	136	36	136	3.22	0.98	7.63
9	831791	M	50	87	3.8	28	0.6	114	117	134	34	56	3.34	0.83	4.06
10	805057	M	55	98	4.5	49	1.1	70	164	221	27	92	4.35	1.18	5.43
11	833010	M	55	115	4.1	35	0.9	99	133	176	33	65	3.1	1.26	8.08
12	632475	M	60	71	3.9	48	1	76	125	143	32	64	4.35	1.18	5.43
13	812941	M	55	90	3.7	38	0.8	102	145	125	29	91	3.1	1.26	8.08
14	814146	M	70	98	3.8	33	1	79	78	78	25	37	4.98	1.15	7.89
15	829809	M	76	110	3.8	23	1.1	69	97	119	28	45	3.73	1.62	10.2
16	763437	M	55	87	4	34	0.9	93	132	151	48	54	1.17	0.867	11.3
17	829804	M	45	122	4.2	22	0.6	155	140	143	44	68	1.37	1.29	8.31
18	739872	M	62	130	4	28	0.7	121	144	134	35	83	1.82	1.28	8.25
19	823127	M	50	123	2.9	36	0.8	109	224	104	58	145	2.12	1.2	9.18
20	725582	M	50	140	3.7	23	0.9	95	255	72	58	183	1.58	1.12	7.01

RBS – RANDOM BLOOD SUGAR, ALB- ALBUMIN, BU – BLOOD UREA, CREAT – CREATININE, eGFR – ESTIMATED GLOMERULAR FILTRATION,CHL – CHOLESTEROL, TG – TRIGLYCERIDES, HDL – HIGH DENSITY LIPOPROTEIN, LDL – LOW DENSITY LIPOPROTEIN, TSH – THYROID STIMULATING HORMONE, TT3 – TOTAL TRI-IODOTHYRONINE, TT4 – TOTAL TETRA-IODOTHYRONINE.

MASTER CHART OF THE CONTROLS

S No	HOSPITAL NO	sex	age	RBS	ALB	BU	CREAT	EGFR	CHL	TG	HDL	LDL	TSH	TT3	TT4
21	830006	M	75	101	2.8	26	1	77	137	126	31	81	1.85	1.13	5.8
22	829798	M	51	128	4	39	0.9	95	166	113	19	124	2.56	1.44	4.63
23	830000	M	82	133	3.1	42	1.2	62	195	244	32	115	0.32	1.12	7.31
24	830003	M	52	88	4.1	34	0.6	150	156	136	26	102	1.5	1.15	7.99
25	823567	M	48	136	4.4	45	0.9	96	110	140	25	57	2.38	1.22	7.56
26	830010	M	60	126	3.6	25	1	81	200	238	43	109	1.21	1.12	7.47
27	742578	F	54	75	4	30	0.3	132	123	98	45	58.4	0.6	0.8	9.9
28	714658	F	45	105	3.6	12	0.4	126	150	120	44	82	3.4	0.9	9.5
29	833829	F	48	85	3.8	19	0.6	93	171	111	33	115	4.2	1.6	10.7
30	819002	F	45	120	4.1	28	0.6	96	145	99	38	87.2	0.8	0.9	8.8
31	829607	F	70	78	3.9	32	0.8	80	158	132	28	103	3.27	1.1	8.5
32	834197	F	50	97	4.1	25	0.6	95	130	131	33	79	1.4	1.11	7.26
33	834274	F	35	65	3.9	14	0.9	73	181	128	44	112	2.17	1.33	5.58
34	803073	F	60	88	4	20	0.5	134	189	297	28	102	3.24	1.33	6.12
35	834804	F	63	96	3.9	34	0.6	107	158	170	36	88	1.56	1.08	7.05
36	670206	F	75	110	4	26	0.9	65	173	166	25	115	3.78	1.9	11.2
37	835222	F	64	134	4.4	24	1	59	169	150	35	103	3.7	1.14	8.9
38	835414	F	58	109	3.8	36	0.7	91	111	92	16	77	2.15	1.45	11.1
39	783117	F	60	111	4.2	39	0.8	78	199	277	30	113	0.98	1.18	6.8
40	835847	F	55	122	3.7	22	1	61	140	160	28	80	1.78	0.9	8.8

RBS – RANDOM BLOOD SUGAR, ALB- ALBUMIN, BU – BLOOD UREA, CREAT – CREATININE, eGFR – ESTIMATED GLOMERULAR FILTRATION, CHL –CHOLESTEROL, TG – TRIGLYCERIDES, HDL – HIGH DENSITY LIPOPROTEIN, LDL – LOW DENSITY LIPOPROTEIN, TSH – THYROID STIMULATING HORMONE, TT3 – TOTAL TRI-IODOTHYRONINE, TT4 – TOTAL TETRA-IODOTHYRONINE