STATUS OF THYROID HORMONE PROFILE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

A THESIS

SUBMITTED TO

JAIPUR NATIONAL UNIVERSITY

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

In

LIFE SCIENCES (BIOCHEMISTRY)

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AUGUST 2016

CANDIDATE DECLARATION

I, Samir Singh, hereby declare that work, which is being presented in the Thesis entitled ***"Status of Thyroid Hormone Profile in Patients with Chronic Kidney Disease"*** *submitted in fulfillment of the requirements for the degree of* ***Doctor of Philosophy in Life Sciences (Biochemistry)*** *is my original work and has not, to the best of my knowledge, been presented by any other individual at any other institution of higher learning.*

This proposal represents an original study; and has not been presented to any other institution for review and approval.

It is also declared that I have taken course work of one semester at School of Life and Basic Sciences, Jaipur National University, Jaipur.

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SUPERVISOR DECLARATION

This Thesis work entitled ***"Status of Thyroid Hormone Profile in Patients with Chronic Kidney Disease"*** *submitted in fulfillment of the requirements for the award of the degree of* ***Doctor of Philosophy in Life Sciences (Biochemistry)*** *is a bonafide record of the work carried out by Mr. Samir Singh with our approval as University Supervisor and Co-Supervisor.*

It is also certified that the candidate Mr. Singh have attended the course work of one semester at School of Life and Basic Sciences, Jaipur National University, Jaipur.

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DEDICATION

This Ph.D. thesis is dedicated to my beloved father and mother

and

to my wife and daughters

and

to all who believe in me

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to all the people who helped me to accomplish my task successfully. Their continuous wholehearted support, encouragement, advice and enthusiasm inspired me to complete my Ph.D. thesis work.

My deepest gratitude goes to my amiable Supervisor ***Dr. Aman Verma*** *for her lenient, merciful and ever mesmerizing supports, words of inspiration, precious advice and guidance, prolific and absolute suggestion during my entire Ph.D. work period. It has been a great honor for me to be her Ph.D. student. Her positive energy, dedication, and enthusiasm throughout this project are commendable. I appreciate all her patience and contributions throughout this work. Her genuine scientific knowledge not only encouraged me, also inspired to think beyond boundaries. She has transformed me into a meticulous researcher and provided me with a toolbox full of skills and experiences.*

I would like to express my sincere gratitude to ***Prof. Dr. Gopi Aryal****, Co-Supervisor for my Ph.D. work for his valuable support and suggestions. Special thanks to you sir for useful discussions and helping me in every possible way by encouraging me with optimistic suggestions, corrections which help this thesis to complete.*

I am especially indebted to the Nephrologists (Prof. Dr Sudha Khakurel, Dr. Kalpana Shrestha, Dr. Santosh Thapa and Dr. Sarju Raj Singh) of KIST Medical College Teaching Hospital, Lalitpur, Nepal for their deep support throughout the Ph.D. work. My sincere gratitude also goes to the technicians and technologists (especially Liladhar Acharya) of Clinical Laboratory Services.

I would also like to express my warm regards to ***Prof. Dr. Balman Singh Karki****, Principal of KIST Medical College Teaching Hospital for his valuable advice and cordial cooperation admiration to complete this work. I also express my thankful words to my colleagues Mrs. Rashmi Jha, Mr. Yashad Dongol, Dr. Amit Singh, Mrs. Shipra Parmar, Dr. Nisha Jha, Dr. Shiv Raj K.C, Mr. Mahesh Chaudhary, Dr. Nikki Jha and Mrs. Omi Bajracharya for their incessant and unforgettable support in every moment of my research work.*

I am very much thankful to Mr. Bikesh Shrestha of East West Trading Private Limited, Kathmandu for proving me free thyroid function test reagent kit. I am highly obliged to my

colleagues of Clinical Biochemistry Department. I am highly gratified to Prof. Dr. Amita Pradhan and Padam Dahal of Community Medicine Department for their statistical support regarding analysis of my data in my thesis work.

I also express my sincere thanks to ***Honorable Sandeep Bakshi****, the Chancellor of Jaipur National University,* ***Prof. K.L. Sharma*** *(Honorable Pro-Chancellor) and* ***Prof. H.N. Verma*** *(Honorable Vice-Chancellor) for guidance and counseling for the completion of this work. Thanks are also due to Dr. Divya Shrivastava, Joint Director, School of Life Sciences, Jaipur National University for steady prop up throughout my research. I am also grateful to respected faculty members of School of Life Sciences Dr. Nihamohan Kulshreshtha, Dr. Nehi Sinha, and Dr. Kapilesh Jadav for continuous official support, prompt communications and helping me to do my best.*

I would also like to thank ***Er. Ashish Thapa****, Managing Director of KIST Medical College for his continuous support by providing creative environment and space during my research period. I am also thankful to the patients visiting Nephrology and Dialysis unit of KIST Medical College for providing samples for my work.*

Especially, I owe a debt of gratitude from inner-core of heart to my beloved parents, Late Prof. Shubhchandra Kumar Singh and Late Mrs. Nirmala Singh, who always taught me the value of education and nurtured me to try harder and to be a better person. Their heavenly blessing are always with me. I also wish to thank my great educationalist and philosopher Uncle ***Prof. Dr. Mahendra Prasad Singh****, Honorable Ambassador of Qatar from Nepal and my elder brothers Prof. Dr. Ashok Singh, Mr. Sudhir Singh, Er. Amul Singh, Adv. Satish Singh and Shashank Singh (younger brother) for their immense love and support for this work. Special thanks to my sweet daughters, Shubhani and Aarohi who use to cheer me on with a very bright smile. I am also thankful to my wife Mrs. Lalani Singh, she was with me through the entire PhD work, giving perspective, cheering me on, and helping in many ways, making personal sacrifices, through this long process. Besides this, several people have knowingly or unknowingly helped me in the successful completion of this project. I am thankful to all of them.*

Place: Jaipur ***(Samir Singh)***

Date:

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CHAPTER: ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

The term 'Chronic Kidney disease (CKD)" is now taking the place of chronic renal failure (CRF) or insufficiency. CKD infers long-standing, and usually progressive, impairment in renal function (Kumar & Clark, 2012). CKD is turning into an important general health issue around the world (Abraham et al., 2016; De Nicola & Zoccali, 2016). Chronic glomerulonephritis (CGN) was the one of the main cause of kidney diseases quite a few years back (Barsoum, 2006; Ikechi, 2016; Khakurel *et al*., 2015). These days, infections have turned into a less vital reason for kidney diseases. Besides, current evidence recommends that diabetic nephropathy (DN) and hypertension (HTN) are among the main leading causes for CKD overall (Ghaderia & Beladi-Mousavi, 2014; Hahr & Molitch, 2015; Malekmakan *et al*., 2016).

Patients with CKD are at increased risk for progression of end stage renal disease (ESRD) which requires renal replacement therapy (RRT) or renal transplantation for long-term survival (Hanif, et al., 2016; Levey *et al*., 2007; Zhang *et al*., 2008). Though, there are very few data related to the prevalence of pre-dialysis CKD in developing countries, the overall prevalence of CKD established on a urinary albumin/creatinine ratio or glomerular filtration rate (GFR) in urban areas of Nepal is 10.6% (Sharma *et al*., 2013).

Nepal is a federal republic and landlocked country of South Asia with a population of approximately 28 million. In current scenario, country has only 30 nephrologists. Till 1980, there were no services for RRT in Nepal. Peritoneal dialysis (PD) and renal biopsies were started by the late Dr. Puskar Raj Satyal at Bir Hospital (oldest Hospital of Nepal), Kathmandu in 1980 AD. With the assistance of India, Government of Nepal had established country first hemodialysis (HD) unit in the same hospital with two working HD machines in 1986 AD. The present nephrology services are still inadequate and must be extended throughout the nation. Moreover, only the major cities have the optimal facilities for nephrology care (Abraham *et al*., 2016).

National Kidney Foundation- Kidney Diseases Outcomes Quality Initiative (NKF-K/DOQI) of USA define and classify CKD in 2002, which is still internationally accepted. This classification

defines CKD as kidney damage or GFR <60.0mL/min/1.73m^2 for three months or more irrespective of the cause (National Kidney Foundation, 2002).

Thyroid hormone (TH) is released by the thyroid gland and is directed by the anterior pituitary hormone named thyroid stimulating hormone (TSH), which is under the control of the hypothalamic thyrotropin-releasing hormone (TRH). Thyroxine (T4) is formed basically by the thyroid gland and is changed over to the biologically active form triiodothyronine (T3) (Mariani & Berns, 2012; Mullur *et al*., 2014; Ross, 2016).

Thyroid hormone profile includes the test parameter such as free triiodothyronine (fT3), total free triiodothyronine (TT3), free thyroxine (fT4), total thyroxine (TT4) and TSH. T3 and T4 are present in both bound and free form. The free hormones are really active molecules. The free fractions of the hormones can be measured accurately by Chemiluminiscence Immunoassay (CLIA) method (Vasudevan & Sreekumari, 2013).

TH and Kidney are interconnected by numerous mechanisms (Ramirez *et al*., 1996). During embryogenesis, thyroid hormone is involved in the growth and development of several components of the kidney. TH influences the kidney by systemic on the other hand local hemodynamic changes and by an immediate impact on the function of this organ. TH are essential for the electrolyte and water maintenance. Likewise, TH are also involved in the stimulation of renin that is formed by the juxtaglomerular cells of Kidney. This process is completely independent of the ouabain sensitive sodium pump and protein synthesis and also effect kidney angiotensinase activity. Kidney, on the other hand plays an important role in the regulation and removal of TH (Dousdampanis *et al*., 2014; Miulescu *et al*., 2014).

The etiology of thyroid dysfunction in CKD patients is multifactorial (Iglesias & Díez, 2009). Renal tubular function and GFR are highly influenced by thyroid function abnormalities. Out of several thyroid dysfunctions, hypothyroidism is commonly observed in CKD patients (Miulescu *et al*., 2014; Rhee *et al*., 2014). The frequency of clinical and subclinical hypothyroidism and low T3 syndrome rises with the advancement of CKD, most likely because of the diminished action of the enzyme T4-5′ - deiodinase (Dousdampanis *et al*., 2014).

Low activity of TH is escorted by a failure to excrete an oral water overload and due to reduction in the GFR. The first and the furthermost common thyroid function defect in CKD patients is a

low T3 level. This low T3 disorder happens in CKD because of a multiple reasons. Fasting, chronic metabolic acidosis and protein malnutrition influence iodothyronine deiodination, and protein binding of T3, reducing the chances of conversion of T4 to T3. Furthermore, inflammatory cytokines, for example, tumor necrosis factor (TNF)-α and interleukin (IL)-1 hinder the expression of 5'- deiodinase, which is the main enzyme responsible for peripheral conversion of T4 to T3 (Basu & Mohapatra, 2012).

Several studies showed a low T4 level in CKD patients. The free T4 levels may be low to normal in CKD due to and defective binding of T4 in CKD. This impelled the thought of a "sick euthyroid state" in CKD, which is nowadays named as Non-thyroidal illness (NTI) (Basu & Mohapatra, 2012; Mohamedali *et al*., 2014; Singh *et al*., 2006).

One of the most common problem associated with CKD, is the derangement of mineral metabolism commonly observed during early stages of CKD. The minerals such as calcium and phosphorus are the mainly affected in the progression of CKD (Singh et al., 2012). The main role of healthy kidney is the conversion of inactive vitamin D to its active metabolite, 1,25-dihydroxy cholecalciferol also known as calcitriol. Due to the reduction in renal function, the production of calcitriol decreases resulting low plasma concentration of calcium (hypocalcaemia). So, there is less absorption of dietary calcium through the intestine. Additionally, the renal clearance of phosphorus decreases with a deteriorating kidney function that cause the elevation of plasma phosphorus concentrations known as hyperphosphatemia. Hypocalcaemia stimulates the synthesis of parathyroid hormone (PTH) from parathyroid glands (Walker et al., 2014).

For a long time, nephrologists have been worried about the legitimacy of the biological markers used to assess nutritional status in CKD and ESRD patients. The most ideal and trustworthy clinical marker is serum albumin, commonly used by the kidney consultants. Previous studies have demonstrated that serum albumin is a dependable indicator of nutritional status which additionally shows an outstanding response to nutritional mediation (Chung et al., 2012). Elevated albuminuria/proteinuria signifies deteriorating CKD and CVD risk, particularly in DN, HTN and patients with glomerular ailments. The worldwide prevalence of proteinuria in CKD population is 4-8% and 10-20% in hypertensive, obese, and diabetic peoples. Persons at high risk for CKD must go through testing for proteinuria (Henry Ford Health System, 2011).

1.2 RATIONALE FOR THE STUDY

CKD is a major public health problem and its prevalence has reached epidemic proportions in some countries. It is an important cause of morbidity and mortality. Impaired kidney function can affect TH metabolism. Hypothyroidism, NTI as well as hyperthyroidism have been reported in CKD patients. Thyroid dysfunction may worsen the morbidity in CKD patients and increase cardiovascular mortality. Low T3 has been found to be an independent predictor of cardiovascular mortality in CKD patients.

There is very few documented data on CKD in Nepal and the prevalence of CKD in Nepal is estimated to be 10.6%. This prevalence is approximately same as compared to global prospects. CKD patients either on HD or on conservative management showed biochemical features of hypothyroidism. This study sought to describe the nature and magnitude of thyroid hormone derangements in CKD patients, given the global increase in CKD prevalence. The information can provide evidence on the value of including thyroid hormone estimations in CKD patients.

1.3 SIGNIFICANCE OF THE STUDY

1. The information available about thyroid function and its relation with CKD in developing countries including Nepal is scare. Therefore, this study will be among few studies that will be conducted in Nepal according to the knowledge of the researcher.

2. The clinical significance of the study will to see the extent to which serum fT3, fT4 and TSH are related to CKD so that the description of thyroid functions will become clearer.

3. This study will help the Nephrologists of Nepal to revise the treatment protocol of CKD patients.

4. The prevalence of CKD in Nepal is increasing, so this study will generate awareness among CKD patients with thyroid function disorders.

1.4 OBJECTIVE OF THE STUDY

1.4.1 General objective

The general objective of the research was to evaluate the status of thyroid hormones profile, minerals and malnutrition indicator in patients with chronic kidney disease visiting Nephrology and Dialysis Unit of KIST Medical College Teaching Hospital (KISTMCTH), Imadol, Lalitpur district, Nepal.

1.4.2 Specific objectives

1. To estimate serum creatinine of CKD patients visiting Nephrology and Dialysis Unit of KISTMCTH, Imadol, Lalitpur district, Nepal

2. To estimate GFR values by using Cockcroft-Gault equation based on the creatinine clearance for the categorization of CKD stages.

3. To determine the levels of serum fT3, fT4 and TSH in CKD patients in relation with the different stages.

4. To describe the types of thyroidal dysfunctions in CKD patients

5. To assess a possible association between fT3, fT4 and TSH with CKD.

6. To assess the mineral derangement in CKD patients

7. To evaluate the albumin as a nutritional marker in CKD

CHAPTER: TWO

LITERATURE REVIEW

2.1 KIDNEY STRUCTURE AND FUNCTION

2.1.1 Kidney

The kidneys are bean shaped paired organs specialized in performing a wide varieties of role in vertebrates. They remove the nitrogenous wastes from the blood via urine. They are essential part of urinary system furthermore serve homeostatic functions, for example, the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure. Every adult kidney weighs about 150 grams which is about the size of clenched fist (Guyton & Hall, 2012).

2.1.2 Structure of Kidney

Each kidney of adults measuring about 11-14 centimeters (cm) in length, 5-6 cm in width and 3-4 cm in depth in adults. Both kidneys lie at the retroperitoneal position on either side of the vertebrae at the level of T12 to L3 as shown in **Figure 1**. The renal parenchyma consist of an outer cortex and an inner medulla (Kumar & Clark, 2012). The renal medulla contains 8-10 pyramid shaped masses called the renal pyramids, isolated by renal columns. Each cone shaped pyramid has base that typically originates from the lining present in between cortex and medulla that continued into space of renal pelvis. The outer lining of pelvis has two distinct part called major calyces and minor calyces, which involve in the collection of urine from the tubules of each papilla. Approximately one million of nephrons are present in the kidney which is regarded as the functional unit of the kidney. Glomerulus, proximal tubule, loop of Henle, distal tubule and collecting duct are the part of nephron. The renal capsule and ureters are innervated through T10-12 and L1 nerve roots, and renal pain is fondled over the corresponding dermatomes. Renal blood flow is about 22% of total cardiac output *i.e.* 1100 milliliters/minutes. Renal arteries supply the arterial blood to the kidneys. As compared to right vein, the left renal vein is longer and due

to this reason, the left kidney is selected for live donor transplant nephrectomy (Guyton & Hall, 2012; Kumar & Clark, 2012).

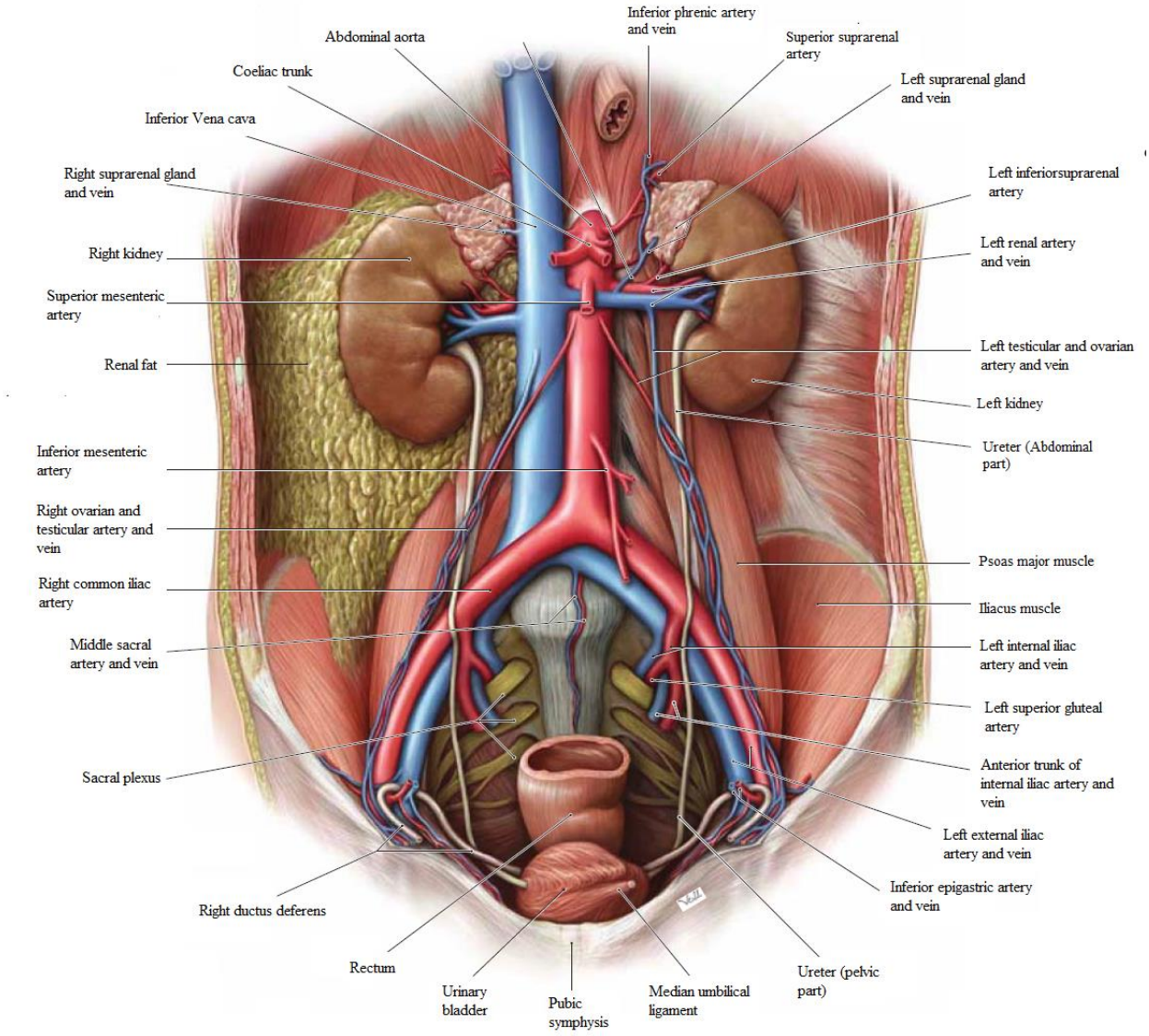


Figure 1: Structure of Kidney Disease (Gilroy et al., 2016)

2.1.3 Physiology of Kidney

The kidneys play a vital role in the maintenance of normal body function. The basic function of the kidneys is the formation of urine through complex mechanisms that involved filtration, reabsorption and secretion. Moreover, the kidneys also excrete urea and uric acid which are the

end products of protein and nucleic acid metabolism. The kidneys regulate fluid, electrolyte and acid base balance of the body and create a steady environment for the metabolic processes of tissues and cells. This function is essential for life and it is realized by balancing solute and water transport, excreting metabolic waste products, conserving nutrients, and regulating acid-base balance in the body (Scott & Quaggin, 2015).

The kidney also produces three main hormones; erythropoietin which stimulates the production of red blood cells, renin which regulates blood pressure and calcitriol (the active form of vitamin D) which helps in regulation of plasma calcium levels (Kurt & Kurtz, 2015).

2.2 DEFINITION, CLASSIFICATION AND STAGING OF CHRONIC KIDNEY DISEASE

Kidney disease results in the loss or reduction of functional nephrons. CKD, a new terminology that has replaced CRF was defined in 2002 by the National Kidney Foundation Kidney Disease Quality Outcome Initiative (NKF/KDOQI). The aims of this clinical practice guidelines are to

1) define chronic disease and classify its stages, regardless of underlying cause
2) evaluation of laboratory measurements for the clinical assessment of kidney disease
3) association of the level of kidney function with complication of CKD
4) stratification of the risk for loss of kidney function and development of chronic vascular disease

CKD is defined as structural damage or GFR<60 ml/min/1.73m^2 for more than three months. Kidney damage is defined by the NKF/KDOQI as pathological abnormalities or markers of kidney damage, including abnormalities in urine or blood tests or abnormal imaging tests (NKF/KDOQI, 2002). In developing countries, there is high population of undetected early-stage CKD. The goal of NKF is to disseminate the simple definition and five-stage classification system of CKD, to recapitulate the major recommendations on early detection of CKD in adults and to analyze some of the issues concerned with recommendations. This information is particularly important for the clinicians to screen the early-stage CKD to prevent the human and economic cost of CKD (Levey *et al*., 2003).

The level of kidney function in all patients with chronic kidney disease can be uniformly measured regardless of the fundamental cause of the disease (Levey *et al*., 2003). In the past, there has been a lack of agreement on how the progression of chronic kidney disease should be defined and classified. This may have contributed to under-diagnosis and under-treatment of early kidney disease resulting in lost opportunities for slowing or preventing disease progression. In the literature, it is widely agreed that starting treatment at the right stage in the progression of CKD is essential to help slow disease progression and prevent adverse outcomes (Levey *et al*., 2003; Pereira, 2000; NKF/DOQI, 2002; St Peter *et al*., 2003). In an attempt to reach a consensus and provide a common ground on which to base future treatment and research, the American NKF/KDOQI work group developed a classification system that separated the period from very early kidney disease to ESRD into five stages (NKF/DOQI, 2002). Definitions were based on renal function as measured by the GFR of the patient. Normal kidney function is said to equate to a GFR of 120-130 ml/min with deterioration in kidney function correlating with a reduction in the glomerular filtration rate. **Table 1** describes the five stages of chronic kidney disease.

Stage 1 of CKD is described as the very early period of the disease where only minor kidney damage has occurred. Usually, clinical symptoms are absent at this point, which make diagnosis very difficult. This is the ideal time to provide treatment for the underlying kidney disease, along with appropriate management of allied conditions like hypertension and diabetes.

Patients who are classified as having **Stage 2** CKD have a glomerular filtration rate of between 60 and 89 ml/min/ 1.73 m^2 and suffer from a mild degree of kidney damage. Aggressive management of the underlying causes of the disease and emerging manifestations, for example, calcium and phosphate imbalance, hyperglycemia and anaemia, are recommended (Silverberg, 2003; St Peter *et al*., 2003).

Stage 3 CKD indicates a further decline in kidney function with possibly some clinical signs beginning to appear. As mentioned previously, it is not uncommon for a patient to reach this stage of the disease without knowing that they have a problem. Again ongoing specialist treatment and follow up of these patients is essential to try and maintain kidney function and prevent such complications as cardiovascular disease, anaemia, malnutrition and bone disease.

Stage 4 of CKD means that end stage failure is imminent and preparation for renal replacement therapy (dialysis or transplantation) is required.

Stage 5 CKD is defined as ESRD where dialysis or transplantation is mandatory to sustain life.

The need to provide a common language for communication among providers, patients and their families, investigators and policy-makers was the reason the American National Kidney Foundation developed the five-stage classification system. Defining CKD this way provides opportunities to direct the most effective treatment at a particular stage of the disease process (Compton *et al*., 2002).

In addition, classification seeks to provide a framework for developing guidelines for clinical practice, clinical performance measures, and improvement of continuous quality tasks (Parker *et al*., 2004). The classification of the stages of kidney disease by the American National Kidney Foundation has been integrated in some recent American and British literature in association with policies for prevention and early discovery of CKD (Compton *et al*., 2002; Parmar, 2002; St Peter *et al*., 2003). As this classification system has only been available for almost a decade it is difficult to predict the extent to which it will be utilized internationally. Kidney function and the outcome of kidney disease have been outlined along with a way of defining the loss of kidney function into stages. The five stages of chronic kidney disease, as described were developed in an attempt to provide a common language for nephrology health care professionals to use to promote international best practice in the management of CKD.

Table 1: Stages of CKD (Kumar & Clark, 2012)

Stage	GFR (mL/min/1.73 m^2)	Description
1	≥90	Normal or increased glomerular filtration rate (GFR), with other evidence of kidney damage
2	60–89	Slight decrease in GFR with other evidence of kidney damage
3A 3B	45–59 30–44	Moderate decrease in GFR with or without other evidence of kidney damage
4	15–29	Severe decrease in GFR with or without other evidence of kidney damage
5	<15	Established renal failure

2.3 GLOMERULAR FILTERATION RATE (GFR)

Glomerular filtration rate is defined as the rate at which filtered fluid flows through the kidney. GFR provides the best reliable measure to access renal function. The normal values of GFR varies with age, gender and body mass index. Normal values of GFR in young adults ranges from 120-130 ml/min/1.73m^2 and declines with age. A GFR value less than 60 ml/min/1.73 m^2 indicates the loss of approx. half of the adult level of normal kidney function. Prevalence of the complications of CKD increases below this level. Age-related decline of GFR is considered as normal aging process. But significantly decreased GFR in elderly is alarming and is and independent predictor of adverse outcomes such as CVD. Additionally, declining of GFR in elderly necessitates adjustment in drug doses as in other patients with CKD. Hence, the definition of the chronic kidney disease is the same regardless of the age. Because the GFR decreases with age, the occurrence of CKD increases with age. It is found that approx. 17% of people over 60 years of age has GFR less than 60 ml/min/1.73 m^2.

The guidelines given by NKF to define kidney failure as either

1. GFR less than 15 ml/min/1.73 m^2 which is accompanied by signs and symptoms of uremia in most of the cases or
2. A need to start kidney replacement therapy (dialysis or transplantation).

Creatinine clearance (CCr or CrCl) refers to the amount of blood plasma cleared of creatinine per unit time and is a convenient measure for estimating the GFR. Together, GFR and CCr may well be accurately calculated by relative measurements of substances in the blood and urine, or calculated by formulas using only a blood test result (GFR and CCr). These test results are important in the assessment of the excretory capabilities of the kidney. For example, classification of CKD and dosage of drugs that are excreted mostly in urine are based on GFR or creatinine clearance (Lesley *et al*., 2006)

Various methods are available for estimating GFR recommended by the nephrologists are briefly described here:

2.3.1 Clearance method

The idea behind renal clearance was proposed as a way of expressing the relationship between the excretion per unit time and the concentration in the plasma which is obviously an index of the kidney's ability to clear the blood of any substance (Harvey, 1980). Measurements of GFR are by tradition based on the renal clearance of a plasma marker, expressed as the volume of plasma wholly cleared of the marker per unit time. If the marker has no extra-renal elimination, tubular reabsorption or secretion then the clearance is defined by the formula

GFR = UV/P, where

U = Urinary Concentration of the substance

V = Urine flow rate (urinary volume/time)

P = Average plasma concentration

The perfect marker should be endogenous; in addition it must be filtered freely by glomerulus. Furthermore it should neither be reabsorbed nor secreted by the renal tubule and eliminated solely by the kidney. Such a marker is not yet recognized. A variety of markers used to measure GFR include exogenous (inulin, iothalamate) or endogenous (urea, creatinine) substances (Ferguson & Waikar, 2012; Sirwal *et al*., 2004).

2.3.2 GFR prediction from plasma creatinine

An estimate of bedside GFR is often obtained from plasma creatinine concentration alone in clinical practice though with some level of accuracy (Chen, 2013). A formula that will permit an immediate estimation of GFR from plasma creatinine has been considered by a number of researchers. Approximation of GFR from plasma creatinine may give erratic results because plasma creatinine is dependent on GFR as well as on muscle mass which varies with gender, age and weight. Cirrhosis and muscle wasting diseases lead to a reduction in plasma creatinine; conversely ingestion of high amounts of protein can result in increase in plasma creatinine levels of up to 10% (Hull *et al*., 1981; Parke *et al*., 2015).

Furthermore, a marked reduction in GFR can be present before it shows in the concentration of plasma creatinine beyond the upper limit of the reference range. The value of these formulae for GFR prediction is likely to increase when there is an accurate plasma creatinine measurement in addition to inhibition of tubular secretion of creatinine by cimetidine. To improve the estimation of GFR from plasma creatinine concentration, formulae have been derived which incorporate variables like weight, height, age, and gender (Azar, 2013; Choi *et al*., 1993).

2.3.3 GFR estimation by new endogenous markers

a) **β2-Microglobulin** (M.W 11815 D) is filtered at glomerulus like water. Afterwards almost the entire substance is reabsorbed and broken down in the renal tubule. The plasma concentration in health is often low because it is filtered so freely (average 1.5 mg/L). The plasma concentration increases as the GFR declines reaching about 40 mg/l in terminal uremia. Plasma β-microglobulin concentration logarithm is linearly related to the logarithm of glomerular filtration rate through the whole range so that it serves as a good marker of renal dysfunction. The plasma concentration of β-microglobulin is neither affected by muscle mass nor by the sex of an individual. The estimation of this substance entails the use of expensive radioimmunoassay and this has limited its use in clinical practice. Rise in plasma concentration could be due to increased production rather than reduced clearance in patients with some tumors and inflammatory diseases (Dajak et al., 2010; Purde *et al*., 2016; Sirwal *et al*., 2004)

b) **Cystatin C** is a 13-KD protease inhibitor which is produced generally by nucleated cells. It is neither affected by the muscle mass nor sex of an individual. Its production, unlike β2-microglobulin is not affected by states of inflammation or malignant conditions. Cystatin C is usually excreted by filtration through the glomerulus and metabolized in the cells of the proximal tubules. Its measurement has been projected as an alternative and more precise marker of GFR compared to creatinine especially among patients with slight to moderate reductions in GFR (Dajak *et al*., 2010; Newman *et al*., 1995; Purde *et al*., 2016).

2.4 HEALTH CARE SYSTEM IN NEPAL

Nepal is a federal republic and small country with landlocked territory in South East Asia bordered by China to the north and by India to the south, east and west having an area of 147,181 square kilometers. Nepal is known as the land of Mount Everest, the tallest mountain on earth, as the birthplace of Lord Buddha and Goddess Janaki. The country lies between the two economic giants India and China. The nation-state of Nepal was the formation of King Prithvi Narayan Shah. The leader of the small realm of Gorkha, battled to join the different kingdoms that spotted the land area characterized by modern Nepal. The triumph of Kathmandu Valley, which took a total of ten years of arranging, attack and discretion, was the highlight of his successes (1769). The work started by King Prithvi Narayan was proceeded by his descendants. At the best degree the Nepali (then known as the Gorkhali), the present Empire was secured an area that was no less than a third more than its present limits. Nepal as shown in **Figure 2** can be separated comprehensively into three ecological zones: the lowland, the midland and the highland (http://www.nationsonline.org /oneworld/nepal.htm).

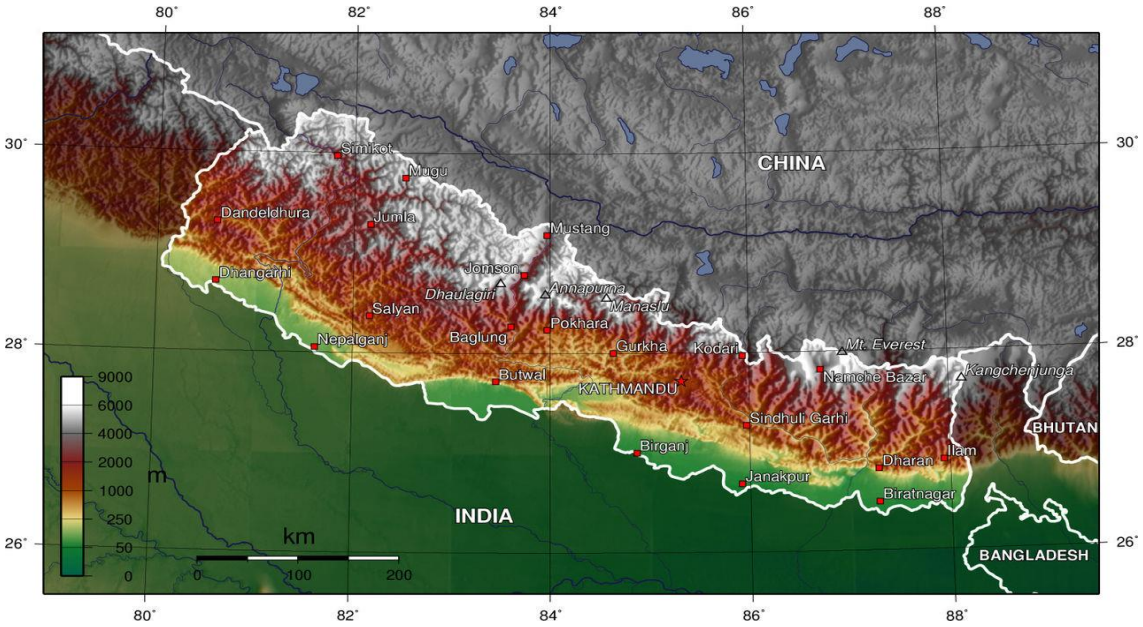


Figure 2: Map of Nepal showing the different ecological divisions (https://en.wikipedia.org/wiki/Nepal)

The altitude of the Himalayan Region (the highland) ranges between 4877 m to 8848 m, It incorporates 8 of the highest 14 summits in the world, which surpass altitude of 8000 meters including Mount Everest. The mountain region represents around 64 percent of total area, which is shaped by the Mahabharat range that takes off up to 4877 m and the lower Churia range. The lowland Terai, the level river plain of the Ganges with a belt of marshy grasslands, savannas, and forests, possesses around 17% of the total area of the nation. Adult literacy rate was 65.9% in Nepal. Nepal is divided into seven provinces according to new constitution as shown in **Figure 3** (Ghimire, 2014; Constitution of Nepal, 2015; http://www.nationsonline.org/oneworld/nepal.htm).

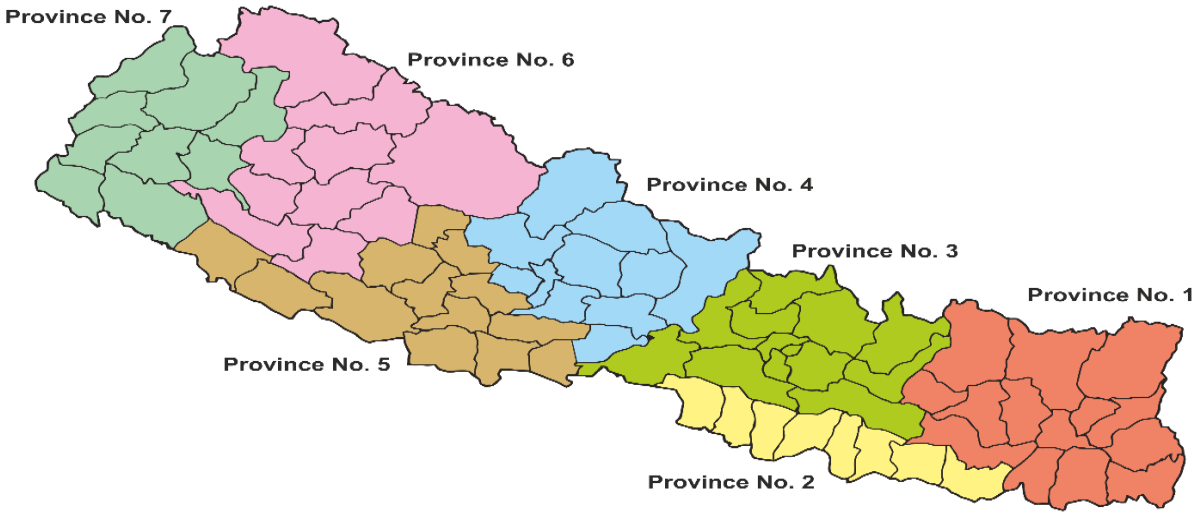
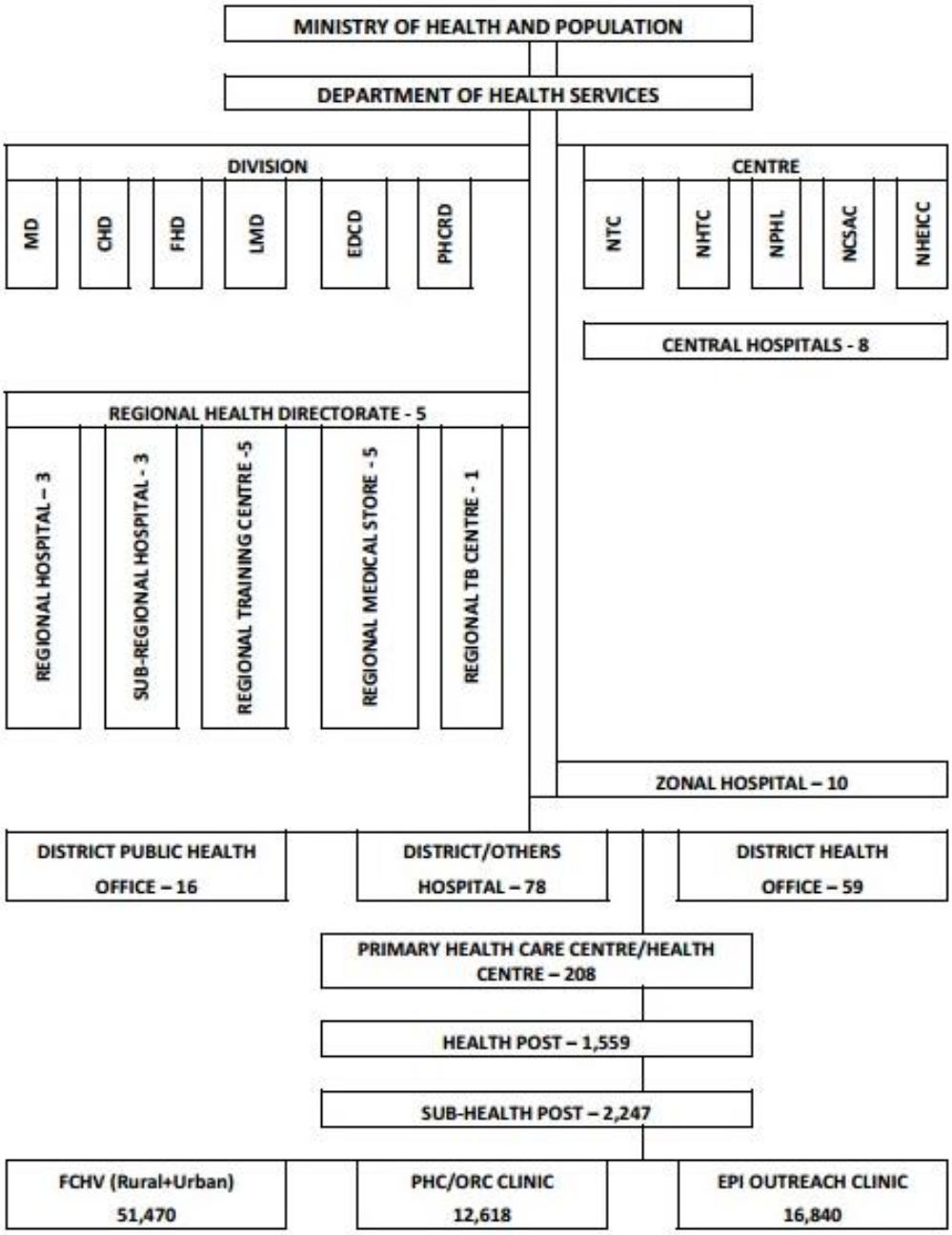


Figure 3: Provinces of Nepal (https://en.wikipedia.org/wiki/Nepal)

Population of Nepal as of the census day (June 22, 2011) stands at 26,494,504 showing population growth rate of 1.35 per annum. Similarly, Total number of households in the country is 5,427,302 with 5,423,297 individual households and 4,005 institutional households (Barracks, Hostels, and Monasteries etc.). Census is done in Nepal in every ten years duration. During the census, all the factors of population in different categories like age, religion, gender, employment are determined (National report on National Population and Housing Census, 2011).

Citizen's health and development of the nation are inter-related and the progresses made in the health sector are considered as the major indicators for development. Interim constitution of Nepal has established health as the fundamental right of people. Ministry of Health and Population of Nepal is trying to maintain the achievements made in the control of communicable diseases and reduction of infant and child mortality rate, and move towards controlling of non-communicable diseases, better management of medical emergencies and ensuring quality health services to all people including old, physically and mental disabled, single women, poor, marginalized and at risk communities. National Heath Policy of Nepal was established in 2048 B.S. for the motive to protect the achievements made so far and to adequately address prevalent and new challenges faced within health sector by developing people-centered and efficient management through optional utilization of available means and resources. To provide promotive, preventive, curative and rehabilitative health services, National Health Policy 2048 has been updated and National Heath Policy 2071 (2014) has been formulated as described in **Figure 4** (http://www.mohp.gov.np/index.php/publication-1/policy).

Department of Health Services (DoHS) is responsible for delivering preventive, promotive, diagnostic and curative health services throughout Nepal. Director General (DG) is the organisational head of the DoHS. The current organizational structure of the DoHS includes six Divisions: i) Management Division with infrastructure, planning, quality of care, management information system and free medication & treatment for very severe disease to impoverished Nepalese citizens. ii) Child Health Division covering Nutrition and Newborn care. iii) Family Health Division with the responsibility of reproductive health care, including safe motherhood and neonatal health, family planning and Female Community Health Volunteers (FCHVs). iv) Logistics Management Division covers procurement, supplies and management of logistics, equipment and services required by DoHS and below level v) Epidemiology and Disease Control Division with the responsibility of controlling epidemics, pandemic and endemic diseases as well as treatment of animal bites. vi)Primary Health Care Revitalization Division with the responsibility of carrying out activities for primary health care (http://dohs.gov.np/wp-content/uploads/2014/04/ Annual_Report_2070/71).



MD: Management Division CHD: Child Health Division EDCD: Epidemiology and Disease Control Division FHD: Family Health Division LMD: Logistic Management Division PHCRD: Primary Health Care Revitalization NTC: National Tuberculosis Center NHTC: National Health Training Center NPHL: National Public Health Lab NCSAC: National Center for AIDS and STD Control NHEICC: National Health Education, Information and Communication Center

Figure 4: Organogram of Health Policy of policy of Nepal (http://dohs.gov.np/wp-content/uploads/2014/04/ Annual_Report_2070/71)

2.5 HISTORY OF CKD IN NEPAL

The current census of Nepal estimated by Worldometers algorithm, which processes data collected from the United Nations Population Division on July 17, 2017 is approximately 29 millions (http://www.worldometers.info/world-population/nepal-population/). Population of Nepal, according to the census done by Central Bureau of Statistics in 2011 was 26,494,504 (http://cbs.gov.np/sectoral_statistics/population/national_report.pdf). A total number of Nephrologists in Nepal is 30 only till now. Until 1980, the country was running lack of renal replacement therapy but in the early 1980s, late Dr. Puskar Raj Satyal took pioneer action in development of nephrology department and initiated intermittent peritoneal dialysis and renal biopsies at Bir Hospital, Kathmandu. With the support by Government of India in 1986, the first hemodialysis (HD) unit was established in the same department of Bir Hospital with two functioning dialyzers (HD machines).

A second dialysis unit was established in 1996 in Institute of Medicine (Tribhuvan University Teaching Hospital), Kathmandu ensued by a few other private hospitals and dialysis centres within as well as outside the capital. With the support of Nepal Kidney Foundation, Dr. Rishi Kafle has established a large dialysis unit that started on a small scale in 1997 and provides free or at a very subsidized rate dialysis to a large section of the Nepali CKD population. Dr. Sanjib Sharma's work, on early detection and prevention of renal disorder in Dharan in eastern part of Nepal, is also appreciable, was endorsed by the International Society of Nephrology (Abraham et al., 2016). In August 2008, the first renal transplantation was performed by Dr. David Francis at Tribhuvan University Teaching Hospital, with the support of Nepalese surgeon Dr. Dibya Singh Shah. Till date, more than 200 renal transplantation have been done successfully in this hospital. Two more centers for renal transplantation are also functioning in Nepal and a total of approximately 400 successful renal transplantation have been done in the country till now. Now, continuous ambulatory peritoneal dialysis (CAPD) service is also being operated and around 100 patients are on CAPD. Nearly 1500 patients are on HD in 41 HD centers with 252 HD machines (Abraham, 2008).

Table 2: Prevalence of decreased eGFR, albuminuria and CKD, adjusted for country, ethnic origin and sex (Ene-Iordache et al., 2016)

	Participants (n)	Men (%)	Age (years)	Prevalence (95% CI)		
				eGFR <60 mL/min per 1·73 m²	ACR >30 mg/g	Chronic kidney disease
General population cohorts						
Eastern Asia	..	..	..	..	..	..
China	7340	25·9%	53·4 (10·2)	13·0% (12·2–13·8)	19·6% (18·7–20·5)	29·9% (28·9–31·0)
Mongolia	832	22·8%	41·1 (13·6)	9·6% (7·7–11·9)	11·3% (9·3–13·7)	18·0% (15·5–20·8)
Southern Asia	..	..	..	..	..	..
India	3196	42·9%	50·1 (13·4)	2·5% (2·0–3·1)	15·4% (14·1–16·7)	16·8% (15·5–18·1)
Nepal	21066	38·6%	40·8 (15·6)	16·2% (15·7–16·7)	5·8% (5·5–6·2)	20·1% (19·6–20·6)
Middle East	..	..	..	..	..	..
Iran	31615	98·4%	43·8 (11·3)	5·8% (5·5–6·0)	0·6% (0·5–0·7)	6·3% (6·1–6·6)
Africa	..	..	..	..	..	..
Nigeria	1912	36·6%	44·3 (13·2)	20·7% (18·9–22·6)	3·9% (3·1–4·9)	23·0% (21·2–25·0)
Eastern Europe	..	..	..	..	..	..
Moldova	1403	29·4%	50·7 (14·3)	11·2% (9·6–13·0)	17·1% (15·2–19·2)	25·5% (23·3–27·9)
Latin America	..	..	..	..	..	..
Bolivia	3410	36·1%	41·6 (13·7)	1·7% (1·3–2·2)	4·5% (3·9–5·3)	5·5% (4·7–6·3)
Ethnic origin	..	..	..	..	..	..
Eastern Asian	8168	25·6%	52·1 (11·2)	12·6% (11·9–13·4)	18·8% (17·9–19·6)	28·7% (27·7–29·7)
Southern Asian	24244	39·1%	42·0 (15·7)	14·4% (13·9–14·8)	7·1% (6·8–7·4)	19·7% (19·2–20·2)
Black African	1934	37·1%	44·3 (13·2)	20·5% (18·7–22·4)	4·0% (3·2–5·0)	23·0% (21·1–24·9)
White	33022	95·5%	44·1 (11·5)	6·0% (5·8–6·3)	1·3% (1·2–1·4)	7·1% (6·9–7·4)
Other	3406	36·1%	41·6 (13·7)	1·7% (1·3–2·2)	4·5% (3·8–5·2)	5·5% (4·7–6·3)
Sex	..	..	..	..	..	..
Men	45041	..	44·2 (13·1)	7·3% (7·1–7·6)	3·0% (2·9–3·2)	9·7% (9·5–10·0)
Women	25733	..	44·1 (14·3)	14·2% (13·8–14·6)	9·9% (9·5–10·3)	22·2% (21·7–22·7)
Overall	70774	63·6%	44·2 (13·5)	9·8% (9·6–10·0)	5·5% (5·4–5·7)	14·3% (14·0–14·5)

A remuneration of up to $1890 to renal transplant patients for transplant surgery and immunosuppressive drugs and up to $ 2460 for HD and CAPD are provided by government of Nepal yearly (Reddy *et al*., 2013). Even though, the current nephrology services are not sufficient fulfil the need of large scale of CKD patients and must be explored throughout the country. Till now, the facilities for Nephro care is limited to the major cities only. The services of nephrology

care should be affordable and accessible to all Nepalese population. Till now, our program offers only live related donors for renal transplantation, it is right time to introduce a deceased donor transplantation program in Nepal (Abraham *et al*., 2016). Ene-Iordach and colleagues analyzed data from 21066 Nepalese people covering both general and high-risk population and reported that the prevalence of CKD in Nepal was 20.1% in both groups which was described in **Table 2** (Ene-Iordache *et al*., 2016). In summary, awareness, early detection and timely management of renal disorder would help to minimize the burden of this disease in Nepal (Abraham *et al*., 2016).

A total of CKD in Nepal is estimated to be 10.6% in urban areas only (Perico & Remuzzi, 2012; Sharma *et al*., 2013) and the major predisposing factors that leads to end stage renal disease (ESRD) in Nepal are supposed to be Diabetes mellitus, GN and HTN (Ghimire *et al*., 2014).

2.6 GLOBAL PREVALENCE OF CKD

Prevalence of this disease is increasing exponentially thus constitutes a major health priority worldwide. The expenses of this growing epidemic represents a huge burden on health sector worldwide (World Kidney Day: Chronic Kidney Disease, 2016). 10% of the world population i.e. approx. 500 million people have been estimated globally having CKD, among which millions die each year because of unaffordable treatment (World Kidney Day: Chronic Kidney Disease, 2016; Matsha *et al*., 2013). According to the 2010 Global Burden Disease study, CKD was ranked 27th in the list of causes of total number of deaths worldwide in 1990, but rose to 18th in 2010. Rocketing of the disease was only second to Human Immuno defficiency Virus (HIV) and Acquired Immune Deficiency Syndrme (AIDS) (Jha *et al*., 2013). Eventually, the occurrence of the disease is high in developing countries of South Asia, Eastern Europe and Latin America as shown in **Figure 5**. Precipitating factors for CKD in developing countries are Diabetes Mellitus (DM), HTN, environmental factors, low socioeconomic status and intrauterine growth retardation. Scarcity of infrastructure, modern equipment and expert manpower limits early screening for detection of CKD in low and middle income countries (LMICs) (Ene-Iordache *et al*., 2016). The ESRD necessitates kidney replacement therapy or kidney transplant which incur enormous cost to individuals as well as national health budgets. High expenditure of dialysis for long term is a problem for people of affluent countries also. To prolong life, it is estimated that

over 1.4 million people receive kidney replacement therapy worldwide with the incidence with increasing incidence by 8% annually (Schieppati & Remuzzi, 2005).

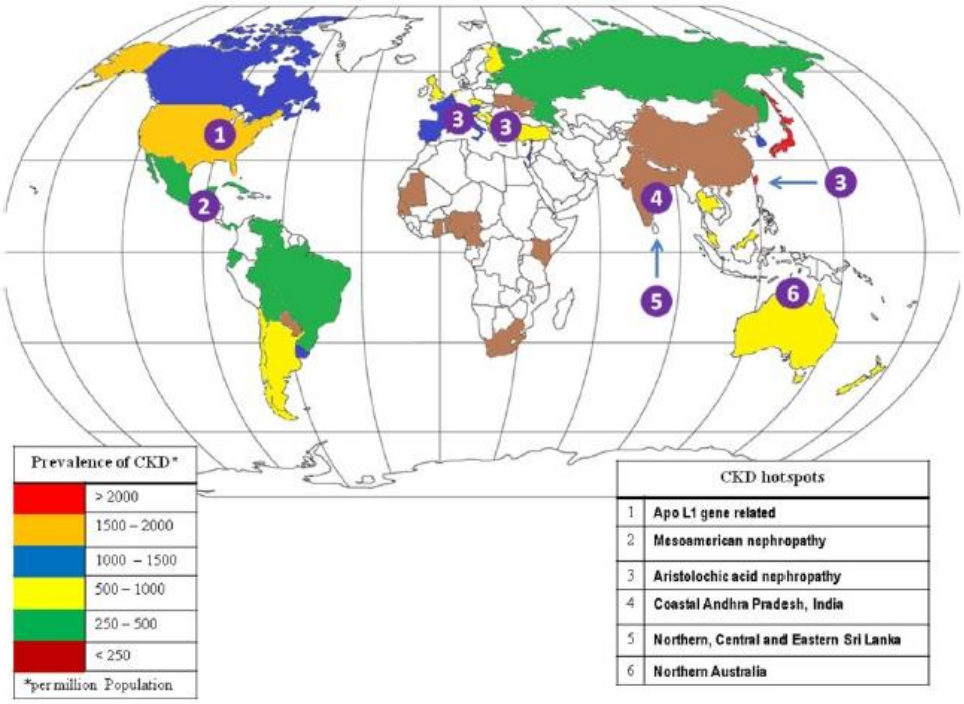


Figure 5: Global CKD hotspots and prevalence (Abraham *et al*., 2016)

CKD is a main root of morbidity and mortality worldwide. According to study done by Global Burden of Disease study 2013, 956 200 people were died from CKD, a 134% increase from 1990, one of the major upswings among the top causes of death (Ene-Iordache *et al*., 2016; Global Burden of Disease Study, 2015). Up to 14% of adults in the United States aged > 18 years, speaking to an estimated 31.4 million individuals, were found to have some level of CKD in 2007–2010. In Australia, CKD was common present in around 1 in 7 persons aged equal to 25 years. Notwithstanding being common in developed countries, CKD is likewise profoundly pervasive in developing nations. A cross-sectional study demonstrated that the across the nation pervasiveness of CKD in China was 10.8%, influencing an estimated 119.5 million (95% CI, 112.9-125.0 million) patients, same like the level saw in the United States in 2003 (Xue *et al*., 2014). In a current multicenter study in India, the prevalence of CKD was seen to be 17.2%, with

around 6% having CKD stage 3 or more awful. These reports propose that much should be done to concentrate on kidney diseases in India. Kidney diseases may turn into a noteworthy risk to the health of the number of inhabitants in developing countries and especially India soon (Trivedi *et al*., 2016). In 2009, Afolabi and his colleagues put the prevalence among Nigerians in a family practice population at 10.7% (Afolabi *et al*., 2009). The prevalence of CKD in Ghana has varied over the years; from 1.6% per million people to 4% among hypertensives in the Greater Accra region as documented in the study by Addo and co-workers (Addo *et al*., 2009). Furthermore, a prevalence of 46.9% has been recorded among hypertensives in Ghana (Osafo *et al*., 2011).

2.7 RISK FACTORS OF CKD

Several clinical and epidemiological reports have provided a relationship between numerous factors and the initiation and progression of CKD. These have been grouped into two well defined classes: those that cause the CKD (risk factors) and those that are associated with CKD in the absence of established causal relations (risk markers) (Staples & Wong, 2010; Tsai *et al*., 2016).

Virtually, all kidney diseases progress to terminal renal failure relatively independent of the initial disease. Diabetic nephropathy, chronic glomerular diseases and hypertensive nephrosclerosis are among the most widespread causes of CKD (Malekmakan *et al*., 2016; Haroun *et al*., 2003; Remuzzi *et al*., 1997). A primary disease eventually leads to secondary glomerular injury and nephron loss that is clinically characterized by proteinuria and hypertension, which leads to inflammation or scarring which causes kidney failure and ultimately a gradual elevation in the plasma creatinine concentration and a progressive decline in GFR (Jacobson, 1991). Apparently, the excessive protein filtration, caused by the glomerular hypertension, might per se have toxic effects on the kidneys and increase the rate of progression (Remuzzi & Bertani, 1998; Tryggvason & Pettersson, 2003). Studies in rats have suggested that hyperfiltration and glomerular hypertension may play important roles (Brenner et al., 1982). Hyperfiltration is observed in diabetes and obesity, but also in any condition associated with a reduced number of nephrons (Brenner *et al*., 1996). To compensate for this nephron loss, the glomerular plasma flow rate and glomerular hydrostatic pressure increase in the surviving nephrons, thus raising the single nephron glomerular filtration rate. Initially, these changes are adaptive because they maintain the overall GFR. However, the glomerular hypertension has

negative long term effects and causes progressive renal sclerosis in a self-perpetuating vicious cycle, whereby nephron loss due to sclerosis further increases flow and pressure in the remaining glomeruli leading to a gradual progress of CKD (Brenner *et al*., 1996). The central mediator of this observed glomerular haemodynamic changes seems to be angiotensin II, but it also controls other factors that might be of importance in the progression of kidney disease, such as the production of reactive oxygen species, the regulation of cytokines and profibrotic growth factors, among others. Inappropriate activation of other systems, such as the sympathetic system, the endothelin system and of aldosterone, has also been implicated in the progression of CKD (Gross & Amann, 2004).

2.7.1 Diabetes Mellitus

Diabetes contributes substantially to the burden of ESRD (Narres *et al*., 2016; Ronald, 2016). Thus, the rapidly rising trend in type II diabetes prevalence throughout the world is of major concern (Zimmet, 2003). There are indications that genetic susceptibility to nephropathy development may be in operation in both type I and type II diabetes, although gene hunting studies have been unable to identify any particular mutations which could explain why diabetic nephropathy is mostly associated with diabetic patients (Bergrem & Leivestad, 2001). Changing environmental or behavioral factors appear to be of importance for the development of diabetic nephropathy beside the genetic factors (Ma, 2016; Murea *et al*., 2012; Wu *et al*., 2014).

Pathological quantity of urine albumin secretion, diabetic glomerular lesions and diminished GFR are the characteristic features DN or diabetic kidney disease. DN is a substantial cause of CKD and end-stage renal failure. Several consequential researches have been conducted by scientists and clinicians, which elaborated the understanding of the pathophysiology of diabetic nephropathy and augmented the number of potential therapies available. DN is characterized by structural and functional alteration of kidney. Various structural modifications occurring in glomeruli are mesangial expansion, thickening of the basement membrane and characteristic nodular glomerulosclerosis (Kimmelstiel-Wilson nodules). In the early stages of DN, tubular hypertrophy is seen but sooner or later interstitial fibrosis with tubular atrophy develops, in addition to arteriolar hyalinosis. There is an infiltration of macrophages and T-lymphocytes on advancement of DN. Ultrastructural alteration such as podocyte loss and reduced endothelial cell

fenestration takes place. Early glomerular hyper-filtration and raised albumin excretion are significant initial functional alteration; and with advancing nephropathy, aggravating proteinuria and diminishing GFR (Butt *et al*., 2010; Taniwaki *et al*., 2000).

2.7.2 Hypertension

There is compelling evidence from the epidemiological studies that HTN causes a decline in renal function and increases risks of ESRD (Ishikura *et al*., 2016; Kovesdy *et al*., 2016). However, some investigators have questioned whether non-malignant HTN (in contrast to malignant hypertension) is an important initiator of kidney disease (Hsu, 2002; Kincaid-Smith, 2004). Although evidence that HTN accelerates the progression of already existing renal failure is overwhelming, there is lack of conclusive data from clinical trials that aggressive treatment of HTN reduces risk of kidney disease onset (Judd & Calhoun, 2015).

2.7.3 Glomerular disease

The compensatory reaction to nephron loss of hyperfiltration in the saved nephrons, trying to look after GFR, prompts glomerular damage and the finding on renal biopsy examination of auxiliary glomerulosclerosis. Glomerular cell expansion, macrophage invasion and the progressive accumulation of extracellular grid (ECM) segments all may add to the advancement of the glomerular sclerotic injury, extending of the slender tuft additionally extends the nearby mesangial cells and this affects mesangial multiplication. How these progressions happen is not surely knew, but rather cytokines, for example, changing development component β (TGF-β) and platelet-derived growth factor (PDGF) might be in charge of at any rate part of the grid gathering (Boor, 2014; Metcalfe, 2007). There are two types of glomerular disease namely glomerulonephritis and glomerulopathies (Kumar & Clark, 2012; Stephany, 2010).

2.7.4 Obesity

Obesity, a component of the metabolic syndrome, has become a key worldwide problem. (Han & Lean, 2016) Although this phenomenon may result from altered dietary patterns and a

sedentary lifestyle among people in affluent developed countries, it is now a rapidly emerging problem in developing countries. Worldwide obesity has increased 3-fold since 1980 and according to reports from the World Health Organization (WHO), over one billion adults are overweight (body mass index [BMI] less than or equal to 25 kg/m^2) with at least 300 million being obese (BMI less than or equal to 30 kg/m^2) (Ellulu *et al*., 2014). There are further, great concerns about the rising prevalence of overweight and obesity among adolescents and children of school going age. Obesity contributes significantly to the burden of chronic diseases such as cardiovascular disease, cancers, type 2 diabetes, and hypertension, among others (Han & Lean, 2016; Locke *et al*., 2016; O'Brien & Dixon, 2002; Panwar *et al*., 2015).

The alarming increment of obesity worldwide has been paralleled by a steadily increasing incidence of ESRD as a result of type 2 diabetes and hypertension (United States Renal Data System, 2015). Unquestionably, much of the excess risk for CKD observed among people with obesity (El-Atat *et al*., 2003; Hall *et al*., 2004) is linked to the increased prevalence of hypertension and/or type II diabetes (El-Atat *et al*., 2003; Mokdad *et al*., 2003). However, it also appears that obese individuals diagnosed with hypertension and diabetes are at a higher risk of developing nephropathy, compared with leaner subjects with these conditions, independent of blood glucose concentration and other factors. In epidemiological studies, a high BMI was independently linked to proteinuria among diabetics (Savage *et al*., 1995; Spangler & Konen, 1996), and hypertensive subjects (Ribstein *et al*., 1995). Obesity may also aggravate existing nephropathies and is also associated with increased risk of graft failure after renal transplantation. Further evidence for a link between obesity and kidney damage is provided by the fact that weight loss in the obese reduces proteinuria and hyperfiltration (Chagnac *et al*., 2003; Hall *et al*., 2014; Morales *et al*., 2003).

2.7.5 Genetic Susceptibility

There are indications that a generalized genetic susceptibility contributes to the development of ESRD (Li, 2015; Ma, 2016). The observation that there is a clear familial aggregation of ESRD due to diabetes, HTN and GN, initiated the search for specific “candidate genes” that might be involved in renal diseases. It has been suggested that from various types of genetic association studies that genes of the renin angiotensinogen system and genes coding for cytokines and growth

factors might be of interest, among others (Buraczynska & Ksiazek, 2001). In the previous decade, podocyte research has been incredibly supported by the advancement of strong new molecular, cellular and animal tools, prompting explanation of an expanding number of proteins required in podocyte function and identification of mutated genes in hereditary glomerulopathies. Gathering proof demonstrates that podocyte disorders may underlie these hereditary glomerulopathies as well as assume critical part in a wide spectrum of acquired glomerular diseases. Hereditary susceptibility, environmental impact and systemic response are all required in the intercession of the pathogenesis of podocytopathies (Cheng & Harris, 2010). Studies using the genome scan approach, which has the potential for a more comprehensive evaluation of inheritance throughout the genome and to locate previously unknown genes related to diseases, have recently found evidence of susceptibility loci for diabetic nephropathy (Freedman, 2007; Lu *et al*., 2016).

2.7.6 Analgesics

Chronic analgesic nephropathy (AN) is a gradually progressive renal disease caused due to its excess use of blends containing no less than two analgesics (e.g., aspirin, paracetamol, pyrozolones, phenacetin) and caffeine, codeine, and/or barbiturates for a long time, which may prompt psychological reliance and abuse. The disease is portrayed by capillary sclerosis, renal cortical atrophy, chronic interstitial nephritis, and papillary sclerosis/putrefaction/calcifications. In various cases, not identified with the movement or phase of renal failure, the uroepithelia can create transitional cell carcinoma (De Broe & Elseviers, 2009).

A few studies likewise indicated out the relationship between the utilization of single-ingredient analgesics containing acetaminophen or aspirin and CKD. A Swedish study directed in 2001 found that the over utilization of acetaminophen or aspirin on daily basis was connected with a risk of renal failure that was 2.5 times as high as that for nonusers. An American study found that patients use non-steroidal anti-inflammatory drugs (NSAID) in day by day bases were connected with twofold build hazard for CKD. NSAID have been connected with nephrotic syndrome, interstitial nephritis and ESRD, regardless of that patient with CKD ought to evade the utilization of NSAID, still studies found that CKD mindfulness was not connected with decrease of NSAID use (Banaga *et al*., 2015; Waddington *et al*., 2015).

2.7.7 Metabolic syndrome

The metabolic syndrome (MetS) is defined as a constellation of risk factors of CVD and type 2 diabetes (Grundy *et al*., 2004). It is mostly characterized by central obesity, dyslipidaemia (raised triglycerides and low high-density lipoprotein [HDL] cholesterol), hyperglycaemia and hypertension. The MetS increases mortality (Thomas *et al*., 2007) and it also associated with conditions like non-alcoholic fatty liver disease (Abdeen *et al*., 2006). MetS had also been linked with kidney dysfunction. The incidence of MetS globally has reached epidemic levels. In the United States the prevalence of MetS is between 30-35% in both males and females and around 20-30% in the United Kingdom (Tillin *et al*., 2005; Cheung *et al*., 2006). In Ghana, the prevalence of the MetS is about 14% as reported in a recent study by Owiredu and colleagues (Owiredu *et al*., 2011).

Age has been identified as a factor that affects the MetS causing an increase in the prevalence especially in people who are >60 years in the United States (Ford *et al*., 2002). Possible culprits in the occurrence of this disorder include irregular timing of meals, urbanization, western lifestyle and westernization of diets.

A number of organizations including the WHO, the US National Cholesterol Educational Program Adult Treatment Panel (NCEP ATP III), the European Group for the Study of Insulin Resistance (EGIR) and the International Diabetes Federation (IDF) have proposed definitions and sanctioned clinical criteria for the definition of MetS (Alberti *et al*.,2005). Altogether, the definitions and criteria give a catalogue with straightforward beneficial markers that are likely causes of cardiovascular disease such as dyslipidaemia, hypertension, obesity and diabetes. The NCEP ATP III criteria is the most commonly used and it has helped in the identification of components of the metabolic syndrome and has considered obesity as largely responsible for the increasing prevalence of the MetS (Grundy *et al*., 2004; NCEP, 2001). Whereas, insulin resistance as well as microalbuminuria are essential for the WHO criteria, upper body adiposity is vital for meeting the IDF criteria (Lin *et al*., 2009).

2.7.8 Socio-economic status

It is evident that socio- economic status is linked to the development of ESRD since both low income and low educational level have been associated with elevated risk (Abraham *et al*., 2016; Nicholas *et al*., 2015).

2.7.9 CKD -associated Mineral and Bone Disorders

CKD-associated mineral and bone disorders implies bone and mineral metabolism abnormalities and/or extra-skeletal calcification secondary to the consequences of CKD (Gal-Moscovici & Sprague, 2007; Moe *et al*., 2006). Renal osteodystrophy (ROD) is an array of histological changes, which arise in bone architecture of CKD patients. The primary site of phosphate excretion and 1-α-hydroxylation of vitamin D is the kidney. CKD patients develop hyperphosphataemia as a result of reduced 1, 25 dihydroxycholecalciferol levels that indicate decreased synthesis as a result of parenchymal scarring. Moreover, excretion of phosphate by the kidney is reduced. Consequently, serum calcium levels fall resulting in an increase in the rate of production of parathyroid hormone (secondary hyperparathyroidism). One prominent function of Parathyroid hormone is to increase phosphate excretion in the urine. In addition, it also increases plasma calcium levels by promoting bone resorption and increasing 1-α-hydroxylation of 25-hydroxy vitamin D produced in the liver (limited effect because of reduced kidney reserve from scarring). Rising phosphate levels are generally observed in stage 3 CKD patients. Conversely, bone architecture is distorted quite early by secondary hyperparathyroidism just before serum phosphate level is noted to be abnormal. This gives an indication that treatment with phosphate binders should start when estimated GFR (eGFR) have declined below 50 mL/min per 1.73 m^2. A high or low bone turnover can result in changes in bone architecture. Four types of bone phenotypes ROD can be diagnosed in CKD patients namely osteitis fibrosa cystica (with high bone turnover due to secondary hyperparathyroidism), osteomalacia (resulting in low bone turnover and inadequate mineralization, often associated with reduced synthesis of vitamin D), a dynamic bone disorder (with low bone turnover due to over-suppression of the parathyroid glands), and lastly mixed osteodystrophy (with elements of both high and low bone turnover). The major type of ROD and CKD-mineral and bone disorder varies between pre-dialysis and end stage kidney disease patients. High bone turnover bone disease is most common

in in pre-dialysis patients. Conversely, low bone turnover is common in dialysis patients. Majority of incidents of ROD is found in CKD patients with low turnover disease (Joy *et al*., 2007). This predominant condition is due to the over suppression of parathyroid hormone and high levels of calcium in the dialysis solutions (Hruska & Teitelbaum, 1995). The ability of phosphate retention to stifle the renal synthesis of 1, 25 dihydroxyvitamin D, acidosis and the lack of the physiologic inhibitory effect of vitamin D on parathormone secretion also contribute, albeit small, to the low turnover bone disease in CKD patients (Llach, 1995). CKD-associated mineral bone disorders significantly increase mortality in patients with CKD. In reality, hyperphosphatemia has been identified as the most significant risk factor associated with cardiovascular disease in CKD patients (Lee *et al*., 2007). The precise mechanism underlying this relationship is still unclear. It is believed to be related to hyperparathyroidism (El-Kishawi & El-Nahas, 2006) and vascular calcification due to elevated phosphate levels (Hutchison, 2007). The use of calcium based binders and excessive vitamin D therapy (Moe, 2006) influence vascular calcification and the associated cardiovascular mortality. Patients on HD with plasma phosphate level above the K/DOQI guideline objectives have a 40% higher rate of mortality compared to those having lower target levels (Noordzij *et al*., 2005). The main objective of therapy of CKD-associated bone and mineral disorders is to reduce phosphate levels (Coresh *et al*., 2007). When phosphate or parathyroid levels begin to rise, the primary therapy is to restrict dietary phosphate intake. Serum phosphate concentrations should be maintained between 2.7 and 4.6 mg/dL among patients with CKD stages 3 and 4, and between 3.5 and 5.5 mg/dL for those with stage 5 CKD according to KDOQI guidelines. Various groups of phosphate binders can be applied to achieve this goal. For the treatment of chronic conditions calcium-based formulations for the management of hyperphosphataemia due to CKD are the most widely used and have replaced aluminium binders since aluminum-associated toxicities have been established. However, calcium-based phosphate binders can induce hypercalcaemia, which increases the tissue calcium deposition, especially in the presence of hyperphosphatemia (Cozzolino *et al*., 2014; Rivera & Faye Smith, 2013).

2.7.10 Malnutrition in CKD

Kidney disease is nearly connected with protein–calorie malnutrition. The WHO characterizes malnutrition as "terrible support" described by "lacking or abundance admission of protein, vitality, and micronutrients, for example, vitamins, also, the successive diseases and disorders that result (WHO: Malnutrition, 2009)." The definition suggests that protein–calorie malnutrition (consequently alluded to as "malnutrition") will move forward while missing nutrients are given. Serum albumin is the chief wholesome marker used to recognize malnutrition in patients with CKD. Through supports by nephrologists, renal dietitians, the exploration community, government organizations, health care payers, and substantial dialysis associations, it has additionally turned into an accepted file of clinical execution. The utilization of serum albumin as a nourishing and quality care marker includes the accompanying cause: a) Serum albumin is a dependable factor of malnutrition; b) since serum albumin is generally low in patients with CKD, these patients ought to be viewed as malnourished; c) replacing missing nutrients will raise low albumin levels; and d) in light of the fact that hypoalbuminemia is unequivocally connected with mortality, supplanting missing nutrients to raise albumin will likewise move forward persistent results. This survey develops past viewpoints by fundamentally looking at these presumptions and offering an option vision to translating serum albumin (Friedman & Fadem, 2010).

2.8 THYROID HORMONE ANATOMY AND PHYSIOLOGY

The thyroid gland in an adult weighs 15-40 grams and consists of two lateral lobes connected in the middle by a broad isthmus as shown in **Figure 6**. It is closely attached to the thyroid cartilage and to the upper end of the trachea and thus moveable on swallowing. It is often palpable in healthy adult women (Ross, 2016).

The thyroid is composed of lobules of colloid-filled spherical follicles called acini. The lobules are enclosed by fibrovascular septa. The follicles, the main functional unit of thyroid, are lined by cuboidal epithelium with numerous fine microvilli that contains the iodinated glycoprotein, thyroglobulin. The TH are synthesized and stored in thyroglobulin. Calcitonin-secreting C-cells or parafollicular cells are dispersed within the follicules. Embryologically, the thyroid gland originates from the base of the tongue and descends in front of the trachea and thyroid cartilage.

The gland has a rich blood supply from superior and inferior thyroid arteries (Kumar & Clark, 2012).

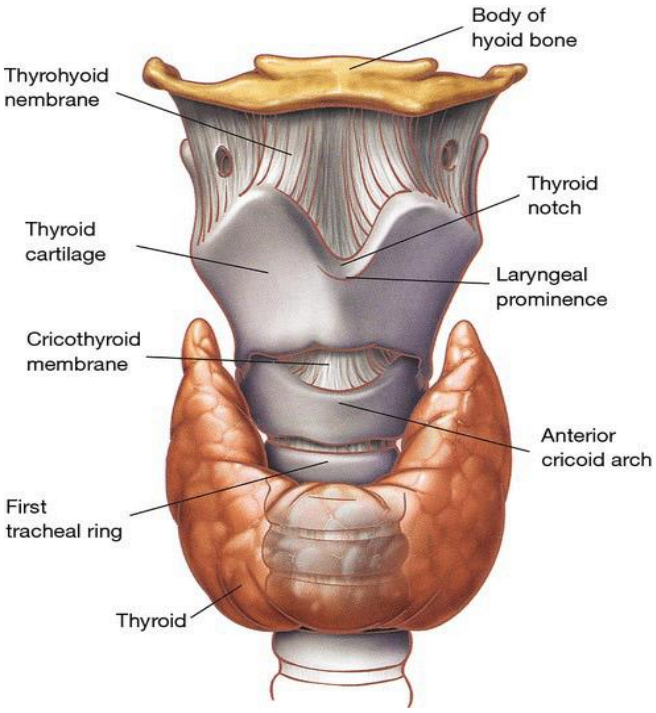


Figure 6: Anterior view of the laryngeal cartilages and membranes (Deslauriers, 2007)

The thyroid gland synthesizes and secretes two hormones:

- **L-thyroxine (T4) which is the prohormone and**
- **Triiodothyronine (T3) which acts at the cellular level**

Inorganic iodide is trapped in the gland by an enzyme dependent system, oxidized and then incorporated into the tyrosine residues of the glycoprotein thyroglobulin so as to form mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) which are then converted into T3 and T4. Formation of T4 is greater than T3 in thyroid gland but T4 is converted in some peripheral tissues like liver, kidney and muscle to the more active form T3 by 5'-monodeiodination. An alternative 3'-monodeiodination of T4 yields the inactive reverse T3 (rT3) which occurs particularly in non-thyroidal illness. In plasma, more than 99% of T3 and T4 are transported by hormone binding

proteins i.e. thyroxine binding globulin (TBG), thyroid binding prealbumin (TBPA) and albumin. Only less than 1% free hormone is available for action in target tissues where T3 binds to specific nuclear receptors within target cells. Production of T3 and T4 in the thyroid is controlled by a known mechanism called 'feedback mechanism'. Many drugs and other factors affect the binding affinity of hormones with TBG which gives vague result of total T3 and T4 levels. Thus most laboratories therefore now estimates free T3 and T4 levels. (Dousdampanis *et al*., 2014; Kumar & Clark, 2012)

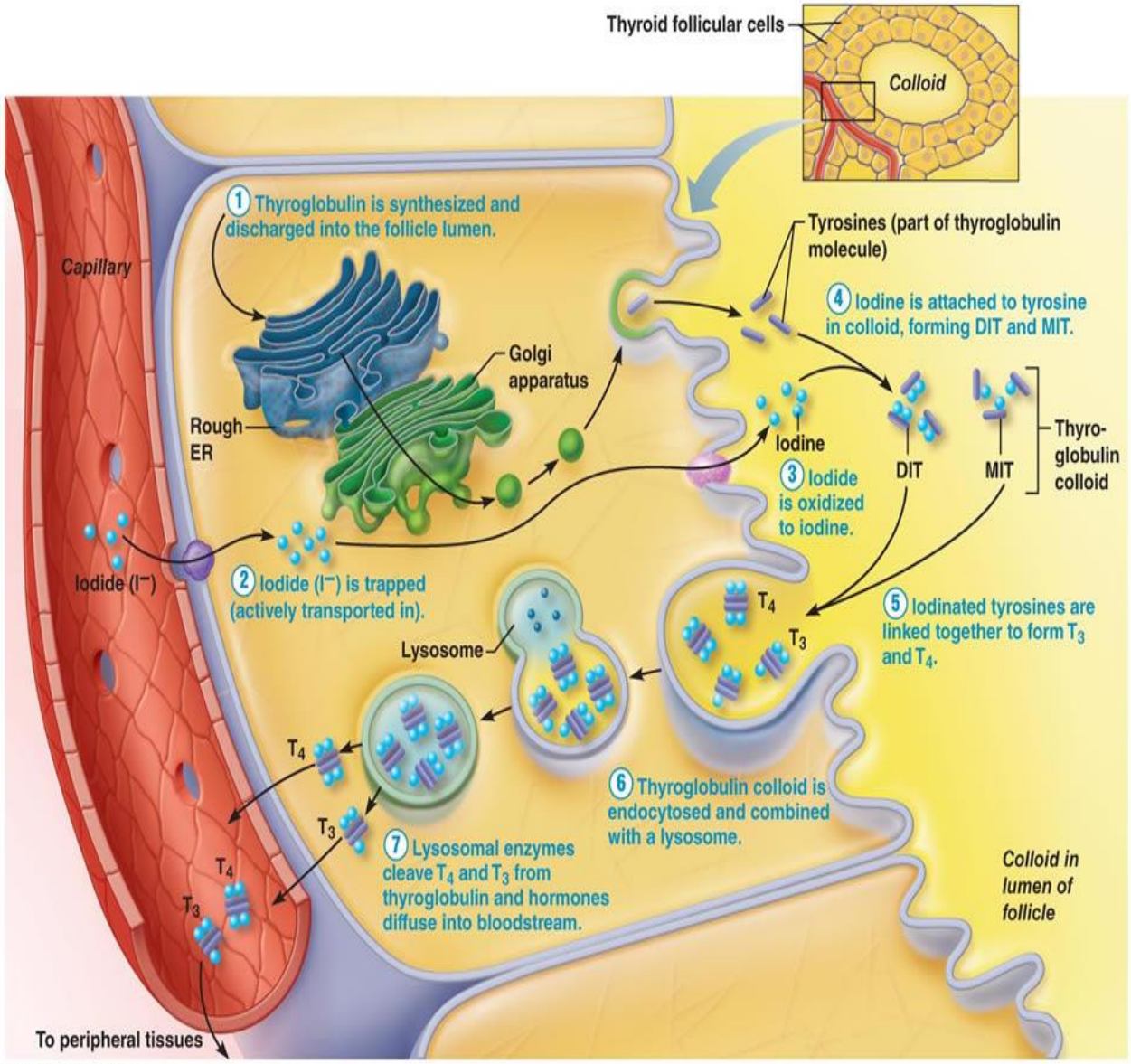


Figure 7: Biosynthesis of thyroid hormone (http://classes.midlandstech.edu/carterp/ Courses/bio211/chap16/chap16.htm)

2.9 CONTROL OF HYPOTHALAMIC-PITUITARY-THYROID AXIS

Thyrotropin-releasing hormone (TRH), a peptide hormone produced and secreted by hypothalamus, stimulates the pituitary to secrete TSH which in turn stimulates growth and activity of the thyroid follicular cells via the G-protein coupled TSH membrane receptor. This causes release of T3 and T4 into the circulation which exert negative feedback on hypothalamus (**Figure 8**). Circulating T4 is deiodinated in the peripheral tissues to form T3 which binds to the specific thyroid hormone nuclear receptors (TR) on the target cells ensued by modification in gene transcription. There are two different kind of TR receptors – TR-α and TR-β and the tissue specific response of T3 varies according to the local availability of these TR receptors.TR-α knockout mice show retarded growth, bradycardia and hypothermia while TR-β knockout mice show thyroid hyperplasia and elevated T4 levels (Kumar & Clark, 2012).

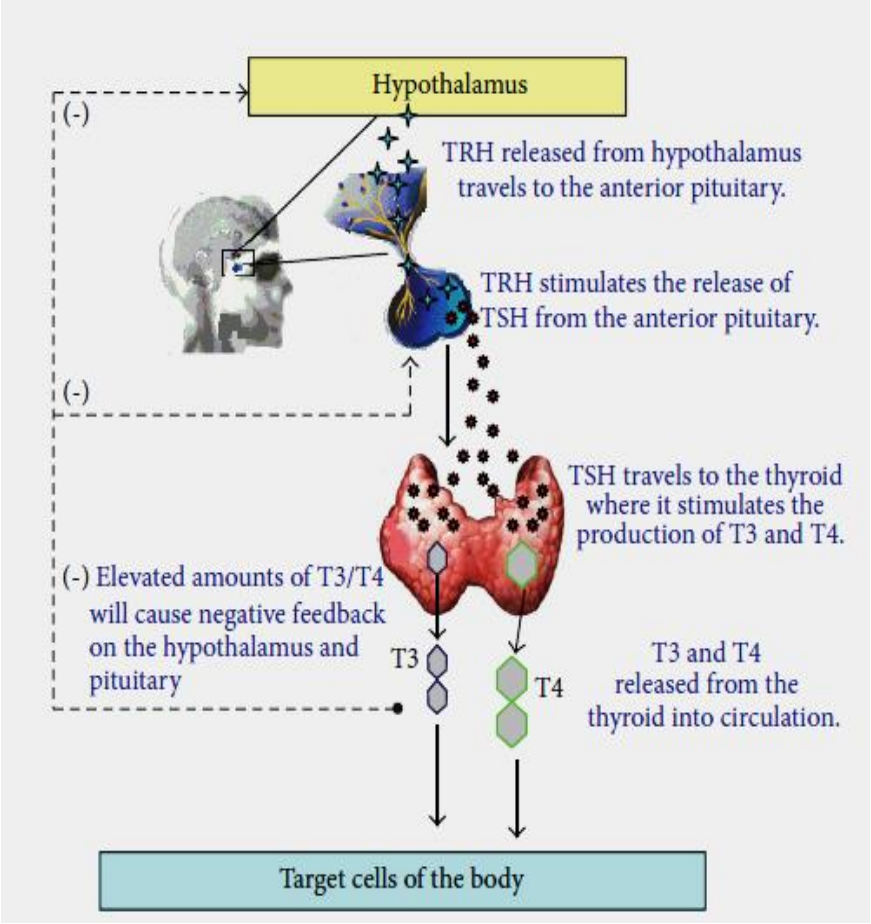


Figure 8: Control of thyroid hormone synthesis (Mohamedali *et al*., 2014)

The physiological effects of thyroid hormone are summarized in the **Table 3**.

Table 3: The physiological effects of thyroid hormone (Kumar & Clark, 2012)

Target	Effect
Cardiovascular system	Increases heart rate and cardiac output
Bone	Increases bone turnover and resorption
Respiratory system	Maintains normal hypoxic and hypercapnic drive in respiratory centre
Gastrointestinal system	Increases gut motality
Blood	Increases red blood cell 2,3-bisphosphoglycerate facilitating oxygen release to tissues
Neuromuscular function	Increases speed of muscle contraction and relaxation and muscle protein turnover
Carbohydrate metabolism	Increases hepatic gluconeogenesis/glycolysis and intestinal glucose absorption
Lipid metabolism	Increases lipolysis and cholesterol synthesis and degradation
Sympathetic system	Increases catecholamine sensitivity and beta-adrenergic receptor numbers in heart, skeletal muscle, adipose cells and lymphocytes

Dietary Iodine Requirement: Dietary iodine deficiency is a major cause of thyroid disorder worldwide as iodine is an essential micronutrient required for the synthesis of thyroid hormone. The recommended dietary intake for iodine is 140 microgram and dietary supplementation of salt and bread has reduced the incidence of endemic goiter which is still prevalent (Kumar & Clark, 2012).

2.10 THYROID FUNCTION TEST

The serum level of fT4, fT3 and TSH are now measured through immunoassays worldwide. As there is only minor circadian variation, estimation can be made at any time. Particular interpretation of the tests are summarized in **Table 4** with typical findings in most prevalent disorders.

Table 4: Characteristics of thyroid function tests in common thyroid disorders (Kumar & Clark, 2012)

Thyroid disorders	TSH (0.35-5.5 µIU/ml)	Free T4 (0.89-1.76 ng/dl)	Free T3 (2.30-4.2 pg/ml)
Thyrotoxicosis	Suppressed	Increased	Increased
Primary hypothyroidism	Increased	Low/low-normal	Normal or low
TSH deficiency	Low-normal or subnormal	Low/low-normal	Normal or low
T3 toxicosis	Suppressed	Normal	Increased
Compensated euthyroidism	Slightly increased	Normal	Normal

2.11 EFFECT OF THYROID HORMONE ON RENAL GROWTH AND DEVELOPMENT

One of the vital action of TH is that it influences protein synthesis and cell growth. Acceleration of renal development by TH have been seen in studies on neonatal rats. Serum level of TH influences the functioning renal mass (measured as the kidney to body mass ratio); hypothyroidism decreases the ratio while hyperthyroidism increases it (Basu & Mohapatra,

2012). But, severe hyperthyroidism has adverse effect on protein; it results in protein breakdown and even renal atrophy. Children with congenital hypothyroidism have shown a high incidence of congenital kidney disorder. Neonatal kidney function is also affected by TH. TH level affects the enzymes involved mitochondrial energy metabolism within cells of proximal convoluted tubules (PCT). Increased activity of the Na^+/K^+ co-transporter (NaPi), Na^+/H^+ exchanger (NHE) as well as the Na^+/K^+ ATPase has been seen in PCT in response to TH. In conclusion, TH has positive effect on the renal development and early renal function (Basu & Mohapatra, 2012; Baum *et al*., 1998; Dosdampanis *et al*., 2014; Mariani & Berns, 2012).

2.12 EFFECT OF THYROID HORMONE ON RENAL PHYSIOLOGY

TH have both pre-renal and direct renal effects on renal functions.

1. Pre-renal effects of TH are mediated by its effect on the cardiovascular system and the renal blood flow (RBF)
2. The direct renal effect of thyroid hormone are mediated by its influence on:
   a) GFR,
   b) Tubular secretory as well as reabsorptive processes, as well as
   c) Hormonal effect on renal tubular physiology

TH regulate renal clearance of water load by their influence on GFR. The importance of Na^+/K^+-ATPase in the transport of Na^+ and K^+ (the two solutes) in the PCT is remarkable. TH regulate reabsorption of Na^+ at the PCT mainly by increasing the activity of Na^+/K^+-ATPase and tubular potassium permeability. Similarly, the tubular reabsorption of Calcium is also regulated but the mechanism of regulation of Magnesium is alike. TH also influence the adrenergic receptors and dopaminergic activation of renal tubular cells. Their effect has been observed on renin-angiotensin-aldosterone axis by adrenergic regulation, renin release as well as affecting angiotensinase activity (Basu & Mohapatra, 2012).

Water and electrolytes on different compartments of the body is regulated by TH. The kidneys also influence the regulation of metabolism and elimination of TH and thus is a significant target organ for TH actions. The diminished activity of TH is ensued by an inability to excrete oral water overload. This effect of TH is not because of a partial suppression of vasopressin synthesis

or lowering of reabsorptive efficiency in the dilutor segment of the renal tubule, but rather to a reduction in GFR (Iglesias & Diez, 2009).

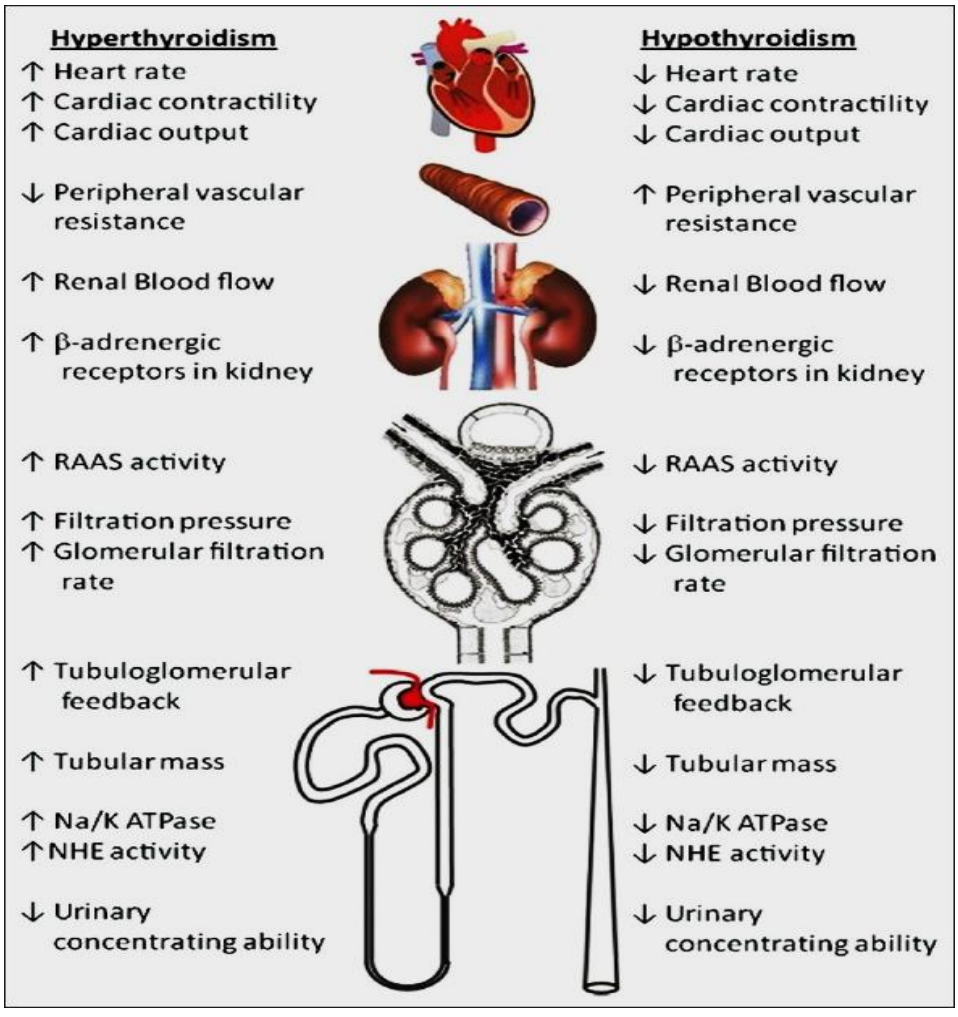
TH have effects on nearly every organ system of the body. They are synthesized and secreted by thyroid gland under the controlling activity of anterior pituitary hormone TSH which, in turn, controlled by hypothalamic TRH. T4 is synthesized only in the thyroid gland while the more biologically active form, T3 is produced in many of the peripheral tissues including kidney by local deiodination of T4 with the help of enzyme T4-deiodinase which have local availability. The isoform D1 is found in the kidney whose activity decreases during uremia. The effect of TH is exerted by binding of the hormone to its specific nuclear receptors, which regulate the rate of gene transcription by binding to TH response elements of target genes (Cheng *et al*., 2010; Mariani & Berns, 2012).

2.13 THYROID AND RENIN-ANGIOTENSIN SYSTEM (RAS)

Various significant hemodynamic, structural and functional changes of the renal and cardiovascular system (CVS) mediated by renin-angiotensin system (RAS) have been marked in thyroid disorders (Dousdampanis *et al*., 2014; Vargas *et al*., 2012). However, renal tissue mainly synthesizes renin, thyroid hormone may influence alteration in RAS. TH maintains the normal blood pressure (BP) with two mechanisms- either by enhancing the response of CVS to the action of sympathetic nervous system or/and by stimulating directly the RAS. Experimental animal models with hyperthyroidism have demonstrated increment in density and activity of β-adrenergic receptors in the cortex of kidney (Haro *et al*., 1992). Furthermore, Atlas in 2007, suggested a clear connecting link between RAS and β-adrenergic receptors by reporting that blockade of β-adrenergic receptors leads to depletion in plasma renin activity (PRA) while stimulation of β-adrenergic receptors increases PRA (Atlas, 2007). Excitingly, on administration of ACE-inhibitors in hyperthyroid animals in combination with HTN, blood pressure (BP) declined to normal levels with increased heart rate and cardiac output (CO) indicating that RAS is the predominant factor of HTN in hyperthyroidism not the increased CO (Atlas, 2007; Dousdampanis *et al*., 2014).

2.14 THYROID DISORDER AND GFR

Renal blood flow, GFR, kidney structure, tubular function, and electrolyte homeostasis are effected by Thyroid dysfunction (**Figure 9**). Various studies have shown that subclinical and clinical hypothyroidism is common in patients with estimated GFR 60 ml/min/1.73 m^2. These studies raises a question whether thyroid dysfunction has direct impact on renal function *i.e.* whether hypothyroidism has any role in lowering GFR in some of these patients (Chonchol *et al*., 2008).



RAAS: Renin-angiotensin-aldosterone system
NHE: Sodium-Hydrogen exchanger

Figure 9: Effects of thyroid dysfunction on GFR ((Basu & Mohapatra, 2012)

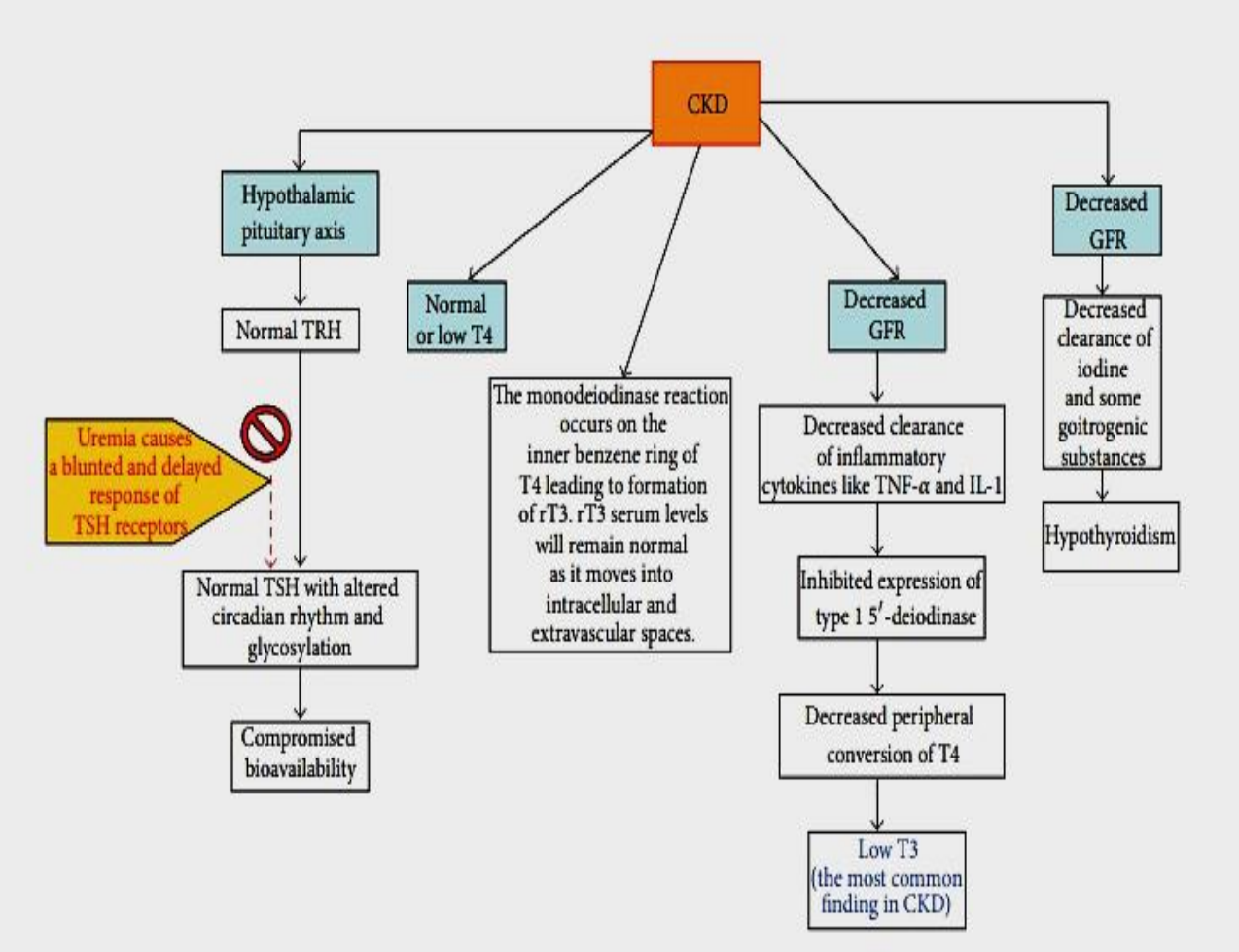
In various studies serum creatinine have shown its link with thyroid disorder. In the excess of 6 mg/dl of serum creatinine level have been found to cause hypothyroidism, with a few patients even described as having ESRD in spite of the creatinine levels were reported in the range of 1.5-2.5 mg/dl in most cases. Raised levels of serum creatinine can take place within a short period of 2 week's significant hypothyroidism. The raised levels typically normalize speedily when thyroid hormone replacement in given after short period of hypothyroidism (Kreisman & Hennessey, 1999). However the recovery becomes tardy and also incomplete when hormone replacement therapy is given after long period of severe hypothyroidism. Analogous to this, several human and animal investigations have demonstrated a decreased serum creatinine in the setting of hyperthyroidism which can also be reversed upon treatment (den Hollander *et al*., 2005; Mariani & Berns, 2012)

2.15 THYROID DISORDER AND GLOMERULAR DISEASE

Both of the thyroid diseases- hypo- and hyperthyroidism are associated with various types of glomerulonephritis. In thyroid disorder, the category of GN prevalent is membranous, IgA, mesangiocapillary, membranoproliferative and minimal change GN. Out of which, membranous GN is the most frequent (Iglesias & D´ıez, 2009; Mohamedali *et al*., 2014). Histologically, two significant alterations perceived are a thickened glomerular basement membrane (GBM) due to immune complex deposition and an enhanced mesangial and endocapillary cellularity (Akikusa *et al*., 1984). Thyroid dysfunction and GN are pathophysiologically connected by appearance of proteinuria and formation of immune complexes. This association is very frequent in autoimmune thyroiditis. Prevalence of immune complexes is seen in almost 50% of autoimmune thyroiditis. These complexes are mainly accountable for changes in renal function by depositing on the basement membrane of the glomeruli. Moreover, some other studies have also demonstrated a deposition of thyroglobulin in the basement membrane of the glomeruli. Apart from thyroid disorder, parallel effects are also seen in various other autoimmune diseases such as systemic lupus erythematosus (SLE) and DM (Iglesias & D´ıez, 2009; Mohamedali *et al*., 2014).

2.16 ASSOCIATION OF THYROID DISORDER WITH CKD

Hypothalamus-pituitary axis as well as the peripheral metabolism of thyroid hormone are markedly influenced by chronic kidney disease. In CKD patients, decreased levels of T3 is the most common laboratory finding and subclinical hypothyroidism is the most common thyroid disorder (Basu & Mohapatra, 2012). Although TSH level is usually normal, there is altered circadian cycle (manifestation of TSH bioactivity). In uremia, the response of pituitary receptor for TRH is diminished causing a decline in TSH release. Because of depleted clearance and raised half-life of TSH, the response of TSH to TRH is delayed. In uremia, abnormal serum constituents are found which can also replace T3 and T4 from normal protein binding sites (Basu & Mohapatra, 2012).



TNF-α: Tumor necrosis factor α **IL-1: Interleukin 1**

Figure 10: Effect of chronic kidney disease on thyroid profile (Mohamedali *et al.*, 2014)

Normal or decreased levels of T4 may be due to the action of enzyme mono-deiodinase, taking place in the inner benzene ring instead of outer ring which lead to the formation of reverse T3. Even so, levels of reverse T3 are found normal in CKD patients as it moves from vascular space to extra vascular and intracellular space. After HD, transient increment in the T4 levels are normally seen due to the use of heparin as an anticoagulant which inhibits T4 binding to proteins (Mohamedali *et al*., 2014). In CKD, the level of T3 is also decreased due to diminished activity of the enzyme iodothyronine deiodinase (synthesizes T3 from T4) which is adversely affected by fasting, chronic metabolic acidosis and protein malnutrition usually seen in CKD. These factors affect the binding of proteins to T3 (Basu & Mohapatra, 2012).

In CKD, due to diminished rate of clearance of the inflammatory *e.g.* tumor necrosis factor (TNF)-α and (Interleukin) IL-1 conversion of T3 from T4 decreases by peripheral (extrathyroidal) tissues and thus level of T3 is declined as explained in **Figure 10**. These cytokines inhibit genetic expression of 5'-deiodinase that help in the conversion of T4 to T3 (Lim *et al*., 1977). Declined levels of free T3 have been suggested to be an independent indicator of mortality in hemodialysis patients (Zoccali *et al*., 2006). Decreased T3 levels are also associated with renal post-transplant risks of graft loss and therefore the clinicians are suggested to measure T3 levels before renal transplantation. Low T3 levels observed in CKD is incapable to raise the level of TSH. In uremia, evidence from a number of experiments suggest that the sensitivity of thyrotrophs is raised. This may be responsible for the resetting of the central thyrostat which indicates that lowered level of serum thyroid hormones and, in turn, influence the negative feedback inhibition. In CKD, due to physiological compensation for low T3 and T4 (with normal TSH) the rate of protein catabolism is decreased which leads to an increase in nitrogen waste overload (Mohamedali *et al*., 2014).

2.17 NON-THYROIDAL ILLNESS

Various anomaly in TH secretion, metabolism and action have been described in patients without previous finding of intrinsic thyroid disorder during critical illness and are collectively called as "Non-thyroidal illness" (NTI); this term is now commonly used replacing "euthyroid sick syndrome" and "low T3 syndrome". A decreased level of total T3 which is most prevalent thyroid disorder, can be detected very early, within two hours after the onset of severe physical stress.

But lowering of T3 is only one of the endocrine picture suggested in such a situation, hence the term NTI is seems more suitable and also consolidating its extrathyroidal source (Mancini, 2013). During this condition, intrinsic abnormality of hypothalamus-pituitary-thyroid axis is not seen and occurs due to secondary adaptive changes. After the onset of NTI, changes in T3 and TSH levels is diagnosed as early as 24 hours in patients with chronic liver disease, CKD, post-surgery, myocardial infarction, malignancy, sepsis, burns and person with malnutrition (Shiv Raj, 2014).

In CKD patients, the earliest and most frequent thyroid function abnormality seen is decreased T3 level (Basu & Mohapatra, 2012; Wiederkehr *et al*., 2004). There are various factors that causes this "low T3 syndrome" *e.g.* fasting, chronic metabolic acidosis and chronic protein malnutrition affect iodothyronine deiodination as well as protein binding of T3, lowering the peripheral conversion of T4 to T3 and its protein binding. Besides, genetic expression of type 1,5'-deiodinase which is accountable for peripheral conversion of T4 to T3, is inhibited by inflammatory cytokines such as TNF-α and IL-1 (Zoccali *et al*., 2005). Moreover, impaired renal handling of iodine raises serum iodine levels, leading to a prolonged Wolff-Chaikoff effect (Bando *et al*., 2002). There is controversy regarding the clinical significance of this decreased T3 level syndrome. Several researchers have correlated the low levels of T3 (particularly total, T3 not free T3) in CKD patients with increased levels of markers of inflammation such as highly sensitive C-reactive protein (hsCRP), IL-6 *etc*., increased endothelial dysfunction, diminished cardiac function and poor survival as well as cardiovascular mortality (Carrero *et al*., 2007; Zoccali *et al*., 2005). Certain studies were enfeebled because they could not detect the association or could not exclude the confounders appropriately (Tripepi & Zoccali, 2003). In some other studies, the decreased free T3 not the total T3 is found to be associated with increased mortality (Basu & Mohapatra, 2012; Ozen *et al*., 2011).

2.18 EFFECT OF DIALYSIS ON THYROID HORMONE

Most of the CKD patients on HD are clinically euthyroid (Iglesias & D´ıez, 2009; Mohamedali *et al*., 2014). In about 20% of uremic patients, mild elevation of TSH levels (5-20mU/l) are observed, usually not considered as hypothyroidism in this selected group of patients. Although the total T4 levels are low, heparin inhibits T4 binding to protein, thus elevating free T4 fraction in CKD patients ensuing heparin dialysis (Basu & Mohapatra, 2012). Compensatory effect on

cellular transport of TH are seen in CKD patients on HD. It helps to maintain the normal erythroid state in spite of decreased serum thyroid hormone levels (Rodrigues *et al*., 2004). Because of all these reasons, regardless of decreased serum thyroid hormone profile, supplementation of thyroid hormone is not suggested without substantial increment in TSH level and pragmatic consideration (Basu & Mohapatra, 2012).

There is substantial increment in prevalence of hypothyroidism (particularly subclinical) and decreased T3 levels among the patients of peritoneal dialysis (PD) (Kang *et al*., 2008). TBG, T4 and T3 loss in PD patient is fluent. In spite of continuous and significant loss of protein, TBG levels in these patient are normal. The T4 and T3 losses are minimum and thus easily compensated for. Hence, thyroid hormone replacement therapy is not required in CKD patients on PD (Basu & Mohapatra, 2012).

2.19 DRUGS IN THYROID AND RENAL DISEASE

Drugs taken in thyroid and kidney disease may adversely affect other organ's functions, such as thionamides *e.g.* methimazole, carbimazole, propylthiouracil cause hypothyroidism as well as kidney dysfunction by immune mechanisms causing various glomerular diseases such as vasculitis, lupus nephritis or necrotizing GN with pulmonary hemorrhage (Basu & Mohapatra, 2012; Iglesias & D´ıez, 2009; Yu *et al*., 2007).

Alemtuzumab, used in kidney transplantation, has been observed to cause autoimmune thyroid disease (Kirk & Hale, 2006). Interferon-α (IF-α), also used in renal cell carcinoma (RCC) as well as to cure hepatitis B and C virus infections, pre-transplant etc. has been reported to cause hyperthyroidism. Lenalidomide, used in the treatment of renal cell carcinoma for its antitumor and anti-angiogenic properties, causes a subcute thyroiditis and transient thyrotoxicosis (Stein & Rivera, 2007). Sunitinib, a new drug for the treatment of RCC, causes hypothyroidism which some clinicians believe to be associated with better prognosis. Therapeutic use of lithium causes hypothyroidism as well nephrogenic diabetes insipidus and CKD (Iglesias & D´ıez, 2009). Amiodarone is linked with both hypothyroidism as well as acute renal damage (Marales *et al*., 2003). Rifampicin causes both tubulointerstitial nephritis and hyperthyroidism (Basu & Mohapatra, 2012).

During the therapy of hyperthyroidism patients with CKD, important considerations should be done. Usually, CKD patients need lower doses of (radioisotope of iodine 131) ^{131}I for the treatment of Grave's disease. Normal therapeutic dose of ^{131}I is required by the patients of hyperthyroid on HD due to clearance of ^{131}I by dialysis (Holst et al., 2005). A five-fold reduction in the ^{131}I dose is needed for the treatment of thyroid carcinoma by patients of CKD on PD to avoid excessive radiation (Basu & Mohapatra, 2012).

CHAPTER: THREE

METHODOLOGY

3.1. Setting of the study

The present research study was conducted at Out Patient Department (OPD) and In Patient Department (IPD) of Nephrology and Dialysis Unit of KIST Medical College Teaching Hospital located in Imadol, Mahalaxmi Municipality of Lalitpur district. Lalitpur district is one of the three districts of the Kathmandu valley. The other two districts are Kathmandu and Bhaktapur. Kathmandu is the capital of Nepal. The 2011 population census, the total population of Lalitpur district is 466784 (National report on National Population and Housing Census, 2011).

3.2 Study population

The target population was the patients aged 18 years and above with established CKD categorized into five groups according to the stages of CKD (NKF-KDOQI guidelines) attending Nephology and Dialysis Clinic of KIST Medical College Teaching Hospital, Lalitpur, Nepal.

3.3 Study design

Study design was cross sectional and observational for all the study participants.

3.4 Sampling method and Sample size

The sampling method used for the research work was a convenience sampling method for study subjects attending OPD and IPD of Nephrology and Dialysis Unit of KIST Medical College Teaching Hospital. These patients were given specialized form to collect their information as per study requirement.

A total of 163 CKD patients aged between 21 to 87 years were included in present study.

3.4.1 Sample size calculation of study population

To calculate the sample size, the Kish formula of 1965 for cross sectional studies is used (Batte et al., 2016; Kish, 1965).

$$n = Z\alpha^2 PQ/d^2$$

n = no of sample size

Zα= standard normal deviation at 5% level of significance is 1.96

P= prevalence of low T3 syndrome is 88% (Rajeev et al., 2015)

Q= (100-P) % *i.e.* 12%

d= permissible margin of error at 5 % level of statistical significance

Now, $n = (1.96)^2 \times 88 \times 12 / (5)^2$

$= 162.26$

3.4.2 Estimation of GFR using Cockcroft-Gault equation

Cockcroft-Gault equation is the most well-known and frequently-calculated estimates of GFR via Creatinine Clearance (Botev et al., 2009; Cockcroft & Gault, 1976).

$$GFR_{Cockcroft} = \frac{(140 - age) \times mass\ (kg)\ [\times 0.85 \text{ if female }]}{72 \times serum\ creatinine\ (mg/dl)}$$

3.5 Ethical considerations

The necessary approval to conduct the study from Institutional Review Committee (IRC) of KIST Medical College Teaching Hospital, Lalitpur, Nepal was obtained. Official letters of request was sent to the Chairman of IRC of KIST Medical College Teaching Hospital, Lalitpur, Nepal for approval to carry out the study in the hospital and Biochemistry Department of Clinical

Laboratory Service (CLS). All the recruited participants was given a full explanation about the purpose of the research work and assurance about the confidentiality of the information obtained through the written consent and blood analysis. The patients who refuse to give the consent was excluded from the study.

3.6 Research Work Duration

This study was conducted for a period of one year from 15th December 2014 to 15th December 2015.

3.7 Criteria

The level of thyroid hormones concentration can be influenced by multifactor including DM, CVD, pregnancy and certain pharmacological agents. So the selection of appropriate subjects was based on the following criteria.

3.7.1 Inclusion Criteria

Individuals with following criteria were selected for the study;

1. Patients with established CKD attending Nephology and Dialysis Clinic of KIST Medical College Teaching Hospital, Lalitpur, Nepal

2. Patients aged 18 years and above

3. Informed consent

3.7.2 Exclusion Criteria

Individuals with following criteria were excluded from the study;

1. Patients with a visible goiter

2. Decline consent

3. Pregnancy

4. Thyroid dysfunction

5. Thyroid cancer

6. Hormonal therapy with drugs like Levothyroxine

3.8 Medical history

A short interview was used for collecting the data and Medical history of the patients. Only CKD patients were included in the study. Demographic and Clinical data including age, sex, body height and weight, and comorbidities, were recorded. The BMI was calculated and their valid record such as number of dialysis sessions per week and duration of dialysis in hours were collected. The following data concerning the detail medical history was also collected at same time.

3.9 Blood sampling and processing

Blood sample was collected from all the subjects who were agreed to participate in the study as well as who met the inclusion criteria. Five ml blood was obtained from each subject into vacutainer plain tubes and was left for short time to allow the blood to clot, then serum samples was obtained by centrifugation at 5000 rpm for 10 minutes.

3.10 Separation and Storage

The serum of the subjects was separated and pipetted into well labelled eppendorf tubes. These tubes were assigned study number for identification purposes and was kept for refrigeration at -8°C in the laboratory of KIST Medical College Teaching Hospital for assay.

3.11 Biochemical analysis

Serum biochemistry was performed on the Biochemistry analyzer Humalyzer 3000 from Human Diagnostics, Germany. Parameters that were determined include: Albumin, serum Creatinine, serum Calcium and serum Phosphorous.

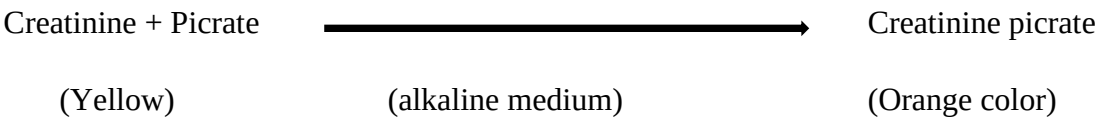
Serum thyroid hormone profile (free T3, free T4 and TSH) were analyzed by using ADVIA Centaur CP Immunoassay System which is a Chemiluminescent Immunoassay (CLIA) technique from Siemens Healthcare Diagnostics (USA).

3.11.1 Creatinine

Creatinine measurements are used in the assessment of renal dysfunction. Elevated creatinine levels are found in renal diseases and insufficiency with decreased glomerular filtration (uremia or azotemia if severe); urinary tract obstruction; reduced renal blood flow including congestive heart failure, shock and dehydration.

Principle and Method

Creatinine is estimated by Jaffe reaction which is a colorimetric method used to determine creatinine levels in blood and urine. Creatinine in alkaline medium reacts with picrate to produce orange color which absorbs light at 490 – 510 nm. The rate of increase in absorbance is directly proportional to the concentration or creatinine in specimen (Jones, 2011).



3.11.2 Albumin

Albumin is the main contributor to the plasma total protein and performs a varieties of functions including regulation of the dissemination of extracellular fluid, acts as a transport agent for a

wide variety of substances such as hormones, lipids, vitamins, calcium and trace metals and forms part of the amino acid pool.

Principle and Method

Serum albumin in the presence of Bromocresol green (BCG) under acidic condition forms a green colored complex. The absorbance of this complex is taken at 600 – 650 nm wavelength and is proportional to the albumin concentration in serum/ plasma (Tietz, 1994).

BCG + Albumin ⟶ Green BCG/Albumin complex

3.11.3 Calcium

Increased serum calcium may be observed in hyperthyroidism, vitamin D detoxification multiple myeloma and some neoplastic diseases of bone. Decreased serum calcium may be observed in hypoparathyroidism, vitamin D deficiency, steatorrhoea, nephrosis, and nephritis**.**

Principle and Method

Calcium reacts directly with O-Cresolphthalein Complexone in alkaline medium forming a reddish violet color. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline.The absorbance is taken at 570 nm (Tietz, 1994).

Calcium + O-Cresolphthalein Complexone ⟶ Reddish violet complex

(alkaline medium)

3.11.4 Phosphorous

Calcium and phosphate in serum usually exhibit a reciprocal relationship. An increase in one of these components is usually accompanied by a decrease in the other. Increased serum phosphorus

levels may be found in hypervitaminosis, hypoparathyroidism and renal failure. Decreased serum phosphorus levels may be found in rickets, hyperparathyroidism, and the Fanconi syndrome, which is associated with a defect in reabsorption of phosphorus from the glomerular filtrate (Tietz, 1994).

Principle of the Method

Phosphorus in serum reacts with ammonium molybdate to form phosphomolybdate, which is then reduced by stannous chloride and hydrazine sulphate to molybdenum blue. The intensity of the colour is measured at 640 nm (Jones, 2011).

Inorganic Phophorous + H_2SO_4+Ammonium+ Molybdate $\longrightarrow$ Unreduced Phosphomolybdate

3.11.5 Thyroid Function Test (fT3, fT4 and TSH)

The ADVIA Centaur analyzer system (Siemens Healthcare Diagnostics, USA) is a stand-alone analyzer giving the elements that are trademark for modern robotized immunoassay systems (also known as Chemiluminiscence Immunoassay technique): arbitrary access and multichannel working mode, adjustment steadiness, positive sample distinguishing proof by a scanner tag peruser, and bi-directional interface to a research center programming system. Sample tubes are discharged inside three minutes in the wake of pipetting for further preparing. All reagents and supply materials (water, cuvettes, pipette tips) might be refilled and waste might be purged amid a run. Reagents are blended physically before stacking and can then stay on-board the assay system for 28 days. Both assay cuvettes and pipette-tips are dispensable single use materials. The system is furnished with a clot-location gadget. Daily support comprises of a robotized washing methodology that takes around 40 minutes and the routine start-up takes around 10 minutes. The greatest throughput is 240 samples for every hour. The analyzer requires a zone of roughly 2.5 square meters for establishment.

Principle

The ADVIA Centaur fT3, fT4 and TSH assay (Siemens Healthcare Diagnostics) is a sandwich immunoassay utilizing paramagnetic microparticles as solid phase and direct chemiluminescence of acridinium ester for discovery of particular sign. Fifty microliters (µl) of sample are administered in a sample cuvette for fT3, fT4 and 200 µl of sample are administered in a cuvette for TSH. A homogenized suspension of the paramagnetic microparticles (225 µl) covered with a polyclonal sheep-against fT3, fT4 and TSH counter acting agent is then included; hatching for 5 minutes permits arrangement of the sandwich. The microparticles are isolated with magnets from the arrangement and washed. Corrosive and base reagent augmentations trigger the chemiluminescent response that is identified by a photomultiplier. The power of the light flag measured more than 5 seconds is directly identified with the fT3, fT4 and TSH fixation in the sample. A predefined master alignment curve of the individual reagent lot is perused from the reagent cartridges by a standardized identification scanner. This master curve is balanced by the client utilizing two calibrators when another reagent lot is initially presented and after a suggested interim of 28 days. The assay is standardized by worldwide standard readiness. The measuring scope of the TSH assay is from 0.004 µIU/ml (logical affectability) to µIU/ml Results are accounted for around 15 minutes subsequent to pipetting the sample. The reference range suggested by Siemens Healthcare Diagnostics for the ADVIA Centaur fT3, fT4 and TSH assay are 2.30-4.2 picogram/ml, 0.89-1.76 nanogram/dl and 0.35-5.5 µIU/ml respectively

3.12 Quality Assurance

Measures were taken to ensure the best quality of laboratory results. Pre-analytical quality measures include exclusion of lipemic, icteric and hemolyzed samples. Analytical measures analysis was done according to manufacturer's specifications. Commercial control materials also called as Internal Quality Control (IQC) from Bio-Rad (Bio-Rad Laboratories, USA) was included in each batch and results were only accepted if the IQC was within acceptable limit. Post analytical measures including data interpretation was done based on the reference ranges given by the manufacturer. The results were counter-checked to ensure that there was no any possibility of data entry error.

The analysis was done by the qualified laboratory personnel using Standard Operating Procedures (SOPs) derived from the manufacturer insert.

3.13 Statistical analysis

Data was computer analyzed using SPSS (Statistical Package for Social Science), version 21 with the following steps:

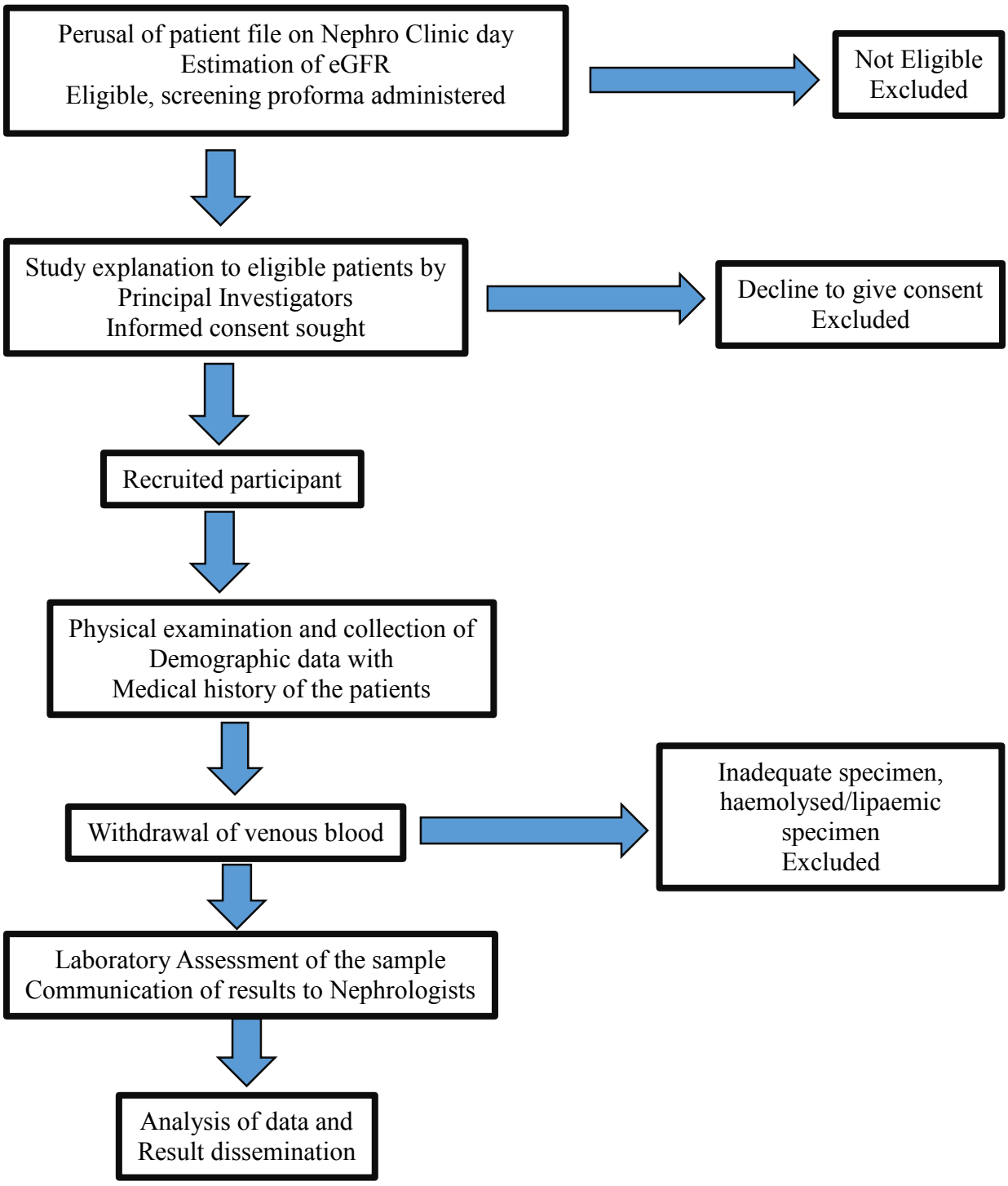
- The following variables such as sex, diagnosis, CKD stages and thyroid status were collected as categorical data that will be presented using percentages and frequencies.

- Continuous variables include age, serum creatinine levels, serum calcium levels, serum phosphorous levels, serum albumin levels, duration of CKD and thyroid hormone profile were presented using mean and standard deviation.

- The non-parametric Kruskal-Wallis test was also done to find the differences in median (interquartile range) among the different stages of CKD as the sufficient sample size was not achieve in some stages.

- ANOVA (Analysis of Variance) was done to determine mean differences of these parameters in different stages of CKD.

3.14 STUDY FRAMEWORK



CHAPTER: FOUR

RESULTS

4.1 DEMOGRAPHIC CHARATERISTICS OF STUDY POPULATION

The study population comprised 163 CKD patients as shown in **Figure 11** with different CKD stages (CKD stages 1-5) aged between 21 years to 87 years. Among 163 participants, 95 (58.3%) were males and 68 (41.7%) were females.

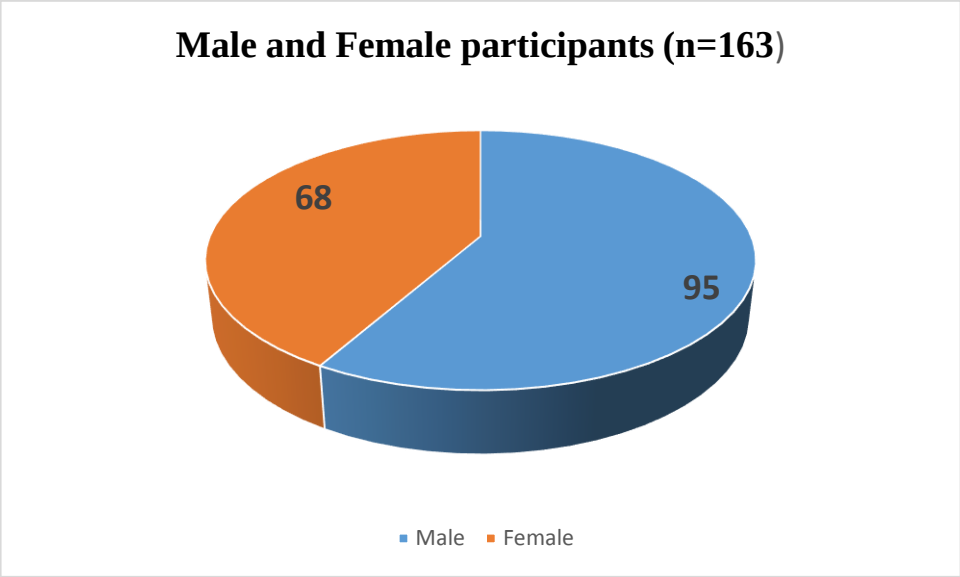


Figure 11: Gender wise distribution of study population

All the study participants were grouped into 6 groups according to their age as distributed in **Table 5**. The age range of first group were between 21 years to 30 years with total 20 (12.3%) participants. Out of 20, 12 (12.6%) participants were males and 8 (11.8%) were females. Second age group consists of 13 (8.0%) participants with age ranging from 31 years to 40 years in which 9 (9.5%) were males and 4 (5.9%) were females. The age of third group were varied between 41 years to 50 years with total 45 (27.6%) study subjects. This highly populated group had 19 (20%) males and 26 (38.2%) females. Fourth group had age ranged between 51 years to 60 years with

32 (19.6%) study participants in which 25 (26.3%) were males and 7 (10.3%) were females. The age varied between 61 years to 70 years were fallen in fifth age group consisting of 28 (17.2%) CKD patients with 19 (20%) males and 9 (13.2%) females. All 25 (15.3%) study participants of sixth age group had age above 70 years in which 11 (11.6%) were males and 14 (20.6%) were females.

Table 5: Age wise distribution of study participants

Age group	Male	Female	Total
21-30	12 (12.6%)	8 (11.8%)	20 (12.3%)
31-40	9 (9.5%)	4 (5.9%)	13 (8.0%)
41-50	19 (20%)	26 (38.2%)	45 (27.6%)
51-60	25 (26.3%)	7 (10.3%)	32 (19.6%)
61-70	19 (20%)	9 (13.2%)	28 (17.2%)
>70	11 (11.6%)	14 (20.6%)	25 (15.3%)
Total	**95 (100%)**	**68 (100%)**	**163 (100%)**

4.1.1 Frequencies of descriptive variables (mean, standard deviation (SD) and median)

The present study described the mean, median and SD of the variables that were included in the study. The mean age of all participants (n=163) was 52.38 ± 15.83 with a median age 52. The mean creatinine values of CKD participants was 5.14 ± 3.44 (**Figure 12**) and the median value was 4.10. Similarly, the mean ± SD values of fT3 (**Figure 13**), fT4 (**Figure 14**) and TSH (**Figure 15**) were 2.41 ± 0.67, 1.14 ± 0.26 and 3.14 ± 2.25 respectively. Median values of these thyroid profile were calculated as 2.45 for fT3, 1.13 for fT4 and 2.56 for TSH. The mean and median values of minerals such as calcium and phosphorous were found to be 8.40 ± 1.01 (median = 8.30) and 4.04 ± 1.24 (median = 3.90) respectively (**Figure 16 & Figure 17**). Lastly, the mean albumin levels of all CKD participants were 3.55 ± 0.66 (**Figure 18**) with a median albumin value 3.50.

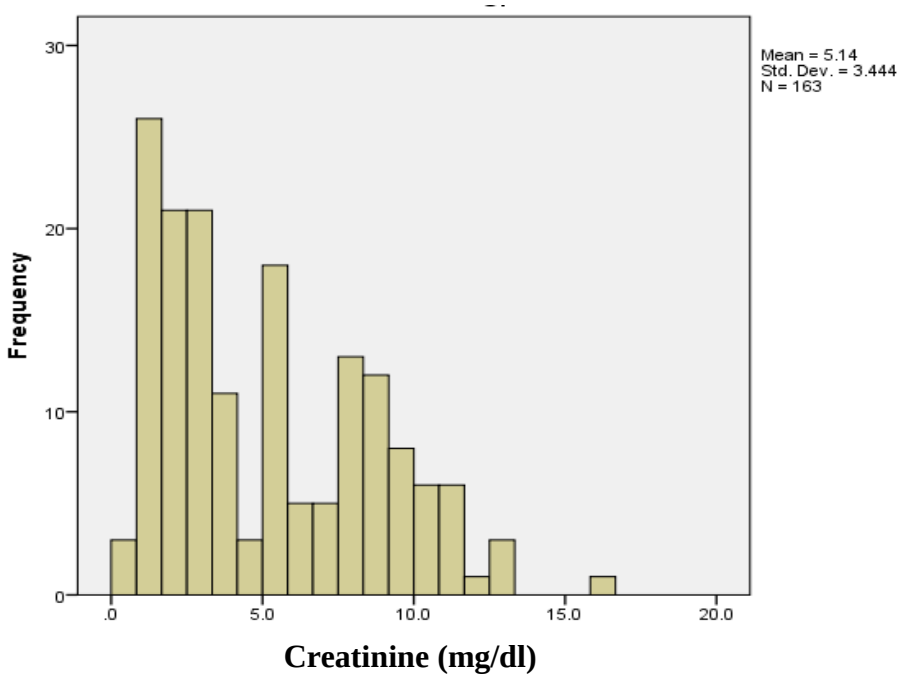


Figure 12: Frequency of Creatinine with mean ± SD

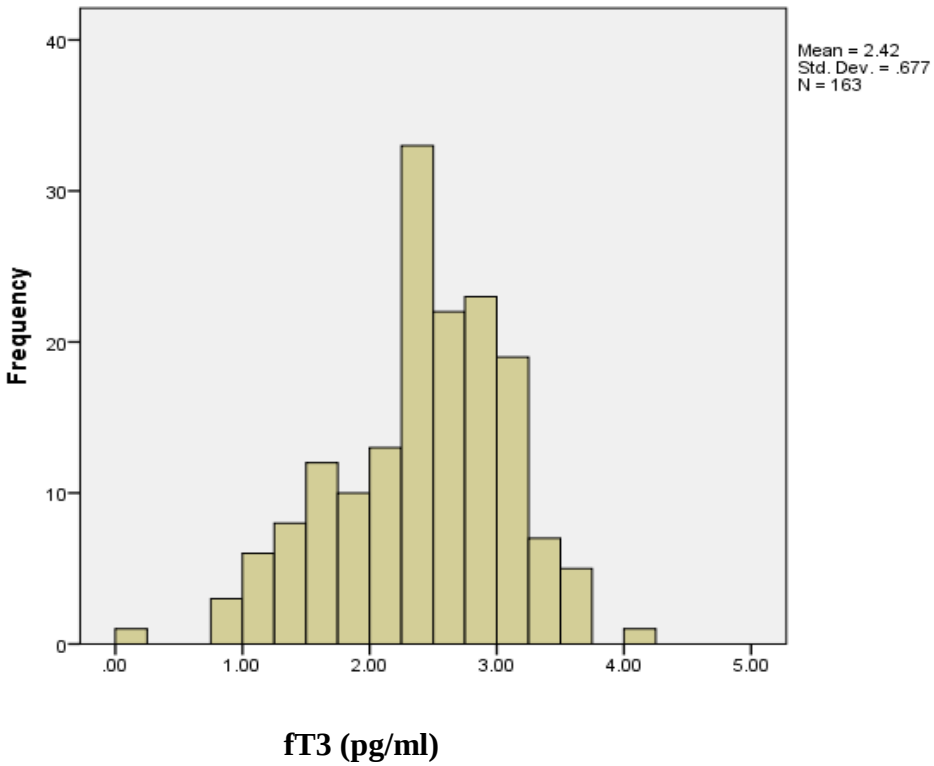


Figure 13: Frequency of fT3 with mean ± SD

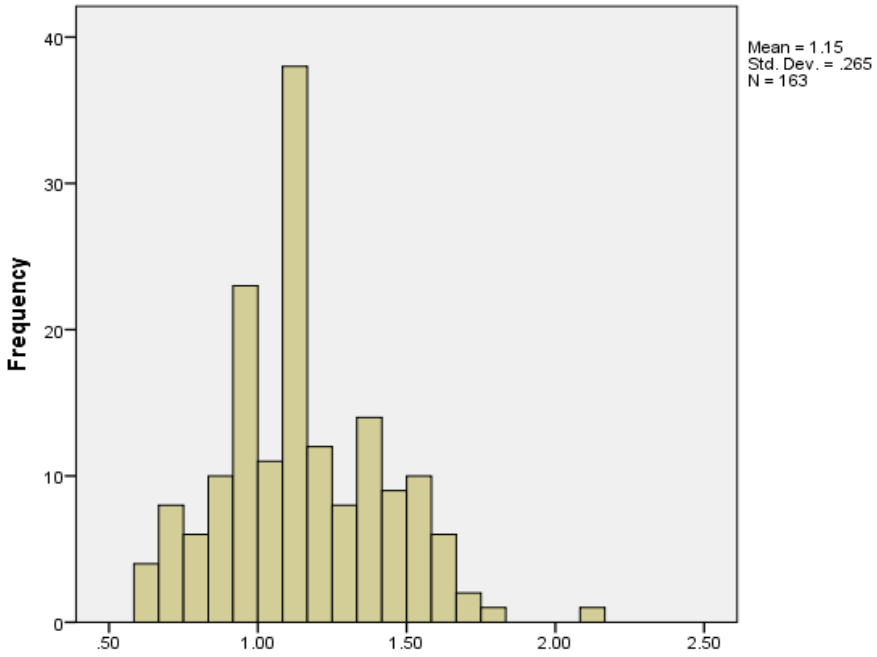


Figure 14: Frequency of fT4 with mean ± SD

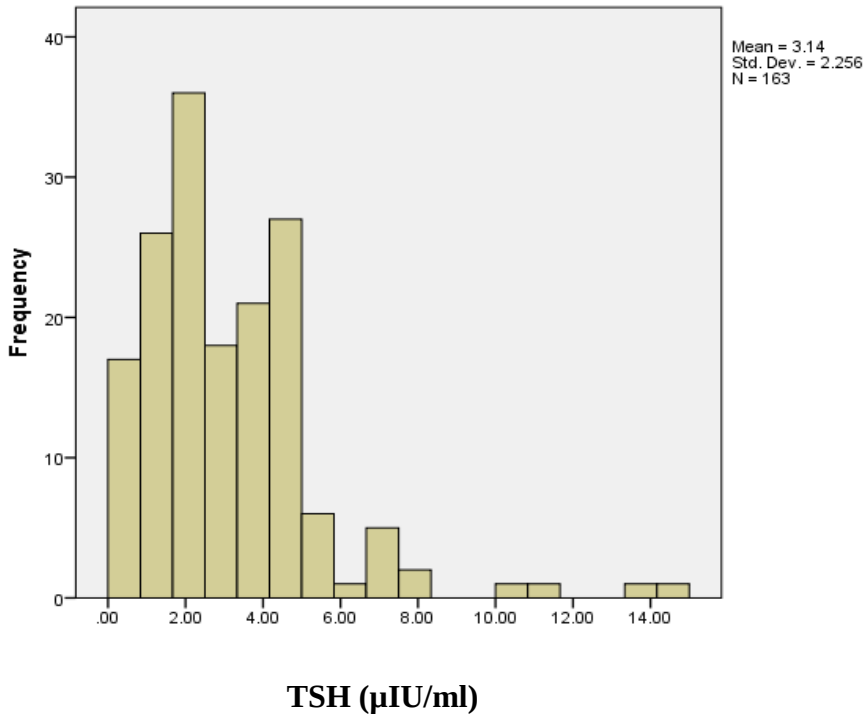


Figure 15: Frequency of TSH with mean ± SD

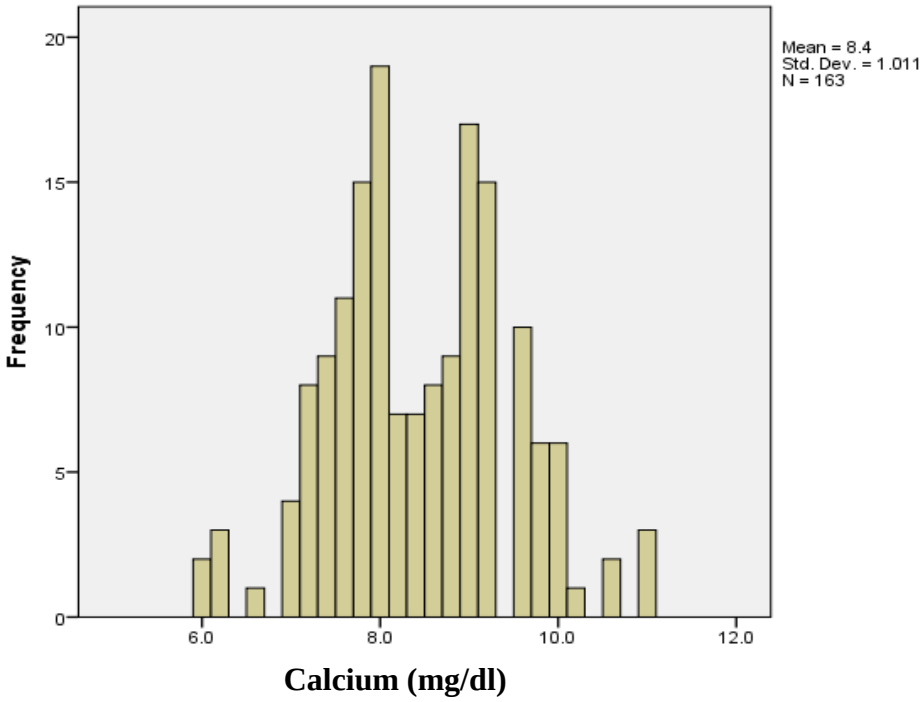


Figure 16: Frequency of calcium with mean ± SD

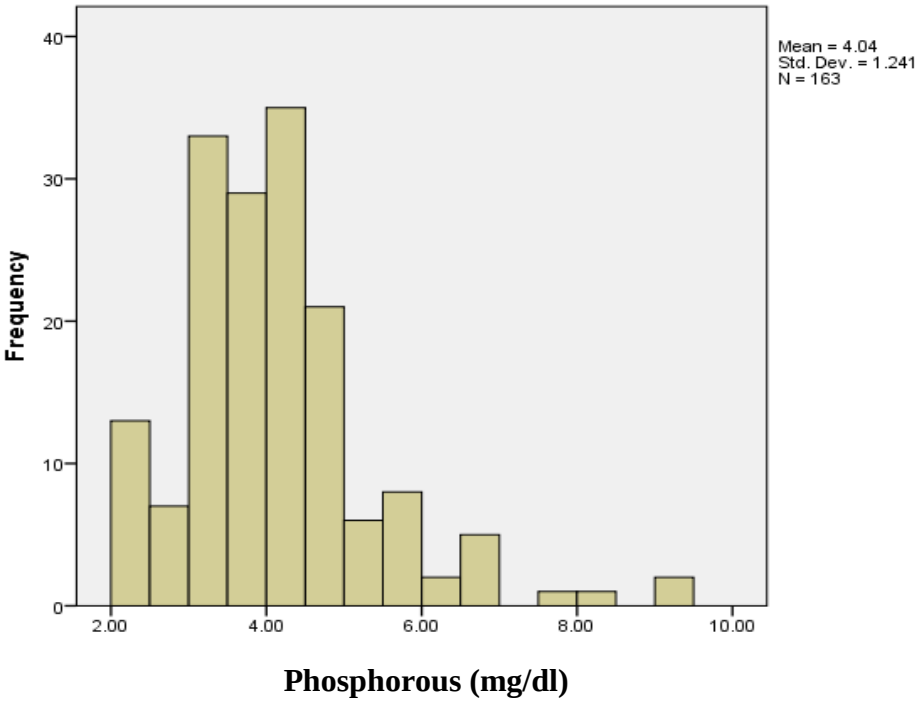


Figure 17: Frequency of Phosphorous with mean ± SD

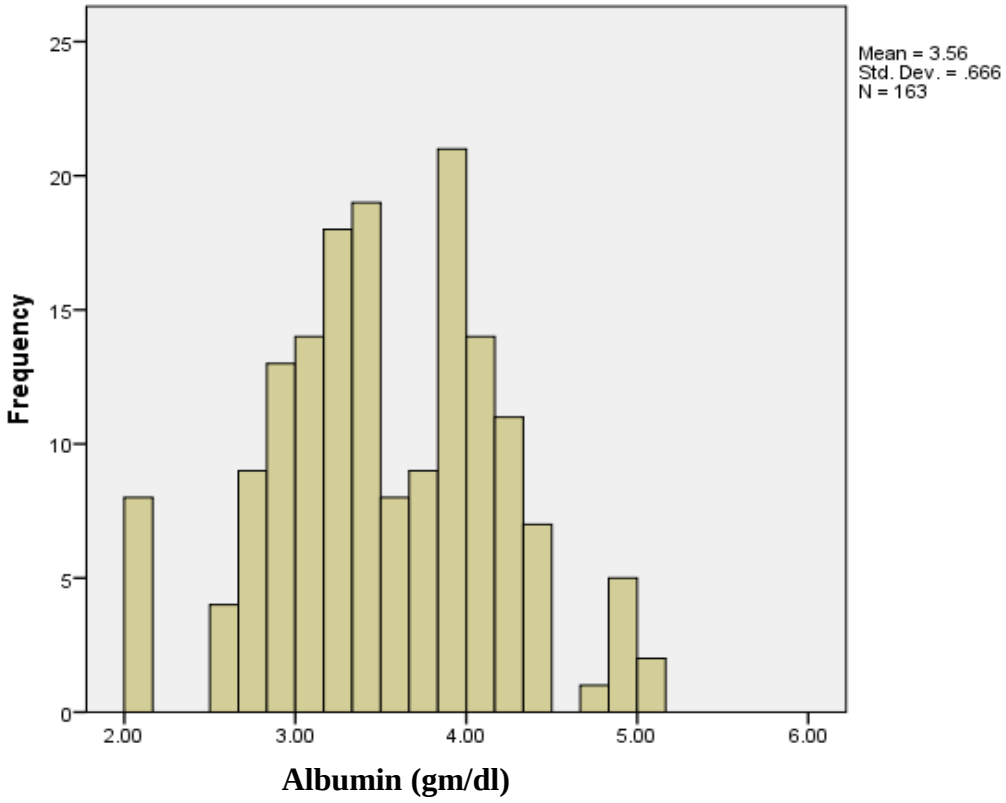


Figure 18: Frequency of Albumin with mean ± SD

4.2 CLINICAL CHARATERISTICS OF STUDY POPULATION

4.2.1 Classification of participants into CKD stages using Cockcroft-Gault formula

The present study (**Table 6**) showed the distribution of 163 study participants in different stages of CKD classified according to NKF-KDOQI clinical practice guidelines based on the GFR calculated by Cockcroft-Gault equation formulated in 1976 on the basis of creatinine clearance. In stage 1 CKD, only 4 (2.45%) participants were present in which 2 (2.10%) were males and other 2 (2.94%) were females. Fourteen (8.58%) participants were present in stage 2 CKD containing 9 (9.47%) males and 5 (7.35%) females. In stage 3 CKD, there were total 39 (23.9%) participants, out of which 22 (23.2%) were males and 17 (25%) were females. Stage 4 contains 20 (12.3%) participants with 14 (14.7%) males and 6 (8.8%) females. Finally in stage 5 CKD, a total number of participants present were 86 (52.8%). This largely populated stage comprised of 48 (50.5%) males and 38 (55.9%) females.

Out of 86 participants of stage 5 CKD receiving RRT, 36 (41.9%) were on conservative management and 50 (58.1%) participants were on HD as shown in **Figure 19**.

Table 6: Distribution of participants in different stages of chronic kidney disease

Stages	Male	Female	Total
1	2 (2.1%)	2 (2.94%)	4 (2.45%)
2	9 (9.47%)	5 (7.35%)	14 (8.58%)
3	22 (23.2%)	17 (25%)	39 (23.9%)
4	14 (14.7%)	6 (8.8%)	20 (12.3%)
5	48 (50.8%)	38 (55.9%)	86 (52.8%)
	95 (100%)	**68 (100%)**	**163 (100%)**

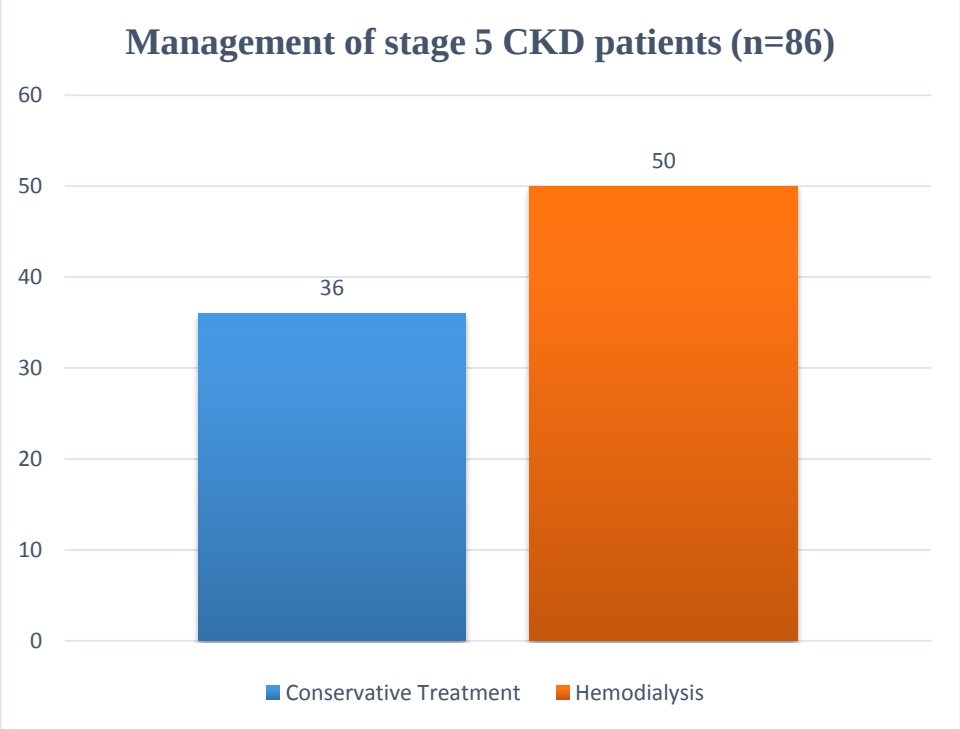


Figure 19: Histogram showing the management of stage 5 CKD patients

4.2.2 Primary risk factors of developing CKD

Regarding factors causing CKD (**Table 7**), DN was in the lead and was present as a primary risk factors in 69 (42.33%) patients. Chronic glomerulonephritis was present in 40 (24.53%) followed by HTN in 36 (22.08%) study participants. Autosomal dominant polycystic kidney disease, obstructive uropathy, focal segmental glomerulosclerosis and membranous lupus nephritis were present in 7 (4.29%), 6 (3.68%), 3 (1.84%) and 2 (1.22%) CKD patients respectively as a primary causative factors.

Table 7: Factors causing chronic kidney disease

Causes	Frequency (n)	Percentage (%)
Diabetic nephropathy	69	42.33
Chronic glomerulonephritis	40	24.53
Hypertension	36	22.08
Autosomal dominant polycystic kidney disease	7	4.29
Obstructive uropathy	6	3.68
Focal segmental glomerulosclerosis	3	1.84
Membranous lupus nephritis	2	1.22

4.2.3 Duration of chronic kidney disease

The participants had been diagnosed with CKD for varying duration of time, ranging from 3 months to 10 years with a median of 24 months. This is evidenced by the fact that the duration category of 1-5 years had the highest number of patients. Majority of the participants had the disease for 5 years or less; this is shown in **Figure 20**.

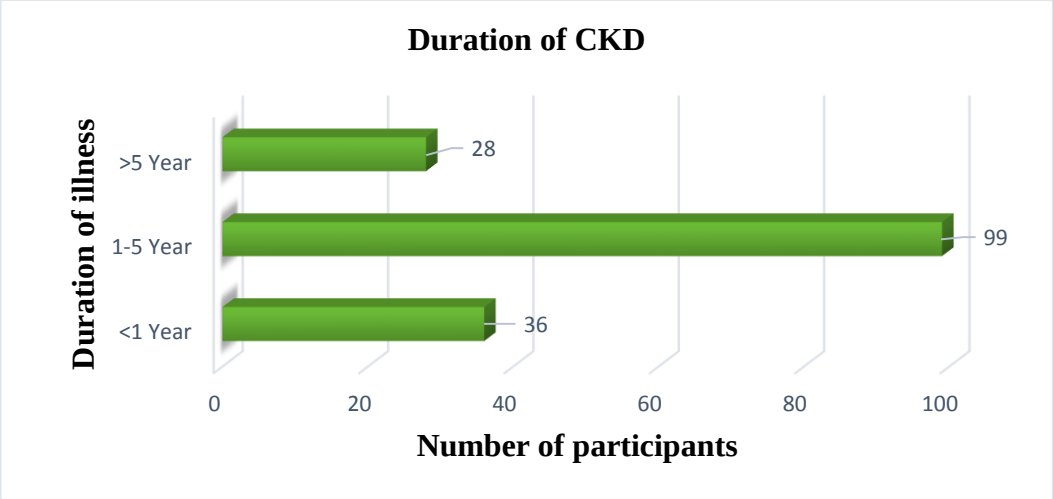


Figure 20: Histogram showing the duration of illness in CKD patients

4.2.4 Thyroid hormone results with mean ± SD of all the participants

Most of the study participants had normal thyroid hormone levels, the percentage normal ranging from 66.9% for free T4, to 91.4% for TSH. Of the participants who had abnormal thyroid hormone levels apart from TSH where 14 (8.6%) participants having its elevated values, 54 (33.12%) had low fT3 values and 19 (11.70%) had low fT4 levels. The mean values of the thyroid hormones were within the normal laboratory (and manufacturer's) reference ranges in all the CKD stages. However, the fT3, fT4 and TSH results showed markedly deranged values as shown in **Table 8** below.

Table 8: Distribution of CKD patients having deranged thyroid profile (n=163)

Thyroid profile	Reference range	Mean ± SD	Low		Normal		High	
			n	%	n	%	n	%
fT3 (pg/ml)	2.3-4.2	2.41± 0.67	54	33.12	109	66.9	0	0.00
fT4 (ng/dl)	0.89-1.76	1.14 ±0.26	19	11.70	144	88.3	0	0.00
TSH (µIU/ml)	0.35-5.5	3.14 ± 2.25	0	0.00	149	91.4	14	8.6

4.2.5 Thyroid hormone profile of participants according to the CKD stages

All the study subjects in CKD stage 1 and 2 had normal hormone profiles. Rest of the participants categorized into stage 3-5 showed deranged thyroid profile. In CKD stage 3, 5 participants had low fT3 and 5 had high TSH values. In stage 4 CKD, 5 participants had low fT3 and 4 had high TSH values. Maximum number of derangement were seen in stage 5 CKD population having low fT3 and fT4 values in 44 and 19 participants respectively. Furthermore, high TSH levels were also among the thyroid dysfunction seen in 5 CKD patients (stage 5) as shown in **Table 9**.

Table 9: Distribution of CKD patients having deranged thyroid profile in each CKD stage

Thyroid profile	Stage 1 (n=4)			Stage 2 (n=14)			Stage 3 (n=39)			Stage 4 (n=20)			Stage 5 (n=86)		
	L	N	H	L	N	H	L	N	H	L	N	H	L	N	H
fT3 (pg/ml)	0	4	0	0	14	0	5	34	0	5	15	0	44	42	0
fT4 (ng/dl)	0	4	0	0	14	0	0	39	0	0	20	0	19	67	0
TSH (µIU/ml)	0	4	0	0	14	0	0	34	5	0	16	4	0	81	5

L: Low; N: Normal; H: High

4.2.6 Minerals and albumin status of CKD population

Derangement of minerals such as calcium and phosphorous were also seen in CKD patients (**Table 10**). Out of 163 CKD patients, 86 (52.8%) had low serum calcium and 5 (3.1%) had high values. Thirty four (20.9%) patients had low phosphorus level whereas 18 (11%) had its high concentrations. The level of albumin in CKD patients were found very low as seen in 78 (47.9%) cases. The mean values of calcium, phosphorous and albumin were 8.40 ± 1.01, 4.04 ± 1.24 and 3.55 ± 0.66

Table 10: Distribution of CKD patients according to serum Calcium, Phosphorous and Albumin

Variables	Low		Normal		High	
	n	%	n	%	n	%
Calcium (mg/dl)	86	52.8	72	44.2	5	3.1
Phosphorous (mg/dl)	34	20.9	111	68.1	18	11
Albumin (gm/dl)	85	52.1	78	47.9	0	0.00

4.2.7 Non parametric distribution of sample among different CKD stages

Kruskal-Wallis test study between the 5 different independent stages of CKD showed the statistically significant outcomes as shown in **Table 11**. The median creatinine value of total CKD patients was 4.0 (2.10-7.90). The median creatinine value showed the highly significant differences among the stages of CKD ($P<0.001$).

The median fT3 value of patients with CKD stages was 2.45 (2.05-2.89). The median value was found to be highly significant ($P<0.001$). The median fT4 value of CKD patients was 1.13 (0.95-1.35). The median value was significant ($P<0.001$). The median TSH value of patients was 2.56 (1.54-4.33). The median value of TSH was not significant ($P>0.05$) indicating that there was no any significant differences between stages of CKD

The median calcium and phosphorous values of CKD patients were 8.30 (7.70-9.10) and 3.90 (3.20-4.50) respectively. The median value were found to be significant ($P<0.05$).

The median albumin value of patients with CKD stages was 3.50 (3.10-4.0). The median value was found to be highly significant ($P<0.001$).

Table 11: Distribution of variables in CKD population

Variables	CKD (n=163)			P* value
	M^D	Q1	Q3	
Creatinine (mg/dl)	4.10	2.10	7.90	<0.001
fT3(pg/ml)	2.45	2.05	2.89	<0.001
fT4(ng/dl)	1.13	0.95	1.35	<0.001
TSH (µIU/ml)	2.56	1.54	4.33	0.838
Calcium (mg/dl)	8.30	7.70	9.10	0.004
Phosphorous (mg/dl)	3.90	3.20	4.50	<0.001
Albumin (gm/dl)	3.50	3.10	4.0	0.110

***Kruskal-Wallis test**

M^D=Median; Q1=Quartiles 1; Q2=Quartiles 2

4.2.8 Summary of the results of the variables in each stage of CKD

In **Table 12**, mean and standard deviation of thyroid hormone profile, serum calcium, phosphorous, albumin and serum creatinine were compared between stages 1 to 5 CKD patients. Stage 1 and stage 2 were grouped into same group. The mean value of serum creatinine in different stages were 1.25 ± 0.29, 1.98 ± 0.44, 3.59 ± 1.22 and 7.75 ± 2.63 which were statistically significant ($P < 0.001$).

Significant differences in the mean level of fT3 and fT4 were seen among different stages of CKD patients. The mean value of fT3 in stages 1 to 5 CKD patients were 3.23 ± 0.32, 2.79 ± 0.48, 2.47 ± 0.23 and 2.06 ± 0.63 respectively. The P value in all group showed the significant relationship (< 0.001) among different stages.

The mean value of fT4 in all stages of CKD patients were 1.28 ± 0.17, 1.24 ± 0.24, 1.20 ± 0.16 and 1.06 ± 0.28 respectively. Here the P value in all group were < 0.05, which were statistically significant.

Mean value of TSH in different stages of CKD patients were 2.79 ± 1.02, 3.47 ± 2.50, 3.97 ± 3.88 and 2.87 ± 1.72. So, TSH value in these groups were not differed significantly (P > 0.05).

The mean calcium values in all stages of CKD patients were 8.63 ± 1.16, 8.86 ± 0.88, 8.35 ± 0.95 and 8.15 ± 0.97 which showed the significant relationship between the stages of CKD. Here the P value in all group were < 0.05.

Mean value of phosphorous in different stages of CKD patients were 3.23 ± 0.66, 3.30 ± 0.88, 3.77 ± 0.84 and 4.60 ± 1.26 that exhibited the significant relationship between the stages of CKD.

The mean albumin values in all stages of CKD patients were 3.83 ± 0.51, 3.54 ± 0.59, 3.48 ± 0.66 and 3.52 ± 0.72 Here the P value in all group were < 0.05. So, phosphorous value in these groups were not differed significantly (P > 0.05).

Table 12: Mean ± SD values of various variables among different stages of CKD along with 'p' values

Variables	Stage 1&2 (n=18)	Stage 3 (n=39)	Stage 4 (n=20)	Stage 5 (n=86)	P value
Creatinine (mg/dl)	1.25 ± 0.29	1.98 ± 0.44	3.59 ± 1.22	7.75 ± 2.63	<0.001
fT3 (pg/ml)	3.23 ± 0.32	2.79 ± 0.48	2.47 ± 0.23	2.06 ± 0.63	<0.001
fT4 (ng/dl)	1.28 ± 0.17	1.24 ± 0.24	1.20 ± 0.16	1.06 ± 0.28	0.003
TSH (µIU/ml)	2.79 ± 1.02	3.47 ± 2.50	3.97 ± 3.88	2.87 ± 1.72	0.067
Calcium (mg/dl)	8.63 ± 1.16	8.86 ± 0.88	8.35 ± 0.95	8.15 ± 0.97	0.011
Phosphorous (mg/dl)	3.23 ± 0.66	3.30 ± 0.88	3.77 ± 0.84	4.60 ± 1.26	<0.001
Albumin (gm/dl)	3.83 ± 0.51	3.54 ± 0.59	3.48 ± 0.66	3.52 ± 0.72	0.312

4.2.8 Categorization of thyroid disorders in all the participants

The thyroid hormone levels (fT3, fT4 and TSH) were used to categorize the participants into euthyroid, hypothyroid and non-thyroidal illness. Majority of the participants 95 (58.28%) were euthyroid, 54 (32.12%) had non-thyroidal illness and subclinical hypothyroidism accounted for 14 (8.58%) CKD patients. **Figure 21** shows the different categories of thyroid status.

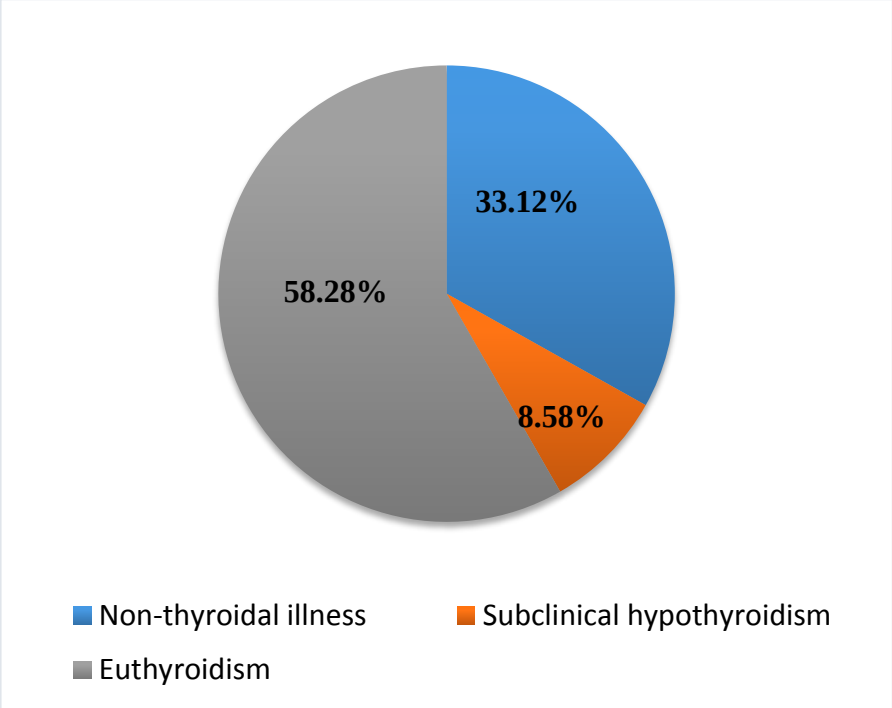


Figure 21: Thyroid dysfunction in CKD patients

CHAPTER: FIVE

DISCUSSION

Chronic kidney disease is now recognized as a main public health burden around the world and is associated with increased risk of morbidity and mortality (Goldberg & Krause, 2016). CKD is also responsible for the enormous economic loss to heath care system of any country (Hill *et al*, 2016). The global incidence and prevalence of CKD is rising exponentially particularly in developing nations (Wachukwu *et al*., 2015). South Asia has a high prevalence of CKD in general as well as high-risk populations. According to World Bank data of fiscal year 2016, Nepal still falls under the category of low income country of South Asian region (World Bank Data of Nepal, 2016). Ene-Iordache and colleagues analyzed data from 21066 Nepalese people covering both general and high-risk population and reported that the prevalence of CKD in Nepal was 20.1% in both groups (Ene-Iordache *et al*., 2016). In 2016, Government of Nepal recognized the urgency of this non-communicable disease and announced that it would provide free lifetime dialysis to all patients with kidney failure despite of several challenges like general awareness, inadequate manpower and poverty (Bhattarai, 2016). As kidney and thyroid gland are interrelated with each other, derangement in thyroid gland function and metabolism of thyroid hormones are common in CKD population (Mohit *et al*., 2014). There are no population based data about the prevalence of thyroid disorders in CKD in Nepal.

5.1 DEMOGRAPHIC CHARACTERISTICS OF CKD POPULATION

The present cross sectional study carried out with a total number of 163 CKD participants recruited for this study in which the prevalence of male participants (58.3%) were greater than that of female (41.7%) as shown in **Figure 11**. The higher proportion of males in this study could reflect the local current change in health seeking behaviour of the male gender. This may also be explained by the fact that men are the breadwinners in most families and hence are more economically empowered to seek health care. Our findings were similar to those of Japanese study done about 6 years ago by Nagata and co-workers on trends in the prevalence of chronic kidney disease and its risk factors in a general Japanese population in which they reported that the prevalence of CKD rises significantly in men as compared to women (Nagata *et al*., 2010).

In a similar study done recently in 2016 on the role of gender in chronic kidney disease in Israel, pointed out that despite the prevalence of CKD has a tendency to be higher in women, the disease is more extreme in men, who additionally have a higher prevalence of ESRD. The greater part of the confirmation in their literature recommends a higher progression rate and mortality risk of CKD in men in contrast to women, with the exception of in post-menopausal women and diabetic patients. However, the reduction in GFR and the expansion in the level of albuminuria are more noticeable mortality risk factors among women. Sex hormones are thought to assume a main part in the biological systems linked with variability in CKD prevalence and characteristics amongst men and women. Animal studies have exhibited the negative impact of testosterone and defensive impact of estrogen on several biological mechanism that are included in kidney injury. Nevertheless, the role of sex hormones in clarifying gender-related contrasts in CKD in human has not yet been built up (Goldberg & Krause, 2016).

The GFR estimation is also dependent upon sex and other variables of the individuals while concerning the differences of CKD in both men and women. On the contrary, evidence based on the recent United States Renal Data System (USRDS) annual data report, predict that the occurrence of CRF between the years 2007 and 2012 was greater in women (15.1%) than in men (12.1%) (USRDS, 2015). Increased prevalence of urinary albumin to creatinine ratio (9.6% versus 8.1% in men) with reduction in GFR (7.6% versus 5.4% in men) in women support the published evidences. Twenty years ago, a French epidemiologic study demonstrated a higher incidence rate of CRF in men (Jungers *et al*., 1996) which was later supported by the Chinese cross-sectional study (Zhang *et al*., 2008) in which they confirmed CKD predominance among men than in women. There may be a geographic inconsistency in the impact of sex on the prevalence of CKD. Taking into an account the latest USRDS data, 57.8% of newly diagnosed ESRD patients were men. Moreover, majority of kidney transplant beneficiaries in the USA (Goldberg & Krause, 2016) were men (59.7%). A few other studies were also discovered the similar findings (Haroun *et al*., 2003; Kastarinen *et al*., 2010; Wakai et *al*., 2004).

As described in **Table 5,** majority of the study participants were aged between 41 to 50 years (27.6%) as compared to prevalence of CKD patients in other age groups. Approximately 80% of the CKD patients were aged above 40 years. These findings were similar to the study done by Muhammad Asif and colleagues in General Hospital of Pakistan on correlation between fT4,

TSH and GFR in CKD. They reported that the prevalence of CKD patients in age group 40-49 years and 50-59 years were 20% and 31% respectively that was greater as compared to the age groups between 20-29 years (8%) and 30-39 (9%) years. Furthermore, Asif and co-workers revealed that the frequency of CKD patients above age 40 years was 83% (Asif *et al*., 2013). A study done by Stevens and colleagues on CKD and ESRD in the Elderly people in 2010 in which they reported that CKD is a major concern in the elder people with high prevalence of acquiring earlier stages of CKD. As the aging is the natural phenomena so, the global population is aging. At present the population of individuals more than 65 years of age is estimated around 420 million or around 7% of the world population. It is anticipated that by 2050 there will be more than 1.5 billion individuals above 65 years of age and more, indicating an increasing rate of elderly people in both developing and developed nations. Moreover, the elder people faces significant challenges regarding their health issues. In the US, individuals more than 65 years old have some chronic illness that accounts for an average number of 3.5 total chronic ailments for every individual, with CVD risk factors (Stevens *et al*., 2010).

An elderly people are more prone of getting non-communicable disease like CKD. The prevalence of obesity in middle-aged and elderly adults is estimated to be more than 30%. Out of which, 11% of middle-aged adults have diabetes and 33% have hypertension. The prevalence of these two conditions will be increased to 23% and 66% by the age of 60 (Flegal *et al*., 2010). In the United States, these rates are most astounding among racial and ethnic minority populaces, and also in individuals with lower financial status. This pattern is comparable in numerous developing nations, where chronic diseases are significant reason for morbidity and mortality (Stevens *et al*., 2010). Zhang and Rothenbacher also studied the prevalence of CKD worldwide in 2008. They reviewed thoroughly 26 studies from the USA, Europe, Asia and Australia and revealed their findings that median prevalence of CKD was 7.2% in a population below 30 years, whereas in persons above 60 years, the prevalence varied from 23.4 to 35.8% (Zhang & Rothenbacher, 2008).

5.2 CLINICAL CHARACTERISTICS OF CKD POPULATION

Classification of CKD into different stages in this study was done as per NKF guidelines (National Kidney Foundation, 2002), with estimated GFR using Cockcroft-Gault formula.

Majority of the participants were in CKD stage 5 (52.8%) followed by CKD stage 3 (23.9%) and stage 4 (12.3%) as shown in **Table 6**. A similar cross sectional study was carried out over a period of 1 year from June 2011 to May 2012 on clinical and demographic characteristics of CKD patients in a tertiary facility in Ghana at the Komfo Anokye Teaching Hospital, Ghana by Amoako and co-workers in which they reported that the majority of patients had CKD stage 5 (79.8%) with stage 4 accounting for approximately 6% (Amoako *et al.*, 2014).

In this current study, out of 163, only 4 CKD participants were present in CKD stage 1 (2.45%) and 14 participants in stage 2 (8.58%). This could be attributed to delay in seeking medical treatment hence patients were seen when the disease had progressed to the more severe stages. The prevalence of the participants between CKD stages 3 to 5 is about 89% indicating that the people of Nepal is unaware about the seriousness of disease. Recently, Ene-Iordache and colleagues studied CKD and cardiovascular risk in six regions of the world and pointed out their finding that the people of South Asia including India and Nepal had very low awareness regarding CKD. In their cohort study, they had estimated awareness of CKD in low-income and middle-income nations and discovered very less CKD awareness (6% in general population and 10% in high-risk population) as compared with the data from developed nations (Ene-Iordache *et al*., 2016). The clinicians use creatinine levels and only estimate GFR when the creatinine levels are elevated. It is recommended that estimation of GFR should be done in all cases after creatinine levels have been determined. It has been estimated from population survey data that at least 6% of the adult population in the United States has CKD at stages 1 and 2. An unknown subset of this group progresses to more advanced stages of CKD. An additional 4.5% is estimated to have stages 3 and 4 CKD (Coresh *et al*., 2007).

In a recent systematic review and meta-analysis of observational studies performed by Hill and co-workers in which they had estimated prevalence of stage 5 CKD (13.4%) in general populations through literature searches in 8 databases comprising 6,908,440 patients (Hill *et al*., 2016). Thomas and colleagues reported that the prevalence of different stages of CKD in the US population was found to be 1.8% for stage 1, 2% for stage 2, 7.7% for stage 3 and 0.35 % for stages 4 and 5. Patients with stages 3 or 4 CKD had maximum chances for the progression to ESRD or stage 5 at a rate of 1.5% per year. The rate of progression of stage 1 or 2 CKD patients to more advanced stages raised by 0.5% per year (Thomas *et al*., 2008). In addition, the NKF

KDOQI provides evidence-based, clinical practice guidelines for all stages of chronic kidney disease to optimize management of related complications. Twelve sets of guidelines have been published and are available on the NKF web site. Each of the complications discussed in this article is addressed by the NKF KDOQI guidelines (National Kidney Foundation, 2002).

5.2.1 Factors responsible for progression of CKD

In this study, majority of study population had a diagnosis of DN, HTN and CGN as the primary risk factors leading to progression of CKD. These can be attributed to the increasing prevalence of DM and hypertension globally. The data recoded in **Table 7** showed the DN (42.33%) in the main lead among the risk factors of CKD followed by CGN (24.53%), HTN (22.08%), polycystic kidney disease (4.29%), obstructive uropathy (3.68%) and others (3.06%) which is concordant with data from a study conducted by Foley reported that DN was the leading cause of ESRD worldwide (Foley & Collins, 2007). Several similar studies were carried out regarding the prevalence of risk factors of CKD. Recently, Malekamakan and co-workers (Malekamakan *et al*., 2016) mentioned the fact findings that DN and HTN was main causative factors for 54.3% of cases of ESRD followed by renal stone (4.9%) and glomerulonephritis (3.7%). A similar study in Saudi Arabia found that HTN (30.4%) and DN (25.2%) were the main risk factors behind renal failure (Eknoyan *et al*., 2004). A study from UK (Ansell *et al*., 2006) found diabetes (18%) and glomerulonephritis (10.4%) as the leading causes of ESRD whereas in Germany, diabetes (34%) and renovascular (22%) diseases were the leading causes. In another study that was carried out in Australia, diabetes (30%), glomerulonephritis (25%), and HTN (13%) were the leading causes of ESRD (Malekamakan *et al*., 2016).

CGN (24.53%) was the second leading cause of CKD in our study that contradicted with the study done recently by Khakurel and colleagues, Amoako and colleagues in which they all reported that CGN was the leading cause of CKD. The prevalence of CGN (33%), HTN (21.2%) and DN (22.2%) were found to be the leading causes of CKD (Amoako *et al*., 2014; Khakurel *et al*, 2015). As this study is a single center based cross sectional study, the finding may differ from other studies conducted in Nepal.

Impairment of renal function corresponds better with the level of tubulo-interstitial injury and fibrosis than with histologically evident glomerular injury. In CKD, the harmony between cell proliferation and apoptosis is affected with increased apoptosis of normal glomerular and tubular epithelial cells. Abnormal apoptotic stimuli, for example, TGF-β, TNF, Fas ligand (FasL) and interferon-α result in expanded cell loss (Metcalfe, 2007). An abundance of cytokines present in kidney of CKD patients initiates macrophages into the kidney; macrophage-colony stimulating factor is over expressed by the tubules as a reaction to injury. Macrophage penetration of the interstitium associates with the renal dysfunction and the cells enhance the reaction by delivering more cytokines further prompting fibrosis and apoptosis. Systemic hypertension is frequent in CKD and its rate increases with declining GFR. Hypertension is because of a blend of sodium and water excess, initiation of the RAAS as well as sympathetic nervous system activation. Systemic hypertension is responsible for glomerular capillary hypertension as well as quick reduction in GFR (Metcalfe, 2007; Muñoz-Durango *et al*., 2016).

Early DN is all around portrayed to be connected with hyperfiltration bringing about an increment of GFR with related glomerular hypertrophy. Essential renal vasodilatation, with a more prominent reduction in afferent in respect to efferent arteriolar resistance results in expansion of intraglomerular pressure and glomerular hyperfiltration. There is significant connection between urinary albumin excretion and glomerular pressure, so, the glomerular HTN in DN engenders a decrease in GFR at any rate because of expanded protein spillage across glomerular capillaries into Bowman's space. Segmental areas of glomerulosclerosis can likewise be incited by intraglomerular HTN as a result of essential renal vasodilatation as it happens in DN (Metcalfe, 2007).

CKD subjects had proteinuria, with high blood pressure and hyperglycaemia. Proteinuria is a sign of increased protein excretion by the kidneys and hence regarded as a traditional marker of declining kidney function (Lopez-Giacoman & Madero, 2015; Vigil *et al*., 2015). In addition to predicting loss of renal function, proteinuria has been linked with CVD and mortality risk in population-based studies (Currie & Delles, 2014). Presence of even minimal albuminuria could reflect generalized endothelial dysfunction in capillaries (e.g. glomeruli) and arteries or abnormalities within the fibrinolytic and coagulation pathways; it may be a marker of inflammatory status and may denote greater severity of end-organ injury or mark the depletion

of certain antioxidants. A higher prevalence of diagnosed diabetes and hypertension and higher body mass index explained the entire increase in prevalence of albuminuria but only part of the increase in the prevalence of decreased GFR. Estimation of GFR from serum creatinine has limited accuracy and a change in mean serum creatinine accounted for some of the increased prevalence of CKD (Lopez-Giacoman & Madero, 2015).

5.2.2 Thyroid dysfunction in CKD

The principal findings of this study is that thyroid hormone dysfunction is very frequent among CKD population with decline in GFR. In this study among 163 CKD patients, 33.12% had low fT3, 11.70% had low fT4 and 8.6% had high TSH values as shown in **Table 8**. In a similar study done by Rajagopalan and co-workers speculated that even though the TSH level remain unchanged significantly, there was significant reduction in T3 and T4 values (Rajagopalan *et al*., 2013). Moreover, Iglesias & Diez reviewed the thyroid abnormalities in different kidney diseases, such as CKD, glomerular disease, tubular disease, nephrotic syndrome etc. and concluded that the thyroid functions were deranged in CKD through several mechanisms (Iglesias & Diez, 2009).

5.2.2.1 Status of fT3 hormone in CKD

In this study, it was found that the number of participants with low fT3 levels were raised with increasing severity of CKD indicating that low fT3 is very common thyroid dysfunction seen in these chronic illness patients. Majority of the low fT3 were seen in stage 5 CKD (26.99%) as shown in **Table 9**. All the study patients with low fT3 level haad normal TSH values. Several authors reported the same findings as this present study and reported that low T3 syndrome is very common in CKD. They also reported that there is increasing incidence for the population of low T3 in accordance with the increased CKD stage (Fan *et al*., 2016; Rajagopalan *et al*., 2013; Song *et al*., 2009). Singh and co-workers recognized low T3 as non- thyroidal illness and classified disorder of thyroid function in several classes as i) low T3 syndrome, ii) low T3-T4 syndrome, iii) high T4 syndrome and iv) other abnormalities (Singh *et al*., 2006).

In a non-parametric analysis (**Table 11**), the median value of fT3 were significantly differ among different stages of CKD. Decreasing pattern of median values was observed with the progression of CKD. Furthermore, the findings in this study are in accordance with the previous investigations by several researchers that the serum T3 levels were below normal range in around one third to one half of the cases of chronic renal failure. Decreased levels of T3 in non-thyroidal cases are due to reduced peripheral conversion of T4 to T3 while thyroid gland production of T3 is normal and T3 clearance rates are normal or lessened. T3 is the most active thyroid hormone produced by 5'-monodeiodination of the prohormone T4 (Basu & Mohapatra, 2012). Two of the functionally distinct enzymes-glutathione peroxidase and 5'-deiodinase require selenium as a cofactor. In patients of CKD, decreased levels of selenium has been reported. Monodeiodination of T4 takes place in virtually all tissues of the body and the reaction is catalyzed by the family enzymes called as the iodothyronine deiodinases. More than 80% of serum T3 are supplied by liver, kidney and muscle. Abnormal conversion of T4 to T3 may be associated with malnutrition and humoral factors like cytokines that are normally associated with CRF (Singh et al., 2006). Brzezinska-Slebodzinska & Pietras had demonstrated that free radicals may affect 5'-monodeiodinase and indirectly decrease plasma T3 level (Brzezinska-Slebodzinska & Pietras, 1997).

Other factors like chronic metabolic acidosis and increased excretion of bound and free T4 in urine of CRF patients were also among the contributors of low T3 concentrations (Padhy & Devi, 2014). In this study we found that thyroid functions were normal in earlier stages of CKD indicating that the low fT3 was commonly seen in advanced CKD stage 3-5 CKD in which GFR is less than 60 ml/min also known as CRF. Thyroid hormone metabolism is disturbed at multiple critical steps in CKD patients including iodine accumulation in the thyroid gland and altered de-iodination. It was also hypothesized that the sub-normal fT3 in ESRD may be due to the accumulation of substances that inhibits binding of T3 to the solid-phase matrices (Zoccali *et al*., 2006).

This study investigate the prevalence of low fT3 in CKD stages 1-5 and thus offers the evidence for demonstrating decreased fT3 levels at advanced stages of CKD subjects in a euthyroid state. In consistent with the previous studies, this study also found a large proportion of stage 5 CKD patients (51.16%) with low fT3 levels as compared to other stages (**Table 12**). Additionally, in

stages 3 and 4 CKD patients, 12.82% and 25% respectively had low fT3 levels. Collectively, these results suggested that while the frequency of low fT3 in CKD stage 3 was lower in comparison to CKD stages 4 and 5, low fT3 can be regarded as early markers of CKD patients. Stages 1 and 2 had low number of patients with normal fT3 levels so, both stages were combined for statistical analysis. ANOVA testing was done to compare means among different stages of CKD. The mean fT3 value varies significantly among groups ($P<0.001$). These results are similar to the findings by Song and co-workers on the prevalence of low triiodothyronine according to the stage of chronic kidney disease in subjects with a normal thyroid-stimulating hormone in which they reported that the prevalence of low T3 in CKD population show the increasing trends with the advancement of stages. They revealed the data of low T3 in CKD stages 3, 4 and 5 which was 28.5%, 60.8% and 78.6% respectively (Song *et al*., 2009).

Thyroid hormones has strong impact on renal development, kidney structure, renal hemodynamics, GFR and sodium and water homeostasis. These impacts of thyroid hormone are mostly due to direct renal activities intervened via cardiovascular and systemic hemodynamic impacts that impact kidney function. As an outcome, both hypothyroidism and hyperthyroidism associate with kidney functions and have significance role in its evaluation. Derangement of thyroid function have similarly been connected to progression of immune mediate glomerular injury, and modifications in thyroid hormones generally encountered in patients with kidney disease. The kidney involve in the clearance of iodine, TSH, and thyrotropin-releasing hormone. Most patients with CKD are clinically euthyroid, with normal TSH and free T4 levels. Patients with CKD may have changes in thyroid function tests reliable with the euthyroid sick syndrome also called as Non-thyroidal illness (Mariani & Berns, 2014).

In addition to the hypothalamic-pituitary-thyroid axis, ESRD alters the peripheral thyroid hormone metabolism. Even with a normal TSH level, T3 which is considered as metabolically active thyroid hormone is found to be reduced in ESRD patients (Abd-Elhafeez *et al*., 2016; Song *et al*., 2009). Long back ago in the year 1988, investigation was done by Kaptein and co-workers reported that out of 287 euthyroid patients with ESRD, 76% had low total T3 levels and 66% had low free T3 levels (Kaptein *et al*., 1988). This study advocates that the great population of ESRD patients have decreased serum T3 levels irrespective of normal TSH levels. Several investigations have suggested that abnormalities in thyroid hormone ranges can occur with hours

of acute illness and the magnitude of these alterations proportionate with severity of disease and survival. In particular, low levels of free T3 has been taken as an independent predictor of mortality in patients on hemodialysis (Fragidis *et al*., 2015). Chang and co-workers recently published their findings in which they reported that decreased T3 is associated with inflammation and cardiovascular damage in ESRD patients (Chang et al., 2015). All these investigations imply that decreased T3 levels can be a marker of prognosis in patients with renal disorder (Song et al., 2009).

Conduction of periodic screening of thyroid function in CKD patients may be essential since the clinical features of thyroid dysfunction are often masked with uremic state. Early diagnosis and treatment of thyroid disorder significantly reduces morbidity and mortality in patients awaiting kidney transplant therapy of CKD stage 5. Various studies have shown that low total T3 and fT3 previous to kidney transplant is associated with decreased graft survival; thus early diagnosis and treatment is considered to better the outcome (Jusufovic & Hodzic, 2011; Thalquotra *et al*., 2014).

5.2.2.2 Status of fT4 hormone in CKD

In this present study, 19 (11.7%) out of 163 CKD patients had decreased fT4 levels. All the patients with low fT4 had also decreased fT3 levels and are from stage 5 CKD only as shown in **Table 9**. However, other stages of CKD patients had fT4 concentration within the normal range. The mean value of fT4 were decreased as the severity of the diseases was progressed (**Table 12**). Thus, the decreasing trends of mean fT4 values showed the statistically significant correlation between thyroid profile and different stages of CKD ($P<0.05$). This reduction in fT4 value may be linked to impaired T4 binding to serum carrier protein like thyroid hormone binding globulin (TBG) and to less extent prealbumin and albumin mainly due to the presence of inhibitors. In the previous studies, it has been reported that many inhibitors of T4 binding to TBG are present in CRF patients which result in decrease level of T3. Several previous investigations have also suggested that the diminished T3 levels can be attributed to the increased excretion of bound and free T4 in urine of CKD patients (Allawi, 2013; Horáček *et al*., 2012; Padhy & Devi, 2014; Rajagopalan *et al*., 2013)

5.2.2.3 Status of TSH hormone in CKD

Among 163 CKD patients, 14 (8.6%) had increased TSH concentration without altering the median normal values (**Table 8**). This elevated TSH levels was seen in CKD stages 3, 4 and 5. The prevalence of high TSH in CKD stages 3-5 were 12.82%, 20% and 5.81% respectively (**Table 9**). The TSH values in this study were normal in 91.4% CKD population. Statistical tool ANOVA test was done to analyze whether the mean TSH values varies among groups (**Table 12**) or not. The analyzed data did not show the significant differences between TSH and different stages of CKD. The underlying mechanism may be mediated by inhibited response to the TRH as a result of TSH glycosylation and altered TSH circadian rhythm. This suggests that thyroid is able to compensate for humoral urinary losses keeping the patient euthyroid. Reduced level of serum TSH have not been reported in euthyroid CRF patients (Allawi, 2013; Horáček *et al*., 2012; Rajagopalan *et al*., 2013).

The high TSH value with normal fT3 and fT4 levels in study population indicates the existence of subclinical primary hypothyroidism among CKD patients beside most prevalent low T3 syndrome (NTI). This findings was similar to those previous studies by several authors in which they reported that subclinical primary hypothyroidism is independently associated with progression of CKD with decreased estimated GFR (Asif *et al*., 2013; Kang *et al*., 2008; Shantha *et al*., 2011).

5.2.3 Minerals and albumin status of CKD population

In this study, prevalence of hypocalcaemia and hypercalcaemia were found in 52.8% and 3.1% CKD patients respectively. The prevalence of low serum phosphate (Hypophosphataemia) was found in 20.9% and hyperphosphataemia in 11% as shown in **Table 10**. In 2012, Singh and colleagues in Nepal analyzed value of calcium and phosphorous in CKD patients and reported that the prevalence of hypocalcaemia in CKD was found to be 29% and Hyperphosphataemia in 42%.

The mean calcium and phosphorous values in different stages of CKD patients showed the significant variations within the CKD stages. Disorders of mineral metabolism begins at early stages of CKD, and is normally escorted by significant changes in minerals levels in blood that prompts clinical issues like bone diseases, musculoskeletal symptoms and growth hindrance. A report from previous studies also revealed strong relationship amongst mortality and disturbed mineral metabolism; probably intervened by vascular calcification (Panichi *et al*., 2010). Actually, the disturbance in mineral metabolism in CKD affect the plasma concentrations of calcium, phosphorous, vitamin D, and PTH homeostasis. Mineral metabolism outcomes in CKD is very significant during the management of ESRD. Furthermore, the alteration in normal phosphate homeostasis can cause renal bone disorder in CKD patients. In our study, the control of serum phosphorous qualities was satisfactory in 53% of patients experiencing HD, while 42% of the patients gave hyperphosphatemia.

According to Mahdavi-Mazdeh and co-workers, hyperphosphatemia occurs as a result of reduced phosphorous filtration and its excretion with the reduction of GFR. Decline in phosphorus excretion can be overcome by lowering the proximal phosphate reabsorption via increase secretion of PTH. Hence, phosphorus level remains within normal range till GFR falls below 30mL/min (CKD stage 4) (Mahdavi-Mazdeh *et al*., 2007).

The prevalence of low serum albumin also known as hypoalbuminaemia in this current study was 52.1%. No statistical differences were recorded between serum albumin and stages of CKD. 47.9% of study population as shown in **Table 10** were found to have normal serum albumin concentration. No cases of hyperalbuminaemia is reported in this study. The mean albumin in CKD participants (n = 163) did not vary among groups. Chung and co-workers reported the albumin as a potential markers of malnutrition in CKD and ESRD population. Varying from normal populations, CKD and ESRD patients experience markedly alteration of total body water supply and experience recurrent changes in plasma volume, both of which are well-known to disturb albumin turnover and subsequently serum albumin values. Besides, a chronic inflammatory condition in CKD and ESRD subjects is known to affect total albumin turnover in the plasma. In spite of certain limitation, serum albumin is routinely evaluated to recognize possibly low protein stores and nutritional status in CKD and ESRD patients (Chung et al., 2012). Friedman and Fadem, (2010) witnessed that serum albumin is a trustworthy index of

malnutrition; since serum albumin is typically reduced in patients with CKD which matches with this study results (Friedman & Fadem, 2010), most of the patients were associated with low albumin level and the mean value of albumin is (3.55±0.66 gm/dl) . Moreover, the findings in this study matches with the recent study done in Egypt by El-Hamid and co-workers in which they reported the high prevalence of hypoalbuminaemia in CKD participants having mean value 2.7 ± 0.55 (El-Hamid, 2016).

5.2.4 Categorization of thyroid disorders in CKD

Finally, the presence of NTI (also known as low T3 syndrome), subclinical hyperthyroidism and euthyroidism (normal thyroid function) were the conclusive findings in this present research study. The prevalence of NTI and subclinical hyperthyroidism reported were 33.12%, and 8.58% respectively (**Figure 21**). The population of euthyroid in CKD was found to be 58.28%. There were no any findings of hyperthyroidism indicating that hyperthyroidism is not very common in patients with signs and symptoms as uremia. The imbalance between hypothalamus pituitary and peripheral response to systemic thyroid hormones leads to thyrotoxicosis. However not many studies have been done on hyperthyroidism in CKD. Pharmacologic agents administered to patients who have CKD may confound the interpretation of thyroid function tests. Glucocorticoids affect the hypothalamic-pituitary-thyroid axis at multiple levels including suppression of TSH secretion, down-regulation of T4 to T3 conversion by 5'deiodinase and decrease of TBG concentration and hormone binding capacity (Wile, 2012). In CKD stage 5 patients awaiting kidney replacement therapy, early diagnosis and treatment of thyroid disease significantly reduces morbidity and mortality. It has been shown that low T3 and fT3 before renal transplant is associated with decreased graft survival thus early diagnosis and may be treatment should be considered to improve the outcome (Reinhardt *et al*., 1997).

Non-thyroidal illness is defined as biochemical alteration in thyroid hormones in the absence of underlying intrinsic thyroid disease and is commonly found in patients with critical illness such as CKD. However, the exact etiopathogenesis and its effective management is still unclear (Xu *et al*., 2014). It is characterized by low T3 and fT3 with an increase in rT3 and normal TSH, T4 may be normal or low. The reduction in T3 levels (low T3 syndrome) is the most frequently observed thyroid alteration in these patients (Chang *et al*., 2015; DeGroot, 2015). In this study,

the levels of fT3 were found to be low and were noted to decrease as the renal insufficiency progresses. This reduction in T3 is primarily related to diminish peripheral tissue conversion of T4 to T3. Chronic metabolic acidosis, the presence of inhibitors of T4 binding to plasma proteins and effects of medication associated with CKD may contribute in this effect. These low fT3 levels have been reported in other studies (Asif et al. 2013; Niemczyk *et al*., 2012). Therefore, reduced fT3 seems to reflect a true selective T3 deficiency due to a defect in T4 to T3 conversion. Patients with chronic renal failure have been reported to exhibit a reduced and delayed TSH response to thyroid releasing hormone (TRH) and thus contribute to low fT3 levels. Inflammation has been shown to play an important role in the causation of deranged thyroid function associated with NTI (Xu *et al*, 2014). Inflammatory cytokines including IL-6 have been implicated in the genesis of low T3 syndrome (Fragidis *et al*., 2015).

The subclinical hypothyroidism is identified purely on biochemical basis and defined as elevated serum TSH levels but normal fT3 and fT4 levels. Several studies have shown that subclinical hypothyroidism is common, especially among older adults. Laboratory tests have shown prevalence of low thyroid function in 4-10% of general population. In patients with ESRD, increased rates of thyroid abnormalities have been reported. Recent studies have shown an elevated rate of subclinical hypothyroidism in CKD patients not requiring HD (Miulecu *et al*., 2014; Yu *et al*, 2016). This study found primary and subclinical hypothyroidism at 8.6% of the participants. This result is in accordance with a local study done about 20 years before on patients on conservative management and dialysis. Chonchol and co-workers also reported that incidence of hypothyroidism was common at 18% of total patients with CKD not requiring dialysis (Chonchol *et al*., 2008). The prevalence of CKD was found to be 12.82% and 20% in patients with stage 3 and stage 4 of CKD respectively. Greater prevalence of hypothyroidism in women is associated with high titer of anti-thyroid antibodies (Cyriac *et al*., 2015); however, this study did not analyze anti-thyroid antibodies. In patients with CKD, there is low iodine clearance due to decreased GFR. Thus the concentration of iodine is found elevated in CKD patients. The thyroid hormone synthesis may be potentially blocked by increased serum inorganic iodine in patients with CKD and explain greater prevalence of diffuse goiter and hypothyroidism in patients of CKD (Ramasubramanian *et al*., 2014).

Moreover, subclinical hypothyroidism have been found to be associated with cardiovascular risk and cardiac impairment in various studies. Any anomaly in the serum TSH level may enhance the development of atherosclerosis (Ling *et al*., 2015). Kovesdy reported significant manifestation of kidney function change is hyponatremia as a consequence of impairment in the diluting capacity of kidney resulting in water retention, in patients with clinically overt hypothyroidism (Kovesdy, 2012).

Hyperthyroidism may contribute to abnormal feedback loop caused by derangement of hypothalamic pituitary thyroid axis associated with pituitary resistance to thyroid hormones (PTRH). Alterations in physiological mechanisms of hypothamic-pituitary-thyroid axis regulation causes PTRH (Suzuki *et al*., 2011). Thyroidotoxicosis results as a consequence of imbalance between hypothalamus, pituitary and peripheral response to systemic thyroid hormones. However, many studies have not been done on hyperthyroidism in CKD patients. The interpretation of thyroid function test may be confounded in patients with CKD when pharmacologic agents are administered. Hypothalamic-pituitary-thyroid axis is affected by glucocorticoids at multiple levels including reduction of TSH secretion, down-regulation of T4 to T3 conversion by 5'-deiodinase and lowering of TBG concentration and hormone binding capacity (Suzanne & Wartofsky, 2007). There are several factors that make the results of thyroid function test vague in CKD patients. A significant factor among them are methodological variation and variation in treatment. Furosemide and heparin are the drugs frequently used in these patients that may enhance the thyroid hormone levels. Other commonly used drugs hypothesized as a suppressor of thyroid hormones like propranolol, glucocorticoids and sulphonyl urea. Heparin, used in hemodialysis, is supposed to sharply raise the levels of both total and free thyroxine in serum. Furosemide act as inhibitor for T4 and T3 binding to serum proteins at high levels and depending on concentration, results in artifactual low, percent free T4 and estimated free T4 levels. At therapeutic doses, furosemide has little or no significant effect on thyroid parameters; but at high doses it leads to transient raise in free T4 and a fall in T4 because of the displacement of T4 from TBG. This alteration depends on serum albumin concentrations which also binds furosemide (Allawi, 2013; Wile, 2012).

CHAPTER: SIX

CONCLUSIONS

CONCLUSIONS

Thyroid disorders and chronic kidney diseases are independently among the most prevalent medical alarming conditions found in Nepal as well as other countries. Because of substantial prevalence of both, it is important to evaluate the physiological association of thyroid dysfunction in relation to renal disorder. The most frequent abnormalities found in CKD in relation to thyroid gland are of low T3 levels and subclinical hypothyroidism. The frequency of subclinical hypothyroidism raises commensurately with decline in GFR. The thyroid volume is often increased as a consequence of low T3, even in normal to reduced T4 levels and normal TSH. While a reduction in renal function also accounts for an inefficacious clearance of harmful serum constituents, inflammatory cytokines, iodide excretion, and an elevation in nitrogen conservation. All of these factors have been clinically proven to influence the normal physiology and metabolism of thyroid hormones. Hyperthyroidism is normally not linked to CKD but is considered to escalate it. It is momentous to consider all clinical features and thyroid manifestations in patients with CKD. As found in numerous data-based researches and current clinical cases, there exist prominent relationships in thyroid dysfunction and kidney disease and vice-versa. Clinicians, including nephrologists, should deliberate the risks of thyroid disease and its compatible treatment when annexed to CKD. Patients who are receiving suitable treatment for thyroid disease have a diminished chance of generating or exacerbating kidney disease. Howsoever, treating patients with mild raises in TSH leads to a negative nitrogen balance by enhanced muscle catabolism. Clinicians should investigate for low fT3 levels in patients prior to kidney transplant as low levels are linked to kidney graft loss.

Thyroid dysfunction may have effects on kidney function and thus restoration of thyroid hormones to normal ranges may setback these alterations. Hence, diagnosis for primary and subclinical hypothyroidism is essential in patients with unexplained elevation of serum creatinine. In addition, thyroid dysfunction has high frequency in CKD patients. In CKD, the pathophysiology of thyroid disorder is multifactorial. Investigations insinuate bidirectional

causal relationship between thyroid disorder and CKD. Low levels of thyroid hormones in CKD patients prognosticate a higher risk of CVD and cumulative effects of all causes mortality in CKD patients with thyroid dysfunction.

This study hypothesize that low T3 syndrome represent an adaptive mechanism as a consequence of decreasing energy demands, which often fails to ensure the survival of these feeble patients. Nonetheless, this hypothesis needs substantial multicenter data and remains to be proven. Probably, low thyroid hormones may imply poor overall health, escalated co-morbidities and higher risk of fragility. The resolution whether to administer thyroid replacement therapy in CKD patients with mild thyroid dysfunction is not candid. It should took into consideration the presence or absence of hypothyroidism and latent risks of iatrogenic hyperthyroidism. Prospective interventional studies are required in order to illuminate the potential benefits of thyroid hormone replacement therapy on morbidity and mortality of CKD patients having subclinical thyroid dysfunction.

In recapitulation, kidney and thyroid function and dysfunction are interdependent through various mechanisms. From diagnostic perspective, in patients with CKD, it is usually sufficient to use thyroid function tests commonly used in clinics. But, to escape mistakes in diagnosis, it is meaningful to acknowledge the effects of hypothyroidism and hyperthyroidism on kidney function, and also the changes in thyroid function tests caused by acute and chronic kidney disease. Medications used in the treatment of thyroid and kidney disorders may induce changes in renal and thyroid physiology consequently. Specific changes in thyroid physiology may be aftereffects of treatment of CKD by HD, PD or kidney transplantation. Some modifications in the general therapy may be necessary in patients with differentiated thyroid carcinoma, especially in the dose of ^{131}I when the kidney function id declined. Moreover, recent investigations have demonstrated riveting relationships in neoplastic diseases influencing thyroid and kidney. A correlation between T3 levels and kidney has been well established in uremic patients; but the relationship between TSH and survival, well established in other population groups, has not been demonstrated in patients with various degrees of renal failure. More investigations in this field will illuminate our understanding of the biological objective of changes in thyroid hormone in patients with kidney disease.

Furthermore, this study also analyze the extent of mineral derangement and malnutrition commonly associated with the progression of CKD.

LIMITATIONS OF THE STUDY

This study had numerous limitations which were listed below.

- At first, the sample size was small as it a single center study done in Nepal in KIST Medical College Teaching Hospital, Imadol, Lalitpur district, Nepal.

- Second, estimation of GFR was done by taking serum creatinine concentrations with Cockcroft and Gault equation, whose accuracy rate may decrease with increased GFR levels, albeit less so with calibrated creatinine measurements.

- Third, the state of diet (calorie intake or dietary composition) or cardiac status influencing thyroid hormonal status were not considered in exclusion criteria.

- Fourth, the sample size for CKD stages 1 and 2 patients were very small.

- Last but not the least Fifth, the patient's comorbidities and the reason behind check-up of the thyroid function test cannot be evaluated fully. These limitations may have induced selection bias in this study.

In spite of all these limitations, this study is precious in the view point of estimating the occurrence of low T3 syndrome in CKD patients having a normal mean TSH value. Even in middle stages of CKD (starting from the stage 3 CKD), marked decrease in T3 is seen. For better elaboration, follow-up studies of T3 are required in early stages CKD patients and the after effect of kidney replacement therapy on T3 level should be illuminated in the viewpoint of prognosis.

RECOMMENDATIONS

- There is need to do thyroid hormone profiles in CKD patients who are in stage 3 and above.
- Further studies should be conducted to find the incidence and prevalence of thyroid function abnormalities in CKD among general population Nepal.
- Study should be conducted to determine the thyroid profiles in lower CKD stages (stage 1 & stage 2).
- Hyperthyroidism in chronic kidney disease in Nepal should be studied further as some documented data regarding its prevalence is available.
- As low T3 is important predictor of mortality in CKD patients, Nephrologists should revise the management protocol of CKD patients.
- Adoption of the policy on estimation of GFR on renal disease patients.
- Future investigations are needed to determine the importance of evaluating or screening for clinical and subclinical hypothyroidism in different stages of CKD.
- Recent studies had shown very low awareness about CKD in Nepal, so CKD awareness programs should be conducted at grass root level in the country.

CHAPTER: SEVEN

SUMMARY

Nowadays, the word "Chronic Kidney Disease (CKD)" is preferred among clinicians comparative to chronic renal failure or insufficiency. CKD infers long-standing, and usually progressive impairment in renal function developing over a period of days or weeks resulting in decline in GFR. CKD is one of the vital health problem worldwide leading to increased global morbidity and mortality. Prevalence of this disease is increasing exponentially thus constitutes a major health priority worldwide. The expenses of this growing epidemic non-communicable disease represents a huge burden on health sector worldwide. 10% of the world population have been estimated globally having CKD, among which millions die each year because of unaffordable treatment. Though, there is a shortage of data related to the prevalence of pre-dialysis CKD in developing countries, the overall prevalence of CKD established on the basis of urinary albumin/creatinine ratio or glomerular filtration rate (GFR) in urban areas of Nepal is 10.6%.

National Kidney Foundation- Kidney Diseases Outcomes Quality Initiative (NKF-K/DOQI) of USA define and classify CKD in 2002, which is still internationally accepted. This classification defines CKD as kidney damage or GFR <60.0mL/min/1.73m^2 for three months or more irrespective of the cause (National Kidney Foundation, 2002).

Thyroid hormones have impact on renal growth and development, renal hemodynamics, GFR, and sodium and water homeostasis. Kidney also involve in the metabolism and excretion of thyroid hormones. Early identification and management of CKD has been shown to reduce the adverse outcomes which include kidney failure and cardiovascular disease. Thyroid dysfunction including hypothyroidism, hyperthyroidism and non-thyroidal illness has been reported in CKD patients. Non thyroidal illness or low T3 syndrome has been shown to worsen CKD by increasing cardiovascular morbidity and mortality and has been reported as an independent predictor of the cardiovascular mortality in these CKD patients. There is need to determine the thyroid hormone profiles in these CKD patients and thus prevent the adverse outcomes.

The present cross sectional study carried out with a total number of 163 CKD participants, categorized in different CKD stages (CKD stages 1-5) on the basis of GFR calculated by Cockcroft-Gault formula, aged between 21 years to 87 years. The prevalence of male participants (58.3%) were greater than that of female (41.7%). The higher proportion of males in this study could reflect the local current change in health seeking behaviour of the male gender. There was a highest number of study participants aged between 41 to 50 years (27.6%) as compared to age prevalence in other age groups. Approximately 80% of the CKD patients were above 40 years of age. Maximum number of participants were in CKD stage 5 (52.8%). The prevalence of the participants between CKD stages 3 to 5 is about 89% indicating that the people of Nepal is unaware about the disease.

Regarding etiology, majority of study population had a diagnosis of DN, HTN and CGN as the primary risk factors leading to CKD. These can be attributed to the increasing prevalence of DN and hypertension globally. Data showed DN (42.33%) as a leading risk factors of CKD followed by CGN (24.53%), HTN (22.08%), autosomal dominant polycystic kidney disease (4.29%), and obstructive uropathy (3.68%).

Among all CKD patients (n=163), 33.12% had low fT3, 11.70% had low fT4 and 8.6% had high TSH values. The number of participants with low fT3 levels raised with increasing severity of CKD indicating that low fT3 was very common thyroid dysfunction seen in chronic illness patients. Majority of the low fT3 were seen in stage 5 CKD (26.99%). The mean value of fT3 in stages 1 to 5 CKD patients were 3.23 ± 0.32, 2.79 ± 0.48, 2.47 ± 0.23 and 2.06 ± 0.63 respectively. The P value in all group showed the significant relationship (< 0.001) among different stages. Decreasing pattern of median values was observed with the progression of CKD. Decreased levels of T3 in this critical illness were due to reduced peripheral conversion of T4 to T3 while thyroid gland production of T3 is normal and T3 clearance rates are normal or lessened. T3 is the most active thyroid hormone produced by 5'-monodeiodination of the prohormone T4. More than 80% of serum T3 are supplied by liver, kidney and muscle. Abnormal conversion of T4 to T3 may be associated with malnutrition and humoral factors like cytokines that are normally associated with CRF.

The mean value of fT4 in all stages of CKD patients were 1.28 ± 0.17, 1.24 ± 0.24, 1.20 ± 0.16 and 1.06 ± 0.28 respectively. Here the P value in all group were < 0.05, which were statistically

significant. All the patients with low fT4 had also decreased fT3 levels and were from stage 5 CKD only. However, other stages of CKD patients had fT4 concentration within the normal range. The mean value of fT4 were decreased as the severity of the diseases was progressed. Thus, the decreasing trends of mean fT4 values showed the statistically significant correlation between thyroid profile and different stages of CKD. This reduction in fT4 value may be linked to impaired T4 binding to serum carrier protein like thyroid hormone binding globulin.

Mean value of TSH in different stages of CKD patients were 2.79 ± 1.02, 3.47 ± 2.50, 3.97 ± 3.88 and 2.87 ± 1.72. So, TSH value in these groups were not differed significantly ($P > 0.05$). The mean TSH values were normal in 91.4% population indicating no statistical significant differences between thyroid hormone profile and severity of renal disease. The underlying mechanism may be mediated by inhibited response to the TRH as a result of TSH glycosylation and altered TSH circadian rhythm. The high TSH levels seen in CKD stages 3, 4 and 5 were 12.82%, 20% and 5.81% respectively. Despite of high TSH, the fT3 and fT4 levels were within the normal range in these patients indicating subclinical primary hypothyroidism is also among the thyroid function abnormalities commonly persists in CKD patients. The subclinical hypothyroidism is identified purely on biochemical basis and defined as elevated serum TSH levels but normal fT3 and fT4 levels.

Prevalence of hypocalcaemia was seen in 52.8% and hypercalcaemia in 3.1% CKD participants. Hypophosphataemia and hyperphosphataemia was found in 20.9% and 11% respectively. The mean calcium values in all stages of CKD patients were 8.63 ± 1.16, 8.86 ± 0.88, 8.35 ± 0.95 and 8.15 ± 0.97. Mean value of phosphorous in different stages of CKD patients were 3.23 ± 0.66, 3.30 ± 0.88, 3.77 ± 0.84 and 4.60 ± 1.26. The mean calcium and phosphorous values in different stages of CKD patients showed the significant variations within the CKD stages. Disorders of mineral metabolism begins at early stages of CKD, and is normally escorted by significant changes in minerals levels in blood that prompts clinical issues like bone diseases, musculoskeletal symptoms and growth hindrance.

Hypoalbuminaemia in this current study was found in 52.1%. The mean albumin values in all stages of CKD patients were 3.83 ± 0.51, 3.54 ± 0.59, 3.48 ± 0.66 and 3.52 ± 0.72. No statistical differences ($P > 0.05$) were recorded between serum albumin and stages of CKD. 47.9% of study

population as shown in were found to have normal serum albumin concentration. No cases of hyperalbuminaemia was reported in this study.

From the current study it was established that CKD is associated with thyroid dysfunction characterized by low serum fT3 and fT4 with high TSH in some cases. Low T3 syndrome and subclinical hypothyroidism were the most common thyroid disorders present in CKD patients.

CHAPTER: EIGHT

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CHAPTER: NINE

APPENDICES

APPENDIX-1: ARTICLE OF RESEARCH WORK (ACCEPTED)

Singh S, Verma A, Aryal G, Thapa S**. Prevalence of thyroid hormone abnormalities in stage 5 chronic kidney disease: a tertiary care center study of Nepal**

(This article was accepted on 29th July, 2016 at 3:03 PM. This article will publish in International Journal of Research in Medical Sciences, Volume 4, Issue 9 in September 2016.

The details of article is given below:

Prevalence of thyroid hormone abnormalities in stage 5 chronic kidney disease: a tertiary care center study of Nepal

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ABSTRACT

Background: Chronic Kidney Disease (CKD) implies progressive, long-standing and irreversible impairment in renal function that results in end stage renal disease (ESRD). ESRD is a frequent cause of Non-thyroidal illness (NTI) with low free triidothyronine (fT3), usually elevated reverse T3 (rT3), normal or low thyroid stimulating hormone (TSH), and if prolonged, low free thyroxine (fT4), despite patient remaining clinically euthyroid. Present study aimed to estimate the prevalence of thyroid hormone abnormalities in stage 5 CKD patients and also to compare these changes with healthy controls.

Materials and Methods: The present cross-sectional observational study was conducted on thirty eight stage 5 CKD patients and 38 age-sex matched healthy volunteers as control. The demographic data, medical history, etiology, physical examination and laboratory results were recorded on a special form developed by the researchers.

Results: Among thirty eight stage 5 CKD patients, 21 (55.26%) were males and 17 (44.73%) were females. 44.73% of the stage 5 CKD patients had low fT3 whereas 28.94% had low fT4 values below the reference range. 5.26% patients had increased TSH values above the normal reference limit. The mean TSH values were not significantly differ among diseased and control groups. Among the risk factors for CKD, diabetic nephropathy (44.73%) was found to be the lead primary cause followed by chronic glomerulonephritis (26.31%) and hypertension (21.05%).

Conclusion: From this study, it was concluded that the prevalence of thyroid hormone abnormalities especially low fT3 and fT4 is very common in stage 5 CKD patients. Diabetic nephropathy was among the lead cause of stage 5 CKD.

Keywords: Chronic kidney disease, Free thyroxine, Free triiodothyronine, Thyroid hormone abnormalities, Thyroid stimulating hormone

INTRODUCTION

The term "Chronic Kidney disease (CKD)" is now replacing terms such as chronic renal failure or insufficiency.[1] CKD implies progressive, long-standing and irreversible impairment in renal function that results in end stage renal disease (ESRD).[2]

Thyroid hormone profile includes the test like plasma triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH). T3 and T4 are produced from thyroid gland whereas TSH is produced by anterior pituitary gland. T3 and T4 are present in both bound and free form. The free hormones are really active molecules. The free fractions of the hormones can be measured accurately by Chemiluminiscence Immunoassay (CLIA).[3]

Kidney and thyroid function are interconnected through several mechanisms. Thyroid hormones are necessary for the maintenance of electrolyte and water. Similarly, kidney is also involved in the regulation of thyroid hormones metabolism.[4]

Failure of metabolic, excretory and synthetic functions are commonly associated with the irreversible loss of renal function that ultimately results in uremia or azotemia i.e. retention of urea and other non-protein nitrogenous substances in the blood. CKD affects thyroid function in several ways that include low circulating thyroid hormone concentration, alteration in peripheral hormone metabolism, disturbance in binding them to carrier proteins, possible reduction in tissue thyroid hormone content and increased iodine store in thyroid gland resulting in the reduction of serum triidothyronine (T3) and thyroxin (T4).[5]

In 2009, Iglesias P and Diez JJ published a review article entitled "Thyroid dysfunction and kidney disease" in a European Journal of Endocrinology in which they report that "Serum TSH

concentration are usually normal or elevated in CKD. Free and total T3 and T4 concentrations are usually normal or low in patients with CKD. The reduction in T3 (low T3 syndrome) is most frequently observed thyroid alteration in these patients. This reduction in T3 concentration has been linked to a decrease in the peripheral conversion of T4 to T3.[6]

ESRD is a frequent cause of non-thyroidal illness (NTI) that refers to a syndrome found in seriously ill or starving patients with low fT3, usually elevated reverse T3, normal or low TSH and if prolonged, low fT4 despite patient remaining clinically euthyroid.[7]

The aim of the present study was i) to estimate the prevalence of thyroid hormone abnormalities in stage 5 CKD patients and ii) to compare these changes with healthy controls.

MATERIALS AND METHODS

The present cross-sectional observational study was conducted in the Department of Biochemistry in collaboration with the Department of Nephrology and dialysis unit of KIST Medical College and Teaching Hospital, Lalitpur, Nepal. This study was conducted for a period of six months from July 2015 to December 2015. Study population consisting thirty eight stage 5 CKD patients (21 men and 17 women) with different causes with age range 21 to 77 years. The demographic data, medical history, etiology, physical examination and laboratory results were recorded on a special form developed by the researchers. Patients had different duration of chronic illness ranging from 6 months to 4 years. Out of thirty eight, 24 patients were on HD and 14 were on conservative treatment. Thirty eight age-sex matched healthy volunteers with normal GFR were taken as control. Patient with stage 5 CKD were confirmed by serum creatinine level, clinical features of uremia and glomerular filtration rate (GFR) estimation. GFR was measured by using Cockcroft-Gault formula. Written informed consent were taken from the study subject. Patient with known history of thyroid function abnormalities and pregnancy were excluded from the study. Five ml of blood sample were collected from cubital vein in a vacutainer plain tubes and were allowed to clot and centrifuged to separate serum. Serum level of creatinine were measured by creatinine kit supplied by Accurex Biomedical, India. Serum fT3, fT4 and TSH were measured by using Immunoassay system which is a CLIA technique from Siemens Healthcare Diagnostics, USA.

The normal range of fT3, fT4 and TSH were 2.30-4.2 pg/ml, 0.89-1.76 ng/dl and 0.35-5.5 µIU/ml respectively.

Statistical analysis

All variables were presented as number and frequency and were arranged in tables. Data were expressed as mean ± standard deviation. The independent sample t test was used to compare stage 5 CKD patient with healthy controls group. Statistical analysis were done by SPSS software (Statistical Package for the Social Sciences, version 21.0, SPSS Inc, Chicago, USA). The level of significance (P) was set to be < 0.05.

RESULTS

Table 1: Frequency and percentage of male and female chronic renal failure

Gender	Frequency (n)	Percentage (%)
Male	21	55.26
Female	17	44.73
Total	38	100.0

Table 2: Factors causing chronic renal failure

Causes	Frequency (n)	Percentage (%)
Diabetic nephropathy	17	44.73
Chronic glomerulonephritis	10	26.31
Hypertension	8	21.05
Others (Obstructive uropathy, polycystic kidney disease, etc.)	3	7.89

Table 3: Distribution of chronic renal failure having normal and deranged fT3, fT4 and TSH

Thyroid profile	Normal		Decreased		Increased	
	n	%	n	%	n	%
fT3 (pg/ml)	21	55.26	17	44.73	0	0.0
fT4 (ng/dl)	27	71.05	11	28.94	0	0.0
TSH (µIU/ml)	36	94.73	0	0.0	2	5.26

Table 4: Comparison of Age, serum creatinine, fT3, fT4 and TSH in Healthy controls with chronic renal failure

Variables	Healthy control (n=38)	CRF patients (n=38)	P* value
Age	51.44 ± 15.34	50.81 ± 17.30	0.867
Serum creatinine	0.88 ± 0.19	7.77 ± 2.87	<0.001
fT3 (pg/ml)	2.96 ± 0.49	2.18 ± 0.56	<0.001
fT4 (ng/dl)	1.37 ± 0.48	0.99 ± 0.22	<0.001
TSH (µIU/ml)	2.68 ± 1.41	2.71 ± 1.66	0.934

*** Independent sample t test**

Among thirty eight stage 5 CKD patients, 21 (55.26%) were males and 17 (44.73%) were females (Table 1). The mean age in stage 5 CKD patients and healthy control group were 50.81 ± 17.30 and 51.44 ± 15.34 respectively, which was not significantly different in two groups ($P > 0.05$)

As shown in Table 2, diabetic nephropathy (44.73%) was the leading cause of stage 5 CKD followed by chronic glomerulonephritis (26.31%) and hypertension (21.05%). Other causes (7.89%) of stage 5 CKD were obstructive uropathy, polycystic kidney disease etc.

Seventeen (44.73%) stage 5 CKD patients had low fT3 whereas eleven (28.94%) had low fT4 values that were below the reference range. There were no any reduction in TSH level but two patients (5.26%) had increased TSH values compared to normal limit (Table 3).

In Table 4, mean and standard deviation of thyroid hormone and serum creatinine were compared between stage 5 CKD and healthy control. The mean value of serum creatinine in stage 5 CKD patients and control group were 7.77 ± 2.87 and 0.88 ± 0.19 which was statistically significant ($P < 0.001$).

Significant differences in the mean level of fT3 and fT4 were seen among stage 5 CKD patients and healthy control group. The mean value of fT3 in stage 5 CKD patients and control group were 2.18 ± 0.56 pg/ml and 2.96 ± 0.49. The P value in both group were < 0.001, which was significant.

The mean value of fT4 in stage 5 CKD patients and control group were 0.99 ± 0.22 ng/dl and 1.37 ± 0.48 respectively. Here the P value in both group was < 0.001, which was statistically significant.

Mean value of TSH in stage 5 CKD patient (2.71 ± 1.66) and healthy control (2.68 ± 1.41) did not vary with each other. So, TSH value in these two groups were not differed significantly ($P > 0.05$).

DISCUSSION

Thyroid hormones (TH), synthesized by thyroid glands, are necessary for evolution and development of the kidney and for the maintenance of water and electrolyte homeostasis. On the otherside, kidney participate in the metabolism and elimination of TH. Moreover, kidney is recognized as an important target organ for TH actions.[8,9]

The present study was carried out to estimate the prevalence of thyroid hormone abnormalities in stage 5 CKD patients. In our study, there was significant reduction in the level of fT3 and fT4 in stage 5 CKD patients as compared to the healthy control group.

Serum fT3 concentration was lowered in 17 (44.73%) out of 38 stage 5 CKD patients. The mean serum fT3 concentration 2.18 ± 0.56 pg/ml in ESRD patients was significantly lower than the control subjects (2.96 ± 0.49 pg/ml). Similarly serum fT4 was also below the normal range in 11 (28.94%) out of 38 stage 5 CKD patients. The mean value of fT4 in stage 5 CKD patients and healthy control group were 0.99 ± 0.22 and 1.37 ± 0.48 respectively. This difference revealed that the mean value of fT4 in stage 5 CKD patients were significantly reduced as compared to control group. These results justified the findings of other studies.[5,10-13]

Low T4 values in ESRD patients may be related to impaired T4 binding to serum carrier proteins. It has been reported that many inhibitors of T4 binding to serum carrier proteins are present in CRF patients and thus contributing to the decreased level of T4 in CRF.[10] CKD affects thyroid function in several ways that include low circulating thyroid hormone concentration, alteration in peripheral hormone metabolism, disturbance in binding them to carrier proteins, possible reduction in tissue thyroid hormone content and increased iodine store in thyroid gland resulting in the reduction of serum triidothyronine (T3) and thyroxine (T4).[14]

CKD disturbs the hypothalamus-pituitary-thyroid axis and the peripheral metabolism of thyroid hormone. Low T3 concentration is the most common laboratory findings observed in CKD

patients. Normal or low levels of T4 may be due to the mono-deiodinase action taking place in the inner benzene ring despite outer ring of T4, resulting in the biosynthesis of reverse T3. Reverse T3 levels, however, are found to be normal in CKD patients because it moves from the vascular space to extra vascular and intracellular spaces. Low T3 levels in CKD may be due to the iodothyronine deiodinase (helps in T3 synthesis from T4) which is affected by fasting, chronic metabolic acidosis, and chronic protein malnutrition seen in CKD. Such factors influence the proteins binding toT3.[15] Low T3 levels in CKD may also be due to the decreased peripheral (extra thyroidal) conversion from T4 to T3 due to decreased clearance of the inflammatory cytokines such as TNF-alpha and IL-1.[16] In CKD, physiological compensation for low T3/T4 (with normal TSH levels) causes a reduction in protein catabolism which increases the nitrogen waste overload.

In the present study, serum TSH concentrations were within the normal range in 36 (94.73%) stage 5 CKD patients out of 38. Only two patients had TSH value increased above the normal range. Mean TSH value in stage 5 CKD patients (2.71 ± 1.66) were similar to that of control group (2.68 ± 1.41). This means that the TSH values in stage 5 CKD patients was not significantly altered. This results agreed with the previous studies.[5,6,10,11,17] Reduced serum TSH levels were not seen in any subjects. Rajagopalan B et al. published an article entitled "Renal function marker and thyroid hormone status in undialyzed CKD" in which he reported that unchanged status of TSH level in CRF compared to normal patients reflects euthyroid status which suggests that thyroid is able to compensate for hormonal urinary losses keeping the patient euthyroid. Reduced serum TSH levels have not been reported to date in euthyroid CRF patients.[11]

Data from our study revealed the fact that the normal thyroid status was found only in half of our stage 5 CKD subject which will fulfilled the criteria for NTI. DeGroot LJ reported in NCBI Bookself about NTI in which he had described that NTI refers to a syndrome found in seriously ill or starving patients with low fT3, usually elevated reverse T3, normal or low TSH and if prolonged, low fT4 despite patient remaining clinically euthyroid.[7]

Recently KC Shiva Raj reviewed the thyroid function tests and its interpretation and reported that the fT4 and fT3 become low or low-normal while TSH remain normal or even low in NTI.[18] In our study, the pattern of NTI was found with low fT3 particularly common in 44.73% as well as low fT4 in 28.94% with TSH normal in 94.73%. This study is similar to the study done by Horacek J et al.[17]

5.26% stage 5 CKD patients had TSH level increased above the normal range with fT3 and fT4 within the normal limit. These findings suggested that the patients had subclinical hypothyroidism. This result is similar to the study by Chonchol M et al. in which 9.5% of CKD patients had subclinical hypothyroidism.[19]

Diabetic nephropathy (44.73%) was the leading cause of stage 5 CKD followed by chronic glomerulonephritis (26.31%) and hypertension (21.05%). Other causes (7.89%) of stage 5 CKD were obstructive uropathy, polycystic kidney disease etc. This is similar to the study done by Foley RN and Marshall SM in which they reported that diabetic nephropathy is the leading cause of ESRD worldwide.[20,21] In our study, chronic glomerulonephritis was the second leading cause of stage 5 CKD which was differ from the study done by Khakurel S et al. in 2009 in which they reported that chronic glomerulonephritis was the leading cause of ESRD followed by diabetic nephropathy and hypertension.[22]

CONCLUSION

From this study, it was concluded that the prevalence of thyroid hormone abnormalities especially low fT3 and fT4 is very common in stage 5 CKD patients. Diabetic nephropathy was among the lead cause of stage 5 CKD. Therefore, these findings reflects thyroid hormone abnormalities which is common in stage 5 CKD patients and will fulfill the criteria for NTI.

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APPENDIX-2: ETHICAL CLEARANCE LETTER

किष्ट मेडिकल कलेज तथा शिक्षण अस्पताल
KIST MEDICAL COLLEGE AND TEACHING HOSPITAL
Mahalaxmi Municipality-16, Imadol, Lalitpur Nepal,Tel-977-1-5201496,5201682
Email : info@kistmcth.edu.np, Web:www.kistmcth.edu.np
GPO Box 14142,Kathmandu,Nepal

IRC NO: 0048/2013/14

Date: 15th December 2014

Mr. Samir Singh
Lecturer
Clinical Biochemistry
KIST Medical College and Teaching Hospital

Subject: Approval of research proposal

With reference to your application for carrying out your study entitled **"Status of thyroid hormone profile in patients with chronic kidney disease",** it is my pleasure to inform you that your study has been **reviewed and approved with ethical clearance** from KISTMCTH IRC meeting.

Research center: KIST Medical College and Teaching Hospital

Date Start: Date: 15th December 2014
Study duration: Till sample size is achieved or 12 months from the date of approval
Name of Principal Investigator: Mr. Samir Singh

Kindly inform the IRC if any problems that arise during the study and obtain re approval if there are any changes to the study protocol. Kindly submit a hard and soft copy of your study after the completion of the study.

Wishing you best of luck for the research

Dr Suraj Bajracharya
Member Secretary
IRC

Committed to excellence in holistic healthcare, education and research

APPENDIX-3: CONSENT FORM

I …… and being explained to on the study purpose by Prinipal Investigator **Samir Singh**, do hereby give informed consent to participate voluntarily in the study entitled "**STATUS OF THYROID HORMONE PROFILE IN PATIENTS WITH CHRONIC KIDNEY DISEASE**".

I am aware that I can withdraw from the study without any benefits or quality of management of my medical condition being interfered with.

Signature of the participant: ……

Date: ……..

Thumbprint:

Signature of the person obtaining informed consent ……..

Date: ……

APPENDIX-4: PATIENT INFORMATION FORM

			Study number:

A. DEMOGRAPHIC DATA

Name: ..... Age (Years): ……..

Gender: Male/ Female Laboratory No:

(OPD/IPD No.)

B. MEDICAL HISTORY

i) Diagnosis: CKD stage ……

ii) Causes of CKD ……

iii) If on dialysis: Session …… Hours ……

iv) Hb …….gm/dl

v) Duration of illness (months) ……

vi) Renal Transplant YES /NO

vii) Renal Biopsy report if any …….

viii) Radiological report if any ……..

ix) Medications currently on ……

C.PHYSICAL EXAMINATION

Height (cm)		Weight (Kg)	
BMI		BP reading (mmHg)	

D. LABORATORY RESULTS

Test Parameter	Value obtained	Unit	Reference range
Free T3		pg/ml	2.3-4.2
Free T4		ng/dl	0.39-1.76
TSH		µIU/ml	0.35-5.5
Creatinine		mg/dl	0.5-1.2
Albumin		gm/dl	3.5-5.0
Calcium		mg/dl	8.5-11.0
Phosphorous		mg/dl	2.5-5.0

APPENDIX-5: TRAINING DURING RESEARCH WORK

KIST MEDICAL COLLEGE & TEACHING HOSPITAL

CERTIFICATE

Awarded to

Mr. SAMIR SINGH

For the successful participation in

"Training on Advanced Data Analysis Using Statistical Tools"

organized by ***KIST Medical College & Teaching Hospital*** *and supported by* ***University Grants Commission –Nepal,***

held from Ashadh 1-6, 2073 (June 15-20, 2016).

Date of Issue: 20 JUN 2016

Prof. Dr. Amita Pradhan
Co-ordinator & Resource Person

Prof. Dr. Bimala Shrestha
Head
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Prof. Dr. Balman Singh Karki
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Certificate 5.1: Training on using Statistical tools

KIST MEDICAL COLLEGE & TEACHING HOSPITAL

CERTIFICATE OF PARTICIPATION

In recognition of the active participation in
"Research Methodology Training" workshop,
organized by KIST Medical College and supported by University Grants Commission, Nepal,
held from 20th April - 23rd April and 26th April, 2015
Mr. Samir Singh
is hereby presented this certificate of appreciation.

Prof. Dr. Amita Pradhan
Co-ordinator and Resource Person

Prof. Dr. Bal Man Singh Karki
Principal

Imadol, Lalitpur, Nepal, Tel: 977-1-5201496, 5201682,
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Certificate 5.2: Training on Research Methodology

APPENDIX-6: GLIMPSES FROM RESEARCH WORK

Photo 6.1: Technologist performing test on chemistry analyzer

Photo 6.2: Technologist performing test on Immunochemistry analyzer

Photo 6.3: Discussion with MBBS interns regarding outcomes parameter of test

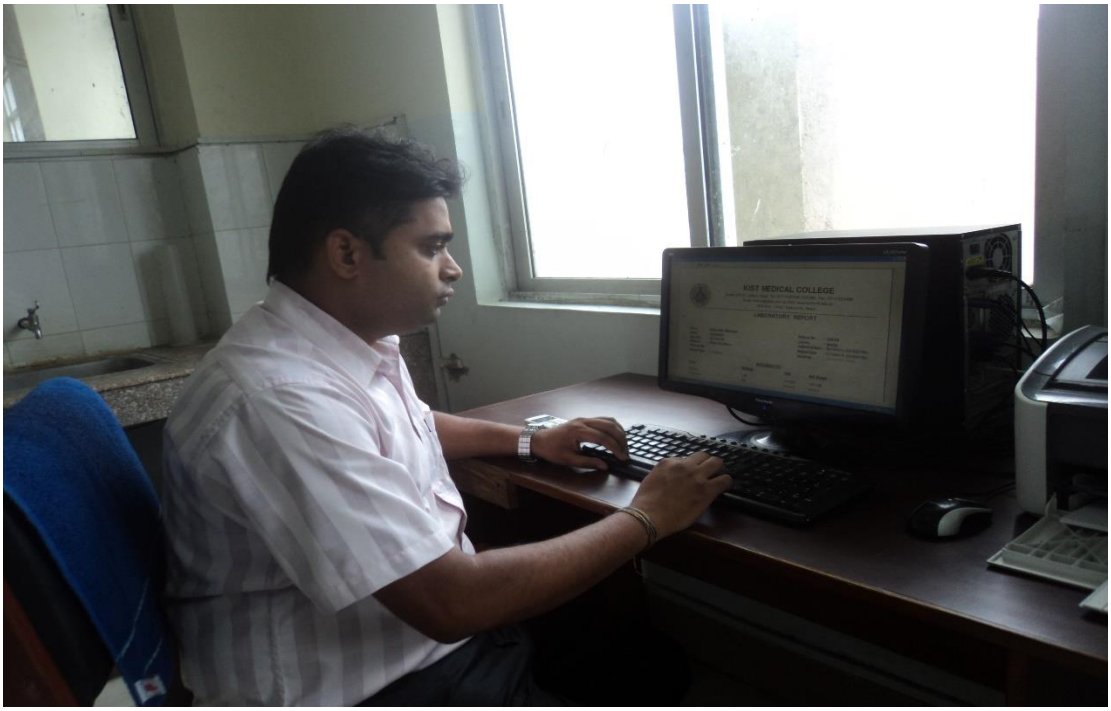

Photo 6.4: Data entry and validation by Principal Researcher