PREVALENCE OF SUB-CLINICAL HYPOTHYROIDSIM AND ITS CORRELATION WITH DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AT KENYATTA NATIONAL HOSPITAL

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STUDENTS DECLARATION

I declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Irari, my siblings Dr. Ken and Carole Wagereka and my friend Peter Njatha. Thank you for your constant support and encouragement all through graduate school.

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ABBREVIATION

ADA - American Diabetes Association

ATA - American Thyroid Association

BMI - Body Mass Index

DM - Diabetic Mellitus

DN - Diabetic Nephropathy

EDTA - Ethylenediaminetetraacetic acid

eGFR - Estimated Glomerular Filtration Rate

ELISA - Enzyme Linked Immunosorbent Assay

ERC - Ethics and Research Committee

ESRD - End Stage Renal Disease

FT_4 - Free Thyroxine

FT_3 - Free tri- iodothyronine

IDF - International Diabetes Federation

KDOQI - Kidney Disease Outcomes Quality Initiative

KNH - Kenyatta National Hospital

NKDEP - National Kidney Disease Education Program

NKF - National Kidney Foundation

NOS - Nitric oxide synthase

OGLA- Oral Hypoglycemic Agent

RAAS - Renin-angiotensin-aldosterone system

SCH - Sub Clinical Hypothyroidism

SPSS - Statistical Package for the Social Science

SVR - Systemic vascular resistance

T2DM - Type Two Diabetes Mellitus

TD - Thyroid Disorders

TFT - Thyroid Function Test

TSH - Thyroid Stimulating Hormone

UACR - Urinary Albumin Creatinine Ratio

UON - University of Nairobi

OPERATIONAL DEFINITION OF TERMS

Type 2 Diabetes Mellitus

A patient with type 2 diabetes mellitus is one attending the diabetes-endocrinology outpatient clinic at Kenyatta National Hospital for management with a documented file diagnosis of type 2 diabetes mellitus

Diabetic Nephropathy

Nephropathy will be defined using the urinary albumin creatinine ratio (UACR) to classify the participants as follows:

Normalbuminuria, <30 mg/g creatinine

Moderate, 30-300 mg/g creatinine (Incipient DN)

Severe, >300 mg/g creatinine (Overt DN) (Gross et al., 2005)

Diabetic nephropathy will be defined as an increased urinary albumin creatinine ratio of ≥ 300mg/g creatinine in the absence of other renal abnormalities or estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m2 according to Kidney Disease Outcomes Quality Initiative recommendation.

Subclinical Hypothyroidism

An elevated TSH >5 (0.25-5mIU /L) and free thyroxine (FT4) level in normal range (10-20 pmol/L).

Estimated Glomerular Filtration Rate

This will be calculated using will be calculated according to equation of the Modification of Diet in Renal Disease.

ABSTRACT

Background: Numerous epidemiological studies show that patients with type 2 diabetes mellitus have a higher prevalence of subclinical hypothyroidism which may be associated with diabetic complications. Controversy however persists about the indications for its treatment and whether individuals with type 2 diabetes mellitus should be routinely screened for this dysfunction.

Objective of the study: To determine the prevalence of Subclinical hypothyroidism and its correlation with Diabetic Nephropathy in patients with type 2 Diabetes Mellitus at the Kenyatta National Hospital.

Methods: This was a descriptive cross- sectional study conducted among type 2 Diabetes mellitus patients attending the diabetes and endocrinology clinic at the Kenyatta National Hospital (KNH). Consecutive sampling was used to recruit 171 study participants without a previous history of thyroid disease. Serum TSH, free T_4 and urinary albumin: creatinine ratio were measured. Estimated GFR (eGFR) (ml/min/1.73 m^2) was calculated according to equation of the Modification of Diet in Renal Disease. Patients with serum TSH levels >5 mIU/L and normal free T_4 were classified as having subclinical hypothyroidism. Diabetic nephropathy was defined as urinary albumin/creatinine ratio ≥300 mg/g and/or an eGFR <60 ml/min/1.73 m^2

Results: The prevalence of subclinical hypothyroidism (SCH) was 6.4% among patients with type 2 diabetes mellitus. Patients with both Diabetic nephropathy and subclinical hypothyroidism were 2.3% of the total population. There is a weak inverse correlation between eGFR with TSH r=-0.021 (p>0.005) and a weak positive correlation between TSH and UACR r=0.034 (p>0.005).

Conclusion and Recommendation: Subclinical hypothyroidism is not associated with diabetic nephropathy in type 2 diabetes mellitus. The study however presents evidence of subclinical hypothyroidism among patients with T2DM. To build up on this findings, exploratory studies are recommended on the causes of Subclinical hypothyroidism in patients with Diabetic Nephropathy.

1.0 INTRODUCTION

In clinical practice, the most common endocrine disorders are Diabetes Mellitus (DM) and Thyroid Dysfunction (TD). Their association is widely known with the first studies published by Papazafiropoulou et al,1979. Thyroid hormones are insulin antagonists. They are both involved in cellular metabolism. Deficit or excess of any one can result in functional derangement of the other (Singh et al.,2011). Their effects are however not well established to date. (Ayman et al.,2018). Worldwide, Type 2 Diabetes Mellitus (T2DM) is becoming increasingly prevalent. It is the third leading cause of mortality thus threatens human health worldwide (Tabák et al.,2009). Many serious complications are associated with diabetes such as microvascular complications and kidney impairment (Liu et al.,2016; Li et al.,2018).

The first cause of renal insult around the world is Diabetic nephropathy. It is one of the most common complications of T2DM and the leading cause of End Stage Renal Disease (ESRD) (Zhang et al.,2014). Moreover, it is considered as the leading cause of DM related morbidity and mortality (Bell et al.,2002). It occurs because of interaction between hemodynamic and metabolic factors. About 20% to 40% of patients with T2DM develop DN. About 40% of these patients will progress to ESRD. It is important therefore to investigate the risk factors that promote the onset of DN (IDF,2018).

Type 2 diabetes mellitus patients have a higher prevalence of thyroid disorders when compared with the euglycemic population (Wu.,2000). A recent meta-analysis that included 61 studies showed a higher prevalence of subclinical hypothyroidism in T2DM patients and a higher prevalence of microvascular complications in patients having the two conditions (Han et al., 2015). Subclinical hypothyroidism is defined as an asymptomatic state, characterized by an elevated serum concentration of thyroid stimulating hormone (TSH) with a normal serum thyroxine level. It has been shown to be an independent risk factor for development of diabetic nephropathy (Furukawa et al.,2014).

Several interactions occur between SCH and the functions of the kidneys. Thyroid hormones affect the development and physiology of the renal system. It is well known that hypothyroidism increases renal blood flow leading to a reduced eGFR (Wang et al.,2018). Serum TSH has also been reported to be an independent risk factor for albuminuria (Radaideh et al.,2013).

Association between SCH as a risk of diabetic nephropathy is still a matter of debate (Bing et al.,2017). Unrecognized thyroid dysfunction may impair metabolic controls in patients with diabetes and in addition may amplify existing complications. Early identification of diabetes and microvascular complications risk therefore provides an opportunity to introduce preventive interventions to stop or delay disease onset (Abbasi et al.,2012). This is important as it would decrease the morbidity and mortality of type 2 diabetes mellitus (T2DM) patients.

2.0 LITERATURE REVIEW

2.1 Overview of diabetes nephropathy

Over the past few decades, the prevalence of Type 2 Diabetes Mellitus has been rising tremendously. According to the International Federation of Diabetes, 425 million cases were reported in 2018 and the prevalence is projected to increase to 629 million by 2045. The prevalence is higher in developing countries at 3.3% in Africa (IDF Atlas Africa, 2018). High rates of macrovascular (Atherosclerotic) and microvascular complications (Nephropathy, Retinopathy, Neuropathy) have been associated with diabetes. Evidence based medicine shows that diabetes morbidity and mortality are attributed to its chronic complications (Khalid *et al*; 2014).

Among these, DN is one of the most common microvascular complications. Along with the diabetes endemic, its prevalence is inevitably increasing rapidly (Harjutsalo, 2014). Approximately 40% of patients with diabetes mellitus develop ESRD. It has also been associated with need for renal replacement, increased premature mortality, cardiovascular diseases and an escalating health care cost (Harjutsalo et al; 2014). In the developed countries, DN is the main cause of ESRD accounting for about 30% of the cases (Barnett *et al*., 2004, Van *et al.*, 2004). It is also expected to become the most common cause of ESRD in the developing world soon (IDF,2018).

2.2 Epidemiology

A dreaded complication of diabetes mellitus, diabetic nephropathy affects approximately 40% of persons with T2DM globally (Viberti *et al*; 1982). It manifests as albuminuria or reduced glomerular filtration rate (GFR) (Eknoyan *et al*; 2003). According to Epidemiology studies of type 2 diabetic patients, prevalence of DN ranges from 7.6% to 55% (Shen et al., 2009). In different international registries prevalence ranges between 11.5% in United Kingdom and 42.9% in Thailand (Magee et al.,2010, Ngarmukos et al.,2006). This large variation in DN prevalence reported by registries may be related to management practice, screening, registry size and can be improved with larger registries, and by implementing standardized practice, especially a longer follow up period. Diabetic nephropathy has been suggested to be more frequent among patients with diabetes in Africa as compared to those in the developed world due to delayed diagnosis, limited screening and diagnostic resources, poor control of blood sugar and other risk factors, and inadequate treatment at an early stage (Kengne et al.,2013; Mbanya et al.,2010). The prevalence of proteinuria in diabetes patients ranges from 5.3% to 53.1% in Africa (IDF Diabetes Atlas.,2013; Noubiap et al., 2015). Data pooled from 54 countries show that more than 80% of ESRD cases are caused by diabetes, hypertension or both. End stage renal disease proportion that is attributable to diabetes varies between 12 to 55%. Its prevalence is also ten times higher in people with diabetes than in the euglycemic population (1DF,2018). In Kenya, diabetes accounts for 9-15% of patients with ESRD and few of these patients are offered adequate renal replacement therapy due to co-morbidity, cost and lack of insurance (Saraladevi, 2009). Unfortunately, patients with T2DM commonly present with albuminuria and overt nephropathy at the time of diagnosis or soon after (IDF,2018). Prevalence of diabetic nephropathy in Ethiopia, from the studies of diabetic complications, is 15.7% to 29.5% (Worku et al.,2010, Abejew et al.,2015, Tefera et al.,2014).

2.3 Pathogenesis of diabetic nephropathy

Diabetic nephropathy, otherwise called Kimmelstiel-Wilson disorder or nodular diabetic glomerulosclerosis or intercapillary glomerulonephritis, was first depicted in 1936 by Clifford Wilson (1906-1997) and Paul Kimmelstiel (1900-1970) (Vujičić et al.,2012).The two doctors were the first to depict the nodular glomerulosclerosis sores in diabetic patients having proteinuria and hypertension.

A progressive kidney disease, Diabetic nephropathy is characterized by three cardinal functional changes (Vujičić et al.,2012) 1) Persistent albuminuria (≥ 300 mg/24 hours or > 200 μg/min) confirmed at two visits 3-6 months apart, 2) Progressive decrease in glomerular filtration rate (GFR), 3) Increase in arterial blood pressure. Diabetic Nephropathy occurs as a result of metabolic alterations and hemodynamic alterations.

2.3.1 Hemodynamic Factors

Decreased resistance in both the afferent and efferent arterioles cause glomerular hyperfiltration. This is because the afferent arteriole has lesser resistance than the efferent. Hemodynamic factors that contribute to the development of diabetic nephropathy include increas ed systemic and intraglomerular pressure,

as well as activation of vasoactive hormone pathways including the renin angiotensin system and endothelin. These Hemodynamic variables that contribute to the improvement of diabetic nephropathy incorporate expanded systemic and intraglomerular weight, as well as enactment of vasoactive hormone pathways counting the renin angiotensin framework and endothelin. These hemodynamic pathways enact intracellular moment couriers such as protein kinase C (PKC), Mitogenactivated protein (Outline kinase), atomic translation variables such as NF-kB and different development variables such as the pro-sclerotic cytokine, TGF-β and the porousness improving development figure, vascular endothelial development figure, VEGF. Glucose subordinate pathways are too actuated inside the diabetic kidney and result in improved oxidative stretch, renal polyol arrangement and the aggregation of progressed glycation conclusion items (AGEs). In combination, these pathways eventually lead to expanded renal albumin porousness and extracellular framework aggregation, resulting in frequent proteinuria, glomerulosclerosis and eventually tubulointerstitial fibrosis.

2.3.2 Hyperglycemia

In vitro investigations have demonstrated that hyperglycemia directly affects mesangial cell multiplication, matrix extension, and glycosylation of glomerular proteins (Heilig et al., 1995, Lin et al., 2006) Proof likewise demonstrates that, high glucose levels causes; debilitation in cell development, impedence in production of growth factors and impedance in gene occurrence. This will eventually lead to increment of the extracellular matrix.

Hyperglycemia may cause lethal products which may lead to cell damage. TGFβ is an example of a toxic product. It expands production of extracellular matrix in the glomerular mesangium. It also causes extracellular matrix removal by inhibiting collagenases synthesis.

2.3.3 Advanced Glycation Products

In chronic hyperglycemia, a partion of the excess glucose binds to free amino acids in blood or tissue proteins. This is a non-enzymatic procedure that produces reversible early glycation items, and afterwards, irreversible advanced glycation end products (AGEs), which accumulate in the tissues and bind to collagen which contributes to the onset of microvascular complications of DM (Vlassara et al.,1996) Because of this process, there is an ascent in the quantity of collagen cross-links and diminished degradation in the extracellular matrix. Cross-linked collagen and other proteins are resistant against degrading enzymes, like metalloproteinases. AGEs may additionally increase synthesis of various cytokines, which activates mechanisms leading to diabetic nephropathy (Czekalski et al.,2004). A study by Viberti *et al*;1994 demonstrated that administration of inhibitors of the formation of AGEs (e.g. aminoguanidine) reduces renal gathering of AGEs and the subsequent mesangial hypertrophy and albuminuria in experimental animals (Viberti et al.,1994).

2.3.4 Protein kinase C

Intracellular increment of glucose expands the synthesis of a molecule known as diacylglycerol (DAG), which is a basic activating cofactor for protein kinase C (PKC). PKC impacts expression of a variety of genes. Its overactivation prompts blood stream variations from the norm, vascular porusness, capillary and vascular occlusion, pro-inflammatory gene expression and oxidative stress (Cooper et al., 1998) Protein kinase C also plays a critical role in cell proliferation and differentiation, and apoptosis. This enzyme ascents cytokines and extracellular matrix and production of endothelin. This changes cause thickening of the glomerular basement membrane and obstruction of arteries and increased glomerular permeability.

2.3.5 Aldolase-reductase pathway

Another vital enzyme in the pathogenesis of diabetic nephropathy is aldose reductase. This enzyme changes over glucose to sorbitol. Long haul hyperglycemia increases the activity of the enzyme and the production of sorbitol. Their accumulation intracellularly causes osmosis thereby allowing entry of water into the cell (Koya et al.,1998). This builds up the extracellular matrix. Aggregation of extracellular matrix in the mesangium and glomerular basement membrane (GBM) ascents the mesangium volume and thickening of GBM. Accumulation of sorbitol also causes a decrease in myoinositol in glomerular cells (Viberti et al., 1998) which causes tissue damage (Frank et al.,1994)

2.3.6 Prorenin

Prorenin binds to a particular tissue receptor prompting the activation of the signal pathway of p38 (Nguyen, 2006). Ichihara et al in 2006 demonstrated a possible role of prorenin in the development of diabetic nephropathy using an experimental model. In their investigation, a prolonged prorenin receptor blockade inhibited the activation of MAPK, which avoided the advancement of diabetic nephropathy despite the increased activity of angiotensin II.

2.3.7 Cytokines and Growth Factors

Hyperglycemia invigorates expanded articulation of various development variables and initiation of cytokines, which overall contributes to increased kidney insult (Hohenstein et al., 2006, Navarro-Gonzalez,2008) In samples of the kidney biopsy from T2DM, a critical increment in platelet derived growth factor (PDGF) expression was found. additionally, the site of expression of this factor is adjoining to the regions of interstitial fibrosis, which is important in the development of fibrosis in kidney injury (Langham et al., 2003) Hyperglycemia also ascents the glomerular expression of TGF-beta; specifically, matrix proteins are stimulated by this growth factor (Wolf et al.,1999) Furthermore, bone morphogenic protein 7 (BMP-7) expression in DM reduces, and the expression of profibrogenic TGF-beta is elevated (Turk et al.,2009)

2.3.8 Reactive Oxygen Species

Increasing evidence shows the importance of reactive oxygen species (ROS) in the pathogenesis of diabetic nephropathy. Although generation of ROS may be influenced by numerous mechanisms, the critical role in their generation is played by superoxide generated by glycolysis and oxidative phosphorylation in the mitochondria. “ROS activates all important pathogenetic mechanisms, such as increased production of AGEs, increased glucose entry into the polyol pathway, and PKC activation” (Dronavalli., 2008). Moreover, ROS causes direct damage endothelial glycocalyx, which leads to albuminuria without the concurrent damage to the GBM itself.

2.4 Risk factors associated with development of diabetic nephropathy

Diabetic nephropathy development has several risk factors. They can be grouped into those that can't be modified (age, hereditary factors and race) and those that can and should be modified (hyperglycemia, hypertension, dyslipidemia, and GFR)

2.4.1 Genetic Factors

Genetic factors establish the occurrence and severity of diabetic nephropathy (Vargas et al 1974). There is a 14% likelihood for an offspring of the parents without proteinuria to have clinical proteinuria, 23% likelihood in situations where one of the parents has proteinuria, and 46% likelihood on the off chance that that the two parents have proteinuria. The risk can't be clarified by duration of DM, expanded circulatory strain or guideline of glycemic status. However, a role could be played by genetic predisposition for arterial hypertension and an increase in salt intake. Despite Chromosomes 3, 7, 18, and 20 being implicated in diabetic nephropathy, the role of specific inclining hereditary determinants can't be affirmed because of inconsistent results of the studies of genetic components vital in the development of DN.

2.4.2 Race

The rate of diabetic nephropathy is expanded in Asian Indian, Mexican American, and African American ethnic groups. DN severity is higher in Blacks (3- to 6-fold in comparison with Caucasians), American Mexicans, and especially in Pima Indians in the North West part of the United States (Smithson et al.,1998). This observation in genetically diverse populations observes that nutrition, socioeconomic factors and poor glycemic control, blood pressure, and body weight, play the key part

2.4.3 Age

Age and duration of DM in patients with T2DM raise the risk for albuminuria A populace investigation of 1586 Pima Indians with T2DM, subjects confirmed to have DM before age 20 had a higher risk of developing kidney failure (25 vs. 5 patients in 1000 incident patients). According to Svensson et al. there was a low risk of kidney failure in T1DM patients if the condition was confirmed by the age of 5.

2.4.4 Increased Blood Pressure

Hypertension prevalence in patients with T1DM (40%) and T2DM (70%) is high, before albuminuria is established. Results from a number of clinical studies that were large (UKPDS, ADVANCE) showed a causative relationship between raised arterial pressure and DN. In addition, three factors have been demonstrated to play a role in the development of raised arterial pressure including raised arterial rigidity, excessive volume of extracellular fluid and hyperinsulinemia. Hyperinsulinemia plays a role in the development of raised arterial pressure via insulin resistance in T2DM or through insulin administration. A study by Randeree et al., (1992) demonstrated that 80 patients with T2DM who begun treatment with exogenous insulin had an increased blood pressure from 132/81 mm Hg to 149/89 mm Hg. This hypertensive reaction could be caused by weight gain along with pro-hypertensive effect of insulin. Hyperinsulinemia might be the interphase between being overweight and raised blood pressure in patients with or without DM, because it ascents sympathetic activity and sodium retention in the kidneys. Insulin causes Sodium and water retention while the increased filtration of glucose is induced by a high glycemic state. The abundant glucose that is filtered is reabsorbed (as long as there is a moderate hyperglycemia) in the proximal tubule by means of sodium-glucose co-transport, which consequently leads to the increase in reabsorption of sodium reabsorption .This increases blood pressure, which may be prevented and regulated by salt-free diet. Patients with DM have increased arterial stiffness. This develops due to the increased glycation of proteins and as a result the development of arteriosclerosis. Decreased arterial elasticity in patients with glucose intolerance or DM contributes to the increased systolic pressure which is an independent mortality risk factor (Ref).

2.4.5 Glomerular Filtration Rate

A risk factor for advancement of diabetic nephropathy is increased GFR at diagnosis. In around half of the patients with T1DM lasting up to five years, the value of GFR DN is about 25-50% above normal range. These patients have a higher risk of developing DN. Structural and hemodynamic changes is influenced by increased intraglomerular pressure which results to glomerular hyperfiltration and hypertrophy and damage to the endothelial wall. Strict glycemic control, a diet low in protein, and control of blood pressure may decrease the progress of renal disease in type 1 diabetes mellitus (T1DM). In T2DM however, more than 45% of patients have GFR that is two standard deviations higher than that in their age-matched no-DM or overweight controls. The hyper filtration rate (117-133 mL/min by and large) is lower than that in T1DM. Being that they are more advanced in age, patients with T2DM in this manner have an increased likelihood of advancement of atherosclerotic vascular changes that impact GFR and glomerular size. Investigations conducted on animal models showed that DM is associated with damage of renal autoregulation. As a result, increased blood pressure does not induce the expected vasoconstriction in the afferent arteriole, which would reduce the influence of systemic hypertension on intraglomerular pressure.

2.4.6 Glycemic Regulation

Diabetic nephropathy regularly occurs in patients with poor glycemic control. The degree of glycemic control is an essential indicator of terminal kidney failure. A study by Krolewski et al (1996) demonstrated that the prevalence of terminal kidney failure was 36% in patients with the poorest glycemic control in comparison with 9% in the group with well-controlled glycaemia. It is has been accepted that the degree of glycemic control is an important risk factor for the development of DN.

2.4.7 Overweight

High body mass index (BMI) ascents the risk of development of chronic kidney disease in patients with DM. Additionally, adequate diet and reduction in body weight decrease proteinuria and improve kidney function in these patients. The role of excess weight as a risk factor for diabetic nephropathy (independent of DM and glycemic control) has not been clearly confirmed

2.4.8 Smoking

Despite studies conducted recently the association between smoking and progression DN, a large prospective study by Hovind et al. (2003) was not able to confirm the association between smoking and reduced GFR rate in patients with DM with or without ACEI therapy

2.4.9 Oral Contraception

Ahmed et al. (2005) demonstrated the association between the use of oral contraceptives and development of DN.

2.5 Sub-clinical hypothyroidism as a risk factor for kidney disease

2.5.1 Thyroid disorders in Diabetes Mellitus

Diabetes mellitus being an endocrine disorder is prone to affect other endocrine functions such as the thyroid function (Singh et al.,2014, Vergara et al., 2013) The association of these two endocrine dysfunctions is known widely with the initial study published in 1979. From then onwards, several studies worldwide have estimated the prevalence of thyroid disorders (TD) in diabetic patients. The worldwide prevalence of TD in diabetes has been shown to vary from 2.2 to 17%, with subclinical hypothyroidism being the most common disorder. (Papazafiropoulou et al., 2010)

A large Europe study, a meta-analysis, indicated that the prevalence of thyroid disorders in the general population, including is 3.82%. This includes SCH. 3.05% is hypothyroidism and 0.75% is hyperthyroidism. Another meta-analysis carried out by Kadilaya et al., 2010, showed a thyroid dysfunction prevalence of 11% in a diabetic population. Several other cross-sectional studies have shown a high prevalence of thyroid disorders that was significant in patients with T2DM.Of these studies, the largest demonstrated that prevalence of thyroid dysfunction in patients with T2DM was 10.9% and 6.9% in females and males respectively (Rebecca et al.,2018) Thyroid disorders prevalence that has been reported in a diabetic population also varies with the characteristics of the population under study. A study carried out in Calabar, Nigeria reported a high prevalence of 46.5%. Similarly, in Ngugi et al, in Kenya showed a 61% prevalence of thyroid disorders in T2DM patients.

In many studies, SCH was the most prevalent finding. Many other studies either performed a sub-analysis of patients with previously known thyroid disease or excluded them. The most common finding in these situations was evidently SCH. This finding indicated that most of the undiagnosed thyroid disorders would be of this class (Ref).

2.5.2 Effect of Thyroid disorders on kidney function

The most common endocrine diseases are thyroid disorders and they affect virtually all physiological systems, with particularly a marked impact on renal and cardiovascular systems. Thyroid hormones are important regulators of renal and cardiac mass, renal sodium handling, vascular function and consequently blood pressure (BP). The renin angiotensin system (RAS) acts globally to control renal and cardiovascular functions, while the components of RAS act systemically and locally in individual organs (Felix et al.,2012). Various authors have implicated the local and systemic RAS in the arbitration of structural and functional changes in renal and cardiovascular tissues due to abnormal thyroid hormone levels (Kobori et al.,2007)

The basic unit of the kidney which the nephron consists of the renal corpuscle and the tubule. A dense network of capillaries known as the glomerulus filtrates blood. Substances that are filtered are reabsorbed from the tubular fluid in segments of the renal tubule. Secretion of components from plasma such as potassium, urea and substances produced by the tubular cell also takes place. During formation of urine, alterations of the tubular fluid takes place. Overall, physiological effects of the thyroid hormone are stimulatory where; basal metabolic rate is increased, which demands for increased gluconeogenesis, glycolysis, lipid metabolism and protein synthesis. The blood flow, cardiac output (CO) and heart rate are stimulated by thyroid hormones as well.

Both hyper- and hypothyroidism cause hemodynamic and vascular changes, which have an influence on kidney function through effects on renal blood flow. These changes are described schematically in (Figure 1.)

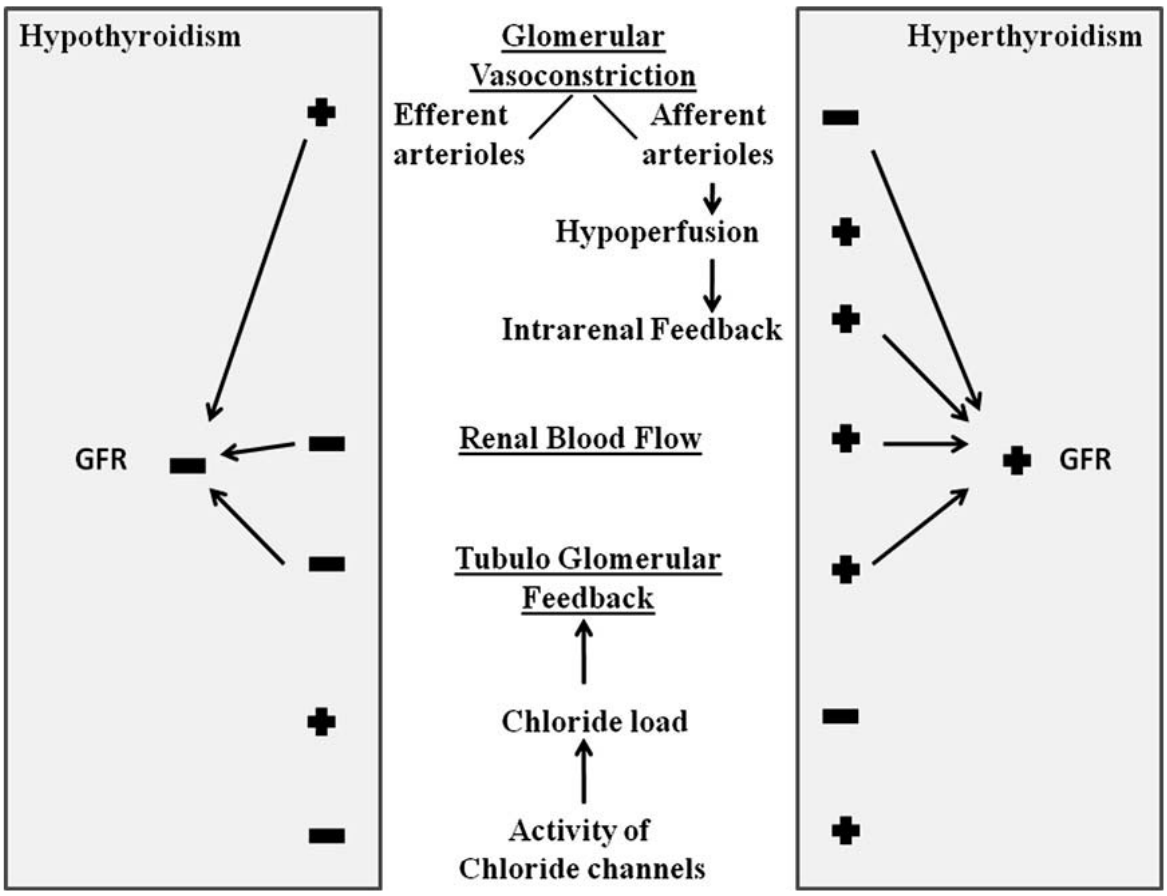

Figure 1 Changes in kidney function in hypo- and hyperthyroidism that have an effect on the glomerular filtration rate (Ingrid et al.,2009)

2.5.2.1 Cardiac output

Thyroid hormones have a positive chronotropic effect caused by a shortened atrio-ventricular conduction time, upregulated β-receptors in cardiac tissue and changes in electrophysiological parameters. This results in tachycardia, (Biondi et al.,2002) and also positive inotropic effect which results from changes in several potassium, calcium and sodium channels as well as activity of myosin isoenzymes. Cardiac output is reduced in hypothyroidism (Udovcic et al.,2017) caused by decreased filling of ventricles, decreased cardiac contraction and bradycardia

2.5.2.2 Renal blood flow

The increased cardiac output that results from positive inotropic and chronotropic effects, raised blood volume by activation of RAAS and decreased vascular resistance cause a raised adrenal blood flow (RBF) in patients with hypothyroidsm. The decreased CO in hypothyroidism leads to a decreased RBF. Glomerular lesions seen in hypothyroidism for example an increased mesangial matrix and basement membrane thickening which might contribute to the decreased RBF (Ref).

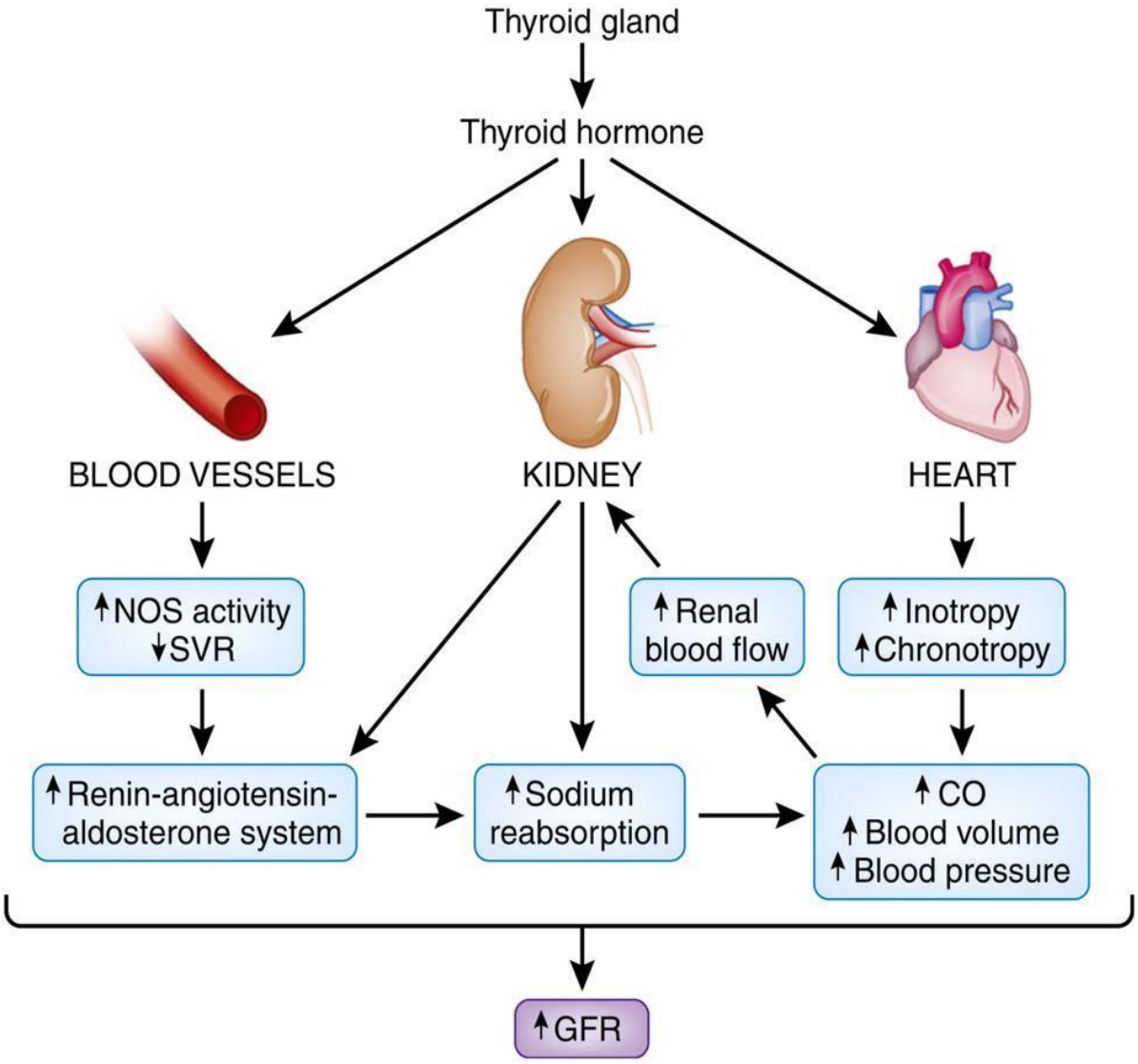

Figure 2 Multiple direct and indirect effects of thyroid hormones on GFR (Laura et al.,2012)

2.5.2.3 Systemic vascular resistance

In a hyperthyroid stat, Systemic vascular resistance (SVR) is decreased. Capillary vessels in the muscle tissue are greater in number and reduced contractility caused by a reduced response to norepinephrine aa well as a direct action of thyroid hormones on vascular smooth muscle cells. An increased responsiveness to the endothelium-dependent vasodilator acetylcholine and an increased release of local vasodilators causes several vascular smooth muscle cells to relax. On the other hand, the endogenous renal vasoconstrictor endothelin activity reduces. Atrial natriuretic factor (ANF) activity is increases due to T4 direct effect of expression of genes or a raised cardiac preload. In the medulla and renal cortex of the kidney, nitric oxide synthase (NOS) activity and production of nitric oxide (NO) is increased. This homeostatic effect is protective in the target organs of hypertension which may be attributed to a direct effect of hormones of the thyroid on NOS activity (Ref).

A high arterial pressure, increased circulation with shear stress on the endothelium that results in increased release of vaso-active substances or expression of NOS may be an indirect effect. Effect of T3 directly on gene expression of renin enhances the RAAS activity. There is increased plasma renin concentration, angiotensinogen synthesis in the liver, plasma angiotensin II, serum angiotensin converting enzyme and angiotensin receptor density and renin release (Ref).

2.5.2.4 Renal tubules

Thyroid hormones have both quantitative and qualitative effects on renal tubules. In hyperthyroidism, renal tubules are hyperplastic and hypertrophic which causes a raised kidney weight, increased tubular mass, increased mitotic index, increased DNA content with a constant protein/DNA ratio, increased renal expression of renin mRNA and increased tubular secretion and resorptive capacity (Ref).

A decreased kidney-to-body weight ratio is seen in hypothyroidism. However, renal hypertrophy that is compensatory occurs. Protein/DNA ratio without changes in DNA content of renal cells increases. Supplementation of thyroid hormones and subsequent doubling of the kidney mass shows ability to reverse the decreased kidney mass (Ref)**.**

Tubular transport processes

Thyroid hormones stimulate active, carrier-mediated tubular transport processes by an increased gene expression, synthesis and activity of carrier proteins, such as Na+ -K + - ATPase across the basolateral membrane, and Na+ /H+ exchanger (NHE) activity in brush border membrane vesicles which leads to an increased uptake of Na+ in exchange for H+. Specifically, NHE-2 and NHE-3 mRNA isoforms levels increase after transition from the hypothyroid to the hyperthyroid state. The increased tubular reabsorption of sodium together with a decreased load of filtered sodium causes a decreased pressure diuresis-natriuresis response, and enhanced Na+Ca2+ exchange activity and Ca^{2+} reabsorption in the basolateral membrane through modulation of the uptake of Ca2+ in brush border membrane vesicles. The influence of short-term hypothyroidism on tubular function is only modest, however tubular transport capacity is below normal and phosphate reabsorption is reduced in the proximal tubule. Urinary acidification is impaired with increased sodium and bicarbonate excretion rates

Ability to concentrate urine

Patients with thyrotoxicosis can have a low capacity to concentrate urine though without clinical importance. This impairment may be due to derangements in metabolism or vasopressin sensitivity to the distal nephrons, decreased sodium ion concentration due to increased RBF or osmotic diuresis caused by an increased filtered solute.

In hypothyroidism, urine concentration ability is impaired. This can be reversed with replacement of thyroid hormones. It is however not associated with a decreased GFR, excretion of solutes, urea or plasma arginine-vasopressin concentration (Ref)**.**

2.5.2.5 Glomerular filtration rate

The ultrafiltrate is formed by the glomerulus into the capsule of Bowman by filtration through the capillary wall of glomerulus. The filtration rate, the glomerular filtration rate (GFR), is the result of the mean net filtration pressure, the permeability of the filtration barrier and the surface available for filtration. The permeability is dependent on the structural and chemical characteristics of the glomerular capillary wall. GFR represents the magnitude of ultrafiltration of plasma in the first steps of urine formation. It is therefore regarded to be the best overall index of kidney function. Hyperthyroidism and hypothyroidism have a respectively increasing and decreasing effect on GFR through several mechanisms (Ref).

2.5.3 Sub-clinical hypothyroidism and Diabetic Nephropathy

Compared with the general population, T2DM patients have a higher prevalence of thyroid disorders. The most common amongst them is Subclinical Hypothyroidism (SCH) (Wu et al.,2007) SCH is an asymptomatic state defined as elevated thyroid stimulating hormone (TSH) levels above the upper limit of the reference range in addition to a normal free thyroxine level (FT4) (American Thyroid Association, 2012) Among T2DM patients, subclinical hypothyroidism prevalence ranges from 2 to 17% (Perros et al.,1995, Chen et al.,2007, Yang et al.,2010, Kim et al.,2011) . A meta-analysis recently that included 7378 diabetic patients from 17 studies, reported a prevalence rate of 10.2% for SCH. (Han et al. 2015)

This prevalence in T2DM patients over the past decade, has drawn much of attention due to the suspected compounding of diabetes-related vascular complications. The association between subclinical hypothyroidism and microvascular complications in type 2 diabetic patients and the association between TSH levels and the microvascular complications in type 2 diabetic patients has been investigated. In a study done in Japan by Yasuda et al., 2011, associated SCH with albuminuria in type 2 diabetic patients. TSH level in this study was also found to be an independent risk factor for the presence of albuminuria. Another study by Sharma et al.,2017 reported that TSH levels in T2DM patients with DN increase with an increase in urinary albumin creatinine ratio. The conclusion was that SCH is an independent risk factor for albuminuria. Similarly, a study by Rasha et al 2017 in Cairo, Egypt concluded that increased TSH is significantly associated with increased serum creatinine, increased ACR and decreased eGFR.

Several studies have also shown an association between SCH and diabetic nephropathy in T2DM patients. A study by Chen et al.,2007, reported an increased risk of DN in T2DM patients with SCH. Furukawa et al in 2014 reported that SCH may be independently associated with DN in Japanese patients with T2DM. An Iranian study in 2016 by Mansournia et al also reported that SCH when compared to euthyroid groups was independently associated with about 3 times higher rates of DN using multivariate logistic regression models.

Hormones from the thyroid gland affect almost all organ systems in the body and are linked to several components that contribute to renal dysfunction. “Hypothyroidism is linked to reduced renal plasma flow, low glomerular filtration rate, decreased sodium reabsorption and inability to dilute urine” (Vargas et al.,2006) Being asymptomatic, subclinical hypothyroidism may therefore

impair renal function. Thyroid hormones have several protective effects on the kidney function therefore replacement therapy can reverse the deterioration of renal function in these patients (Karanikas et al.,2004, Mooraki et al.,2003, Kreisman et al.,) Shinya et al in 2014 therefore concluded that SCH may be a new therapeutic target to prevent development and progression of renal disease in diabetes patients. Studies by Peng et al.,2015 also reported that levothyroxine treatment may decrease urinary albumin excretion rate and exert kidney protection effects in early type 2 diabetic nephropathy and SCH patients.

2.6 Screening and diagnosis of diabetic nephropathy.

Diabetic Nephropathy is a glomerulopathy that is defined by characteristic functional and structural changes. These major structural changes include glomerular basement thickening, glomerular sclerosis and mesangial expansion (Paola et al.,2007) The aim of investigators of DN today is early diagnosis. At present, diagnosis of DN in a clinical setting relies on the assessment of renal injury, usually by assessment of urinary albumin to creatinine ratio and by assessment of kidney function, usually by calculating eGFR (Lin et al.,2016)

2.6.1 Microalbuminuria

Diabetic Nephropathy is a clinical syndrome. It is characterized by a continued increase in excretion of protein; particularly albumin, an early and continuous intra-renal hypertension, a late decline of GFR which eventually leads to ESRD and increased relative mortality from cardiovascular diseases (Rehman and Hamayun,2004) Normal individuals excrete very low amounts of protein in the urine. Persistent excretion of protein in increased levels serves as a kidney damage marker. Depending on the kidney disease that is present, certain types of proteins like albumin are excreted. A raised albumin excretion is a sensitive marker for a disease in the glomerulus. Microalbuminuria was first described as a predictor of nephropathy more than three decades ago. It has been recognized as a sign of abnormal vascular function and increased vascular permeability. Persistent microalbuminuria which may precede overt DN by several years is defined as a stage of incipient nephropathy (Kevin et al.,2000). A high proportion of diabetic patients are seen to have microalbuminuria and overt nephropathy, a short period after the diagnosis of DM.If no specific interventions are made, 20-40% of diabetic patients with microalbuminuria progress to overt nephropathy. After onset of overt nephropathy, 20 years later, 20% of these patients will have progressed to ESRD (Macissac et al.,2014). Urinary albumin measurement therefore has an important role in secondary prevention, to decide treatment and monitor response to treatment of kidney disease (Ref).

2.6.1.1 Screening Microalbuminuria

Microalbuminuria is defined as the presence of a small amount of albumin in the urine, which cannot be detected with the usual urine dipstick methods. The definition however depends on the method of urine collection. (Table 1)

Table 1 Definition of abnormal urinary albumin excretion (adapted from ADA, 2010)

Category	**Spot collection (mg/g creatinine)**	**24 hr collection (mg/24h)**	**Timed collection (µg/min)**
Normal	< 30	< 30	< 20
Moderately increased	30 – 300	30 -300	20 – 200
Severe	> 300	> 300	> 200

The ADA and the Canadian Diabetes Association as well as the National Clinical Guidelines for Management of Diabetes Mellitus in Kenya recommend that screening must be initiated at the time of diagnosis in patients with T2DM. If microalbuminuria is absent, screening should be repeated yearly**.**

In clinical practice, various methods for collection of urine are used to measure albumin. The amount of albumin excreted in urine during a 24-hour period has been considered the gold standard. (CDA, 2008, ADA,2013). '24-hour urine collections however may be associated with significant collection errors, largely due to improper timing and missed samples, leading to over-collections and under-collections.

Timed overnight collections or shorter timed daytime collections may reduce the inconvenience of a 24-hour collection but are still associated with collection errors. In addition, errors due to incomplete bladder emptying are relatively more important in shorter collection intervals' (Assadi et al.,2002).

An easier and more practical alternative is collection of a first morning void or a spot (random) urine sample (Assadi et al.,2002). It has been suggested that a first morning void is to be preferred over a spot urine sample, because the former is less influenced by factors such as hydration status and physical activity, reducing the variability that is caused by these factors. (NKDEP,2010) From a practical point of view, however, spot urine samples are preferred because they can be collected during consultation at the doctor's office and therefore pose the least inconvenience for patients. According to the National Kidney Foundation (NKF), clinical practice guidelines, under most circumstances, untimed spot urine samples should be used to detect and monitor proteinuria in

children and adults (NKF,2002) It is usually not necessary to obtain a timed urine collection (overnight or 24- hour) for these evaluations in either children or adults. First morning specimens are preferred, but random specimens are acceptable if first morning specimens are not available (ref).

Albumin measurement results can be expressed as urinary albumin concentration (mg/l) or as Urine Albumin Creatinine Ratio (mg/g or mg/mmol) (ADA,2013) A spot urine albumin-creatinine ratio (UACR) can also be measured. It is a ratio between two measured substances (albumin and creatinine) A UACR estimates 24-hour urine albumin excretion hence 24-hour collection and timed specimens are not necessary. Albuminuria is present when UACR is greater than 30mg/g.

Engaging in exercise within the last 24 hours prior to testing, infection, fever, congestive heart failure, marked hyperglycemia, marked hypertension, urinary tract infection, hematuria and menstruation can all increase urinary albumin over baseline level and confound the diagnosis of diabetic nephropathy. Owing to this variability in urinary albumin excretion, measurement of albumin to creatinine ratio on random spot urine sample is preferred because it helps to compensate for patients with such underlying conditions (ADA, 2004; Finebarg et al., 2013)

2.6.2 Significance of Microalbuminuria as an early marker for diabetic nephropathy

During the early stages of DN, glomerular hyperfiltration occurs resulting in microalbuminuria. As the disease progresses, macroalbuminuria sets in which is followed by a deterioration in kidney function and eventually ESRD. This may eventually require renal replacement therapy (Remuzzi et al.,2002) Under normal circumstances, a minute quantity of albumin is filtered through the glomerulus. This is accompanied by its near-complete reabsorption in the tubules (Haraldsson et al.,2008) Thus, increased urinary albumin excretion (UAE) is accepted as a well-established biomarker of glomerulopathy (Matheson et al.,2010) and tubulopathy because in the latter, there is reduced reabsorption of the filtered albumin (Birn et al.,2006)

Albuminuria is an important component of DN and different degrees of UAE can occur. Normoalbuminuria refers to UAE of <30 mg/day or 20 μg/min, while microalbuminuria and macroalbuminuria refer to UAE of 30–300 mg/day or 20–200 μg/min, and >300 mg/day or 200 μg/min, respectively (Cohen et al.,2012) Since baseline albuminuria is the strongest predictor of ESRD and cardiovascular morbidity in T2DM (de Zeeuw et al., 2006), the American Diabetes Association recommended yearly UAE screening in all patients with T2DM which commences at the time of diagnosis (ADA,2014) Remarkably, microalbuminuria constitutes a risk factor for ESRD and chronic kidney disease and also serves as a powerful predictor of cardiovascular morbidity and mortality in diabetic subjects (Ninomiya et al., 2009)

Microalbuminuria is therefore considered as a clinically important indicator of deteriorating kidney function in diabetic patients. It serves as an early indicator of DN risk and a predictor of its progression as well as cardiovascular disease risk in diabetes mellitus (Uwaezuoke et al.,2015) Detection of small increases in urinary excretion of albumin is a sensitive marker for development of diabetic nephropathy which is the main cause of mortality and morbidity in patients with diabetes mellitus (de Zeeuw et al., 2006) Its detection is an indication for initiation of appropriate therapy for the purpose of preventing the advance to overt diabetic nephropathy.

Microalbuminuria can be resolved by glycemic control and specific medications such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers (Ghazalli et al.,2003)

2.6.3 Assessment of renal function

Once diagnosis of DN is made, the renal function must be assessed so as to measure progression of DN and to eventually confirm the need for treatment of ESRD (Rosing et al., 2004) Glomerular filtration rate is accepted as the best measure of overall kidney function in health and disease. (Stevens et al.,2006)

2.6.3.1 Glomerular filtration rate

Glomerular filtration rate is accepted as the best measure of overall kidney function in health and disease. In principle, glomerular filtration rate (GFR) is the product of the number of nephrons. Chronic kidney disease progressively destroys the nephrons hence reducing the GFR of the subject (Afkhami-Ardekani et al., 2008). An accurate measure of GFR can be undertaken using low molecular weight markers of kidney function such as inulin, iohexol or technetium (labelled DTPA), however, these methods are time consuming, expensive and generally not available. The regular measurement of serum creatinine levels is simple to perform and is currently the most common method. However, because creatinine is invariably reabsorbed by the renal tubules, serum creatinine and creatinine clearance measurements tend to underestimate the GFR in the context of hyperfiltration and overestimate the GFR in the context of hypofiltration and is subject to errors in urine collection unless great care is taken (Chadban et al., 2010). For optimal approximation of GFR from serum creatinine measurements, allowances need to be made for age, gender, race, height and weight of the individual as in the Modification of Diet in Renal Diseases (MDRD) equation which is a satisfactory index of GFR (Levey et al., 1999). In microalbuminuric patients, GFR may remain stable, but a subset of patients has shown a rapid decline (Gross et al, 2005). Persistent proteinuria results in progressive fall of GFR culminating into ESRD in months to a year if not managed (Hamed et al., 2002). The most convenient method for appraising the kidney function is the estimated glomerular filtration rate (eGFR) which is used to stage the level of chronic kidney disease. The eGFR is usually based on serum creatinine levels, age, sex and race of an individual. The recommended equation by the National Kidney Foundation is that of the MDRD which is the most widely used for computing eGFR for people above 18 years old (Gross et al., 2005). This calculator is available on National Kidney Disease Education Program website at http://www.nkdep.nih.gov/professionals/gfr-18calculator/org-con.html. Although the measurement of albuminuria is essential to diagnose DN, there are some patients who present decreased GFR when urinary albumin excretion values are normal. Based on this, the classification

of National Kidney Foundation can also be used to stage chronic kidney disease in such patients (Zelmanovitz et al., 2009).

2.6.4 Prevention and treatment of Diabetic Nephropathy

According to recommendations by American Diabetes Association, patients with type 2 diabetes mellitus of more than 5 years after diagnosis should undergo annual screening for development of microalbuminuria, for early detection or prediction of nephropathy to enable timely medical interventions (ADA, 2010). The prevention and treatment strategy should focus on lifestyle modification, control of hypertension and dyslipidemia.

Lifestyle modification is a first step in diabetes management, irrespective of presence of chronic kidney disease (CKD) and should comprise of measures to encourage smoking cessation, weight loss, and increased physical activity as well as dietary changes.

Strict glycemic control is one of the logical measures that help to prevent development of DN according to various clinical trials (Zelmanovitz et al., 2009). The ADA (2010) and NKF (2007) recommend achieving HBA1C of 7.0% in patients with diabetes irrespective of presence of CKD. Guidelines from AACE vary slightly, endorsing a more stringent HBA1C goal of 6.5%. Dietary approach and therapeutic intervention involving insulin use is utilized to achieve this goal (Ref).

Intensive blood pressure control is another strategy used to prevent development of nephropathy. Blood pressure goals currently recommended are 130/80 mm Hg for patients with both type 1 and type 2 diabetes irrespective of CKD (NKF, 2007; ADA, 2010). Use of anti-hypertensive drugs such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) which are renin-angiotensin system blockers confer an additional benefit on renal function as several studies have demonstrated that, treatment of hypertension produced a beneficial effect on albuminuria (Gross et al., 2005; Fowler, 2008).

Management of the diabetic patients with respect to dyslipidemia should be focused on raising the ‘good cholesterol’ HDL-C and lowering the ‘bad cholesterol’ LDL-C through dietary modifications which includes reduction in saturated fats, trans fat and cholesterol intake together with increases in omega 3 fatty acids, viscous fiber, plant stanolls/ sterols, weight loss and increased physical activity (Bakris, 2011). If this fails, a combination therapy involving statin and other drugs, such as fibrates or niacin may be necessary to achieve ideal lipid control, but

patients should be monitored closely for possible adverse reactions of therapy (Fowler, 2008; Zelmanovitz et al., 2009). Conventional therapies such as strict glycemic control and antihypertensive treatment do not completely stop the progression of diabetic nephropathy in diabetic patients (Ruggenenti & Remuzzi 1998).

Thyroid dysfunction causes remarkable changes in renal blood flow, glomerular filtration rate, tubular secretory and absorptive capacity, electrolyte pumps, and kidney structure. Hypothyroidism is accompanied by a decrease in GFR and renal blood flow (Iglesias & Diez 2009) and Type II diabetic patients with subclinical hypothyroidism are associated with an increased risk of diabetic nephropathy (Chen et al. 2007) The results of previous studies suggest that overt and subclinical hypothyroidism are both associated with reduced eGFR and high prevalence of CKD and that these abnormalities can be normalized through thyroid hormone replacement therapy (Hollander et al.,2005, Gopinath et al.,2013) Therefore, the screening of thyroid dysfunction in T2DM patients is necessary to reduce the vascular complications and management of diabetes and also reduce the risk of progression to overt hypothyroidism. Replacement of thyroid hormone is fundamental in the treatment of hypothyroidism patients. It alleviates the deleterious effects of hypothyroidism on the kidney and heart

2.7 Problem statement and justification

Diabetes mellitus is a critical health problem affecting major populations worldwide. The prevalence of thyroid disorders is higher in Diabetic patients than in the general population, SCH being the most common. Despite this, the clinical importance of its biochemical abnormalities remains unclear. It is asymptomatic and has been associated with hyperlipidemia, atherosclerosis, cardiac dysfunction and overt hypothyroidism. Several studies have also associated SCH with diabetic nephropathy.

Diabetic nephropathy (DN) is one of the main chronic complications in type 1 and 2 DM and is currently the most common cause of end stage renal disease (ESRD). The disease accounts for a significant number of morbidity and mortality among diabetics. ESRD is the stage of kidney impairment that is irreversible and cannot be controlled by conservative management alone and requires dialysis or kidney transplantation to maintain life. In Kenya, DN is a primary indication for hemodialysis. This exerts a huge social and economic burden on the overall cost of the health care system and the community through large number of hospital admissions and specialized medical care. It is therefore important to generate more information on the risk factors that compound this disorder to encourage preventive care.

These findings imply that SCH may be a new therapeutic target to prevent development and progression of renal disease in DM patients. For a sub-population of diabetic patients found to have SCH, nephropathy should be averted by continuous monitoring using renal function tests at least twice a year. Patients found to have microalbuminuria should be treated to avoid progression to overt nephropathy. It is envisaged that information generated from this study will form a foundation for future studies which can influence thyroid function screening to diabetic patients with diabetic nephropathy. Owing to the paucity of data in the prevalence of SCH and its potential association with diabetic nephropathy, this study will examine the relationship between SCH and Diabetic Nephropathy in patients with type 2 Diabetes Mellitus.

Research question

1. What is the prevalence of sub clinical hypothyroidism among patients with Type 2 Diabetes Mellitus at Kenyatta National Hospital?
2. Is there a correlation between subclinical hypothyroidism and Diabetic Nephropathy in patients with type 2 Diabetes Mellitus?

Hypothesis

There is no correlation between subclinical hypothyroidism and Diabetic Nephropathy in patients with type 2 Diabetes Mellitus

2.8 Broad objective

The main aim of this study is to determine the prevalence of SCH and its correlation with Diabetic Nephropathy in patients with type 2 Diabetes Mellitus at Kenyatta National Hospital

2.9 Specific objectives

1. To classify thyroidal conditions using TSH and FT_4 in T2DM patients
2. To determine the prevalence of SCH in T2DM patients
3. To estimate UACR in patients with T2DM
4. To correlate eGFR with UACR
5. To determine the proportions of T2DM patients with subclinical hypothyroidism and Diabetic Nephropathy
6. To correlate TSH and UACR in patients with T2DM

3.0 METHODOLOGY

3.1 Study design

This was a cross sectional descriptive study

3.2 Study site

This study was conducted at Kenyatta National Hospital (KNH) Diabetic mini clinic. It is a national referral hospital located in the capital city, Nairobi. It thus caters for a diverse population seeking specialist review and follow-up in various fields of medicine. The facility also acts as a primary facility for the population within and around the capital city.

Kenyatta National Hospital also serves as a teaching hospital for the College of Health Sciences, the University of Nairobi and the Kenya Medical Training College. It has a 2000 bed capacity though hosts between 2500-3000 in-patients. Outpatient services cater to over 500,000 patients annually.

Patients with Diabetes are followed up at the Diabetic clinic. The mini clinic runs daily from Monday to Thursday and the main clinic runs on Friday. Ninety percent of the patients attending these clinics have Type 2 diabetes mellitus.

During this study, 5ml of blood and 10ml of urine was collected from the study participants.

The specimens collected from the participants were analyzed for various biochemical profiles namely: TSH, FT4, creatinine and UACR at the Biochemistry laboratory at Kenyatta National Hospital.

3.3 Study population

Type 2 Diabetes Mellitus patients attending the Kenyatta National Hospital Diabetic and Endocrinology mini clinic for management Diabetes Mellitus

3.4 Inclusion criteria

1. Patients that have been diagnosed with Type 2 diabetes mellitus
2. Patients attending the Kenyatta Hospital Diabetic clinic
3. Signed informed consent to participate in the study

3.5 Exclusion criteria

1. Patients with acute inter-current illness
2. Patients with known Chronic diseases
3. Patients with other endocrine disorders
4. Pregnant women
5. Patients with a history of thyroid disease or those on thyroid medication
6. Patients with non-diabetic renal dysfunction or on follow up at the renal clinic

3.6 Sample size

Sample size was calculated using the Cochran formula

Equation 1

$$n_0 = \frac{Z^2 pq}{e^2}$$

Equation 2

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Z=1.96

e=0.05 Margin of error

P= Estimated prevalence (15.8%) Ghazali et al (2010)

q=1-p

N= population of patients at the KNH diabetes and endocrinology center (960 in 2 months)

n= 167

Therefore, 167 participants will be recruited in the study.

3.7 Recruitment and Screening

Recruitment of study participants was done at the Diabetic and Endocrinology clinic by a trained research assistant. The research assistant reviewed the files of patients attending the diabetes outpatient clinics. A screening proforma was used to select the patients who met the inclusion criteria. The patients were then given relevant information about the study and those that gave a written informed consent were be recruited.

3.8 Sampling technique

Consecutive sampling was used to recruit patients who satisfied the inclusion criteria during the study period until the desired sample size is achieved.

3.9 Administration of consent form

The research assistant explained the purpose of the study; the benefits and risks involved and then sought informed consent.

3.10 Questionnaire for demographic details of subjects

Demographic details of age, gender, smoking status, family history of kidney disease, duration of diabetes and medication use of the participants was obtained using a standardized self-report questionnaire. This was administered by the research assistant after consent had been obtained.

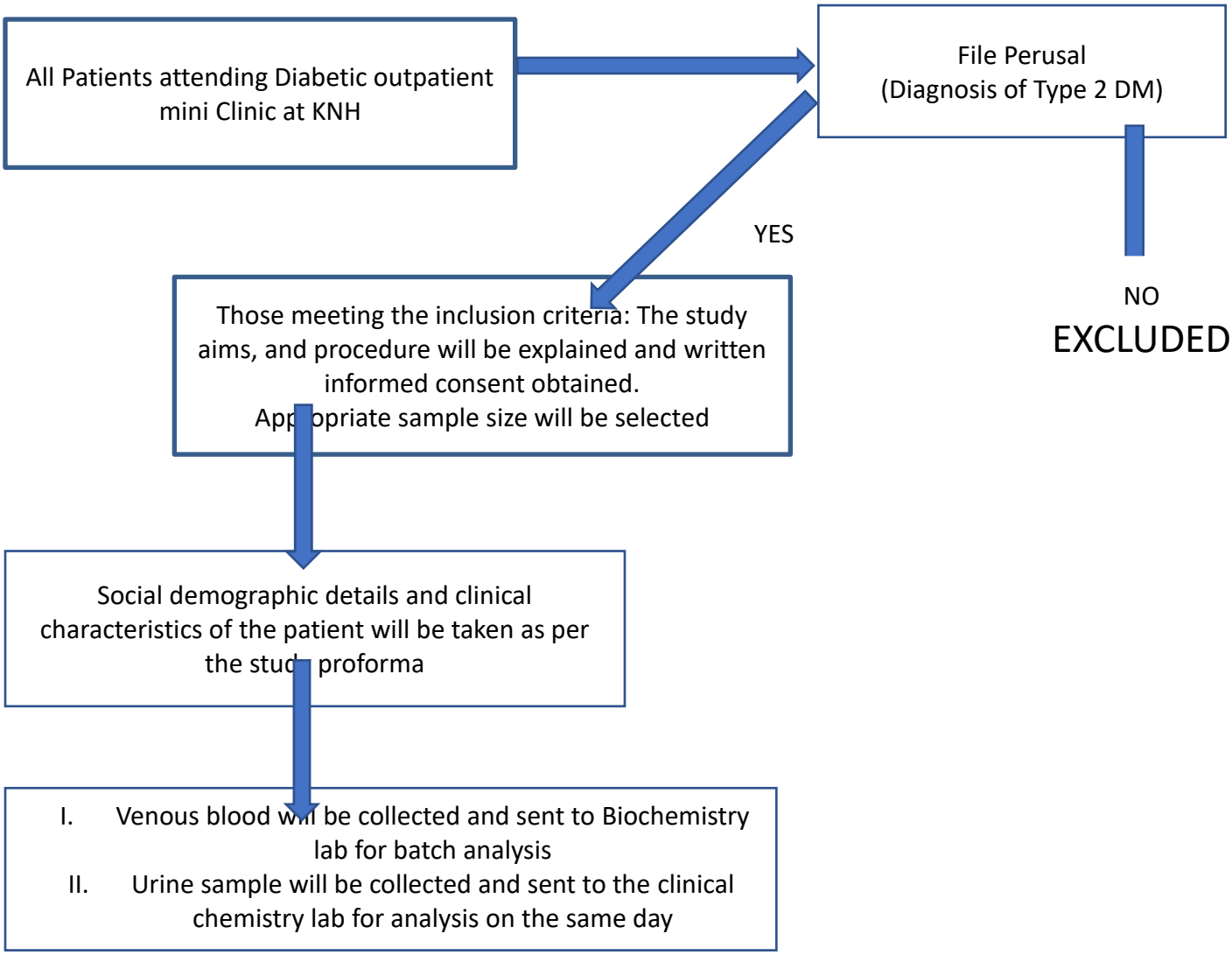

Figure 3 Flow Chart

3.11 Laboratory methods

3.11.1 Specimen collection

3.11.1.1 Blood sample collection

Venous blood was collected aseptically with appropriate infection control including skin sterilization with medical spirit, clean gloves, sterile needles and proper disposal of soiled materials. Four milliliters of venous blood was collected from non-fasting patients into sterile plain vacutainers and delivered to the KNH Biochemistry Laboratory for analysis of creatinine, FT_4 and TSH determination.

3.11.1.2 Urine sample collection

Spot urine samples were collected from the subjects in sterile universal urine collection bottles at random during their medical visit. The samples were thereafter transported to the KNH Biochemistry Laboratory for processing within 2 hours. The measurements of urine albumin to creatinine excretion ratio (UACR) was then be performed.

3.11.2. Handling of collected samples

Cooler boxes with ice packs at approximately 4º C (2-8 ºC) were used for temporary storage to facilitate transport to the laboratory. Serum was separated, frozen & stored in the laboratory.

Batch assaying was done once the required numbers of samples was achieved. Specimens were thawed once. Thawed specimens were homogenized. Particulate matter was eliminated by centrifugation. Urine specimen was analyzed on the same day of collection. In cases of delay in the analysis it will be stored at 2-8ºC.

3.11.3 Specimen analysis

3.11.3.1 TSH

Principle of assay for Thyroid Stimulating Hormone is based on the Electrochemical Chemiluminescent Immunoassay (Cobas E 601).

The reference range for normal TSH is 0.27-5µIU/ml, kit method was followed for analysis of TSH**.**

3.11.3.2 FT_4

Principle of assay for free Thyroxine is Electrochemical Chemiluminescent Immunoassay (Cobas E 601). The reference range for normal FT4 is 10-20 pmol/L. The kit method was followed for analysis of fT_4.

3.11.2.3 Urine Processing

The initial test was a urine dipstick test noting the presence of leucocytes and nitrites as indices of infection. Samples positive for nitrites and leukocytes did not undergo any further analysis as this indicated positive for urinary tract infection**.**

Urine albumin and creatinine excretion rate was then quantified using Clinitek 50® system (Bayer healthcare LLC, USA). It is a semi-automated bench top instrument designed to read microalbumin 2 reagent strips calorimetrically to give results of urinary albumin and creatinine excretion rate.

3.11.2.3.1 Principle of assay

Albumin determination is based on albumin binding a high affinity sulfonephthalein dye at a constant pH to produce a blue color while creatinine reacts with disopropylbenzene dihydroperoxide and 3,3',5,5'- tetramethylbenzibenzidine using a catalyst copper creatinine complex to produce colored complex ranging from orange through green to blue. The intensity of these colors is determined calorimetrically to give the actual concentration of albumin and creatinine (Bayer healthcare LLC, USA).

3.11.2.3.2 Assay of urine albumin-creatinine excretion rate

The strip was dipped in the patient's urine wetting the test pads then removed immediately dragging the edge of the strip against the rim of the urine container to remove excess urine. The start button on the Clinitek 50® system was switched on while blotting the reagent strip on a paper towel after which it was placed on the instrument's test/feed table with the reagent pads facing up. The table then automatically moved into the instrument's reader position where the strip was identified and read, displaying or printing the results as soon as they were available. The details of the chemical reactions were as per the test protocol. A diagnosis of microalbuminuria was be made when the ratio of urinary albumin to creatinine (UACR) is 30-

300, whereas macroalbuminuria was indicated when the UACR was above 300 mg/g. Normoalbuminuria was said to exist if UACR is less than 30 mg/g (ADA, 2012)

3.11.2.4 Serum creatinine determination

3.11.2.4.1 Principle of assay

Serum creatinine concentrations were determined by Jaffe-reaction photometric colorimetric test for kinetic measurements using human creatinine liquicolor reagent. Creatinine forms an orange-red colored complex with picric acid in alkaline solution. The absorbance of this complex is proportional to the creatinine concentration in the sample (Human Gesellshaft fur Biochemica und Diagnostica MBH, German).

3.11.2.4.2 Serum creatinine assay

A 500 µl volume of creatinine working reagent was incubated at 37oC for about two minutes, and then mixed with 50 µl of patient's serum or standard as during calibration. The reactants were incubated within the programmed lag time of 30 seconds, and a read time of 120 seconds after which the clinical chemistry analyzer displayed the concentration of creatinine at a wavelength of 510 nm. The details of the chemical reactions were as per the test protocol.

3.11.2.5 Computation of estimated glomerular filtration rate

Estimated glomerular filtration rate for participants aged 18 years and above was computed using Modification of Diet in Renal Diseases (MDRD) formula available on National Kidney Disease Education Program website at http://www.nkdep.nih.gov/professionals/gfr-calculator/org-con.htm: 33 eGFR (ml/min/1.73m2) = 175 x (serum creatinine level) -1.154 x (age)-0.203 x (1.742 if female) x (1.212 if African American) (Levey et al., 1999). A normal eGFR was taken to be above 90 mls/min/1.73m2 according to Levey et al (1999) and Renal Association (2013).

3.12 Quality assurance

Research assistants were registered nurses and clinical officers. A week prior to conducting the study the principal investigator took the research assistants through the process of proper administration of consent and data collection to ensure proper adherence to laid out procedure. The principal investigator provided direct supervision throughout the study period. Recommended procedures for specimen collection in an aseptic technique, proper labeling and storage were adhered to. Laboratory tests were be done at the KNH biochemistry unit laboratory which put in place the following quality assurance processes; i). Qualified staff duly licensed by the Kenya Medical Laboratory Technicians and Technologist Board ii). All machines and procedures have operation manuals accessible for quick reference. The chemistry analyzer and other machines undergo periodic calibrations as per manufacturer's instructions. The laboratory run quality control tests before samples are analyzed. The laboratory is ISO 9001:2008 certified.

3.13 Data management and statistical analysis

To ensure good quality, data was collected uniformly. A quality assured laboratory was used for blood and urine testing and a statistician assisted in data analysis. Data recorded in the data collection tools was kept confidential and stored safely (under lock and key) by the principal investigator. Data was collected at the clinic and entered into a questionnaire (appendix 1). This was then be entered into a windows Excel data base. Cleaning was be carried out after entry using frequency distributions and cross-tabulations until no more errors were detected. Statistical products and service solutions (SPSS) version 10.0 were used for data analysis**.** Baseline continuous and categorical variables were expressed as mean (SD) and frequency (percentage), respectively. Between-group comparisons will be performed using independent t test or one-way ANOVA for continuous variables and Chi square test for categorical variables. Correlation of numeric data was done by Pearson's correlation (r). Statistical significance was set at $p < 0.05$

3.14 Ethical considerations

The study was undertaken after approval by the Department of Human Pathology and the KNH/UON ethics and research committee.

The objectives and of the study were clearly explained to eligible participants in a language suitable to them and consent was sought before the study commenced prior to inclusion into the study. Only patients who gave informed consent were enrolled. Permission to analyze samples at KNH Biochemistry Laboratory was sought from the laboratory management in accordance with their research activity policy. Information gathered from the study participants was kept confidential. The study results wile disseminated to health care providers to aid in patient care.

4.0 RESULTS

4.1 Social Demographic Characteristics

A total of 171 T2DM patients who met the inclusion criteria were enrolled into the study between July and September 2019. Notably, majority of the participants 102 (59.6%) were of the female gender compared to the male gender 69 (40.4%)

Age was normally distributed with a mean (±SD) age of 55 (12.4) years and a range between 23-81 years. All the study participants were grouped into 4 groups according to their age as shown in Table 2**.** The age range of first group was less than 40 years with a total of 24 (14%) participants. The Second age group consisted of 41 (24%) participants with age ranging from 40 years to 49 years. The third age group consisted of individuals between 50 years to 59 years with a total of 49 (28.7%) study subjects. The age group consisting of individuals aged above 60 years was the most populated having 57 (33.3%) study participants as shown on Figure 4.

Of the population recruited, 68 (39.8%) had attained secondary education. Most of the participants were employed 100 (58.5%) as summarized in Table 2

Table 2 Summary of demographic characteristics of study participants

Variable n=171		Frequency (n)	Percentage (%)
Gender	Female	102	59.6
	Male	69	40.4
Age Groups (years)	<40	24	14
	40-49	41	24
	50-59	49	28.7
	>60	57	33.3
Highest Level of Education	No formal education	16	9.4
	Primary	57	33.3
	Secondary	68	39.8
	Tertiary	30	17.5
Employment Status	Unemployed	54	31.6
	Employed	100	58.5
	Retired	17	9.9

4.2 Clinical profile of Study Participants

4.2.1 Duration of T2DM

The participants had been diagnosed with T2DM for varying duration of time, ranging from 1 Year to 35 years with a median of 8 years. Majority of the participants had the condition for 5 years or less; this is shown in Figure 4.

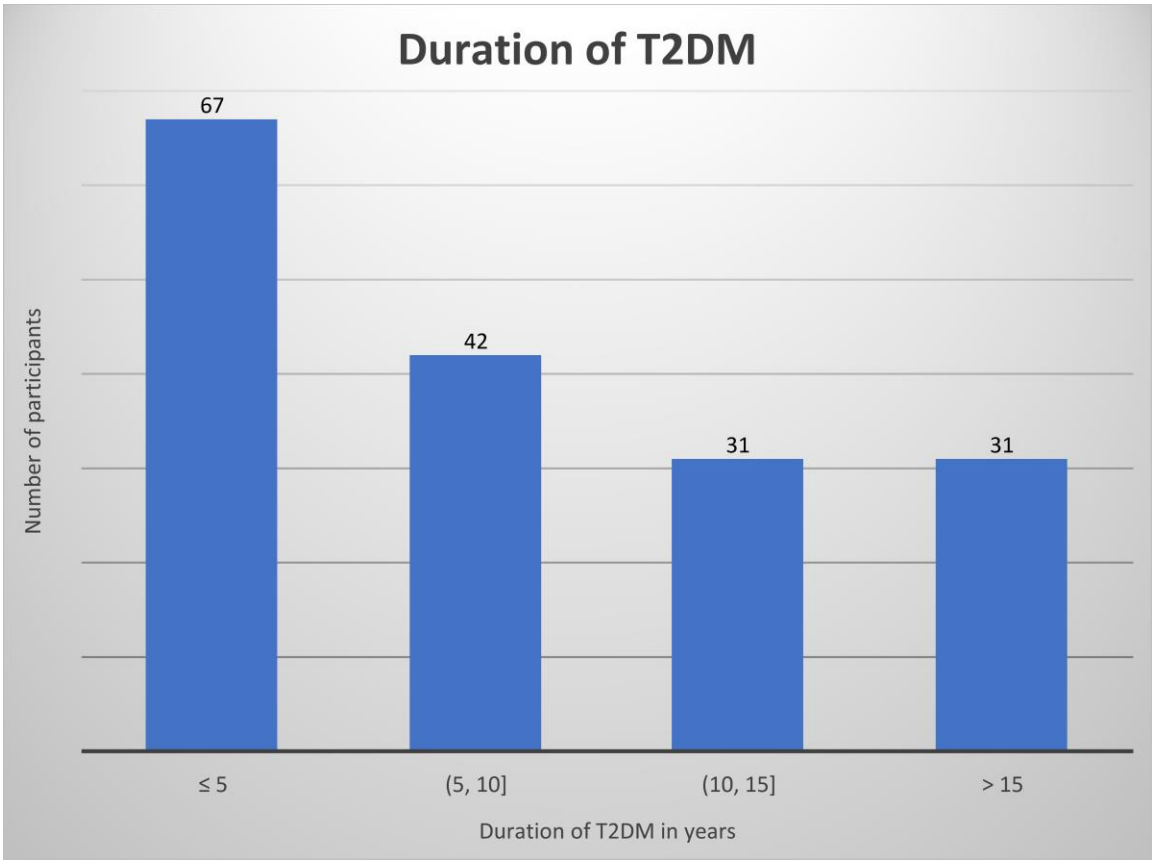

Figure 4 Histogram showing duration of T2DM in years since diagnosis

4.2.2 Type of treatment

Majority 75 (43.9%) of the participants were on Oral Glucose Lowering Agents (OGLA) followed by those on combination therapy consisting of OGLA and insulin at 71 (41.5%). Twenty-five patients (14.6%) were on insulin-based regimen as monotherapy for glycemic control as summarized on Table 3 above.

4.2.3 Family history of T2DM

Out of the 171 study participants, 122 (71.3%) had a family history of T2DM also shown on Table 3 below.

Table 3 Clinical Profile of Study Participants

Variable (n=171)		Frequency (n)	Percentage (%)
Type of Treatment	OHA	75	43.9
	Insulin	25	14.6
	OHA/Insulin	71	41.5
Family History of T2DM	Yes	122	71.3
	No	49	28.7

Laboratory Findings

4.3.1 Thyroid Function tests

The mean (±SD) TSH and FT_4 levels were at 2.41 (1.8) IU/ml and 15.38 (2.84) pmol/l respectively and were within normal reference range. The prevalence of thyroid dysfunction in this study was 10.5% as shown in Table 4.

Table 4 Serum thyroid biochemical profile of the study participants

Variable	Mean (±SD)	Range
TSH (mIU/ml)	2.41 (1.8)	0.20-11.05
FT4 (pmol/l)	15.38 (2.84)	1.69-23.80

Variable	n=171 (%)
Thyroid function status	
Dysfunction	18 (10.5%)
Euthyroid	153 (89.5%)

4.3.2 Thyroid hormone profile of study participants

In the present study a total number of 171 patients with T2DM underwent thyroid function test including serum TSH and free T_4. Based on these biochemical values, the results indicated that 18 (10.5%) out of the 171 T2DM subjects studied had thyroid dysfunction. A hundred and fifty three (89.5%) were euthyroid (serum TSH, serum freeT4 within normal limits), 11 (6.4%) patients had subclinical hypothyroidism (serum TSH > 5mIU/ml with normal levels of serum freeT4), 4 (2.3%) had overt hypothyroidism (serum TSH > 5 mIU/ml, and serum freeT4 < 10 pmol/L). This is summarized on Table 5. This study did not record any subclinical hyperthyroidism or secondary hyperthyroidism (table or figure).

4.3.2.1 Prevalence of subclinical hypothyroidism

Out of the 171 participants enrolled in the study, 11 had subclinical hypothyroidism. This gave a prevalence of 6.4%.

As summarized on Table 5, seven out of eleven participants with subclinical hypothyroidism were females while 4 were males. Therefore, the percentage of subclinical hypothyroidism among female and male patients were 4.1% and 2.3% respectively.

Table 5 Thyroid hormone profile of study participants

Thyroid Profile	Male (n=69)	Female(n=102)	Total (n=171)
Euthyroid	63 (36.8%)	90 (52.6%)	153 (89.5%)
Subclinical hypothyroidism	4 (2.3%)	7 (4.1%)	11(6.4%)
Overt hypothyroidism	0 (0%)	4 (3.9%)	4 (2.3%)
Secondary hypothyroidism	1(0.6%)	1 (0.6%)	2 (1.2%)
Subclinical hyperthyroidism	0 (0%)	0 (0%)	0 (0%)
Overt hyperthyroidism	1(0.6%)	0 (0%)	1(0.6%)
Secondary hyperthyroidism	0(0%)	0 (0%)	0 (0%)

4.3.2.2 Distribution of TSH levels among patients with SCH

Of patients with SCH, 81.8% had a serum TSH between 5 to 10 mIU/L while 18.2% had TSH levels above 10 mIU/L. This is summarized in figure 5 below

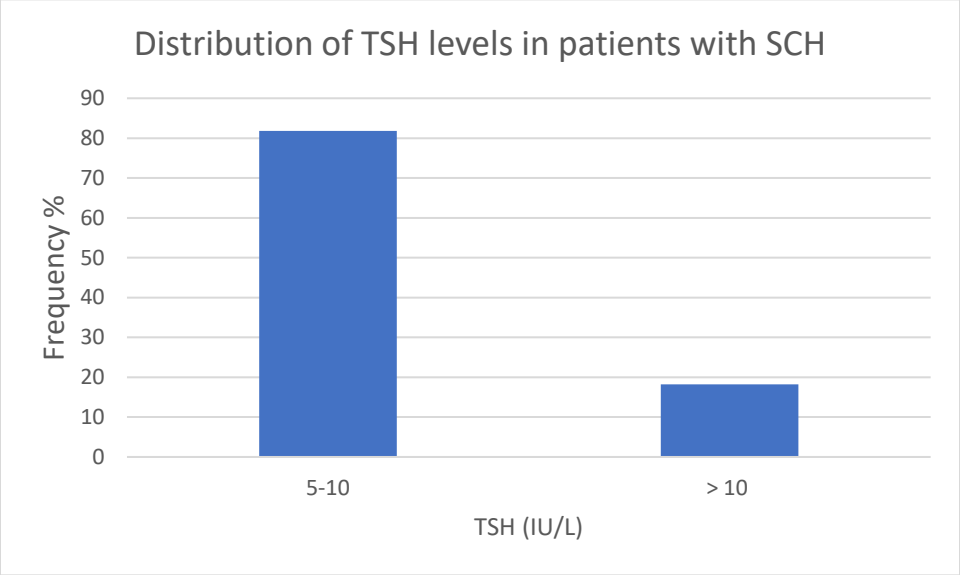

Figure 5 Distribution of serum TSH levels among T2DM patients with SCH

4.3.3 Renal Function tests

Out of the 171 study participants, 160 (93.5%) had normal serum creatinine levels (120 ≤ μmol/L). 11 (6.4%) participants had elevated serum creatinine levels (>120 μmol/L). The mean (±SD) serum creatinine was 85.59 (±22.88) μmol/L.

The estimated glomerular filtration rate was calculated using the MDRD formula and ranged from 22.4 to 147.6 mL/min/1.73m2 with a mean of 93.15(±23.27).

Based on their urinary albumin: creatinine ratio, the 171 participants were divided into three categories: normal albuminuria (<30 (mg/g creatinine), microalbuminuria (30-299 mg/g creatinine), and macroalbuminuria (>300 (mg/g creatinine).

A summary of baseline renal parameters is presented in table 6 below

Table 6 Baseline renal parameters

Variable	Mean (±SD)	Median	Range
Serum Creatinine (μmol/L)	85.59±22.88		60-255
eGFR (mL/min/1.73m2)	93.15±23.27		22.4-147.6
UACR		0-197.1	0-1326.2

4.3.3.1 Prevalence of Diabetic Nephropathy among patients with type 2 Diabetes Mellitus

Diabetic nephropathy was determined by the presence of macroalbuminuria and /or low eGFR of< 60 mL/min/1.73 m2. The prevalence of microalbuminuria was 58 (34%) of the participants and 18 (10.5%) had macroalbuminuria.

Of the total participants screened, 13 (7.6%) had eGFR< 60 mL/min/1.73 m2 as summarized on Table 7.

Of the 171 patients studied, the overall diabetic nephropathy prevalence was 26 (15.2%) of whom 13 (7.6%) patients had macroalbuminuria only, 8 (4.7%) patients had low eGFR and 5 (2.9%) had both macroalbuminuria and low eGFR

Table 7 Prevalence of Diabetic Nephropathy among patients with type 2 Diabetes Mellitus

eGFR ml/min/1.73 m^2	Normal albuminuria <30	Moderate 30-300	Severe >300	Total n=171
≥90	59 (34.5%)	29 (17%)	5 (2.9%)	93 (54.4%)
60-89	31 (18.1%)	26 (15.2%)	8 (4.7%)	65 (38%)
<60	5 (2.9%)	3 (1.8%)	5 (2.9%)	13 (7.6%)
Total	95 (55.5%)	58 (34%)	18 (10.5%)	11 (100%)

4.3.3.2 Prevalence of Diabetic Nephropathy among patients with type 2 Diabetes Mellitus

Among the 11 T2DM patients with SCH, 3 had normal albuminuria, 7 had moderately increased albuminuria and only 1 had severely increased albuminuria.

A total of 4 T2DM patients with SCH had either severely increased albuminuria and/or eGFR <60 mL/min/1.73 m2 as summarized on table 8 below.

4.4. Between group comparisons

4.4.1. Age distribution between euthyroid and subclinical hypothyroidism T2DM patients

Age distribution was studied in subclinical hypothyroid T2DM patients in relation to the euthyroid group. Among the 11 SCH patients, 1 (9%) patient was below 40 years of age, 3 (27.3%) were in the age group of 40 to 49 years, 4 (36.4%) were in the age group of 50 to 59 years and 3 (27.3%) patients were above 60 years of age as shown below on Table 9.

Among the various age groups, analysis revealed that majority of the patients with SCH fell in the age group between 50 to 59 years and the lowest prevalence of SCH was in the participants aged below 40years.

The mean age of T2DM with SCH patients was 51.27 (SD ±9.90) years, whereas the mean age in euthyroid patients was 55.17 (SD ±12.49) years, the difference not being statistically significant (p value: 0.313).

Table 8 Distribution of age in years between the Euthyroid and SCH group

Age group	Euthyroid (n=153)		SCH (n=11)	
	Frequency (n)	Percent (%)	Frequency (n)	Percent (%)
< 40 years	22	14.4	1	9
40-49 years	37	24.2	3	27.3
50-59 years	43	28.1	4	36.4
>60 years	51	33.3	3	27.3
Total	153	100	11	100

4.4.2 Clinical and hormonal profile in euthyroid and subclinical hypothyroidism group

Results of laboratory parameters obtained in euthyroid patients with T2DM were compared with those of the SCH group by statistical analysis using SPSS software and summarized in Table 9 below. Independent 't' test was used to compare the means between two independent groups. A p-value of < 0.05 was considered statistically significant

Table 9 Between group comparisons of the variables between euthyroid and subclinical hypothyroidism

Parameters	Euthyroid (n=153)		SCH (n=11)		P value
	Mean	SD	Mean	SD	
TSH	2.07	1.04	7.75	1.95	<0.05*
FT4	15.36	2.34	14.38	3.60	0.395
Serum Creatinine	84.46	19.27	100.46	53.50	0.346
eGFR	93.62	22.75	90.86	28.79	0.703
Microalbuminuria	143	47.03	190.17	80.80	0.152
Macroalbuminuria	643.57	459.63	528.80	120.63	0.731
UACR	167.29	312.94	199.87	195.96	0.734

4.4.2.1 TSH and fT4 levels in the euthyroid and SCH group

Serum TSH was significantly higher in the SCH group than the euthyroid group (P value <0.05) as shown on graph below. No significant difference was found in Free T_4 between the two groups.

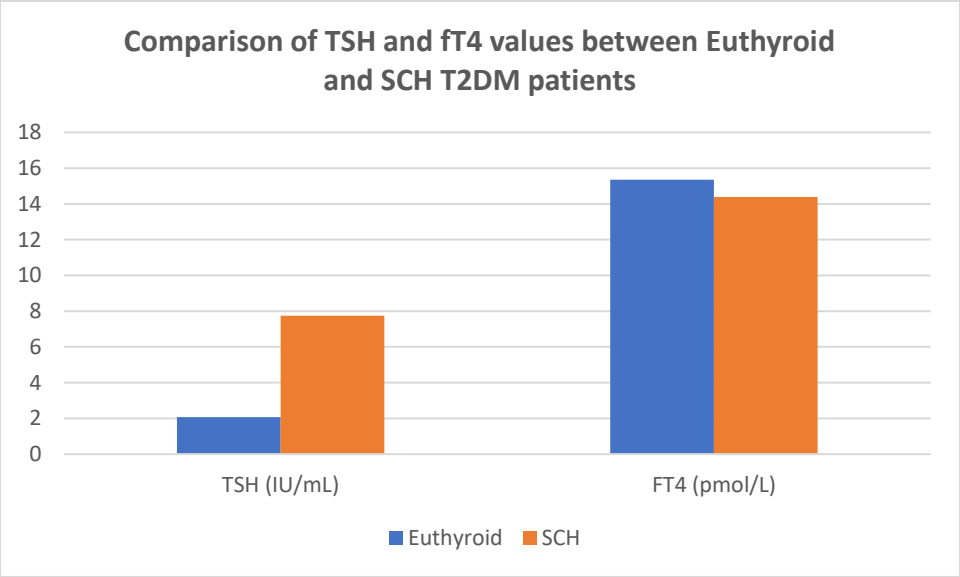

Figure 6 TSH and FT4 levels in the euthyroid and SCH group

4.4.2.2 Comparison of creatinine, eGFR and UACR between the euthyroid and SCH group

As shown on Figure 8 below, Serum creatinine and urinary albumin: creatinine ratio was higher in the SCH than in the euthyroid group though the difference was not statistically significant. eGFR was also found to be slightly lower in the SCH group but the difference was not statistically significant.

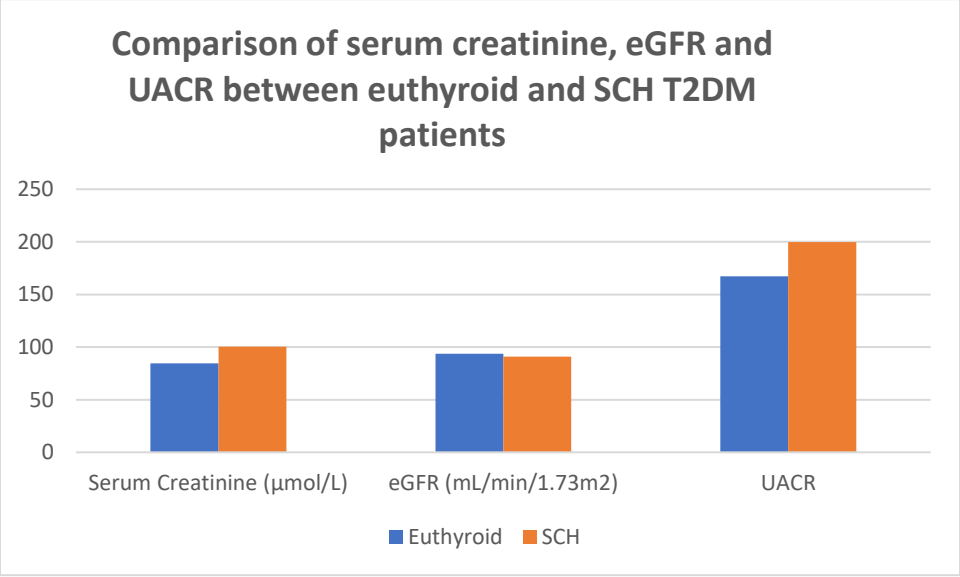

Figure 7: Graph comparing serum creatinine, eGFR and UACR between the euthyroid and SCH group

4.4.2.2 UACR distribution in T2DM Euthyroid patients and patients with SCH

Of the 153 T2DM subjects that were Euthyroid, 92 fell into the normal Albuminuria range, 51 had microalbuminuria and 17 had macroalbuminuria.

Among the 11 patients with SCH, 2 had normal albumin excretion. 7 had microalbuminuria and 2 had microalbuminuria

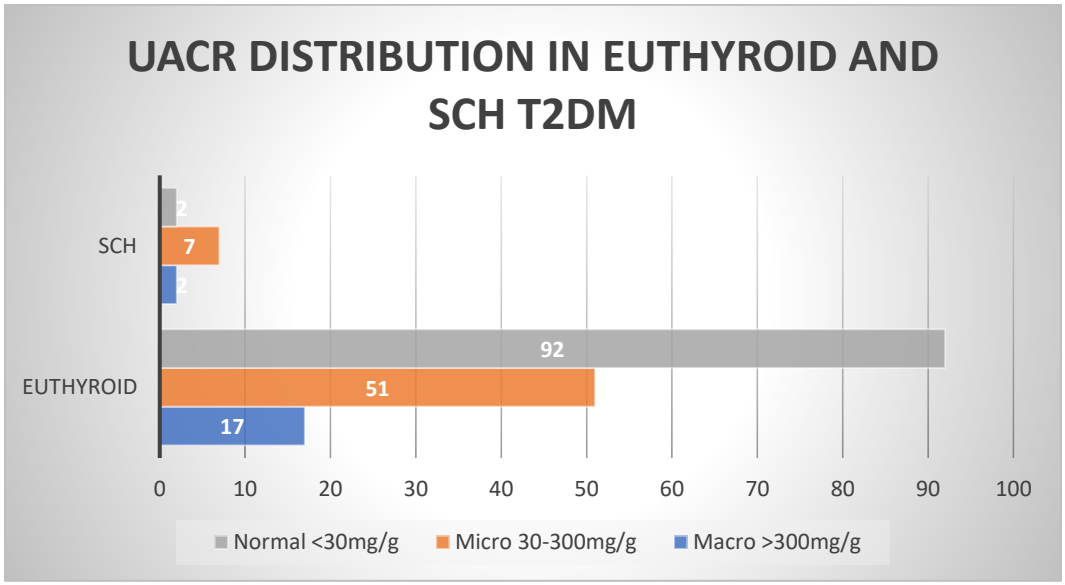

Figure 8: Graph showing UACR distribution between euthyroid and SCH group

4.5 Correlation between TSH and related renal indexes

4.5.1 Correlation between TSH and related renal indexes

A Pearson correlation was conducted to evaluate the null hypothesis that there is no relationship between eGFR and TSH (n=171). Although it was not significant, analysed data shows a weak, negative association between TSH (mean 93.2, SD 23.2) and eGFR (93.15, SD 23.27), r=-0.021 (*p=0.783*). Higher levels of TSH are associated with a low eGFR.

4.5.1 Correlation between TSH and UACR

A Pearson correlation was conducted to evaluate the null hypothesis that there is no relationship between TSH and UACR (n=171). Analysed data conclude there was a weak, positive association between TSH (mean 2.42, SD 1.82) and UACR (166.17, SD 300.63), r=0.034 (*p=0.663*). Higher levels in TSH are associated with higher levels in UACR.

5.0 Discussion

This study was conducted at Kenyatta national hospital diabetes and endocrinology clinic between July 2019 and October 2019. The purpose was to determine the prevalence of subclinical hypothyroidism and its correlation with diabetic nephropathy in patients with diabetes mellitus.

The study population was predominantly mature adults, with a female preponderance at 59.6%. The mean (±SD) age was 55 (±12.44) years and duration of diabetes was 8.6 years on average.

The study evaluated 171 patients overall. 18 (10.5%) of the study participants had thyroid dysfunction and 153 (89.5%) were euthyroid. A study done by Tagoe et al., (2014) which recorded 10.1 % prevalence of thyroid dysfunction among Ghanaian diabetes mellitus population attending the Korle-Bu Teaching Hospital in Accra was in agreement to the results of the current study. However, in Nigeria and India, Udiong et al., (2007) and Maaz et al., (2018) reported 46.5% and 28.0 % respectively among diabetic subjects with thyroid dysfunctions; rates much higher than what was recorded in the current study. The difference in the rate observed in this study and the previous studies cited above could be attributed to difference in population characteristics such as race, sex, and age as well as methodological differences. These factors have been shown to be determinants of a wide range of prevalence reports of thyroid dysfunction among persons with diabetes mellitus (Moayeri and Rabbani, 2004).

Euthyroidism, subclinical hypothyroidism, overt hypothyroidism, secondary hypothyroidism and overt hyperthyroidism were conclusive findings in this present study. The prevalence of SCH and overt hypothyroidism reported was 6.4%, and 2.3% respectively. Only 0.6% had overt hyperthyroidism. There were no findings of subclinical hyperthyroidism or secondary hyperthyroidism indicating that hyperthyroidism is not very common in patients with T2DM. Consistent with the other studies, it was observed that Subclinical hypothyroidism was the most common thyroid dysfunction followed by hypothyroidism. Several studies either excluded participants with previously known thyroid disease (Kim *et al*.,2011) or performed a sub-analysis on this group (Diez *et al*.,2011).

In these circumstances, subclinical hypothyroidism was clearly the predominant finding, suggesting that most of the undiagnosed thyroid disease would be of this type. This observation was consistent with those made in the general population (Garmendia *et al*.,2014).

Over the past 10 years, prevalence of SCH among T2DM patients has drawn much of attention, due to the suspected exacerbation of diabetes-related vascular complications. The prevalence of subclinical hypothyroidism in the present study was found to be 6.4 % in type 2 diabetic patients. The results are comparable to a retrospective study carried out in Calabar, Nigeria which found the prevalence to be 6.3% () In a meta-analysis including 7378 diabetic subjects from 17 studies, Han et al.(2015) reported prevalence rate of 4.69–18.86 % for SCH. Significant heterogeneity and publication bias were observed among the studies; thus, they estimated the pooled SCH prevalence to be 10.2 % (95 % CI 4.7–15.7 %) after the correction.

It is well documented that SCH is more common among women than men, in both general and diabetic populations (Cooper *et al*.,2001) In the present study prevalence of subclinical hypothyroidism was 2.3 % in male patients and 4.1 % in female patients. However, the difference in gender between euthyroid and SCH in T2DM patients was not statistically significant. Results have been inconsistent among the published studies. For example, Furukawa *et al*.2014 did not find any gender differences between the euthyroid and SCH groups, while others found female preponderance (Chen *et al*., 2007, Han *et al*.,2015 Mansournia *et al*.,2017)

The gold standard in the diagnosis of diabetic nephropathy is renal biopsy. In this study, 58(33.9%) patients with microalbuminuria (UACR 30-300 mg/g) were classified as having incipient diabetic nephropathy. Microalbuminuria (MA) is the first clinically detectable stage of renal insult and patients with MA are referred to as having incipient nephropathy (ADA). Reported prevalence rates of MA in Sub Saharan Africa varies widely between 9.8%–45.7% among T2DM (Lutale *et al*.,2007, Ogbu *et al*.,2013, Muddu *et al*.,2019). This variation may be due to differences in the definition of MA, and the methods of collecting a urine specimen (Newman *et al*.,2005). The prevalence of Microalbuminuria in the present study is 33.9%. This finding is clearly lower than rates reported by Ufuoma *et al*.,2016, in Nigeria. Their study reported a prevalence of 57%. The difference could be attributed to social economic disparity and disparities in access to health care.

Among the 58 T2DM patients with microalbuminuria, 7(12.1%) had SCH. This finding is higher than a Japanese study that found a prevalence of 8.4% (Furukawa *et al*.,2014) but much lower than

an Iranian study that found the prevalence to be 39.1%. Majority (7/11) of the T2DM patients with SCH had microalbuminuria. SCH is a well-known risk factor of overt hypothyroidism () Overt hypothyroidism is also a well-known risk factor for renal dysfunction in the general population (). Furthermore, A study by Roy *et al*.,2019 found that persons with poor control of glycemic status with SCH progressed to develop DN when compared to those without SCH. Though glycemic control was not assessed. In this study, poor glycemic control has been reported in Kenyan studies as well as other sub Saharan countries like South Africa and Ethiopia (). Intervention at this stage of microalbuminuria can therefore retard or reverse the progression to overt nephropathy.

Overt diabetic nephropathy in this study was defined as urinary albumin creatinine excretion rate > 300mg/g creatinine or eGFR<60 mL/min/1.73m2. The prevalence of DN among the study participants was found to be 15.2%. A similar definition was used by Furukawa *et al*., (2014) who found the prevalence of DN among T2DM patients to be 7%. Ayman *et al*., (2018) from Egypt reported a prevalence of 8.22%. However, their study did not include patients with an eGFR below 60 mL/min/1.73m2 in the DN definition. A much higher prevalence was reported in studies that used a urinary albumin creatinine excretion rate > 30mg/g creatinine as the definition of DN. For example, a study in Iran and China reported a prevalence of 41.2%, and 40.6% respectively. (Mansournia *et al*.,2016, Zhao *et al*.,2018) A local study reported a prevalence of 42.5% in the same study population (Nyamai *et al.*,2014) This large disparity in the prevalence of DN could also represent methodological differences as well as differences in the genetic make-up of the population.

Among the 26 T2DM patients with DN 4 (15.4%) had SCH. The results were comparable to Jing *et al.*, (2016) who found a prevalence of 14.5%. A higher prevalence was reported by Furukawa *et al.*, (2014), Mansournia *et al.*, (2016) who found a prevalence of 20.7% and 27.6% respectively. Zhao et al., (2018) reported a lower prevalence compared to the present study of 10.8%.

Vascular endothelial dysfunction is believed to be an important factor in the pathogenesis of microvascular and macrovascular complications in diabetes. Endothelial dysfunction in Type 2 DM occurs as a result of hyperglycemia, growth factors, vasoactive agents, and components of metabolic syndrome (Schalkwijk *et al.*,2005). Endothelial dysfunction has also been reported in subclinical hypothyroidism as well as well as in those with upper normal TSH values (Vargas *et al*.,2006, Vignera *et al*., 2012, Ghada *et al*.,2016). The association between diabetic nephropathy

and subclinical hypothyroidism has not been extensively investigated. Among the few studies done, the findings have been contradictory. A Chinese and a Japanese study reported an association between subclinical hypothyroidism and diabetic nephropathy (Chen *et al*.,2007, Furukawa *et al*.,2014). Yasuda *et al*, (2016) found an association between subclinical hypothyroidism and albuminuria. Kim *et al*., (2011) did not find an association between subclinical hypothyroidism and diabetic nephropathy. An Iranian study failed to show a correlation between subclinical hypothyroidism and diabetic nephropathy (Shokoufeh *et al*.,2017)

In the present study, no correlation was found between subclinical hypothyroidism and DN in type 2 diabetes. The present study showed that there was a weak inverse correlation between TSH and eGFR. In addition, analysed data concluded there was a weak, positive association between TSH and UACR. Discrepancy in results may depend on characteristics of enrolled subjects, genetic make-up of the population and differences in management of T2DM. For example, the mean age of participants with SCH in this study was (51.27±9.9) years which is younger than the Chinese and Japanese study (67.2±10.8 and 63.7±11.1). The mean duration of diabetes mellitus in patients with SCH in the present study was also shorter (7.36±6.83) than the two studies that had a mean duration of (10.3±9.2) and (9.7±7.6) years respectively.

5.1 Limitations

- This is a cross-sectional survey and doesn't allow for review of long-term outcomes
- The study was done on patients from a single hospital only, therefore the results may not fully describe the entire population of patients with type 2 diabetes mellitus

5.2 Conclusions

1. The prevalence of sub clinical hypothyroidism in type 2 diabetes mellitus patients attending Kenyatta National Hospital Diabetic and Endocrinology clinic is 6.4%.
2. The most common thyroid dysfunction among patients with type 2 diabetes mellitus is sub clinical hypothyroidism
3. Out of the 11 type 2 diabetes mellitus study participants with sub clinical hypothyroidism, 4 had diabetic nephropathy
4. The study did not find a correlation between sub clinical hypothyroidism and diabetic nephropathy among type 2 diabetes mellitus patients at Kenyatta National Hospital

5.3 Recommendation

The study presents evidence of subclinical hypothyroidism among patients with type 2 diabetes mellitus at Kenyatta National Hospital Diabetic and Endocrinology. The percentage of Subclinical hypothyroidism found in this study is however not sufficient for screening to be recommended in diabetic patients.

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APPENDICES

APPENDIX I: SCREENING PROFOMA

STUDY NUMBER …….

PREVALENCE OF SUB-CLINICAL HYPOTHYROIDSM AND ITS CORRELATION WITH DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Tick as appropriate for positive response	**Yes**	**No**
1. Have you ever been diagnosed with a thyroid disorder?	☐	☐
2. Have you ever had a thyroid gland surgery?	☐	☐
3. Have you ever been on radio iodine therapy?	☐	☐
4. Are you on any medication for thyroid disorder?	☐	☐
5. Have you been diagnosed with any of the following?		
a. Non-diabetic Renal disease	☐	☐
b. Myocardial infarction	☐	☐
c. Neuropathy	☐	☐
d. Eye problems	☐	☐
e. Diabetic foot/un-healing ulcer	☐	☐

Others (specify)_____

For official use

Recruited ☐ Yes ☐ No

APPENDIX II: QUESTIONNAIRE

PREVALENCE OF SUBCLINICAL HYPOTHYROIDSM AND ITS CORRELATION WITH DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Study No…… Date……

Social demographic characteristics

1. Gender
   a. Male ☐
   b. Female ☐
2. Age (years) ☐☐☐
3. Level of education
   a. No formal education ☐
   b. Primary ☐
   c. Secondary ☐
   d. Tertiary ☐
   e. Others (specify) _____
4. Occupation
   a. Self employed ☐
   b. Formal employment ☐
   c. Casual employment ☐
   d. unemployed ☐

Past medical history

5. What is your current smoking status?
   a. Never Smoked ☐
   b. Ex-smoker ☐
   c. Current smoker ☐
      i. How many sticks do you smoke? ☐1-15/day ☐>15/day
6. Duration of Diabetes (in years) ☐

7. For how long have you been attending this clinic? _____months

8. Are you currently on any medication? ☐Yes ☐No

9. If yes, please specify
   a. Oral hypoglycaemic agent ☐
   b. Insulin formulation ☐
   c. Blood pressure lowering drugs ☐
   d. Others (specify) _____

Family history

10. Did or do any of your relatives suffer from diabetes? ☐Yes ☐No
11. If yes, please specify ☐Parents ☐Sibling ☐other
12. Did or do any of your relatives suffer from Kidney disease? ☐ Yes ☐ No
13. Do you know anyone in your family that has a thyroid disease? ☐ Yes ☐ No

    If yes, how are they related to you? _____

Physical examination

Signs and symptoms of thyroid disorders

1. Presence of a goitre Yes ☐ No ☐
2. Sensitivity to cold Yes ☐ No ☐
3. Puffy face Yes ☐ No ☐
4. Poor memory Yes ☐ No ☐
5. Constipation Yes ☐ No ☐
6. Weight gain Yes ☐ No ☐

7. Depression Yes ☐ No ☐

8. Dry skin Yes ☐ No ☐

9. Hoarse voice Yes ☐ No ☐

10. Fatigue Yes ☐ No ☐

11. Protruding eyes Yes ☐ No ☐

14. Laboratory findings

TEST	UNITS	REF RANGE	VALUE	NORMAL	ABNORMAL
1. Microalbumin	(mg/g creatinine)	30-300			
2. Creatinine	(µmol/l)	50-110			
3. eGFR	(mL/min/1.73m^2)				
4. TSH	(mIU/L)	0.27-4.2			
5. FT4	(pmol/L)	12-22			

14.Laboratory findings from the file

HbA1C levels (%) ☐

15. Diagnosis (Tick appropriately)

i. Euthyroid ☐

ii. Primary hypothyroidism ☐

iii. Secondary hypothyroidism ☐

iv. Sub clinical hypothyroidism ☐

v. Primary hyperthyroidism ☐

vi. Secondary hyperthyroidism ☐

vii. Sub clinical hyperthyroidism ☐

viii. Atypical profile ☐

DODOSO II

PREVALENCE OF SUBCLINICAL HYPOTHYROIDSM AND ITS CORRELATION WITH DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

DODOSO

Nambari maalum…… Tarehe……

Habari za Jumla

1. Jinsia
   c. Mwanaume ☐
   d. Mwanamke ☐
2. Umri (miaka) ☐☐☐
3. Level of education
   f. No formal education ☐
   g. Primary ☐
   h. Secondary ☐
   i. Tertiary ☐
   j. Others (specify) _____
4. Occupation
   e. Self employed ☐
   f. Formal employment ☐
   g. Casual employment ☐
   h. unemployed ☐

Taarifa kuhusu hali ya afya ya mshiriki

5. Je unavuta sigara?
   a. Sijawahi vuta sigara ☐
   b. Mvutaji wa zamani ☐
   c. Mvutaji sasa ☐
      i. Unavuta sigara ngapi kwa siku? ☐1-15 kwa siku ☐>15 kwa siku

6. Umeugua ugonjwa wa sukari kwa muda gani (kwa miaka) ☐

7. Umekua ukihudhuria hii kliniki kwa muda gani? _____miezi

8. Mshiriki anatumia madawa yoyote? ☐Ndio ☐Apana

9. Kama ndio taja
   e. Oral hypoglycaemic agent ☐
   f. Insulin formulation ☐
   g. Blood pressure lowering drugs ☐
   h. Zingine (taja) _____

Historia ya familia

10. Mshiriki ana jamaa walio na ugonjwa wa sukari? ☐Ndio ☐ La
11. Kama ndio, taja ☐Wazazi ☐Ndugu ☐Wengine
12. Mshiriki ana jamma walio na ugonjwa wa figo? ☐ Ndio ☐ La
13. Je mshiriki anafahamu mtu yeyote kwa familia anaye ugua ugonjwa wa tezi?

☐ Ndio ☐ La

Kama ndio, mna uhusiano gani? _____

Physical examination

Signs and symptoms of thyroid disorders

12. Presence of a goitre Yes ☐ No ☐

13. Sensitivity to cold Yes ☐ No ☐

14. Puffy face Yes ☐ No ☐

15. Poor memory Yes ☐ No ☐

16. Constipation Yes ☐ No ☐

17. Weight gain Yes ☐ No ☐

18. Depression Yes ☐ No ☐

19. Dry skin Yes ☐ No ☐

20. Hoarse voice Yes ☐ No ☐

21. Fatigue Yes ☐ No ☐

22. Protruding eyes Yes ☐ No ☐

16. Laboratory findings

TEST	UNITS	REF RANGE	VALUE	NORMAL	ABNORMAL
1. Microalbumin	(mg/g creatinine)	30-300			
2. Creatinine	(µmol/l)	50-110			
3. eGFR	(mL/min/1.73m^2)				
4. TSH	(mIU/L)	0.27-4.2			
5. FT4	(pmol/L)	12-22			

13. **Laboratory findings from the file**

HbA1C levels (%) ☐

17. Diagnosis (Tick appropriately)

i. Euthyroid ☐

ii. Primary hypothyroidism ☐

iii. Secondary hypothyroidism ☐

iv. Sub clinical hypothyroidism ☐

v. Primary hyperthyroidism ☐

vi. Secondary hyperthyroidism ☐

vii. Sub clinical hyperthyroidism ☐

viii. Atypical profile ☐

APPENDIX III: CONSENT FORM

PREVALENCE OF SUBCLINICAL HYPOTHYROIDSM AND ITS CORRELATION WITH DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

INVESTIGATOR: ANNETTE WANGUI IRARI

Position: Post graduate student, Department of Human Pathology, University of Nairobi

Introduction

The investigator is conducting a research study to investigate the prevalence of subclinical hypothyroidism in patients with type two diabetes mellitus.

Objective of the study: The study will provide information

Benefits – the benefit involved is that the laboratory test will be done at no cost to you. The findings of the laboratory tests will form part of your usual care. Copies of the test results will be made available to your physician who will use it to institute appropriate management.

Risks- the only risk involved is a slight discomfort at the needle prick site. Rarely swelling or bleeding may occur at the site. My assistant will however make sure that bleeding has stopped before you leave.

Compensation: There will be no compensation for participating in this study. The tests will be done free of charge.

Participation is voluntary: Taking part in this study is completely voluntary. If you decide not to participate, it will not affect the quality of care you receive at the clinic and there will be no victimization. If you decide to participate, you can withdraw at any time.

Confidentiality – information obtained from this study will be kept private. The records will be kept in a secure lock cabinet/ password protected computer only the researchers will have access to the records.

Procedures: You are being asked to participate in this survey that will take about 30 minutes. If you agree to participate, I will ask you to sign a consent form. I will ask you a series of questions in confidence and all your responses shall be noted down. Most of the questions have a yes or no answer and will require you to remember somethings from the past.

My assistant will conduct a physical examination to look for signs and symptoms of thyroid disorders. Thereafter she will collect a blood sample from you of about 5ml that will be taken to the laboratory for evaluation of TSH, FT4 and creatinine levels. A sample of urine measuring about 5ml will also be collected to determine protein excretion levels in your urine.

The test results will be revealed to you (recorded in your file) as soon as possible for your continued care. The results shall remain confidential.

If you have any questions: you may ask the following:

Name: Annette Irari

Mobile number: 0702266336

Email: wanguirari@gmail.com,

CONSENT DECLARATION

I have read the above information and have received answers to any questions I asked. I consent to take part in the study.

Name of participant_____

Signature of participant_____ Date_____

APPENDIX III: FOMU YA IDHINI

PREVALENCE OF SUBCLINICAL HYPOTHYROIDSM AND ITS CORRELATION WITH DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

MTAFITI: ANNETTE WANGUI IRARI

Cheo: Mwanafunzi wa shahada kuu katika chou kikuu cha Nairobi, Idara ya Pathologia

Kwa mhusika,

Mtafiti mkuu angependa kufanya utafiti kuhusu uwiano wa ugonjwa wa tezi na ugonjwa wa kisukari wa figo. Mgonjwa wa kisukari anaweza kuendeleza ugonjwa wa tezi na anavyozidi kuishi anaweza kupata matatizo kama ugonjwa wa kisukari wa figo. Utafiti huu utaweza kugundua kama kuna uwiano wa ugonjwa wa tezi na ugonjwa wa kisukari wa figo.Hii itasaidia kupata matibabu mapema kabla ya ugonjwa huu kuenea

Faida ya utafiti: Faida inayohusika ni kwamba mapimo kwa maabara itafanyika bila gharama. Matokeo yatayopatikana kutoka utafiti huu yatasaidia madaktari kugundua kama mgonjwa wa kisukari ambaye anaugua ugonjwa wa tezi ako kwa hatari ya kupata ugonjwa wa kisukari wa figo ili awekwe kwa matibabu mapema iwezekanavyo kuzuia kuenea kwa ugonjwa huu.

Hatari ya utafiti: Hatari tu itayohusika na utafiti huu ni usumbufu mdogo kutokana na pigo la sindano. Mara chache uvimbe au kutokwa damu huweza kutokea. Msaidizi wangu atahakikasha kwamba damu haitoki kabla ya kuondoka.

Fidia: Hakuna fidia ya kushiriki katika utafiti huu. Vipimo vyote vitafanywa bila malipo.

Kushiriki ni kwa hiari: Kushiriki kwa utafiti huu ni kwa hiari. Unapo amua kuacha kushiriki, haitaathiri ubora wa huduma unayopata kwenye kliniki na hakutakuwa na unyanyasaji. Unapo amua kushiriki, unaweza kuondoa wakati wowote.

Usiri– taarifa zitazopatikana kutoka utafiti huu zitahifadhiwa binafsi. Rekodi zitahifadhiwa kwa usalama.

Taratibu: Nitakuomba kushiriki katika utafiti huu ambao utachukua muda wa dakika thelathini. Unapo kubali kushiriki, nitakuomba kusaini fomu ya idhini. Nitakuliza mfululizo wa maswali kwa ujasiri na majibu yako yote yatafahamika. Maswali mengi yana ndiyo au La. Pia itahitaji kukumbuka mambo yaliyopita.

Msaidizi wangu atafanya uchunguzi wa kimwili ili aone ishara na dalili za matatizo ya tezi. Kisha atakusanya sampuli ya damu kutoka kwako kama 5ml ambayo itachukuliwa kwenye maabara kwa ajili ya tathmini ya viwango vya TSH, FT4 na viumbe vya ubunifu. Sampuli ya mkojo yenye kipimo cha 5ml pia itakusanywa ili kuangalia viwango vya protini vilivyo kwenye mkojo wako.

Utafahamishwa matokeo ya vipimo (itaandikwa kwenye faili yako) haraka iwezekanavyo kwa huduma yako ya kuendelea. Matokeo yatabaki siri.

Kama una maswali yoyote: unaweza kuuliza wafuatao:

Name: Annette Irari

Nambari ya simu: 0702266336

Email: wanguirari@gmail.com,

RIDHAA TAMKO

Nimesoma maelezo na nimepata majibu kwa maswali yote niliyoyauliza. Ninakubali kushiriki katika utafiti.

Jina la mshiriki_____

Saini ya mshiriki_____ Tarehe_____