ADDIS ABABA UNIVERSITY

COLLAGE OF HEALTH SCIENCES

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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ASSESSMENT OF RENAL FUNCTION IN PATIENTS WITH THYROID DYSFUNCTION ATTENDING AT ARSHO ADVANCED MEDICAL LABORATORY, ADDIS ABABA, ETHIOPIA.

BY: NARDOS ABEBE [BSc]

ADVISOR: MISTIRE WOLDE (PhD FELLOW)

ASSISTANT PROFESSOR

A Research thesis to be submitted to the School of Graduate Studies of Addis Ababa University, College of Health Science , Department of Medical Laboratory Sciences for the Partial fulfillment of Masters of Science (MSc) degree in Clinical Laboratory Sciences, Clinical Chemistry Specialty Track.

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Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented in Addis Ababa University or any other universities. I also declare that all sources of materials used for this thesis have been duly acknowledged.

Name of the candidate: Nardos Abebe (BSc)

Signature_____

Place: Addis Ababa University Department of Medical Laboratory Sciences, Addis Ababa; Ethiopia

Date of submission_____/_____/_____

This research thesis has been submitted with my approval as university advisor.

Name of advisor: Mr. Mistire Wolde(MSc, PhD Fellow)

Signature _____

Place: Addis Ababa University Department of Medical Laboratory Sciences, Addis Ababa; Ethiopia

Date of submission_____/_____/_____

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Abstract

Background: Studies demonstrated that abnormal thyroid functions may result decreased or increased kidney size, kidney weight, and affect renal functions. In this regard, studies on the association of abnormal thyroid functions and renal function tests are scarcely found in Ethiopia.

Objective: to assess renal functions among patients with thyroid dysfunction attending at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Methodology: cross sectional study was conducted from March 21/2015-May 27/2015 at Arsho Advanced Medical Laboratory. During the study period, 71 thyroid dysfunction patients were eligible, and socio demographic data collected by structured questionnaire. Then blood sample was collected for thyroid function tests, renal function and blood electrolyte analysis. The collected data was analyzed by SPSS version 20. ANOVA and binary logistic regression were employed to evaluate the mean deference and associations of thyroid hormone with renal function and electrolyte balances.

Result: Of the total 71 study participants, 54(76.1%) females, and mean age of the study participants were 41.9±9.7years of age. Among the study participants, euthyroid participants were relatively higher 29(41%) followed by hypothyroid participants 25(35%). Of the renal function tests, serum uric acid, and creatinine mean values were significantly decreased in hyperthyroid participants; whereas, eGFR mean value was significantly increased in hyperthyroid study participants($P<0.05$). Meanwhile, from electrolyte balance employed, only serum sodium mean value was significantly increased in hyperthyroid study participants. Binary logistic regression analysis on the association of thyroid dysfunction with electrolyte balance and renal function tests indicated that serum sodium, creatinine, eGFR values and hyperthyroidism have a statistical significant association at AOR 95% CI of 0.141(0.033-0.593, P=0.008); 16.236(3.481-75.739, P=0.001), and 13.797(3.261-58.67, P=0.001) respectively.

Conclusion: The current study reveals is, there is a significant association between thyroid abnormalities with renal functions and electrolyte balance. Knowledge of this significant association has worthwhile value for clinicians, and it may help to improve the health status of patients with thyroid abnormality.

Key word: Thyroid dysfunction, renal function tests, blood electrolyte.

List of abbreviations

ADH	Anti diuretic hormone
ANOVA	Analysis of variance
AOR	Adjusted odds ratio
ARF	Acute renal failure
ATPase	Adinosine triphosphase
BUN	Blood urea nitrogen
Ca^{++}	Calcium
CKD	chronic kidney disease
CI	Confidence Interval
Cl^{-}	Chloride
CNS	Central Nerves System
Cr	Creatinine
eGFR	estimated glomerular filtration rate
EPO	Erythropoietin
HPTA	Hypotalamic-pituitary-thyroid axis
ID-I	Iodothyronine deiodinase –I
ID-II	Iodothyronine deiodinase –II
ISE	Ion selective electrode
ISO	International standard organization
K^{+}	potassium
KDa	Kilo Dalton
Na^{+}	Sodium
NAD^{+}	Nicotinamide-Adenine-dinucleotide
NADH	Reduced Nicotinamide-Adenine-dinucleotide

Na^{+}-H^{+}	Sodium hydrogen
Na/k ATPase	Sodium potassium adinosine triphosphase
PVC	Polyvinyl chloride
RAAS	The rennin-angiotension-aldestrone system
RBF	Renal blood flow
RFT	Renal function test
RPM	Revaluations per minute
SD	Standard deviation
SST	Serum separator tube
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine binding globulin
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
UA	Uric acid
UAOR	Un-adjusted odds ratio
4VMDRD	four variables modification of diet in renal disease

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1 Chapter one

1.1 Introduction

Thyroid gland weights about 15-25gm, has structured as two lobes, and located at either side of the neck. Each lobe is composed of 20-40 follicles separated by highly vascular connective tissue (1). The follicles are ring-shaped structure, in which a single cell band of follicular cell surrounds a closed cavity containing colloid, thyroid hormone and thyroglobulin(2). The follicles are site of thyroid hormone synthesis and storage. The synthesis and secretion of thyroid hormones triiodothyronin (T3) and thyroxin (T4) are stimulated by TSH from the anterior pituitary gland, which is regulated by thyrotropin- releasing hormone (TRH) from the hypothalamus. Thyroxin (T4) is produced only by the thyroid gland, whereas tri-iodothyronine (T3), the more biologically active form of thyroid hormone, is produced primarily through local 5′-deiodination of T4 (1, 2). Under normal circumstances iodothyronine deiodinase -I in liver and kidney provides the main source of T3 to the circulation, whilst iodothyronine deiodinase –II is largely responsible for local T3 production in the CNS, brown adipose tissue and pituitary. In some circumstances ID-II in brown adipose tissue and ID-I in the thyroid may provide a significant source of plasma T3, and ID-I in the pituitary may be important for local T3 production in this gland. (3)

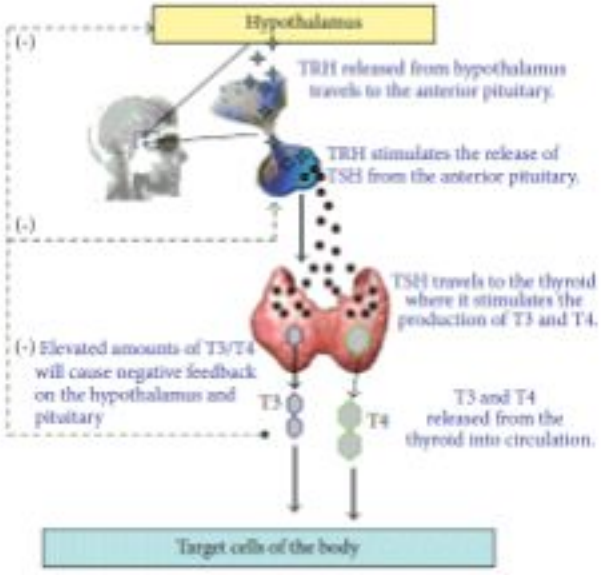

Figure 1 shows Thyroid Physiology (4).

The thyroid hormones play important role in human body. Thyroid hormones through alpha and beta receptors in each body cell, play a critical role in cell differentiation, development of the body, and help to maintain thermogenic and metabolic homeostasis in the adults. They are also necessary for growth and development of the kidney and for the maintenance of water and electrolyte homeostasis (4, 5).

Abnormalities in thyroid hormones synthesis, secretion or actions usually categorized as hypothyroidism, euthyroidism, or hyperthyroidism, depending on the concentrations of TSH, T3 and T4 hormones in serum. These thyroid function abnormalities may be associated with different complication, including disturbances in renal function tests and electrolyte balances. (1,6, 7, 8).Decreased hormone production (T3 and T4) will result a state of hypothyroidism, which is associated with many biochemical abnormalities including serum creatinine and uric acid (4). It was also marked by histological changes in glomerular structure and physiological changes including reduced renal blood flow, glomerular filtration rate (GFR) and absorption of sodium chloride and water (5, 8). And increased hormone production (T3 and T4) will be result in state of hyperthyroidism which is associated with many biochemical changes including urea (9). In addition, it was marked by histological changes in glomerular structure and physiological changes including increasing renal blood flow, glomerular filtration rate (GFR)(8, 9).

The kidney is the chief regulator of all body fluids and is primarily responsible for maintaining homeostasis, or equilibrium of fluid and electrolytes in the body. The removal of potentially toxic waste products such as nitrogenous wastes: urea, creatinine and uric acid are a major function of the kidneys and are accompanied through the formation of urine. The basic processes involved in the formation of urine are filtration, reabsorption and secretion. It also functions as endocrine functions: in the production and release of rennin and erythropoietin hormones (10).

On the removal of waste products of the body through renal system, each minute 1000-1500ml of blood pass through the kidney nephrons afferent arterioles into efferent arterioles. During this process substances filtered through the semi permeable membrane from glomerulus (structure found between afferent and efferent arterioles)into bowman's capsules at a rate of approximately 130ml/min, and this filtration rate known as glomerular filtration rate, GFR. Cells greater than 7 micro meter diameter and the large molecular size (greater than 70 kda) plasma proteins are unable to pass through the semi permeable membrane The GFR is an extremely important in both

the study of kidney physiology and the clinical assessment of renal function. Serum concentration of urea, creatinine uric acid, electrolytes: sodium, potassium, chloride and calcium are used to assess renal function (10).

The interplay between kidney and thyroid in each other's function is known for many years. Thyroid dysfunction affects renal physiology and development, whereas kidney disease could result in thyroid dysfunction. Disorders of the thyroid and kidney may co-exist with common ethological factors. Kidney is involved in the metabolism and elimination of TH. The decline of kidney function is accompanied by changes in the synthesis, secretion, metabolism and elimination of TH, causing dysfunction. Moreover, kidney disease may predispose to alterations in regulation of HPTA, as well as changes in TH uptake and action. (11,12)

The interactions between kidney and thyroid hormones have been demonstrated for several years (8, 13). Although the exact mechanism not clearly known, experimental animal model studies demonstrated the role of thyroid hormones in the determination of kidney size, weight, and structure during childhood and adulthood periods. Histological studies document the effect of thyroid hormone on cortical and outer medullary tubular segment, particularly involving the proximal tubule, distal convoluted tubule, and medullary thick ascending limb (6).

The rennin-angiotension- aldosterone system (RAAS) plays a crucial role in the cross link between the thyroid and the kidney (8).Which is activation of RAAS by the thyroid hormones (6) The RAAS functions as a neuro-hormonal regulating mechanism for body sodium and water content, arterial blood pressure, and potassium balance (13). Renin secretion by cells in the juxtaglumerular bodies of the kidney is increased by decreased renal perfusion pressure, and decreased sodium concentration in the fluid of the distal tubule. Renin converts angiotensionogen to angiotension-I. In the kidney angitension-I is converted to angiotension-II, which is a potent vasoconstrictor. In addition, angiotension-II stimulates secretion of aldosterone and antidiuretic hormone (ADH) (13).

Thus, the current study assessed renal function derangements in patients with thyroid dysfunction at Arsho Advanced Medical Laboratory.

1.2 Statement of the Problem

Thyroid dysfunctions affect renal function at pre-renal and renal stages. Pre-renal effects are mediated by the influence of thyroid hormones on the cardiovascular system and the renal blood flow (RBF). The direct renal effects are mediated by the effect of thyroid hormones on the glomerular filtration rate (GFR), tubular secretary and re-absorptive processes. They also affect renal clearance of water load by their effects on the GFR (9, 14).

Hypothyroidism contributes to renal parenchymal growth retardation, which limits glomerular surface area for filtration. (8) In more than 55% of adults with hypothyroidism, the GFR is reversibly reduced by about 40 %.(8, 9, 12)in addition, the decrease of tubular transport capacity was remarked by Na/K ATPase and Na-H exchanger activity, first in the proximal tubules then almost all segments of nephrone(8), thus there is a remarkable decrement in sodium. There is also a significant increase in serum creatinine, hyperuricemia and gout than in the general population (4).

The effects of hyperthyroidism on the kidney are usually opposite to the effects of hypothyroidism. The GFR increases by about 18-25% among hyperthyroid patients. In sever hyperthyroidism protein breakdown increases and results eventual renal atrophy (14).

Hyperthyroidism can result in/accelerate chronic kidney disease (CKD) by several mechanisms. Firstly, hyperthyroidism results in intra- glomerular hypotension (increased filtration pressure) and consequent hyper-filtration. Secondly, hyperthyroidism predisposes to proteinuria, which is known to cause direct renal injury. Thirdly, hyperthyroidism-induced mitochondrial energy metabolism along with down- regulation of superoxide dismutase contributes to the increased free radical generation and consequent renal injury (3, 14).In addition; hyperthyroidism contributes to anemia in CKD patients and is considered as one of the cause of resistance to recombinant human erythropoietin (EPO). Conversely, hypothyroidism doesn't contribute to progression of CKD except by mild to moderate reduction in GFR (14).

1.3 Literature Review

However there are a few reports on thyroid and kidney, literature search did not show any formal studies reporting the association of thyroid abnormality and renal function on Ethiopian population.

The study conducted in Ethiopia on the Prevalence of chronic kidney disease and associated risk factors among diabetic patients in southern Ethiopia. They found that, from the total participants, the estimated prevalence of chronic kidney disease, defined by eGFR <60 ml/min/1.73 m2, in this study was 18.2% (CI 95% = 12.3% - 23.2%) and 23.8% (CI 95% = 17.5% - 29.9%) when defined by according to the MDRD and C-G equations, respectively. By stages, the prevalence was; 17.3% with stage 3 CKD (14.0% stage 3A and 3.3% stage 3B), and 0.9% with stage 4 CKD by MDRD; whereas 22.9% with stage 3 (15.0% stage 3A and 7.9% stage 3B) and 0.9% with stage 4 by C-G (15).

On the pattern of thyroid diseases in adult Ethiopians and experience in management between February 1986 and July 1991, study conducted at Tikur Anbesa Hospital a total of 373 thyroid patients (68.3% of the total endocrine cases) were seen in the weekly endocrine referral clinic of Tikur Anbessa Hospital (TAH); 258 (69.2%) came from Addis Abeba, 41 were males and 332 females (M: F = 1:8.1) and 71.9% were below 40 years of age. Thyrotoxicosis was seen in 43.7% of the patients, followed by euthyroid solitary nodules (23.6%) and simple goitres (22.3%) (16).

Thyroid dysfunction affects RBF, GFR, tubular function, electrolyte homeostasis, and kidney structure (14). The various effects of hypothyroidism and hyperthyroidism on renal function have been summarized below.

Hyperthyroidism and Renal function: hyperthyroidism is characterized by increased RBF, urea and GFR (4, 9, 14). There is contradicting information on serum creatinine. Some says serum creatinine is decreased (13, 14, 17).And no change on serum creatinine level (9).Hyperthyroidism is associated with a decreased in total body water and exchangeable potassium but not sodium. However, for most part, the serum concentration of sodium and potassium remain normal. Occasionally, hyperthyroidism is associated with hypokalemia (13,14).

Hypothyroidism and renal function: most effects of hypothyroidism on kidney are usually

opposite to the effects of hyperthyroidism. RBF and GFR are reduced (4,13, 14).serum creatinine, uric acid is elevated in hypothyroid patients. (5, 9) hyponatremia is twice as common among those with normal serum creatinine (16).

The effects of hypothyroidism and hyperthyroidism on serum creatinine are always opposite to one another and showed by different researches. study done on the effects of the hyper and hypothyroid state on changes in serum creatinine on 17 patients with hypothyroidism and 19 patients with hyperthyroidism showed that, hypothyroid patients had a mean serum creatinine of 1.02 mg/dl while the hyperthyroid patients had a mean serum creatinine of 0.67mg/dl(18).

On the study done in Pakistan on the effects of thyroid dysfunction on systolic or diastolic blood pressure, pulse rate and creatinine. They found that male creatinine level in hyperthyroid and hypothyroid patients (0.44±0.10, 2.12±0.06 mg/dl) and female (0.39±0.03, 1.72±0.05mg/dl) respectively and there was a significant difference in the level of serum creatinine in the control group male (0.68±0.06mg/dl) and female (0.78 ±0.03mg/dl) (17).

Many researches were done on two or more renal function markers for example blood urea nitrogen with creatinine, eGFR, uric acid and vice versa. Study done in Sudan on evaluation of renal function in Sudanese patient with thyroid disorder found that, the blood urea concentration in patients with hypothyroidism is not differed compared with control (M±SD =23.6±10) and 24.2±6.0, respectively, P=0.7) both creatinine and creatinine clearance showed significant difference (M±SD= 1.04±0.15mg/dl and 93±13.6ml/min, respectively) compared to euthyroid group (M±SD=0.97±0.17mg/dl and 101±10.9ml/min, P=0.033 and 0.004 respectively (9).

study done on the thyroid stimulating hormone, serum creatinine and uric acid levels in patients with hypothyroidism, showed that increased level of serum creatinine (1.8±0.03mg/dl) and uric acid (6.92±0.047mg/dl) in hypothyroid patients compared with control patients (0.86±0.24mg/dl) and (5.78±0.6mg/dl) with P<0.001 respectively(5).

On the case- control hospital based study conducted on Indian population on dynamic changes in biochemical markers of renal function with thyroid status showed that, no significant difference was observed between case and control groups. But a significant increase in creatinine and uric acid levels (0.85 ±0.03mg/dl) and (6.73±0.19mg/dl, P<0.05) were observed in the case group than the control group (0.69±0.01mg/dl) and (5.87±0.18mg/dl) respectively in hypothyroid

patients (19).

Kreisman.SH, etal studied on 29 episodes with paired prior euthyroid and hypothyroid serum creatinine values, the hypothyroid value was greater in 26(89.7%) and equal in 3(10.3%), less in none; the mean hypothyroid value was significantly greater 1.17 versus 0.87 mg/dl with P<0.001. they also conducted that there is consistent and reversible elevation of serum creatinine values in hypothyroid state (20).

Dragovic.T studied on reversal deterioration of renal function accompanied with primary hypothyroidism. There was increased in serum creatinine 180mmol/l with reference range 80-120mmol/l and slightly elevated serum urea levels of 11.6mmol/l with reference range of 3.2-8.2mmol/l. creatinine clearance assessed using Cockroft-Gault formula, was reduced to the value of 35ml/min with reference range 88-120ml/min. after four months of the therapy, thyroid hormone levels returned to normal levels. At the same period, plasma creatinine values decreased to 81mmol/l while creatinine clearance increased up to 72ml/min (21).

Decreased in GFR produces a diminished water delivery to distal tubular segments that is partly responsible for the hyponatremia. Hyponatremia appears in 45% of hypothyroid patients who have elevated serum creatinine levels and in about 21% of these with normal creatinine levels (21).

Chandhury.HS, etal studied on renal function impairment in hypothyroidism. They found that, male uric acid level was higher in hypothyroid patients (5.854±1.27mg/dl than control 5.08±0.67mg/dl, P<0.050. and among the female the mean uric acid level was higher 6.17±1.48mg/dl than that of control 4.28±0.61mg/dl, P<0.00 (22).

A significant correlation between thyroid function and purine nucleotide metabolism has been established in hypothyroidism. Giordano, et al studied on 28 patients with primary hypothyroidism and found a significant increase in the incidence of both hyperuricemia and gout in the hypothyroid patients (19).

The level of potassium in blood serum of patients with hypothyroidism and in clinically healthy individuals was within the normal range and not significantly different. When studying the levels of sodium in the blood serum in patients with hypothyroidism, it was within the reference range 135-145mmol/l, but the median concentration of sodium ion was statistically differed

significantly compared to control (22).

Impaired kidney function is not only has effect on thyroid gland function but also alter thyroid structure (21).Kidney disease may predispose to alteration in regulation of HPTA, as well as changes in thyroid hormone uptake and action (24).

One study done on acute renal failure [ARF] during the oliguric/ anuric and post polyuric phase. Serum T4 and T3 concentration were shown a significant decrease in oliguric/anuric phase as compared with the mean value and with control (25).

Another study done on cross sectional population-based studies shown that higher TSH is associated with lower eGFR and higher prevalence of CKD(<60ml/min) independent of confounding factors such as age, sex, body mass index, smoking and co morbidities such as hypertension diabetic etc (24) .

CKD is associated with a higher prevalence of hypothyroidism, but non with hyperthyroidism.(4) a recent study shown a prevalence of 7% hypothyroidism with estimated GFR> 90ml/min and increased to 17.9% in subjects with GFR <60ml/min(13).

Study done by Jan G Dan Hollander on the Correlation between severity of thyroid dysfunction and renal function in 37 consecutive hypothyroid and 14 hyperthyroid patients renal function as measured by plasma creatinine and glomerular filtration rate (GFR) [based on the modification of diet in renal disease (MDRD) formula] was determined before treatment and after regaining euthyroidism. It was shown that renal function improved significantly during treatment of hypothyroidism and decreased during treatment of hyperthyroidism. There was a strong correlation between the change in thyroid status determined as the ratio log (10) (fT4 post-treatment/fT4 pretreatment) and the change in renal function as a result of therapy expressed as serum creatinine ($r(2) = 0.81$, $P < 0.0001$) and estimated GFR (0.69, $P < 0.0001$) (26).

In another study conducted in India in 2012 on changes in electrolyte and lipid profile in hypothyroidism. They found that phosphorus and magnesium levels in serum were significantly elevated in patients with hypothyroidism when compared to controls ($p<0.001$). The levels of calcium and sodium were significantly decreased in cases when compared to controls ($p<0.001$). However, serum potassium levels in hypothyroid patients were found to be less than that of

controls but the difference was not statistically significant (27).

In the case of study done in Korea on unresolved subclinical hypothyroidism is independently associated with progression of chronic kidney disease. They compared the slope of eGFR decline between the three groups (euthyroid, n=127, unresolved subclinical hypothyroidism n= 20, resolved subclinical hypothyroidism n= 21) with different thyroid status. the slope of eGFR decline was comparable in euthyroid patients and those with resolved subclinical hypothyroidism ($p = 0.971$). The mean eGFR decline/year was -11.00 ± 13.05 in patients with unresolved subclinical hypothyroidism, which was significantly greater than in euthyroid patients or those with resolved subclinical hypothyroidism (all $p < 0.05$). The eGFR decline rate before resolution of subclinical hypothyroidism was identified in 7 patients with resolved subclinical hypothyroidism, and it was similar to one of patients with unresolved subclinical hypothyroidism (-11.7 ± 13.8 ml/min/1.73 m^2/yr; $p = 0.907$) (28).

Study conducted on the hyperuricemia and gout in thyroid endocrine disorders, the biochemical parameters in the 7 hyperuricemic hypothyroid patients under basal conditions and 2 months after L-thyroxin treatment. They indicated that among the hypothyroid patients, 9 (32.1%) presented stable hyperuricemia, a decrease in uric acid excretion (in 3 consecutive tests), increased creatinine, and decreased creatinine clearance. Six (66.6%) of these 9 patients were female, and 3 (33.3%) were male. A statistically significant difference was found between the 9 hyperuricemic hypothyroid patients and the 19 normouricemic hypothyroid patients as regards TSH, fT4, serum and urinary uric acid, serum creatinine, and creatinine clearance (29).

On the study of assessment of plasma uric acid level among indian females with thyroid dysfunction, which Covered 300 subjects in Kota, Rajasthan state, 200 test subject, (100 subjects with hypothyroidism, with age range (21-61) years and duration (1month-4Years), 100 subjects with hyperthyroidism, with age range (21-62 years)and duration of (6month –6 years)and 100 subjects volunteers (21-62) years as control group, to determine the serum uric acid level. They documented that, there is significant increase in the level of uric acid in both hyper and hypothyroidism test groups when compare with control group (6.8±1.67 mg/dl and 6.5±1.68 mg/dl versus 4.0±0.87 mg/dl respectively), ($p<0.05$), in table 1 & 2. Whereas there is no significant difference between the hypo and hyperthyroidism ($p>0.05$) (30).

Majeed MH et al studied on the association between chronic renal failure and thyroid hormone Mean serum levels of fT3 values in patients were decreased comparing to the control group $P < 0.001$ 4.774±1.006 , 2.210±1.030 and 3.035±0.963 P mol / L for conservative , haemodialysis and control groups respectively. There was significant increase in the level of fT3 in haemodialysis compared with conservative groups. fT4 values were also decrease in patients comparing to control group $P < 0.01$ but there was no significant differences between the patients groups. Mean serum TSH levels in patients were slightly higher than control group but no significant differences between groups (31).

Study conducted by Ali KL on the effect of chronic renal failure in thyroid hormone had shown that highly significant reduction in T3 and T4 concentration in patients' serum with CRF compared to control group ($P \leq 0.05$). Serum TSH was measured in CRF and control groups.CRF group had TSH above normal range (32).

Sarvghadi F et al conducted a study which consists of thirty-two patients (22 males, 10 females) with mean±SD of age 38.2±12.6 years were evaluated. Of the assessed, TT3, TT4 and RT3U levels significantly in-creased by improvement of graft function ($p< 0.05$) but in 7 patients with delayed graft function those values remained at lower levels. No cases with hyperthyroidism or hypothyroidism were detected. Thyroid volume decreased and echogenicity in-creased after transplantation ($p<0.05$). Six patients had thyroid nodules and cysts before surgery and 2 new cysts were detected after surgery. There was no relationship between age, sex, type and duration of dialysis and thyroid function after transplantation (33).

Spector AD et al conducted a study which comprises of thirty-eight patients with chronic renal insufficiency who were in a dialysis program underwent studies of thyroid function and metabolic status. And they found that mean values for serum total and free thyroxine (T_4) concentrations and thyroxine-binding globulin capacity were within normal limits. Although mean serum total triiodothyronine (T_3) concentration was normal, 43% of the group had low serum T_3 and 54% had low serum free T_3 concentrations. Serum thyrotrophin (TSH) concentrations were normal in all but four subjects who had very slight elevations. Metabolic status was assessed by various metabolic tests; mean values for each of these tests were normal, and the clinical index scores indicated that all patients were euthyroid. Results of metabolic testing were similar in patients with low and those with normal serum T_3 concentrations. Low

serum T_3 measurements did not accurately reflect metabolic state in patients with chronic renal failure, whereas serum free T_4 and TSH concentrations were reliable indicators of thyroid state(34).

Study conducted by Ahmed MM et al, which consists of 486 patients, of whom, approximately half were female, and the median (range) age was 61 (17-90) years. According to creatinine measurements, renal function was normal in 48 participants, 290 had mild renal failure, 122 had moderate renal failure and 26 had severe renal failure. No significant relationships were observed between renal failure and cardiac or pulmonary dysfunction. Free triiodothyronine (FT3) levels were significantly reduced (P =0.005) and both free thyroxin (FT4; P = 0.034) and parathyroid hormone (PTH; P = 0.028) significantly increased with increasing severity of renal failure. Patients with moderate to severe renal failure displayed reduced hemoglobin levels and were significantly more likely to be anemic (P <0.001). Highly significant increases in alkaline phosphatase (P<0.001), uric acid (P<0.001) and low-density lipoprotein-cholesterol (P =0.014) levels were also observed with increasing renal dysfunction (35).

In Africa, there were a very few research has been conducted on the evaluation or assessment of renal function tests on thyroid dysfunction patients. When it comes to Ethiopia, we can hardly find a research conducted in this very area.

1.4 Significance of the study

As far as the principal investigator's knowledge, research conducted on the assessment of renal function with thyroid abnormalities on Ethiopian patients, are scarcely found. Thus Findings of the present study may indicate possible renal complication that follows abnormal thyroid functions, and so improving health of patients. Moreover, this study may help to establish

baseline information for other similar researches, conducted in the future. It will also help the clinician to manage thyroid function abnormalities optimally.

2 Chapter two

2.1 Objective of the study

- **General objective:** to assess renal function in patients with thyroid dysfunction attending at Arsho Advanced Medical Laboratory.

- **Specific objective:**
  - To determine renal function among euthyroid patients attending at Arsho

Advanced Medical Laboratory.

- To determine renal function among hypothyroid patients attending at Arsho Advanced Medical Laboratory.
- To determine renal function among hyperthyroid patients attending at Arsho Advanced Medical Laboratory.
- To evaluate thyroid abnormalities in compared to electrolyte balance patients attending at Arsho Advanced Medical Laboratory.

3 Chapter three
Materials and Methods

3.1 Study design, and study area: A cross-sectional study was conducted at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

3.3 Study period and study Subjects: All patients attending during the study period, from

March 21/15-May 27/15, who fulfill the eligibility criteria was recruited as a study participant.

3.4 .Eligibility criteria:

3.4.1 Inclusion criteria:

- Patients aged between 18-60years.
- Patients who have a thyroid function test.

3.4.2 Exclusion criteria:

- Patients with known renal failure
- Patients who don't sign on the consent form.
- diabetes Mellitus
- hypertension
- cardiac problem
- treatment on steroid, antihypertensive drugs

❖ Clinical data was obtained from the participants' history on the laboratory request form the rest clinical data were obtained from their health facility by contacting their physician. And recorded on a questionnaire sheet. Clinical assessment of the study group was done by clinicians and they were not suffering from other disorder like renal failure, cardiac problem and hypertension.

3.5 Sampling technique and sample size

Convenient sampling technique was used

Sample size was determined by using the formula of hypothesis testing for two population means (36).

Sample size required (n) =

$$\text{Pooled variance } (\sigma^2) = \frac{s_1^2 - s_2^2}{2}$$

$$n = \frac{2\sigma^2\left[Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right]^2}{(\mu_1 - \mu_2)^2}$$

Anticipated values of the population means: μ1 and μ2

Standard deviation: s1, s2

Level of significance

Power of the test: 100 (1- β) %

Based on Hamed SA and Abdrabo AA studies on eGFR

μ1=125ml/min and μ2= 101ml/min

s1= 15 and s2 = 10

Z for 90% power =1.28 and $Z_{1-\alpha/2} = 1.96$

n= 71

3.7 Variables

- **Dependant variables**

Serum blood urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, calcium and eGFR.

- **Independent variables**

Serum Total T3, Total T4 and TSH

3.8. Principle of the tests

Equipments and Analytical methods: All tests were done on Beckman Coulter. The detail is annexed.

- Serum Urea nitrogen concentration by an adaptation of the enzymatic method of Talke and Schubert.(Kinetic UV test)
- Serum Calcium concentration by Arsenazo IIImethod.
- Serum creatinine concentration by means of the kinetic modification of Jaffe method.
- Serum Chloride ion concentration by measuring electrolyte activity in solution.
- Serum Sodium by measuring sodium ion activity in solution.
- Serum Potassium ion concentration by measuring electrolyte activity in solution.
- Serum Uric acid concentration by modification of Fossati method.
- Serum Total T3 concentration by chemiluminescens method.
- Serum Total T4 concentration by chemiluminescens method.
- Serum TSH concentration by chemiluminescens method.
- GFR was calculation using 4V MDRD:

  The simplified 4VMDRD formula (mL/min/1.73m^2) based on four variables: serum creatinine, age, race and sex (37, 38, 39).

  eGFR (mL/min/1.73 m^2) = 186 X Serum creatinine (mg/dl)$^{-1.154}$ X Age$^{-0.203}$

  X0.742 (*if female*) X 1.212 (*if black)*.

Quality Assurance: samples with lipemic, Icteric and hemolyzed were rejected. And tests were done in accredited Laboratory. (ISO 15189)

Classification of thyroid dysfunction: based on clinical laboratory results classified as follows.

- Euthyroid: classified by serum T3, T4 and TSH level. All were within the biological reference range.(Euthyroid patients were taken as a reference/control)
- Hypothyroidism: classified as an elevated TSH level above the biological reference range and low serum T3 and T4 level below the biological reference range.
- Hyperthyroidism: classified as decreased TSH level below the biological reference range

and high serum T3 and T4 level above the biological reference range.

3.9 Data management and analysis:

Data was collected using a structured questionnaire. The data was entered in to SPSS version 20 software. The following variables were collected as categorical data: sex, thyroid status presented using percentage. Normally distributed/Continuous variables: age, renal function and thyroid profile were presented using mean and standard deviation. ANOVA was used to assess the mean difference between continuous variables. The level of significance was at 0.05 and finally binary logistic regression was used to assess the association of thyroid hormone with renal function and electrolyte balance test.

3.10 Ethical clearance

The study was approved by "Department Research and Ethical Review Committee" of the Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University. Permission was also obtained from the Arsho Advanced Medical Laboratory; Addis Ababa. Patients' Left over blood sample from the routine clinical services was used. Patients' samples were coded and the name of the patient was not used. In addition, confidentiality was kept. Any patient who is not volunteer was not enforced to be included in the study. Finally the study was commenced after having informed consent for all patients.

4. Chapter 4

4.1. Result

Of the total 71 study participants included in the study, proportion of Euthyroid patients were relatively high 29 (41%), followed by hypothyroid cases 25(35%). Moreover54 (76.1%) of the study participants were females, and mean age of the study participants were 41.9±9.7years of age, as shown in table-1.

Table 1. Sex and age category of study participants with thyroid dysfunction attending at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Variable		Thyroid status		
		Hypothyroid(n=25)35 %	Euthyroid(n=29)41 %	Hyperthyroid(n=17)24 %
Age	18-27	1(1.4%)	3(4.2%)	1(1.4%)
	28-37	6(8.5%)	5(7.0%)	6(8.5%)
	38-47	10(13.8%)	8(11.3%)	7(9.9%)
	48-60	8(11.3%)	13(18.5%)	3(4.2%)
Sex	Male	5(7.0%)	5(7.0%)	7(9.9%)
	Female	24(33.8%)	12(16.9%)	18(25.4%)

On the distribution of thyroid hormones, hyperthyroid patients had relative high mean values of (T3=3.6±1.87µg/dl)and (T4=16.11±2.83µg/d)l hormones, whereas TSH was relatively high in hypothyroid patients, (28.3 ±22.39 µg/dl), as shown in table-2.

Table 2.Distribution of T3, T4, and TSH hormones among Hypothyroid, euthyroid, and hyperthyroid patients attending at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Thyroid hormone	Classification of thyroid dysfunction		
	Hypothyroid(n=17) mean ±SD µg/dl	Euthyroid (n=29) mean ±SD µg/dl	Hyperthyroid (n=25) mean ±SD µg/dl
T3	0.58±0.23	1.3±0.26	3.6±1.87
T4	4.18±1.80	9.6±1.58	16.11±2.83
TSH	28.3±22.39	1.68±1.04	0.46±0.027

The renal function test of thyroid dysfunction patients was assessed by measuring serum creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA), sodium (Na), Potassium (K), and estimated Glomerular Filtration Rate (eGFR) values. Of the total RFT test parameters, the mean concentration of Na, BUN, and eGFR (138.5±2.3 mmol/L, 24.2±14.7 mg/dl, and 202.2±103.9ml/min respectively) were higher in hyperthyroid patients; whereas, the mean concentrations of K, Cr and UA (4.4±0.8 mmol/L, 0.8±0.2 mg/dl, 5.5±1.9 mg/dl respectively) were higher in euthyroid patients. Though the mean concentration of all Na, Cr, UA, except eGFR in hyperthyroid participants, were within the biological reference range, all the mean concentration had statistically differed significantly(P< 0.05 when compared to euthyroid participants. as shown in table 3.

Table 3.Mean concentration of renal function test parameters among thyroid dysfunction patients attending at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Renal function	**Type of thyroid abnormalities**			**P-value**
	Hypothyroid (Mean±SD)	**Euthyroid (Mean±SD)**	**Hyperthyroid * (Mean±SD)**	
Sodium	135.7±3.6mmol/L	136.2±3.2 mmol/L	138.5±2.3 mmol/L	0.016
Potassium	4.2±0.4mmol/L	4.4±0.8 mmol/L	4.3±0.6 mmol/L	0.391
Urea	22.9±9.3mg/dl	21.9±10.6 mg/dl	24.2±14.7 mg/dl	0.911
Creatinine	0.7±0.07mg/dl	0.8±0.2 mg/dl	0.6±0.3 mg/dl	0.001
Uric Acid[1]	4.4±1.1mg/dl	5.5±1.9 mg/dl	4.9±1.4 mg/dl	0.042
eGFR	136.5±28.3ml/min	117.8±41.6ml/min	202.2±103.9ml/min	0.001

*statistical significance was indicated between hyperthyroidism and euthyroid.

[1] uric acid was statistically significant for all thyroid abnormalities.

Electrolyte balance was assessed by measuring mean serum K, Na, Calcium (Ca), and Chloride (Cl) values. Of the measured serum electrolytes, the mean concentrations of Na, Cl, Ca,(138.5±2.3 mmol/L, 106.4±4.0 mmol/L, 9.5±0.5 mmol/L respectively) were higher in hyperthyroid patients; meanwhile, mean concentration of K (4.4±0.8 mmol/L) was higher in euthyriod patients. Though the mean concentration of Na was within the biological reference range, it was statistically differed significantly compared to euthyroid participants at (P<0.05) shown in table 4.

Table 4. Mean concentration of serum electrolyte with respect to thyroid dysfunction, among study participants attending at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Electrolyte balance(Renal function)	Type of thyroid abnormalities			P-value
	Hypothyroid (Mean±SD)	Euthyroid (Mean±SD)	Hyperthyroid* (Mean±SD)	
Sodium	135.7±3.6mmol/L	136.2±3.2 mmol/L	138.5±2.3 mmol/L	0.016
Chloride	103.8±3.5mmol/L	104.2±2.7 mmol/L	106.4±4.0 mmol/L	0.377
Calcium	9.3±0.5mmol/L	9.2±0.5 mmol/L	9.5±0.5 mmol/L	0.474
Potassium	4.2±0.4mmol/L	4.4±0.8 mmol/L	4.3±0.6 mmol/L	0.391

*statistical significance was indicated between hyperthyroidism and euthyroid.

Binary logistic regression analysis demonstrated that the mean concentrations of Na, Cr and eGFR were significantly associated with hyperthyroid cases, [UAOR=0.146(0.036-0.598), 13.282(3.446-51.187), 13.281(3.446-51.187) respectively]. These association of hyperthyroidism and the selected abnormal renal function and electrolyte balance were not significantly changed after adjusting for when adjusted for sex, age, renal and electrolyte test parameters e [AOR at 95% CI 0.141(0.033-0.593),95% CI 16.236(3.481-75.739) and 13.797(3.261-58.67) at P <0.05, and P< 0.001 respectively.] as shown in table 5..

Table 5. Unadjusted and adjusted effect of thyroid dysfunction on the electrolyte balance and renal function tests of the study participants with thyroid dysfunction attending at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

Variables	Hyperthyroid		Hypothyroid	
	UAOR(95% CI)	AOR(95% CI)	UAOR(95% CI)	AOR(95% CI)
Serum sodium	0.146(0.036-0.598)	0.141(0.033-0.593*	0.584(0.170-2.085)	0.532(.144-1.967)
Serum uric acid	1.182(0.216-6.457)	1.008(0.162-6.259)	3.611(0.739-17.644)	3.466(0.596-20.172)
Serum creatinine	13.282(3.446-51.187)	16.236(3.481-75.739)**	1.923(0.413-8.965)	1.189(0.215-6.569)

eGFR	13.281(3.446-51.187)	13.797(3.261-58.67)**	4.375(1.046-18.295)	3.243(0.709-14.837)

*statistical significant at P<0.05 and ** statistical significant at P< 0.001

4.2. Discussion

The association of thyroid dysfunction and abnormal renal function tests has been demonstrated in different studies. On the current study, renal function test were assessed by measuring blood urea nitrogen, serum creatinine, eGFR, sodium, uric acid and potassium values. In hyperthyroid cases, the mean values of creatinine, and uric acid were significantly decreased, and, sodium, and eGFR values were significantly increased. Moreover, Serum creatinine, sodium and eGFR mean concentration values have a significant association with hyperthyroidism. On the other hand, electrolyte balance was assessed by measuring serum sodium, chloride, calcium, and potassium values. Even though, mean values for serum sodium and chloride indicated an increment; statistical significance was achieved only for sodium in hyperthyroid study participants.

Hyperthyroidism is associated with decreased mean values of serum creatinine, and uric acid. Whereas eGFR, serum sodium and urea mean values indicate an increment. Similar changes in eGFR, Urea and Creatinine with hypothyroidism have been reported in few studies involving different number of hyperthyroid subjects (9, 40). The effect of hyperthyroidism on the alteration of renal hemodynamic of circulatory system including increased renal blood flow, renal plasma flow, and GFR (41,42). Consequently, serum creatinine value decreased (14). Moreover, for the increment of eGFR in hyperthyroid cases, Insulin like growth factor type-I (43,44), and activation of rennin- angotensin- aldosterone system (RAAS) (14) also have contributions.

In the present study serum uric acid is significantly decreased in study participants with hyperthyroidism, which contrasts with that obtained by Gulabkanwar et al 2014, hyper secretion attribute to increased level of thyroid hormones (T3 and T4), cause increased in the metabolic rate of the metabolites such as purine, which result in increased production of uric acid in the blood, that exceed the renal capacity to excrete uric acid, which may accumulate in joints causing gout, or deposited in the renal causing renal stone. On contrary to the finding of Gulabkanwar et

al, hyper secretion of T3 and T4 attribute to increased in GFR and renal plasma flow. Consequently, serum uric acid value decreased (14).

In euthyroid case , the assessed renal function tests mean values were found within the biological normal reference values and this finding also go in line with other similar studies (5, 9, 17,). These study findings may suggest the normal concentrations of thyroid hormones on maintaining of homeostatic conditions; and these conditions had a direct effect on myocardial cells, and maintain of normal eGFR on each nephron (21). In addition, other normal renal physiological activities, including maintaining water balance, and hemodynamic of circulatory system, may associated with availability of thyroid hormone at normal values (21).

Hypothyroidism, another main classification of thyroid dysfunctions assessed on the current study, has been associated with decrease mean values of creatinine and uric acid, and increase mean value of eGFR. The decrement of serum creatinine in the present study with other studies (5,21) argues against the previously held notion of increased serum creatinine value due to a decrease in eGFR or alteration in RAAS. The study of Nagarajappa K.J, et al demonstrated that elevated serum creatinine levels in hypothyroid patients were due to reduction in GFR and renal plasma flows, and as well secondary to decreased renal plasma flow and urate excretion. On the Other hand, an impaired endothelial- mediated vasodilatation in hypothyroidism increases peripheral and renal plasma flow and GFR, resulting in free water overload and decrease in creatinine clearance (5). Consequently, an elevation of plasma creatinine level might result (21).
But in the current study, increased eGFR and decreased serum creatinine values might be due to, abnormality in creatin kinase level mascular weakness, dystrophy, and poor physiological activity, or according to Kreisman SH, et al, it might be due to the net unchanged creatinine value due to a balance between the decrease in renal clearance and a decrease in creatinine generation (20).

In the current study serum uric acid is significantly decreased in study participant with hypothyroidism, which contrasts with that obtained by Nagarajappa K.J, et al 2012 (21). Hypo secretion characterized by decreased thyroid hormones level (T3 and T4). Deficiencies of these hormones cause significant changes in metabolism such as decrease in purine metabolism. The cause of decreased uric acid is believed to be mainly due to decreased metabolic activity in

hypothyroidism.

The current study also demonstrated that hyperthyroidism was associated with increase Serum Sodium values, whereas calcium, Chloride, and potassium mean values were not changed. As far as the principal investigators knowledge, scarce research was conducted before on electrolyte balance and hyperthyroidism. This significant increment of sodium ions in the case of hyperthyroidism of could possibly due to availability of increased thyroid hormones, the Na-H exchanger and Na-Pico-transporter activity will also increase first in proximal tubules then almost all segments of nephron(45,46,47). Another possibility for the increment of serum sodium value might be of the direct relation of hyperthyroidism with plasma rennin activity, and plasma level of angiotensinogen, angitension II and aldosterone (14).

In euthyroid cases, the present study demonstrated all assessed electrolytes, were in the biological reference range, and no significant change has been indicated. These might be due to the optimal availability of thyroid hormone balances maintaining of the expression and/or the activity of the Na-H exchanger and Na-Pico-transporter (45,46,47). In addition it might be due to potentials normal thyroid hormones on balancing and regulation role in rennin activity and plasma level of angiotensinogen, angitension II and aldosterone values (14).

In the case of hypothyroidism, almost all the assessed electrolyte balance indicates a decrement; however the decrement is not statistically significant. This study finding was different from similar studies conducted by Chaudhury HS et al (22). Decreased thyroid hormones, decreases the plasma rennin, angiotensin II, and serum angiotensin converting enzyme levels. In addition, there is a net decrease in the RAAS activity. This results in afferent arteriolar vasoconstriction and efferent arteriolar vasodilatation. This could result in hypoperfusion of PCT and consequent lukewarm Na and Cl reabsorption in PCT. In addition, there is a decreased activity of basolateral NA/K ATPase, apical Na-H exchanger (NHE), and the Na-Pi co-transporter. Deactivation of these transporters decreases the proximal reabsorption. (8, 14, 45, 46, 47,).

4.3. Conclusion and Recommendations

Conclusion and Recommendations

The present study demonstrated that both hypothyroidism and hyperthyroidism conditions have been associated with significant changes in renal function and electrolyte balance, though they are within the biological reference range. Moreover, the current study findings give a clue that, thyroid abnormalities, may lead not only to renal failure but also to disturbance of electrolyte balances for Ethiopian population, in spite of limited number of study participants, and test parameters. Therefore, assessment of renal function and electrolyte balances for patients with thyroid abnormalities may improve the health status of the patients, presenting thyroid dysfunction. Finally, in order to strengthening the current findings on the association of thyroid dysfunctions with RFT and electrolyte balances, further large scale and cohort studies are recommended.

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Annex A

Study questionnaire

Assessment of renal function tests on thyroid dysfunction patients attending Arsho Advanced Medical Laboratory.

Date _____

Study code_____

A. socio demographic data

Gender 1. Male_____

2. Female_____

Age_____

B. Medical History

I. diagnosis

1. diabetes Mellitus Yes_____ No_____
2. hypertension Yes_____ No_____
3. Renal failure Yes_____ No_____
4. other (specify)_____

II. duration of illness(months)_____

III. Medications currently on

1. Anti-hypertensive Yes_____ No_____
2. steroids Yes_____ No_____
3. thyroid drugs Yes_____ No_____
4. other specify ` Yes_____ No_____

1.የጥናት ጥያቄዎች

የታይሮይድ ቅመም "ሆርሞን" መዛባት ባለባቸው ህሙማን ላይ የኩላሊት ምርመራ ውጤት ምን ይመስላል የሚል ጥናት በአርሾ አድቫንስድ ህክምና ላቦራቶሪ አዲስ አበባ ኢትዮጲያ።

ቀን----------

የጥናት መለያ ቁጥር----------

1. ማህበራዊ ዳሰሳ

ዕታ ወንድ----------

ሴት----------

እድሜ----------

2. የጤናዎ ታሪክ

2.1 ምርመራ	አለ	የለም
የስኳር ህመም	____	____
የደም ግፊት	____	____
የኩላሊት ህመም	____	____
ሌላ ካለ/ያብራሩ/	____	____

2.2 የበሽታዎቆይታ (በወር) _____

2.3 የሚጠቀሙት መድሃኒት ካለ

ለደም ግፊት	____	____
ሰቴሮይድ	____	____
የታይሮይድ መድሃኒት	____	____

ሌላ ካለ/ያብራሩ/ ___ ___

Annex B

Laboratory Request and Report Form

Date----------

Study code----------

Socio demographic data

Gender 1. Male----------

2. Female --------

Age----------

Parameter	**Reference range**	**values**	**Classification of thyroid results**
Serum TT3	0.87-1.78µg/dl		
Serum TT4	6.1-12.2 µg/dl		
Serum TSH	0.34-5.6 µg/dl		
Serum Urea nitrogen	7-25mg/dl		
Serum creatinine	Female 0.6-1.2mg/dl Male 0.7-01.3mg/dl		
Serum uric Acid	Female 2.3-6.6 mg/dl Male 4.4-7.6 mg/dl		
Serum sodium	136-145mmol/l		
Serum chloride	98-107mmol/l		
Serum potassium	3.5-5.1mmol/l		
Serum calcium	8.8-10.6mg/dl		
eGFR	90-160 mL/min/1.73m^2		

Annex C

Consent explanation

Assessment of renal function tests on thyroid dysfunction patients attending Arsho Advanced Medical Laboratory.

Introduction and objective of the study:

I, **Nardos Abebe**, a Master's degree(M.Sc.) student in Medical Laboratory science specializing clinical chemistry at Addis Ababa University and conducting a study on thyroid dysfunction and its effects on renal function.

Thyroid glands are formed of two lobes, which are found on either side of the neck that carry out many functions: for cell differentiation, growth and development of kidney and maintaining homeostasis of electrolyte and water. The kidneys are a pair of organs that carry out key functions: removal of body wastes, maintain body fluid and hormone production. Thyroid dysfunction can cause alteration in the renal functions.

The study aims to assess renal function tests on thyroid dysfunction patients attending Arsho Advanced Medical Laboratory.

This study uses the left over sample from your thyroid function tests.

If you consent to participate, you will:

- Sign a consent form.
- Answer a number of questions contained in the study questionnaire.

Confidentiality:

Any information given to me will remain confidential and your privacy will be respected. You may ask me any questions regarding the study now or any time during the study. If you have any question related to the study, you are kindly welcome to contact me. **Nardos Abebe +251926230520**

3. ስለጥናቱ የስምምነት ማብራሪያ

የታይሮይድ ቅመም "ሆርሞን" መዛባት ባለባቸው ህሙማን ላይ የኩላሊት ምርመራ ውጤት ምን ይመስላል የሚል ጥ ናት በአርሾ አድቫንስድ ህክምና ላቦራቶሪ አዲስ አበባ ኢትዮጲያ።

<u>ስለጥናቱ መግቢያና አላማው</u>

እኔ ናርዶስ አበበ እባላለሁኝ። በአዲስ አበባ ዩኒቨርሲቲበህክምና ለቦራቶሪ ሳይንስ(በክሊኒካል ኬሚስትሪ ስፔሻሊቲ) በመደበኛው ፕሮግራም የማስተርስ /የሁለተኛ/ ድግሪ እየተማርኩ ሲሆን ለመመረቂያ ማሟያ የሚሆነኝን ጥናት የ ታይሮይድ ቅመም 'ሆርሞን" መዛባት ባለባቸው ህሙማን ላይ የኩላሊት ምርመራ ውጤት ምን ይመስላል የሚል ጥና ት በአርሾ አድቫንስድ ህክምና ላቦራቶሪ ውስጥ በማጥናት ለይ እገኛለው።

የታይሮይድ እጢ የተሰራው ከሁለት "ሎቦች" ሲሆን የሚገኙትም በአንገታችን በቀኝ እና በግራ በኩል ነው። ይህ የታ ይሮይድ እጢ በሰውነታችን ህዋስ እድገት እንዲዳብር ፤ለኩላሊት እድገት እና ኩላሊት ትክክለኛ ስራ እንዲሰሩ ቅመ ም 'ሆርሞን" ያመርታሉ።

ኩላሊት በሰውነታችን በቀኝ እና በግራ በኩል የሚገኙ ሲሆን ብዛታቸውም ሁለት ነው ። ኩላሊት በደም ውስጥ የሚ ገኙትን መርዛማ ንጥረ ነገሮች ያጣራል ፤ ያስወግዳል እና ቅመሞችን 'ሆርሞን" ያመርታል።

በሰውነታችን ያለው የታይሮይድ ቅመም 'ሆርሞን" መዛባት የኩላሊታችንን ስራ ያዛባል፤ያስተጓጉላል እና ለኩላሊት በሽታም ያጋልጣሉ።

ጥናቱ በግብዓትነት የሚጠቀመው ናሙና ፤በአርሾ አድቫነስድ ህክምና ላቦራቶሪ የታይሮይድ ቅመም "ሆርሞን" ምር መራ ለማሰራት ከሚመጡ ህሙማን መሃከል የሚወሰድ ይሆናል። ስለሆነም እርሶዎ ፈቃደኛ ከሆኑ በስምምነት ቅ ፁ ላይ ፊርማዎን ያኑሩ። ከዚህም ጋር ተያይዞም ጥቂት መጠይቅ የተዘጋጀ ሲሆን ስለፈቃደኝነተዎ እያመሰገንን እር ሶዎም ትክክለኛ ምላሽ እንዲሰጡን በአክብሮት እንጠይቃለን።

ሚስጥራዊነቱ

ለእኔ የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀ ነው። በጥናቱ ላይ የሚጠይቁት ማንኛውም ጥያቄ ካለ በስልክ ቁ ጥር +251926230520 ደውለው መጠየቅ ይችላሉ።

Annex D

Consent form for participants

Assessment of renal function tests on thyroid dysfunction patients attending Arsho Advanced

Medical Laboratory.

After reading and being explained to on the study purpose by Nardos Abebe, do hereby give informed consent to participate in the study on assessment of renal function tests on thyroid dysfunction patients attending Arsho Advanced Medical Laboratory.

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

signed._____

Sign of Principal Investigator_____

4. ስለጥናቱ የስምምነት ፎርም

የታይሮይድ ቅመም "ሆርሞን" መዛባት ባለባቸው ህሙማን ላይ የኩላሊት ምርመራ ውጤት ምን ይመስላል የሚል ጥናት በአርሾ አድቫንስድ ህክምና ላቦራቶሪ አዲስ አበባ ኢትዮጲያ።

ስለ ጥናቱ እንብቤ ተረድቼ ተስማምቼያለው።

ከጥናቱ በማንኛውም ግዜ የኔ ምርመራ ውጤት ተፅኖ በማያስከትል ሁኔታ ከጥናቱ መውጣት እንደምችል አውቃለ ሁኝ።

የተሳታፊው ፊርማ----------

የአጥኚው ፊርማ----------

Annex E

Laboratory techniques

Specimen collection

1. Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
2. Using the tip of the index finger examine the phlebotomy site , feel the vein, and decide exactly where to place the puncture
3. Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab or cotton. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
4. Release the tourniquet
5. Perform the Vein puncture.
6. Enter the skin with the needle at approximately a 15-degree angle to the arm, with the bevel of the needle up. Follow the geography of the vein with the needle. Insert the needle smoothly and fairly rapidly to minimize patient discomfort.
7. As soon as the needle is in the vein, ease the tube forward in the holder as far as it will go, fairly securing the needle holder in place. When the tube has filled, remove it by grasping the end of the tube and pull gently to withdraw.
8. Withdraw venous blood of approximately 4 ml in the serum separator (SST) tube as required.
9. Place a clean sterile cotton ball or gauze lightly over the site.
10. Apply an adhesive bandage strip over the cotton ball or gauze to adequately stop bleeding and avoid a hematoma.
11. Dispose of contaminated material such as needles, syringes, and cotton in a designated hard-cased container (sharps container).
12. A blood specimen should be centrifuged as soon as clot formation is complete (about 20 – 30 minutes at room temperature).
13. Centrifuge blood for 10 minutes at an RPM of 850 to 1000 X in Stoppard container.
14. If analysis is not done before 8 hours store at -20°c.

Procedure to run sample on Beckman Coulter

1. program the test on the instrument
2. on the white rack put the sample cup
3. label the code
4. dispense 200-500µl of serum to the sample cup
5. load the rack on Beckman Coulter, and then run

Test Principle

The Calcium procedure is based on calcium ions (Ca2+) reacting with Arsenazo III (2, 2′-[1, 8-Dihydroxy-3, 6-disulphonaphthylene-2, 7-bisazo]-bisbenzenearsonic acid) to form an intense purple colored complex. In this method the absorbance of the Ca-Arsenazo III complex is measured bichromatically at 660/700 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

Reaction Principle

Ca^{2+} + Arsenazo III $\xrightarrow[\text{pH 6.9}]{}$ Ca-Arsenazo III complex (purple)

Test Principle

Creatinine forms a yellow-orange colored compound with picric acid in an alkaline medium. The rate of change in absorbance at 520/800 nm is proportional to the creatinine concentration in the sample.

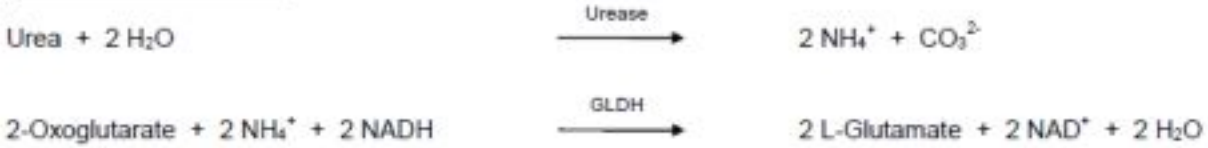
Reaction Principle

Creatinine + picric acid $\longrightarrow$ Creatinine picrate complex

Test Principle

Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced in the first reaction combines with 2-oxoglutarate and NADH in the presence of glutamate-dehydrogenase (GLDH) to yield glutamate and NAD+. The decrease in NADH absorbance per unit time is proportional to the urea concentration.

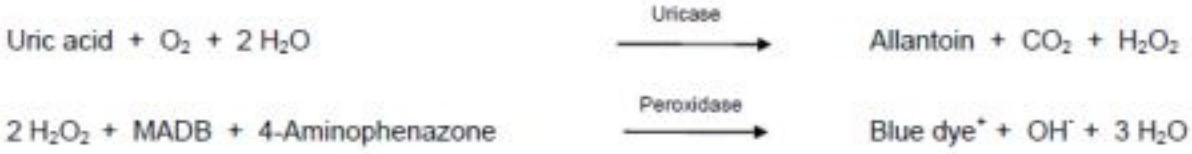
Reaction Principle



Test Principle

Uric acid is converted by uricase to allantoin and hydrogen peroxide. The Trinder reaction is utilised to measure H2O2 (hydrogen peroxide). The formed H2O2 reacts with N,N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) and 4-aminophenazone in the presence of peroxidase to produce a chromophore, which is read biochromatically at 660/800nm. The amount of dye formed is proportional to the uric acid concentration in the sample.

Reaction principle



Test Principle

The ISE module for Na+, K+, and Cl- employs crown ether membrane electrodes for sodium and potassium and a molecular oriented PVC membrane for chloride that is specific for each ion of interest in the sample. An electrical potential is developed according to the Nernst Equation for a specific ion. When compared to an internal reference, this electrical potential is translated into voltage and then into the ion concentration of the sample.

- **Blood Urea Nitrogen**

STANDARD PREPARATION:

Serum calibration: Perform a one-point calibration using a water blank (blue rack) and the appropriate calibrator in a yellow calibration rack. The frequency of calibration is every 14 days. Calibration of this urea nitrogen procedure is accomplished by use of the Beckman Coulter

Chemistry Calibrator material, which is traceable to the National Institutes of Standard and Technology.

CALIBRATION PROCEDURE:

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL:

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

REFERENCE RANGES:

Serum: 7 - 25 mg/Dl

Clinical Interpretation

Blood Urea Nitrogen (BUN) levels are a measure of kidney function and also of prerenal and postrenal conditions. Prerenal causes of elevated BUN include cardiac decompensation, water depletion or increased protein catabolism. Among the renal causes of increased levels are acute glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, and tubular necrosis. Any type of obstruction of the urinary tract is a postrenal cause for elevated BUN levels. Both urea and creatinine are cleared by the renal glomeruli, however, urea is subsequently partially reabsorbed by the renal tubules, while creatinine is not. Consequently, serum urea nitrogen and serum creatinine determinations are frequently performed together in the differential diagnosis of kidney function.

- **Uric Acid**

Measurements of Uric Acid are used in the diagnosis and treatment of numerous

renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

STANDARD PREPARATION:

Perform a one-point calibration using a water blank (blue rack) and the appropriate calibrator in a yellow calibration rack. The frequency of calibration is every 30 days. Calibration of this uric acid procedure is accomplished by use of the Beckman Coulter Chemistry Calibrator material, which is traceable to the National Institute of Standard and Technology.

CALIBRATION PROCEDURE:

If QC recovery drifts during calibration stability period, perform reagent blanking when necessary. Recalibration is required when any of the following conditions occur:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL:

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide. Quality control testing should be performed in accordance with laboratory's standard procedure.

REFERENCE RANGES:

Serum:

Adult Female:	2.3 – 6.6 mg/dL
Adult Male:	4.4 – 7.6 mg/dL

Clinical Interpretation

Measurements of Uric Acid are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

- **Electrolyte Balances**

STANDARD PREPARATION:

Perform a multipoint calibration by using the automated ISE calibration with the appropriate standards placed in the labeled positions on the STAT table or designated ISE Standard Solution area. The frequency of calibration is daily. Calibration of the ISE methods is accomplished by the use of the Beckman Coulter ISE standards for serum.

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL:

During operation of the Beckman Coulter AU analyzer two levels of an appropriate quality control material were tested a minimum of once a day. In addition, controls were performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide. Quality control testing should be performed in accordance with the laboratory's standard procedure.

REFERENCE RANGES:

Serum:

Na^+: 136 – 145 mEq/L

K^+: 3.5 – 5.1 mEq/L

Cl^-: 98 – 107 mEq/L

- **Creatinine**

CALIBRATION PREPARATION:

Perform a two-point calibration using a water blank (blue rack) and the appropriate calibrator in a yellow calibration rack. The frequency of calibration is daily. Calibration of this creatinine procedure for serum determinations is accomplished by use of the Beckman Coulter Chemistry

Calibrator, which is traceable to an isotope dilution mass spectrometry reference method using the National Institutes of Standards and Technology Standard Reference Material.

CALIBRATION PROCEDURE:

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. A fresh bottle of reagent is used for testing.
3. Major preventative maintenance was performed on the analyzer.
4. A critical part was replaced.

QUALITY CONTROL:

During operation of the Beckman Coulter AU analyzer two levels of an appropriate quality control material (Beckman Coulter Quality control) was tested. In addition, controls was performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

References Ranges

Male	0.7-1.3mg/dl
Female	0.6-1.2mg/dl

Clinical Interpretations

Measurements of creatinine are used in the diagnosis and treatment of renal disease. Serum creatinine measurements prove useful in evaluation of kidney glomerular function and in monitoring renal dialysis. Both serum creatinine and BUN are used to differentiate prerenal and postrenal (obstructive) azotemia. An increase in serum BUN without concomitant increase of serum creatinine is key to identifying prerenal azotemia. With postrenal azotemia, both serum BUN and creatinine rise, but the rise is disproportionately greater for BUN.

Serum creatinine varies with the subject's age, body weight, and sex. It is sometimes low in subjects with relatively small muscle mass, cachetic patients, amputees, and in older persons. A serum creatinine level that would usually be considered normal does not rule out the presence of impaired renal function.