ESTABLISHMENT OF REFERENCE INTERVALS TO VALUE OF THYROID FUNCTION TESTS IN CORD BLOOD OF NEONATES BORN IN TWO HOSPITALS, ADDIS ABABA, ETHIOPIA.

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY.

By:

AMAN MEHARI (BSc. MLT)

ID NUMBER GSR/2781/04

November, 2013

ESTABLISHMENT OF REFERENCE INTERVALS TO VALUES THYROID FUNCTION TESTS IN CORD BLOOD OF NEONATES BORN IN TWO HOSPITALS, ADDIS ABABA, ETHIOPIA: A Screening Tool for Congenital Hypothyroidism.

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY

By:
AMAN MEHARI (BSc. MLT)
ID NUMBER GSR/2781/04

UNDER SUPERVISION OF:

1. **DANIEL SEIFU (PhD, Biochemistry)** (Department of Biochemistry, College of Health Sciences, Addis Ababa University)
2. **GOITOM GEBREYESUS (MD, Pediatrician)** (College of Health Sciences, Addis Ababa University)
3. **DEREJE ALEMAYEHU (MD, Gynecologist & Obstetrician)** (Medical Director, Gandhi Memorial Hospital)

November, 2013

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by **Aman Mehari Abraha** entitled: "*Establishment of reference intervals to values of thyroid function tests in cord blood of neonates born in two hospitals, Addis Ababa, Ethiopia"* and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Medical Biochemistry) complies with regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner: _____ Signature: _____ Date: _____

Advisor: _____ Signature: _____ Date: _____

Advisor: _____Signature: _____ Date: _____

Advisor: _____ Signature: _____ Date: _____

Chair of Department or Graduate Program Coordinator

ACKNOWLEDGMENTS

I would like to express my deepest thank for Dr. Daniel Seifu (PhD, Biochemistry), Head of Biochemistry Department, School of Medicine, College of Health Sciences, Addis Ababa University, for his guidance, encouragement, moral support invaluable suggestions and painstaking efforts for completion of this study.

I express my deepest regards for Ato Feyissa Challa (EHNRI, National HIV Laboratory Clinical Chemistry Unit), for his inspiration, suggestion and support whenever I need. Rather it was he who provoked me to start this work and cleared all the hurdlers from beginning to the end in carrying out my M.Sc. thesis work. I am greatly indebted for his unreserved effort, constant encouragement and support during the time of the work.

I am thankful to Dr. Goitom Gebyesus (MD, Pediatrician), College of Health Sciences, Addis Ababa University, and Dr Dereje Alemayehu, (MD, Gynecologist & Obstetrician), Medical Director of Gandhi Memorial hospital, for their assistance in setting the problems, and continuous supervision in recruiting the study participants and allowing me to collect appropriate samples from the hospitals entitled.

I wish to thank the School of Graduate studies of Addis Ababa University for providing me fund for the research. I further want to give my gratitude to the Department of Biochemistry, School of Medicine, college of Health Sciences, Addis Ababa University for provision of laboratory equipments.

Finally, I would like to thank to my Host University, Mekelle University, for allowing me to attend the MSc program.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

BMI	Body Mass Index
CH	Congenital hypothyroidism
CLSI	Clinical Laboratory Standards Institute
DBS	Dry Blood Spot
ECLIA	Electrochemiluminescence immunoassay
EHNRI	Ethiopian Health and Nutrition Research Institute
FT3	Free triiodothyronine
FT4	Free thyroxine
IAEA	International atomic energy agency
IFCC	International Federation of Clinical Chemistry
NCEP	National Cholesterol Education Program
PIOD	Partial iodide organification defects
RAI	Radioactive iodine
RI	Reference Interval
TIOD	Total iodide organification defects
TSH	Thyroid-stimulating hormone
WBC	White Blood Cells
TT3	Total triiodothyronine
TT4	Total thyroxine

ABSTRACT

Introduction:-Reference intervals are ranges of upper and lower limits outside of which values would be considered abnormal. It depends on the type of instrument and reagents, the principle or method and the type of population being served. Therefore it is obvious that it should be established for every population in different regions even within a country.

Objective:-The aim of this study is to establish population specific reference intervals of thyroid stimulating hormone, free thyroxine and free triidothyronine levels of cord blood.

Materials and methods:- A cross sectional study was conducted from July, 2013 to November, 2013 using cord blood sample from 123 new born infants of both sexes born at Tikur Anbessa Teaching Specialized Hospital and Gandhi Memorial Hospital. Thyroid stimulating hormone, free thyroxine and free triidothyronine were measured using Elecsys 2010 immunoassay analyzer at Ethiopian Health and Nutrition Research Institute. The non-parametric method of reference range determination was used.

Results:- 123 Cord blood samples collected from umbilical cord of newborns were analyzed for thyroid stimulating hormone, free thyroxine and free triidothyronine values. The birth weights ranged between 2500g to 4700g with mean (SD) value of 3241.46(459.495)gram. Their gestational age ranged between 37 to 44 weeks with an average of 39.74 weeks. The 2.5^{th} and 97.5^{th} percentiles of values were found to be 3.48 mIU/L and 27.57 mIU/L for thyroid stimulating hormone, 0.89 ng/dL and 1.53 ng/dL for free thyroxine and 1.19 pg/mL and 2.51 pg/mL for free triidothyronine respectively.

Conclusion:- In the present study the reference intervals of thyroid stimulating hormone ,free thyroxine and free triidothyronine were tried to be established and based on the results obtained , were 3.48 - 27.56 mIU/L for thyroid stimulating hormone, 0.89 - 1.53 ng/dL for free thyroxine and 1.19 - 2.51 pg/mL for free triidothyronine. It has been concluded that the result can provide us with an important clue to establish population specific reference intervals using large scale studies.

Key words:-*Thyroid function tests, reference intervals, screening program, Congenital hypothyroidism*

1. **NTRODUCTION**

Reference intervals are the most common decision support tools used for interpretation of numerical pathology reports. And are ranges of upper and lower limits outside of which values would be considered abnormal. As laboratory results may be interpreted by comparison with these intervals, the quality of the reference intervals can play as large a role in result interpretation as the quality of the result itself (Jones & Barker, 2008). Reference value depends on the type of instrument and reagents used, the principle or method for the test that is being performed, the type of population being served and the strength of quality assurance practiced at the laboratories(Norbert *et al*.,1982).

The roll of medical laboratory results in assisting attending physicians in their diagnostic and follow-up endeavors is intimately linked to access to meaningful and reliable reference values. Pediatrics is particularly sensitive to this problem as the processes associated with growth and development is imposing rapid discontinuous changes on the physiology of individuals. Some developmental stages such as the neonatal period are more critical than others, and adequate reference intervals are not available for some analytes. Thyroid function tests are an example for the need of adequate and updated reference intervals in pediatrics (Delvin *et al*., 2006).

The thyroid gland, located immediately below the larynx on each side of and anterior to the trachea, is the largest endocrine gland, normally weighing 15 to 20 grams in adults. Thyroid gland secretes two major hormones, thyroxine and triiodothyronine, commonly called T4 and T3, respectively. Both of these hormones profoundly increase the metabolic rate of the body(Guyton *et al*., 2006). Thyroid function tests are used to evaluate the thyroid's functioning and to diagnose and help determine the cause of thyroid diseases. Thyroid hormone production is regulated by another hormone called thyroid-stimulating hormone (TSH). TSH is made by the pituitary gland, which is located in the brain. From the pituitary gland, TSH travels to the thyroid where it stimulates the production of T3 and T4 and their release into the bloodstream(Zhang & Mitchell, 2011).

Thyroid hormones are of significant importance for regular functioning of almost all body organs. Thyroxine (T4) is the main hormone released from the thyroid gland and it is transformed into biologically active 3',3,5-triiodothyronine (T3) via 5'-deiodinases of thyroid hormone target cells(Brix , 2011). Approximately 20% of triiodothyronine (T3), the more active thyroid hormone, is synthesized by the thyroid gland, while 80% is formed in the peripheral tissues from the 5-deiodination of T4. T3 has a lower affinity for binding to transport proteins and globulins than T4(LaFranchi, 1999).

During fetal life, circulating concentrations of T4 and the active metabolite T3 are low, and the inactive metabolites reverse T3 (rT3) and T3 sulfate are high (figure1.1). This pattern is a consequence of both immaturity of the hypothalamic-pituitary-thyroid axis and coordinated adjustments in the deiodinase system that result in high concentrations of the inactivating deiodinase D3, and low concentrations of the activating deiodinase D1. Despite the low circulating T3 concentration, the amount of T3 in the fetal brain is 60% to 80% of the adult values as early as 20 to 26 weeks' gestation, reflecting the importance of local generation of T3 from T4 (under the influence of a third deiodinase D2).The physiologic rationale for the maintenance of reduced circulating T3 concentrations throughout fetal life is still unknown, but it has been suggested that its function may be to avoid tissue thermogenesis and potentiate the anabolic state of the rapidly growing fetus while, at the same time, permitting highly regulated, tissue-specific maturation in an orderly, temporal sequence. Dynamic changes in thyroid hormone secretion and metabolism occur postnatally that permit the neonate to adapt to the changing demands of postnatal life (Shiri, 2010).

Since reference intervals are affected by different factors such as lifestyle, ethnicity, age/developmental stage, gender, nutrition and other environmental factors **(**Schnabl *et al*., 2008), it is obvious that it should be established for every population in different regions even within a country. However, there are no implemented population specific reference intervals for almost all analytes including the thyroid function tests in Ethiopia, let alone in regional laboratories. Current pediatric reference intervals for TSH, T4, and T3 have been derived predominantly from samples collected on hospitalized infants and children of the western population (Schnabl *et al*., 2008), and may not reflect levels in healthy multicultural populations.

Application of reference ranges specific to other ethnic groups is clinically inappropriate for many biomarkers including thyroid function tests. Another problem with the available paediatric reference intervals, is that they were determined over two decades ago using older/less accurate instrumentation and methodologies that are no longer relevant to testing technology used by clinical laboratories today(Delvin *et al*., 2006).

More recent improvements in thyroid function tests, which is also introduced in our country, is using chemiluminescent technologies. It provided the laboratories with a better detection limit which enabled a better assessment of severely repressed hormone secretion. This study address two issues of the reference intervals of TSH, fT3 and fT4 in our country. These are; population specificity and performed by the most recent chemiluminescent technology.

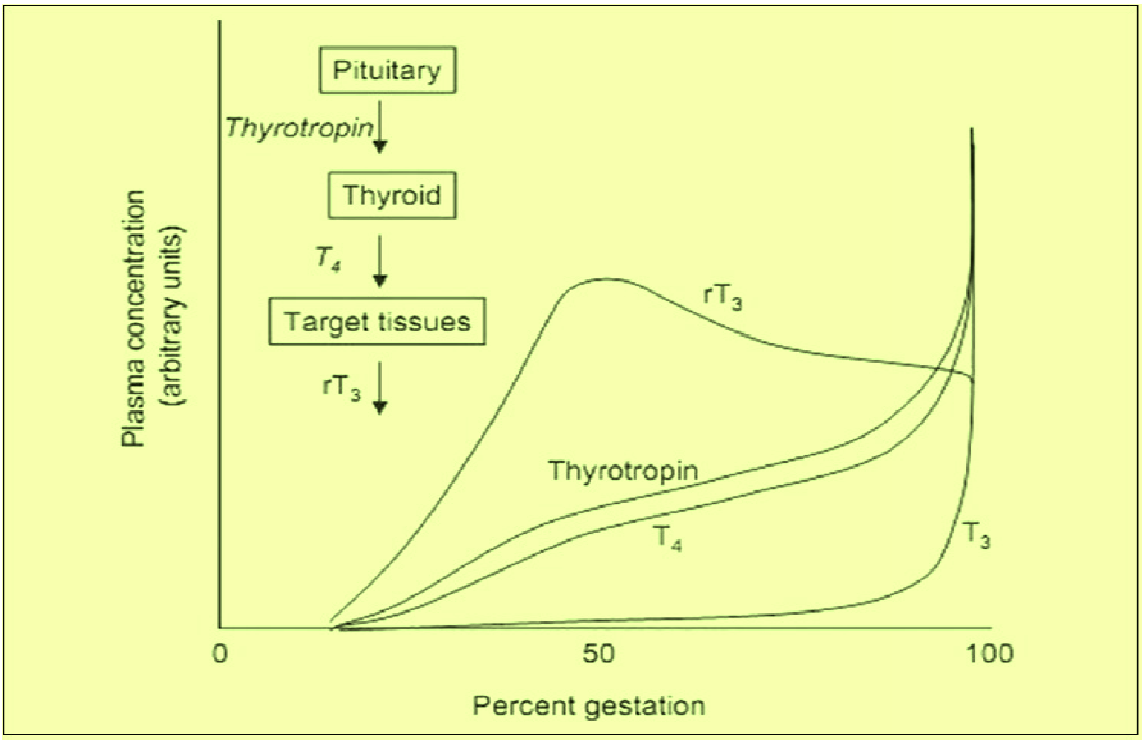

Figure 1.1. The ontogeny of thyrotropin, T4, T3, and rT3 in fetal plasma. The relationship of thyrotropin, T4, T3, and rT3 is represented in the upper left corner of the figure. rT3- Reverse T3(www.ijem.in).

Infants may born with hypo or hyperthyroidism state off different reasons. Hypothyroidism is especially crucial and may lead to irreversible damages unless detected early. Since the 1970's, neonatal screening programs for hypothyroidism have been developed and have become popular worldwide. Specimens from cord blood and filter paper spotted with blood from heel break can be utilized to measure TSH and fT4 for Congenital hypothyroidism screening. Due to early discharges from hospitals in Ethiopia, it is usually difficult to take blood samples from neonates after a few days of life (Yalemtsehay *et al*., 2004).

Therefore, cord blood is the specimen of choice as it is readily available at birth, with adequate volume for supplemental tests when required. Thyroid results should be ready when mothers are fit for discharge to ensure optimal response for recall testing. Besides, heel-prick blood collection is not without side-effects. Therefore cord blood specimen is selected in our study with special interest of the study group to set an indispensible prerequisite of neonatal screening program for congenital hypothyroidism which is part of the postnatal care in the developed countries. In this study it is tiring to establish a reference intervals of thyroid function tests using cord blood which is particularly important to develop regular screening program in our country.

1.1. LITERATURE REVIEW

1.1.1. Reference values

Reference intervals are the most common decision support tool used for interpretation of numerical pathology reports. And are ranges of upper and lower limits outside of which values would be considered abnormal. As laboratory results may be interpreted by comparison with these intervals, the quality of the reference intervals can play as large a role in result interpretation as the quality of the result itself (Jones & Barker, 2008). Reference value depends on the type of instrument and reagents used, the principle or method for the test that is being performed, the type of population being served and the strength of quality assurance practiced at the laboratories. Quality assurance practices have an impact on the comparability of results from different laboratories found at different levels and on the usability of the established reference values (Norbert *et al.*,1982).

The reference interval for many laboratory tests is also defined by threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall. The threshold or limiting values for the reference interval are usually the 0.025 and 0.975 fractiles of the test result distribution in the reference population (Figure 1.2). This definition results in exclusion of the 2.5% of individuals with the lowest results and the 2.5% of individuals with the highest results from the reference interval. In case the clinical interest is only in 'low' results and high test results are not indicative of pathology (or, conversely, if only high test results are of interest), one-sided reference intervals are defined that exclude only the 5% of the population in the 'abnormal' tail of the distribution (Boyd, 2010).

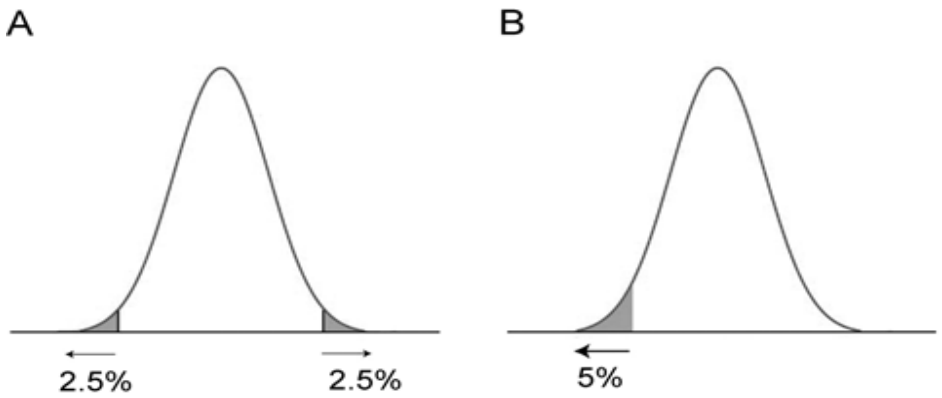

Figure 1.2. The 95% reference intervals are derived by identifying the most outlying 5% of observed values in a reference population (A). one-sided reference interval (B) (Boyd ,2010).

Although this statistical definition of the reference interval has been applied to the majority of laboratory tests, reference intervals have more recently been defined on the basis of analysis of clinical outcomes. The reference limits for cholesterol defined by the NCEP **(**NCEP**,** 2001) provide an example of this approach. Whereas the 97.5th percentile for cholesterol concentration in the general population lies between 280 and 300 mg/dL. The upper reference limits for cholesterol as defined by the NCEP are 200 mg/dL corresponding to approximately the 50th percentile of the population, and 240 mg/dL corresponding to approximately the 75th percentile. These values were chosen by the NCEP expert panel because they were associated with moderate and high risks for the development of cardiovascular disease in epidemiological outcome studies**(**Boyd, 2010).

It is hard to underestimate the importance of clinical laboratory test results. Nearly 80% of physicians' medical decisions are based on information provided by laboratory reports. A test result by itself is of little value unless it is reported with the appropriate information for its interpretation (Huntsman, 2006). Typically, this information is provided in the form of a reference interval (RI) or medical decision limit. An RI also defined as an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others (Ceriotti, 2007).

No RI is completely "right" or "wrong." The majority of RIs in use today refer to the central 95% of the reference population of subjects. By definition, 5% of all results from "healthy" people will fall outside of the reported RI and, as such, will be flagged as being "abnormal." There are many problems associated with the calculation of RI. The latest edition of the Clinical and Laboratory Standards Institute–approved guideline, "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory," recognizes the difficulties and controversies surrounding the establishment of RIs and the verification process. The working group recognizes the reality that, in practice, very few laboratories perform their own reference interval studies, instead of performing a new reference interval study, laboratories and manufacturers refer to studies done many decades ago, when both the methods and the population were very different (CLSI, 2010).

It has been recommended that a RI be established by selecting a statistically sufficient group (a minimum of 120 subjects per group) of healthy reference subjects. However, it is noted in the guideline that "Health is a relative condition lacking a universal definition. Defining what is considered healthy becomes the initial problem in any study. In reality, there will always be some level of uncertainty with a given selection protocol not only because of the definition of health that was selected but also because of the very real possibility that some of the selected subjects may, in fact, have subclinical disease (Katayev *et al*., 2010).

Recruiting a valid group of reference subjects and obtaining informed consent in today's environment is costly, time-intensive, and virtually an impossible task for most laboratories. The challenge is further magnified in establishing RIs for different age groups (eg, pediatric patients and geriatric patients), uncommon sample types (eg, cerebrospinal fluid and aspirations), timed collections, challenge tests, and serial measurements. In light of these difficulties, most laboratories elect not to establish their own RIs, but rather choose to verify RIs that have been reported by the manufacturer or as established by another laboratory. This is a relatively simple study and requires only 20 healthy subjects to recruit (CLSI, 2008).

The underlying assumption here is that the laboratory analytic system is calibrated and producing similar results as the method that was originally used in the published RIs. However, this may not be true because in many cases, the details of the reference study such as its design, the inclusion and exclusion criteria used for selecting the healthy recruits, preanalytic sources of variation, etc, are lacking. A laboratory can elect to "transfer" the RIs that were in use with an older method (or from another laboratory) to a new method. To do this, the laboratory must first demonstrate that the two methods produce comparable results. It is well known that analytic systems drift over time, and there is no guarantee that the method of today is producing results that are comparable to those that were produced at the time of the original RI study. This technique is the main reason why many laboratories today are using RIs that were established decades ago and are out-of-date (Katayev *et al*., 2010).

It is well recognized that pediatricians and pediatric specialists have unique needs and requirements for the laboratory testing of their patients. Age-specific reference intervals and specimen collection issues are particular concerns in pediatric patient care and evaluation. Studies claim that more frequently, adult reference intervals are not appropriate for pediatric patients (Schnabl *et al.*, 2008).

1.1.2. Congenital hypothyroidism

The development and maturation of the brain and central nervous system and other target tissues have a critical dependence on thyroid hormones, beginning before birth and extending through the first 2–3 years of life. Therefore, abnormal thyroid function may lead to different developmental problems. Mental retardation due to congenital hypothyroidism is one of the serious consequences of thyroid abnormalities. Incidence of congenital hypothyroidism ranges from 1 in 3000 to 1 in 4000 newborn infants and is a common preventable cause of mental retardation (Ingrida *et al.*, 2010).

Congenital hypothyroidism is defined as thyroid hormone deficiency present at birth. Thyroid hormone deficiency at birth is most commonly caused by a problem with thyroid gland development (dysgenesis) or a disorder of thyroid hormone biosynthesis (dyshormonogenesis). These disorders result in primary hypothyroidism. Secondary or central hypothyroidism at birth results from a deficiency of TSH. Congenital TSH deficiency may rarely be an isolated problem (caused by mutations in the TSH β subunit gene), but most commonly it is associated with other pituitary hormone deficiencies, as part of congenital hypopituitarism. Peripheral hypothyroidism is a separate category resulting from defects of thyroid hormone transport, metabolism, or action. (Rastogi & LaFranchi, 2010).

Congenital hypothyroidism is classified into permanent and transient Congenital hypothyroidism. Permanent Congenital hypothyroidism refers to a persistent deficiency of thyroid hormone that requires life-long treatment. Transient Congenital hypothyroidism refers to a temporary deficiency of thyroid hormone, discovered at birth, but then recovering to normal thyroid hormone production. Recovery to euthyroidism typically occurs in the first few months

or years of life. In iodine sufficient countries, 85% of congenital hypothyroidism is due to thyroid dysgenesis. This term refers to an aberration of the embryological development of the thyroid gland. The remaining 10-15% of cases can be attributed to the inborn errors of thyroid hormone synthesis, also called dyshormonogenesis, or to defects in peripheral thyroid hormone transport, metabolism, or action (Brown & Demmer, 2002). The main causes of Congenital hypothyroidism has explained in detail as follow.

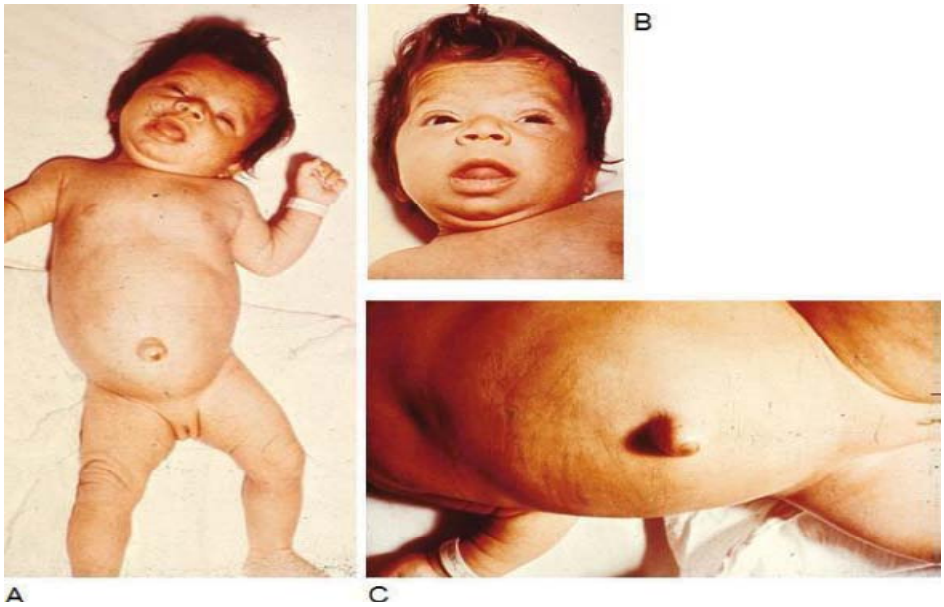

Figure 1.3. *Infant with congenital hypothyroidism.* ***A*** *- 3 month old infant with untreated CH; picture demonstrates hypotonic posture, myxedematous facies, macroglossia, and umbilical hernia.* ***B*** *- Same infant, close up of face, showing myxedematous facies, acroglossia, and skin mottling.* ***C*** *- Same infant, close up showing abdominal distension and umbilical hernia (Rastogi & LaFranchi, 2010).*

1.1.2.1. Thyroid dysgenesis

Thyroid dysgenesis presents in three major forms: thyroid ectopy, athyreosis and thyroid hypoplasia. Thyroid ectopy refers to an ectopic location of the thyroid gland. This accounts for two-thirds of congenital hypothyroidism due to thyroid dysgenesis and is twice as common in females (Castanet *et al*., 2009). In these cases, a thyroid remnant is usually found along the normal pathway of the thyroglossal duct. This represents the path taken by the developing thyroid as it descends from the base of the tongue to its final location in the neck (Castanet *et al*., 2009 & Felice &Lauro, 2004*)*. In one study done on hypothyroid neonates, ectopic thyroid tissue was found inferior and superior to the hyoid bone, and above the thyroid cartilage (Bubuteishvili

et al., 2003*)*. Athyreosis refers to the complete absence of thyroid tissue. Athyreosis and thyroid hypoplasia account for the remaining one third of thyroid dysgenesis. (Rastogi & LaFranchi, 2010).

1.2.1.2.TSH Resistance

There are several forms of TSH resistance. Mutations in the TSH receptor gene leading to thyroid hypoplasia have been found (Biebermann *et al*., 1997). Another form of TSH resistance is dominantly inherited and has been linked to the long arm of chromosome 15(Grasberger *et al*., 2005). Resistance occurs in the absence of a TSH receptor mutation and can again cause thyroid hypoplasia (Grasberger *et al*., 2005). Pseudohypoparathyroidism type 1a, caused by mutations in the alpha subunit of the stimulatory guanine nucleotide binding protein (Gs alpha), results in defective TSH signaling (Lania *et al*., 2006).

1.2.1.3. Thyroid dyshormonogenesis

Hereditary defects in virtually all the steps of thyroid hormone biosynthesis and secretions have been described and account for 10-15% of permanent congenital hypothyroidism. These are generally transmitted in an autosomal recessive manner, but at least one condition has autosomal dominant inheritance. Dyshormonogenesis leads to goitrous hypothyroidism; however, this is rarely seen in babies detected by newborn screening (Rastogi & LaFranchi, 2010). Most commonly, dyshormonogenesis is due to defects of thyroid peroxidase activity (Avbelj *et al*., 2007). Thyroid peroxidase uses hydrogen peroxide to couple iodine to thyroglobulin within the thyroid gland, forming T3 and T4. Severe defects in this enzyme lead to total iodide organification defects (TIOD). This diagnosis is made by showing high radioactive iodine (RAI) uptake of the thyroid gland followed by more than 90% release after sodium perchlorate administration (bakker *et al*., 2002). Less severe mutations cause partial iodide organification defects (PIOD) (Rastogi & LaFranchi, 2010).

1.1.3. Screening of neonate for Congenital hypothyroidism

The term 'newborn screening' is used to describe various types of tests that are done during the first few days of a newborn's life. Screening separates those who might have the disorder from those who probably do not have the disorder. In contrast, diagnostic testing is performed to establish the presence of a condition. Newborn screening that is properly timed and performed has the potential for preventing catastrophic health outcomes, including death. The conditions under which screening is conducted vary. They are usually influenced by factors such as prevalence (population characteristics), testing and treatment availability, outcome, geography, economics (including cost and cost effectiveness), transfer of science and technology, and politics (IAEA, 2005).

The well known fact before many years is that, adequate development of infants, children and adolescents critically depends on the normal functioning of the thyroid gland. The levels of thyrotropin (TSH) and the hormones TT3, TT4, fT3, and fT4 are the most relevant diagnostic indices for hyper or hypo activity of this endocrine organ. Moreover, in the presence of thyroid diseases these parameters provide information about the efficacy of thyrostatic treatment or substitution therapy. More importantly, during childhood and adolescence diagnostic specificity and sensitivity of thyroid function tests are affected by the validity of reference intervals. Covariates such as age and gender contribute to significant differences in hormone levels and have to be considered in the clinical interpretation of an individual patient. Age, in particular, is strongly associated with levels of TSH and thyroid hormones leading to the prerequisite of establishing reference intervals for separate age groups in infants, children and adolescents (Kratzsch *et al*., 2008).

During fetal development, thyroid hormones are important in brain cell migration as well as differentiation of neurons, oligodendrocytes, astrocytes, and microglia (Bernal *et al*., 2003). Neonatal screening can help in the timely diagnosis of newborn thyroid diseases such as congenital hypothyroidism and thus prevent developmental or growth problems (LaFranchi, 1999). Congenital hypothyroidism is one of the major health problems and the main preventable cause of mental retardation in children. If Congenital hypothyroidism is diagnosed promptly and treated early, irreversible mental retardation can be prevented (LaFranchi, 2007.) Because signs

and symptoms of Congenital hypothyroidism are often scarce at birth, newborns are screened at birth in most of the developed countries for early diagnosis of Congenital hypothyroidism under the nationwide neonatal thyroid screening program (O¨ zo¨n ZA, 2008).

Some studies recommend a strict follow up of the newborns to observe a sign and symptoms of Congenital hypothyroidism , if the screening program is not applicable. Symptoms of congenital hypothyroidism are initially nondescript; however, the maternal and pregnancy history may provide some clues. One may also find evidence of maternal autoimmune thyroid disease or an iodine deficient diet. Once home, these babies are quiet and may sleep through the night. Additional symptoms include a hoarse cry and constipation. Neonatal hyperbilirubinemia for more than three weeks is common. This is due to immaturity of hepatic glucuronyl transferase (LaFranchi, 1979 & Grant *et al*., 1992).

Screening of newborns for congenital hypothyroidism is now routinely used in most of the developed world and in an increasing number of developing countries, which has prevented serious intellectual sequelae in a considerable number of patients with Congenital hypothyroidism (Adachi, 2012). The specimen used for newborn screening tests is cord blood and blood from a heel-prick collected on special filter paper cards and plasma or serum of the same source. The specimen from heel-prick is routinely collected between two and five days of age. In addition, some programs also routinely obtain a 2 nd specimen between two and six weeks of age. The filter paper cards are then sent to a centralized laboratory for testing. Early in the experience of screening, most programs undertook an initial T4 test, with a follow-up TSH test on infants below a specified T4 cutoff (Rastogi & LaFranchi, 2010).

With increasing accuracy of TSH measurement, many screening programs now carry out an initial TSH test to detect congenital hypothyroidism. Each program must develop its own T4 and TSH cutoff for recall of infants with abnormal test results. As there are rapid changes in TSH and T4 in the first few days of life, many programs have developed age-related cutoffs. Both of the above screening test approaches can detect the majority of infants with primary congenital hypothyroidism. There are some advantages and disadvantages with each approach in the detection of other thyroid disorders. Both screening test approaches do a good job of detecting infants with primary Congenital hypothyroidism. The primary T4-follow-up TSH test strategy

will detect some infants with secondary or central (hypopituitary) hypothyroidism and infants with “delayed TSH rise”. On the other hand, a primary TSH test strategy will detect infants with mild or “subclinical” hypothyroidism. Neither test approach will detect infants with defects of thyroid transport, metabolism, or action. With a goal of detecting all of these thyroid disorders, some programs have undertaken pilot programs measuring both T4 and TSH on all newborns. These programs tend to report a higher incidence of congenital hypothyroidism (Tijn *et al*., 2005).

Once an infant has been detected with abnormal thyroid screening tests, they should be recalled immediately for examination, and a venapunctuer blood sample should be obtained for confirmatory serum testing. Confirmatory serum is tested for TSH and either free T4 or total T4 combined with some measure of binding proteins, such as a T3 resin uptake. It is important to compare the serum results with age specific reference ranges. In the first few days of life, serum TSH can be as high as 39 mU/L, as a result of the TSH surge that occurs shortly after birth. Most confirmatory serum tests are obtained around one to two weeks of life, when the upper TSH range falls to approximately 10 mU/L (Rastogi & LaFranchi, 2010).

Although levels of all hormones are higher at 1-4 days of age, by 2-4 weeks of age they have fallen closer to the levels typically seen in infancy. The finding of an elevated serum TSH level and a low free T4 or total T4 confirms the diagnosis of primary hypothyroidism. The finding of an elevated serum TSH with a normal free T4 or total T4 is consistent with subclinical primary hypothyroidism. Because of the dependence of the developing brain on optimal concentrations of thyroid hormone, most studies recommend treating infants with subclinical hypothyroidism. It is now recognized that preterm infants or acutely ill term infants with primary hypothyroidism may not show an elevated TSH level on the 1 st screening test. Thus, many programs undertake a routine 2 nd screening test in preterm and acutely ill term infants. Such testing leads to the detection of infants with “delayed TSH rise”, which occurs in approximately 1:18,000 newborns (Mandel *et al*., 2000).

Since the levels of TSH and thyroid hormones are the most substantial diagnostic indices for hypothyroidism, regional neonatal reference values for TSH and thyroid hormones are needed for accurate assessment of Congenital hypothyroidism in the neonatal period (Mutlu *et al*., 2011).

Many studies have done on pediatric reference intervals for thyroid hormone levels from birth to adulthood. Of them one study establishes age specific reference intervals of TSH, fT4 and fT3 for Australian population. And they found that there is significant difference from previously published reference intervals, explained by different analytical assays, differences in ethnicity, population or geographic derived covariates such as lifestyle, salt iodination, and nutrition (Kapelari *et al*., 2008). In other study done in Germany strengthen the importance of age specific reference intervals for thyroid parameters explaining the strong dependency of the parameters on age and slightly on BMI and WBC count (Kratzsch *et al*., 2008). One study also argues that population specific intervals are a tool for interpretation of individual patient laboratory test results. The clinical value of fT4 and other thyroid parameters result depends crucially on the reference intervals with which they are compared (Soldin *et al*., 2009).

The age specificity of the values of thyroid function tests have explained repeatedly through different studies. It explained in different studies that significant changes occur in thyroid physiology at the time of birth in the term newborn and confirmed that, a surge in the serum TSH is seen at 30 minutes after delivery, with peak concentrations as high as 60 to 70 mlU/L. It is believed that the elevation in TSH is to be a consequence of cold exposure in the ambient atmosphere, causes marked stimulation of the thyroid and an increase in the concentrations of both serum T4 and T3. In the first postnatal week, the serum T4 increases to concentrations that are higher than at any other time of life, decreasing thereafter. The T3 concentration rises strikingly at postnatal day 7 and continues to rise for the first 28 days(Soldin *et al*., 2009; Kapelari *et al*., 2008 & Shiri, 2010).

Similar studies have been performed in patterns of thyroid hormones and TSH in the preterm infant. One study by Williams and coworkers reflects, in part, the relative immaturity of the hypothalamic-pituitarythyroid axis. The study shows following delivery, a surge in T4 and TSH occurs that is analogous to that observed in term infants, but the magnitude of the increase is less. In infants born before 31 weeks' gestation, the circulating T4 concentration was not increased and even it falls in the first 1 to 2 postnatal weeks in some of the preterm infants. This decrease in the T4 concentration was particularly significant in very preterm infants, in whom the serum T4 occasionally may be undetectable (Williams *et al*., 2005). It is important to

appreciate that in most cases, the total T4 is more affected than the free T4, a consequence of abnormal protein binding or the decreased TBG in these babies, who have immature liver function. In contrast to the low circulating T4 concentration, serum concentrations of thyroglobulin, the storage form of T4, are higher in the preterm than in the term infant, particularly in those who are sick. In view of the attenuated postnatal TSH rise in the latter babies, it is likely that impaired clearance or degradation of this glycoprotein from the circulation rather than increased secretion plays an important role (Shiri, 2010).

Many hospitals perform newborn screening at 5–6 days of age, and the reference values reflect this postnatal age. In term healthy newborns, there is an initial physiologic surge of TSH (up to 60 mIU/L within 30 minutes of delivery), followed by a rapid decline over the first five days of life to,10 mIU/L (McElduff et al., 2005). Currently, a large number of healthy term newborns are discharged early (before 48 hours of age). Thyroid screening during this time is associated with an increasing number of false-positive results, due to this neonatal TSH surge. In addition, it is difficult to call back infants for thyroid testing once discharged. Some programs have responded by increasing their threshold value for TSH within the first day of life. A potential problem with this practice is the possibility of missing newborns with a slowly rising TSH. All of these factors make use of umbilical cord blood a practical alternative for thyroid screening purposes. Interestingly, some countries revert to cord blood screening as the method of choice, when facing difficult patient recall for initial thyroid testing (Abduljabbar *et al*., 2009).

In Ethiopia, Addis Ababa, one study has reported and 97.8th percentile of the TSH value was 15.4 mIU/L. The study evaluates the TSH values of cord blood using radio immune assay and conclude, the results demonstrated that cord blood samples were easy to collect and applicable for blood spot assay and emphasize that in the given situation in Ethiopia where hospital discharges are in about 24 hrs, cord blood samples for congenital screening should be put in practice given all other necessary infrastructures put in place (Yalemtsehay *et al*., 2004). Another study was done by Feleke and coworkers at the same study area using neonatal serum to establish a reference values of TSH and the cut-off value was reported 29.4mIU/I (Feleke *et al*., 2000). However, this study was conducted using samples taken immediately after delivery to one day of age ,at the time in which the serum TSH value is not stable. A study conducted in 56

healthy adult Ethiopians also tried to establish a reference ranges of thyroid function tests. Mean values for adults Ethiopians was reported as for triiodothyronine (T3) values for 20 males and 36 females were 1.42 +/- 0.32 nmol/L and 1.51 +/- 0.25 nmol/L, and thyroxine (T4) values were 119 22 nmol/L and 116 +/- 21 nmol/L respectively. The mean thyrotropin (TSH) values for males and females were identical at 1.85 +/- 0.94 mIU/L (Wassie & Abdulkadir, 1990). Even though, screening program of Congenital hypothyroidism is not yet started in Ethiopia, the present study can be a crucial starting point to establish the reference range of cord blood values as a prerequisite to the screening program.

1.1.4. Factors affecting levels of TSH and thyroid hormones

The level of TSH, fT4 and fT3 can be affected by different fetal and maternal factors. Maternal age, Birth weight and gestational age reported as a major factors in different studies. Reports show that maternal age and gestational age were related to neonatal TSH levels in newborns without Congenital hypothyroidism in the region of mild Iodine deficiency. It found older maternal age in pregnancy and longer gestation at birth were associated with elevated newborn bloodspot TSH levels (Ingrida *et al.*, 2010).

It also concluded by another study that gestational age is independently associated with lower cord TSH, higher cord total T4, and higher neonatal and subsequent bloodspot total T4 (Herbstman *et al.*, 2008). From the obstetrical point of view, the risk for congenital malformations and chromosomal abnormalities increases with advanced maternal age. Childbearing period in the reproductive life cycle is generally defined in the studies as between the ages of 15 and 44 years in the studies (Boivin *et al.*, 2007). Maternal age as influencing neonatal TSH levels is of particular interest, especially over the recent decades, since the demography of parenting has changed due to delayed decisions to motherhood (Boivin *et al.*, 2009).

2. OBJECTIVE

2.1. GENERAL OBJECTIVE

The aim of this study is to establish reference intervals of thyroid function tests for neonates born in Tikur Anbessa Teaching Specialized Hospital and Gandhi Memorial Hospital, Addis Ababa , Ethiopia.

2.2. SPECIFIC OBJECTIVES

I. To establish population specific reference interval of TSH in cord blood.
II. To establish population specific reference interval of fT3 in cord blood
III. To establish population specific reference interval of fT4 in cord blood.

3. MATERIALS AND METHODS

3.1. STUDY PERIOD AND STUDY AREAS

The study was conducted from July, 2013 to November, 2013 at Addis Ababa University College of Health Sciences Department of Biochemistry, Tikur Anbessa Teaching Specialized Hospital, Gandhi Memorial Hospital and Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, Ethiopia.

3.2. STUDY SUBJECTS

The study involves consecutive births of new born infants from Tikur Anbessa Hospital and Gandhi Memorial Hospital selected using a set of inclusion and exclusion criteria with the help of experienced pediatrician and Gynecologist.

3.3. INCLUSION AND EXCLUSION CRITERIA

3.3.1. Inclusion criteria

- Healthy full term newborns
- Singleton

3.3.2. Exclusion criteria

- Preterm infants
- Multiple births
- Inflamed infants

3.4. STUDY DESIGN

A cross sectional study on healthy newborns from Tikur Anbessa Teaching Specialized and Gandhi Memorial Hospitals was conducted.

3.5. SAMPLING DESIGN AND SAMPLE SIZE

A convenient sampling technique was used to take 132 study participants. Sample size determination was according to recommendations of international organizations which states that the preferred method as a priori nonparametric determination of reference intervals of single

group population should be established at least from 120 reference individuals. In this study 138 samples was taken with 10% contingency plan for non respondents and participants who may excluded after blood collection. The Clinical Laboratory Standards Institute (CLSI) and International Federation of Clinical Chemistry and laboratory medicine(IFCC) were the main references to decide the sample size of 120. According to those international organizations 120 is the minimum sample size required to determine reference intervals for the 95^{th} percentile reference limits (2.5^{th} and 97.5^{th} percentiles).

3.6. SPECIMEN COLLECTION AND STORAGE

5ml Cord blood samples were collected immediately after birth from the umbilical cord of the neonates of volunteer parent. Cord blood was obtained after an uncomplicated vaginal delivery and the umbilical cord was ligated from the placental side. BD vacutainer EDTA tubes were used to collect blood sample and the plasma was separated as soon as possible through centrifugation at 4600rpm for 4 minutes. Plasma samples were stored in deep freezer at -20^{o}C in the department of Biochemistry Laboratory until it transports to EHNRI. Finally the samples were transported using the cooled box and stored in deep freezer at -80^{o}C in EHNRI Clinical Chemistry Laboratory until the hormone analysis was done.

3.7. ASSAYS OF LABORATORY VARIABLES

TSH, fT4 and fT3 were measured using Elecsys 2010 immunoassay analyzer which is a fully automatic run-oriented analyzer system for the determination of immunological tests using the electrochemiluminescence immunoassay "ECLIA"process. All components and reagents for routine analysis are integrated in or on the analyzer. Specific test principles of the hormones to be measured are explained as follow.

3.7.1. TSH Assay

Sandwich immunoassay with total duration of assay of 18 minutes was used. It has two step incubation periods. During the first incubation period 50µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex reacts to form a sandwich complex. At the second incubation streptavidin-coated microparticles was added and the complex becomes bound to the solid phase via interaction of

biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances then removed with ProCell/ProCell M. Application of a voltage to the electrode induces chemiluminescent emission which was measured by a photomultiplier(detailed procedure is explained at annex 3).

3.7.2. fT3 Assay

Competitive immunoassay system with total duration of assay of 18 minutes was used. It has two step incubation periods. During the first incubation period 15µL of sample and an anti-T3-specific antibody labeled with a ruthenium complex was mixed. And the second incubation period starts with addition of biotinylated T3 and streptavidin-coated microparticles, and the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex bounds to the solid phase via interaction of biotin and streptavidin. The reaction mixture then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances were removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier (detailed procedure is explained at annex 3).

3.7.3. fT4 Assay

Competition immunoassay system with total duration of assay of 18 minutes was used. It has two step incubation periods. During the first incubation period 15µL of sample and an anti-T4-specific antibody labeled with a ruthenium complex was mixed. And the second incubation period starts with addition of biotinylated T4 and streptavidin-coated microparticles, and the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex was bounded to the solid phase via interaction of biotin and streptavidin. The reaction mixture then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances were removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier(detailed procedure is explained at annex 3).

Results of all of the hormones of thyroid functions test TSH, FT4, FT3 were determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode (SOP of EHNRI C. Chemistry Lab).

3.8. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data was analyzed using the SPSS version 20. Prior to the calculation of reference intervals data was cleaned. Records with missing descriptive information and outliers were removed before statistical analysis. Outliers was excluded using the following formulas which are suggested by most literatures.

Outlier excluding formula:

$$\text{Upper Limit} = Q_3 + (2.2 * (Q_3-Q_1)$$

$$\text{Lower Limit} = Q_1 - (2.2*(Q_3-Q_1)$$

Where Q stands for Quartile

Once the upper and lower limits are obtained, results higher than the upper limit and lower than the lower limit were removed from the data . A net of 123 participants were used for obtaining the reference intervals. It should be noted that outliers were not excluded from all data analysis. The effects of maternal age, gestational age, gender and birth weight on newborn TSH, FT4 and FT3 levels were assessed by analysis of variance (ANOVA), multiple correlation coefficient and Spearman‘s correlation coefficients. Multiple linear regressions were performed to quantify the associations between the above parameters and changes in TSH, FT4 and FT3 levels. Histogram and Q-Q plot for visual and Kolmogorov-Smirnove (K-S)Test for numeric assessment of distribution of data were used. A probability level of $p < 0.05$ was taken as significant.

Since the numerical values of TSH do not have normal Gaussian distributions, log transformation was required and the non-parametric method was used which is recommended by IFCC and CLSI for such type of data with a minimum of 120 individuals required per partition. Reference limits were calculated using non-parametric ascending rank order statistics. The conventional 95^{th} percentile reference limits were determined by calculating the rank numbers for the 2.5^{th} and 97.5^{th} percentiles.

3.9. QUALITY CONTROL AND QUALITY ASSURANCE

The quality of the our results was maintained by following the standard operational procedures for analysis of TSH, fT4 and fT3 prepared by the senior staffs of EHNRI clinical chemistry Laboratory. The Normal and pathological controls, Preci control Universal, Roche, 11731416122 PC U1 and PC U2 was also performed with every run to control the performance of the overall procedure and the instrument under use. The specified laboratory is currently participating in international quality assurance programs which make our result more reliable.

3.10. ETHICAL CONSIDERATION

Ethical Clearance was given by the Department Research and Ethics Review Committee (DRERC) of Biochemistry. And consent was assured before interview of the mothers and sample collection from the umbilical cord of the neonates using an informed consent form, which was signed by the volunteer study participants' parent and counselor (Midwife's) on behalf of the principal investigator. The form was designed to identify by serial number rather than name of the participant. Participants' parent were informed not to sign full names in order that identities are not reveal. Thus, the confidentiality was confirmed. If any abnormality regarding thyroid function is detected in the course of the study, it was assured to be reported to the clinicians for further investigation and management.

4. RESULTS

123 Cord blood samples collected from umbilical cord of newborns with male to female ratio of 0.83 (56 males and 67 females) were analyzed for TSH, fT4 and fT3 values. Their birth weight ranged between 2500 g to 4700 g with mean (SD) value of 3241.46 (459.495) gram and 47 (38.8 %) of them have birth weight in the range of 3000-3490 gram. Table 4.1 presents the distribution of study participants in ranges of birth weight. Their gestational age ranged between 37 to 44 weeks. 74.8 % of them were born at the range of 39-42 weeks of gestational age. Table 4.2 shows the Gestational age distribution of the newborns and figure 4.2 presented it in histogram. The maternal age is displayed in figure 4.1 in which 82.9% of them were under the age of 30 years.

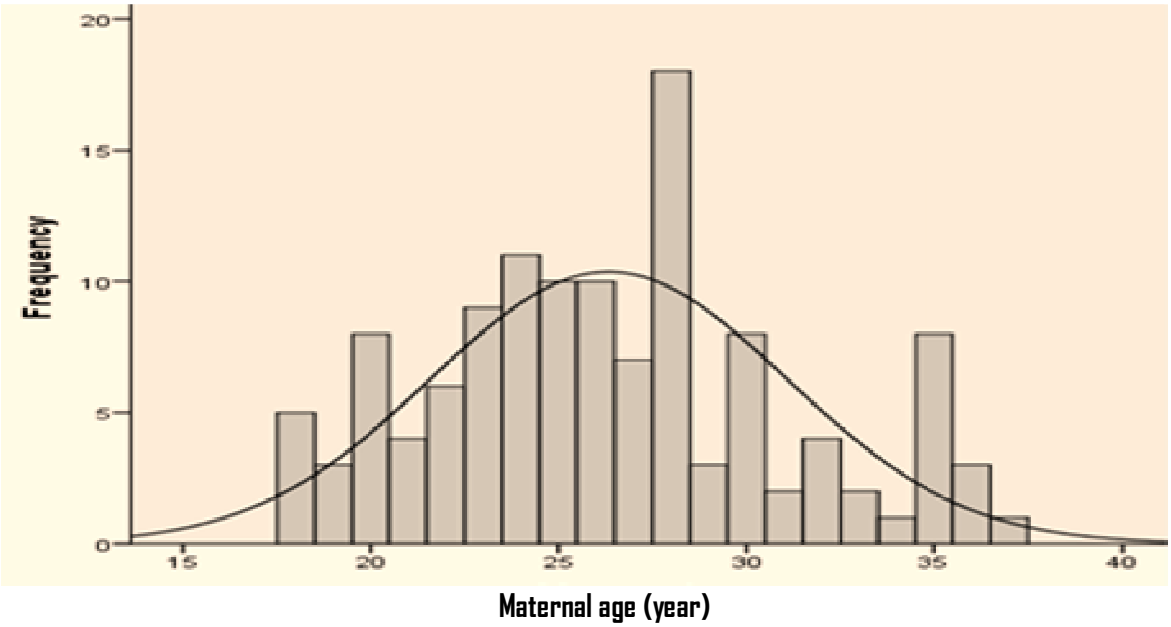

Figure 4.1. Frequency distribution of maternal age of the study participants.

Table 4.1. Birth weight wise distribution of samples

Birth Weight in gram	Number of Samples (%ages) N = 121
2000-2990	30 (24.8)
3000-3490	47 (38.8)
3500-3990	36 (29.8)
>4000	8 (6.6)

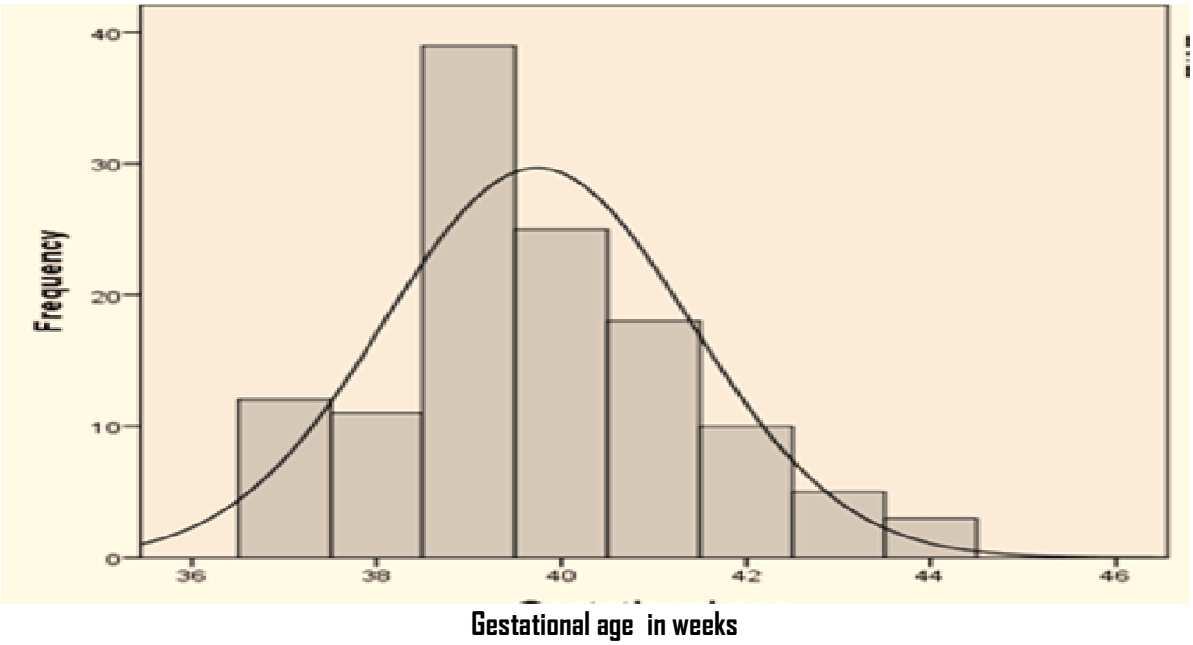

Figure 4.2. Frequency distribution of Gestational age of study participants.

Table 4.2. Distribution of study participants in ranges of Gestational ages

Gestational age in weeks	Number of samples (%ages)
37-38	23 (18.7)
39-42	92 (74.8)
>42	8 (6.5)
Total	123

Table 4.3. Mean values of cord blood TSH, fT4 and fT3 in male and female newborns

Variables	Mean± SD males	Mean± SD females	Mean± SD Total	P-value
TSH (mIU/L)	11.17±6.6	9.52±6	10.27±6.31	0.151
fT4 (ng/dL)	1.16±0.14	1.19±0.14	1.17±0.14	0.195
fT3 (pg/mL)	1.67±0.28	1.73±0.34	1.70±.034	0.302

4.1. TSH RESULTS

The Minimum and Maximum values after exclusion of outliers was 2.40 mIU/L and 30.15 mIU/L respectively. The mean (SD) and median values were 10.27 (6.31) and 8.37 mIU/L respectively. The 2.5th and 97.5th percentiles of TSH values were found to be 3.48 mIU/L and 27.56 mIU/L respectively. The combined and separate mean values for both sexes displayed in table 4.3. Table 4.4 depicts the TSH values of the entire participants.

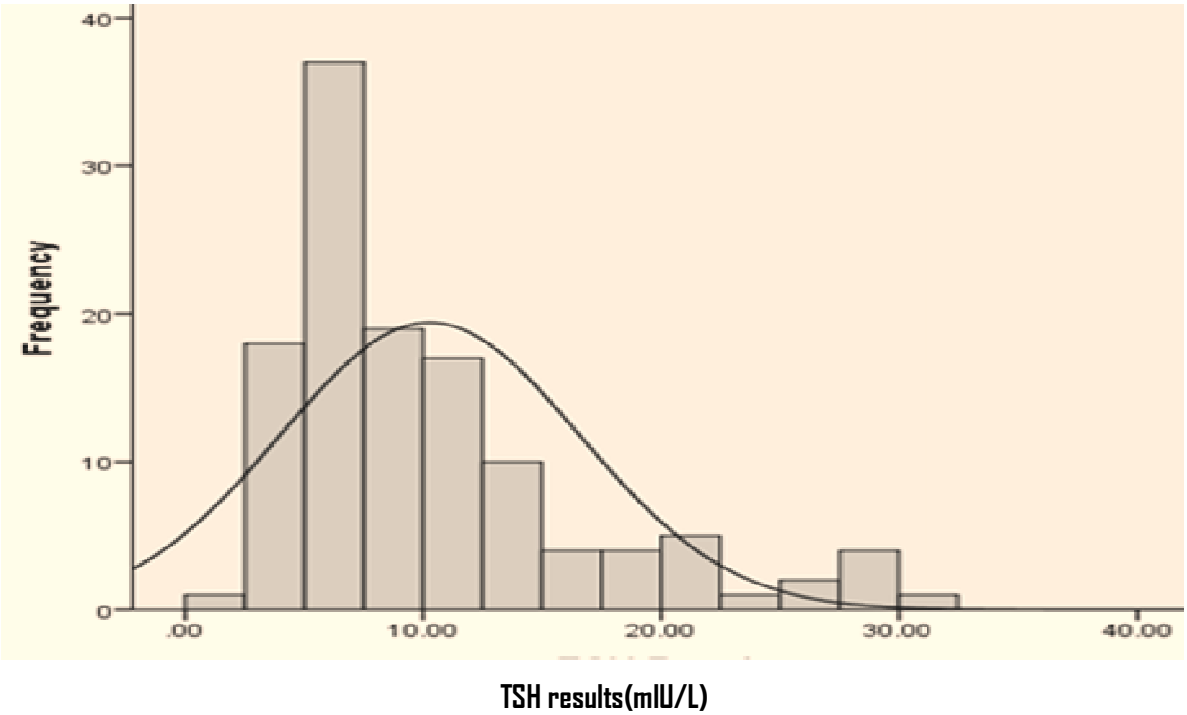

Figure 4.3. Distribution of TSH results among all the study participants.

4.2. fT4 RESULTS

The Minimum and Maximum values of fT4 were 0.71 ng/dL and 1.57 ng/dL with mean (SD) and median values of 1.17 (0.14) and 1.17 ng/dL respectively. 1.16 ng/dL and 1.19 ng/dL were the mean values for males and females respectively. The 2.5th and 97.5th percentiles were 0.89 ng/dL and 1.53 ng/dL.

Table 4.4. Summary of umbilical cord blood TSH levels

TSH result in mIU/L	No of Samples (%ages)
<4	6 (4.9)
4-7.99	52 (42.3)
8-11.99	33 (26.8)
12-15.99	13 (10.6)
16-19.99	6 (4.9)
20-24.99	6 (4.9)
25-29.99	6 (4.9)
30-34.99	1 (0.8)
Total	123

4.3. fT3 RESULTS

The Minimum and Maximum values of fT3 were 1.08 pg/mL and 3.66 pg/mL with mean (SD) and median values 1.70 (0.34) and 1.67 pg/mL respectively. The 2.5th and 97.5th percentiles were 1.19 pg/mL and 2.51 pg/mL respectively.

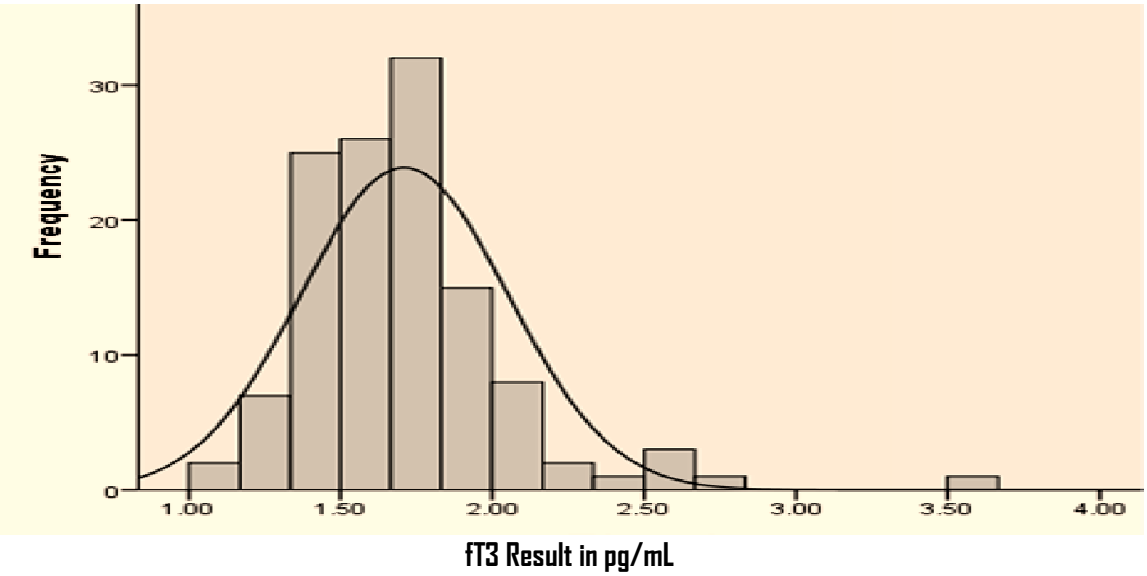

Figure 4.4. Frequency distribution of fT3 results

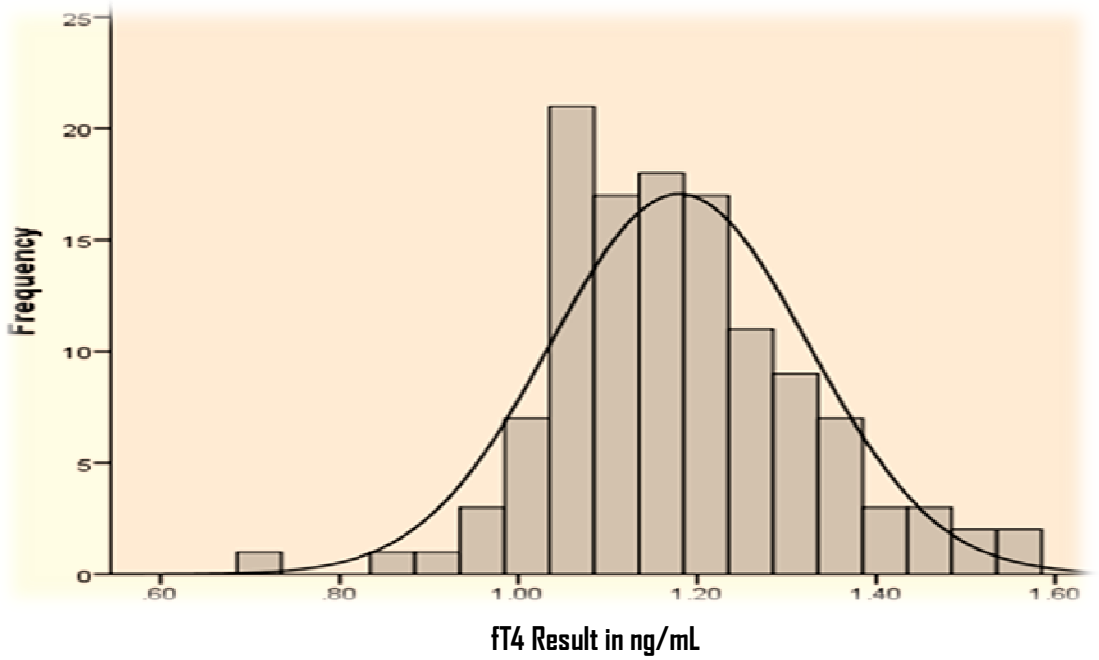

Figure 4.5. Distribution of fT4 result among all study participants.

Table 4.5. 2.5th and 97.5th percentiles of TSH, fT4 and fT3 values

Variables	Prior to log normalized		Log normalized	
	2.5th	97.5th	2.5th	97.5th
TSH (mIU/L)	3.48	27.57	3.48	27.56
fT4 (ng/dL)	.89	1.53	0.89	1.53
fT3 (pg/mL)	1.19	2.51	1.19	2.51

5. DISCUSSION

Congenital hypothyroidism often causes irreversible mental retardation if thyroid hormone replacement therapy is not begun during the first few months of life. The successful introduction of screening in the 1970's has enabled North America, Europe, to a limited extent Asia, Latin America and a few African countries to combat the ill effects of Congenital hypothyroidism and saved lives. Those screening programs have successfully helped in early diagnosis and treatment of congenital hypothyroidism (Mutlu *et al*., 2011). It has not been able to implement in Ethiopia because of several factors, like cost, lack of reliable laboratories on a large scale, and non availability of baseline data in our population. Use of cord blood TSH or combined with fT4 as a screening tool is an attractive preposition because of its simplicity and accessibility. Although several investigators have measured TSH and T4 in cord and serum samples from both premature and term infants, every reference laboratory needs to establish its own normal values in order to validate its own data and technical expertise. Population-specific reference intervals are an important prerequisite for interpreting thyroid hormone measurements. In addition to that, the clinical value of TSH, free thyroxine and free triiodothyronine analysis depends on the reference intervals with which they are compared (Schnabl *et al*., 2008). Therefore, it is important to have population specific normal values for this age group to avoid misdiagnosis and incorrect treatment. The present study was undertaken to establish standard reference values for better evaluation of thyroid function in cord sera in selected hospitals, Addis Ababa, Ethiopia.

Net of 123 study subjects were participated in the study. The demographic data, which are the maternal age, birth weight, gestational age and gender of the newborn, were obtained from the hospital records and interview of the mothers. The maternal age displayed in figure 4.1 shows that the mothers age was concentrated around the age of 23-32 years with mean values 26.33 years and age of 28 years was highly frequent. Since there was not written evidence about the age of the mothers, there is a probability to found biased data which have an effect on the test of association of maternal age with other variables. The birth weight of study subjects displayed by table 4.1, where 63.6% of them were born less than 3500gram with mean (SD) value of 3241.46 (459.495) gram.

The conventional 95th percentile reference limits (2.5th and 97.5th percentiles) method was used to determine the reference ranges of TSH, fT4 and fT3 values of the cord blood. Prior the establishing of the reference intervals the data was tested for normal distribution. The overall distribution of TSH result was positively skewed (figure 4.3), with skewness and kurtosis of 1.41 and 1.46 respectively. TSH values were further tested for normal distribution using Kolmogorov-Smirnove (K-S = 0.007) test and were not normally distributed. Based on the K-S test result obtained the lognormalization was necessary. After logarithmically normalized (figure 5.1.) and 2.5th and 95.7th percentile values were computed, the antilog of these values are 3.48 mIU/L and 27.56 mIU/L, respectively (Table 4.5).

The TSH results of the whole participants summarized in table 4.4. have shown a comparable trend as with the normative data for cord blood TSH values as reported by various workers across the globe. 74.0% of the cord blood results are less than 12 mIU/L, slightly higher than reports from India where 85.75% of results found to be less than 12 mIU/L (Manglik *et al*., 2005). Comparison of means of both sexes was performed using one way ANOVA. Based on the results obtained from this investigation, there is no statistically significant difference between the values for males and females in TSH (*p value 0.151*,), fT4 (*p value 0.195*) and fT3 (*p value 0.302*). This implies that there is no need to establish gender specific reference intervals.

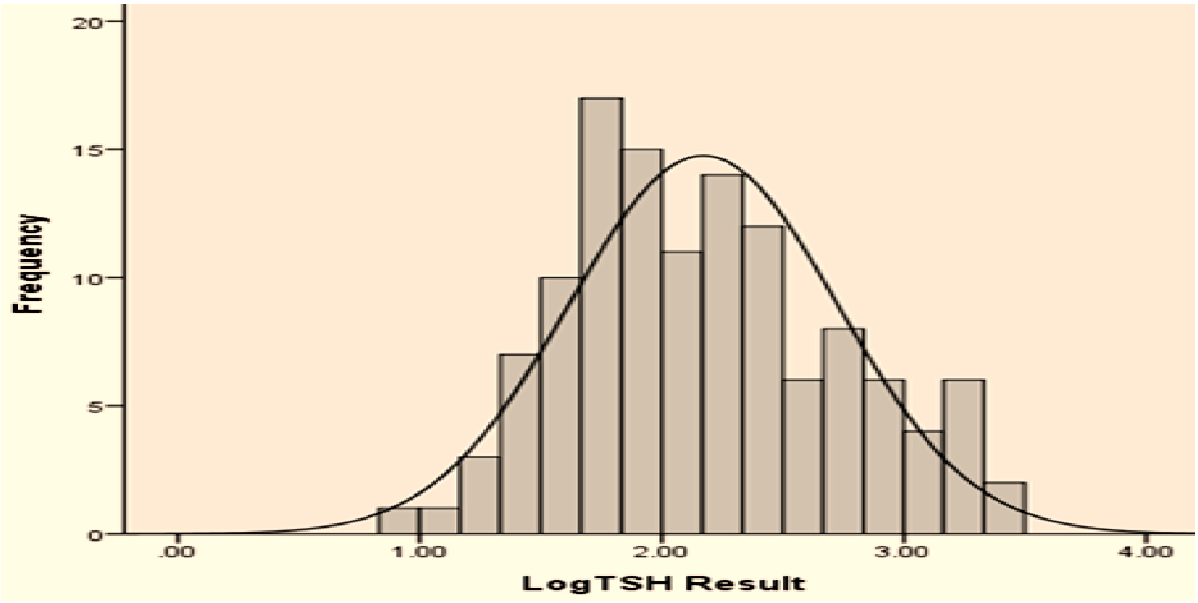

Figure 5.1. Distribution of TSH results among all the study participants after Log. Transformed

Previously few studies have reported in establishing cut-off values of cord blood TSH in Ethiopia. One study from yalemtsehay and co-workers was performed, at the same study area with the current study, and they determined the cut-off value of cord blood TSH (the 97.8th percentile in this case) was 15.4 mIU/L (Yalemtsehay *et al*., 2004). Another study, which was not on cord blood sample, had measured TSH values of blood from heel-pricks in neonates from six hours of life up to seven days old infants. They determined the cut- off point for TSH to be 29.4 mIU/l (Feleke *et al*., 2000). In the present work the 97.5th percentile of cord blood TSH was 27.56mIU/L which shows higher than reports from yalemtsehay and co-workers. This can be explained by the difference in method of analysis. Immunoradiometric assay was used in the previous study which had low sensitivity as 16.1% of males and 30.5% of females were reported for undetected results (Yalemtsehay *et al*., 2004) whereas the lowest values was 2.40 mIU/L in the present study using the Electrochemiluminescence Immunoassay Method. Other possible explanations were the previous study includes all the preterm and full term infants which can lower the cut-off values of TSH. Whereas only full term infants was included in the present work as if it had small sample size relative to the mentioned study.

Many studies reported that cord blood TSH can be used as a screening tool for congenital hypothyroidism from all over the world. A study from Japan had shown that mixed cord blood is a good sampling technique for screening for Congenital hypothyroidism (Fuse *et al*.,1991). And it was concluded that cord TSH had a better specificity and sensitivity as compared to cord or filter paper T4 at 3-5 days of age. A study from Iran shows the reference range of TSH concentration ranged from 0.77 to 24.91mlU/mL with a mean value of 7.09 (Karamizadeh & Amirhakimi, 1997) which had lower values comparing to the present study. However, the time of establishment shows it was done with older methods which may have lower sensitivity. This implies us the importance of method specific and timely updated reference values. One study from India reported the 97th percentile result of TSH as 25.8 mIU/L (Manglik *et al*. 2005) which was comparable but lower than the result of the present study. Both of the above studies had lower cut-off values of TSH relative to the current result obtained which can be explained by the timing of the research as old methods were used and the difference in the target population.

It have not yet encountered a studies with the issue of establishing reference ranges cord blood values for fT4 and fT3 in Ethiopia. Possibly the result of this study could be the first population specific base line data. The 2.5^{th} and 97.5^{th} percentiles are 0.89 ng/dL and 1.53 ng/dL for fT4 and 1.19 pg/mL and 2.51 pg/mL for fT3 respectively. Figure 4.5. presents the distribution of fT4 result among all study participants. The data were slightly skewed with skewness and kutosis value 0.262 and of 0.679 respectively but K-S test result shows fT4 values were normally distributed. Figure 4.4. presents the distribution of fT3 result among all study participants. And the data was slightly skewed data with skewness and kurtosis values of 1.973 and 8.24 respectively. K-S test result indicates fT3 result were normally distributed but have a close test value with the level of significance (0.053). The results of test of normally distribution for fT4 and fT3 shows that result were normally distributed. Therefore, the reference ranges were calculated directly from the untransformed data. The results were compared with studies from abroad and were comparable but slightly lower than results of one study from Turkey as it reported the 2.5^{th} and 97.5^{th} percentiles to be 1.07 ng/dL and 2.02 ng/dL for fT4 and 1.15 pg/mL and 2.81 pg/mL for fT3 (Mutlu *et al*., 2011).

TSH, fT4 and fT3 value were tested using the nonparametric test, kruska - wallis test, of correlation with Maternal age, Birth weight and Gestational age. Despite the reports which concluded that Maternal age and Gestational age were related to neonatal TSH level and another study showed that Gestational age was independently associated with lower cord TSH and high TT4 (Ingrida *et al*., 2010 & Herbstman *et al*., 2008), there was no statistically significant association of the variables with any of the listed maternal and fetal factors.

Even though they were not recalled for conformation, 6 new born had TSH values greater than the upper limit, 27.56 mIU/L, and 2 of the were 51.33 and 57.42 mIU/L. However higher TSH values were not supported by corresponding decrease of fT4 values. Therefore, they simply excluded as outliers from the analysis.

6. CONCLUSION

In the present study the reference intervals of TSH, FT4 and FT3 were tried to establish.The results obtained are 3.48 - 27.56 mIU/L for TSH, 0.89 - 1.53 ng/dL for fT4 and 1.19 - 2.51 pg/mL for fT3. Even though setting of cut-off values need its own expertise panel discussion, it is safe to use the conventional cut-off value of TSH , 20mIU/L, for recall purpose in the target population based on the reference range of cord TSH obtained in this study. Additionally it found that there is no correlation of cord blood values of thyroid function tests with maternal age, gestational age, birth weight and sex. However, Considering the vast concepts of reference ranges, this result only can provide us with an important starting point to establish population specific reference intervals using large scale studies from the whole country.

The present study, similar to other reports, suggests that TSH levels in cord blood might be a feasible alternative specimen for a congenital hypothyroidism screening program in countries where neonatal blood is not easily attainable. Previously it was emphasized by other study that in the given situation in Ethiopia where hospital discharges are in about 24 hrs, cord blood samples for congenital screening should be put in practice given all other necessary infrastructures put in place (yalemtsehay et al., 2004). As it also confirmed that cord blood could be used to prepare blood spots and the present study added with its assets of cut-off values using improved method, this theme of study issue seems progressive towards launching the screening program in our country.

7. RECOMMENDATIONS

We recommend that values of this study should be taken as a piece of brick to build the nationwide reference intervals of thyroid function tests in Ethiopia. Now a days there are central and regional laboratories in Ethiopia that are equipped with automated machines for hormone analysis. Therefore, it is possible to screen newborns for congenital hypothyroidism using cord blood sample.

Further studies are needed and better to include reference values of TSH, fT4 and fT3 from dry blood spot sample as it is the simplest sample transportation from remote areas to central laboratory and we recommend to use antibody test of maternal blood as exclusion criteria.

Considering the problems in this study where some of the demographic data were not properly responded, care should be given to the registration of demographic data especially the maternal age and gestational age which are important factors of cord blood values of TSH and thyroid hormones. Brief explanation about the importance of the data should be given to mothers before starting registration of their personal demography.

REFERENCES

Abduljabbar M., Al Shahri A., & Afifi A., (**2009),** Is umbilical cord blood total thyroxin measurement effective in newborn screening for hypothyroidism?. Journal of Medical Screening, 16(3), 119-123.

Alex Katayev, Claudiu Balciza, and David W. Seccombe, **(2010),** Establishing Reference Intervals for Clinical Laboratory Test Results, Am J Clin Pathol;133:180-186.

Anne Geffre, Kristen Friedrichs, Kendal Harr, Didier Concordet, Catherine Trumel, Jean-Pierre braun, **(2009),** Reference values: a review, veterinary clinical pathology,.

Arun Kumar Manglik, Nandita Chatterjee and Gautam Ghosh, (**2005),** Umbilical Cord Blood TSH Levels in Term Neonates: A Screening Tool for Congenital Hypothyroidism, Indian pediatrics ,volume 42

Avbelj M, Tahirovic H, Debeljak M, Kusekova M, Toromanovic A, Krzisnik C and Battelino T., **(2007),** High prevalence of thyroid peroxidase gene mutations in patients with thyroid dyshormonogenesis. Eur J Endocrinol, 156(5):511-519.

Bakker B, Bikker H, Vulsma T, de Randamie JS, Wiedijk BM, De Vijlder JJ., **(2000),** Two decades of screening for congenital hypothyroidism in The Netherlands, TPO gene mutations in total iodide organification defects (an update). J Clin Endocrinol Metab, 85(10):3708-3712.

Bernal J, Guadano-Ferraz A, Morte B. **(2003),** Perspectives in the study of thyroid hormone action on brain development and function. Thyroid 13:1005–1012.

Biebermann H, Schoneberg T, Krude H, Schultz G, Gudermann T & Gruters A (1997), Mutations of the human thyrotropin receptor gene causing thyroid hypoplasia and persistent congenital hypothyroidism. J Clin Endocrinol Metab, 82(10):3471-3480.

Boivin J, Bunting L, Collins JA, and Nygren KG., **(2007),** International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod ;22(6):1506-12.

Boivin J., Rice F., Hay D., Harold G., Lewis A. and van den Bree MM, **(2009),** Associations between maternal older age, family environment and parent and child wellbeing in families using assisted reproductive techniques to conceive. Soc Sci Med;68(11):1948-55.

Brown RS and Demmer LA**., (2002)**,The etiology of thyroid dysgenesis-still an enigma after all these years. J Clin Endocrinol Metab, 87(9):4069-4071.

Bubuteishvili L., Garel C., Czernichow P., and Leger J., **(2003),** Thyroid abnormalities by ultrasonography in neonates with congenital hypothyroidism. JPediatr, 143(6):759-764.

Castanet M., Polak M., Bonaiti-Pellie C., Lyonnet S, Czernichow P., and Leger J., **(2001),** Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. J Clin Endocrinol Metab, 86(5):2009-2014.

Ceriotti F., **(2007),** Prerequisites for use of common reference intervals. Clin Biochem Rev.;28:115-121.

Clinical and Laboratory Standards Institute (**2008**), Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition CLSI document C28-A3 (ISBN 1-56238-663-8). Wayne: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute (**2009),** Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd ed.Wayne, PA: CLSI

Clinical and Laboratory Standards Institute (**2010**), Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition CLSI document C28-A3 (ISBN 1-56238-663-8). Wayne: Clinical and Laboratory Standards Institute.

De Felice M., Di Lauro R., **(2004),** Thyroid development and its disorders: genetics and molecular mechanisms. EndocrRev, 25(5):722-746.

Edgard E. Delvin , Vijay Laxmi Grey & Zulfikarali Vergee, **(2006),** Gap analysis of pediatric reference intervals related to thyroid hormones and the growth hormone–insulin growth factor axis, Clinical Biochemistry 39 588–594.

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP, **2001**)) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Jama; 285: 2486–97.

Feleke Y., Enquoselassie F., Deneke F., Abdulkadir J., Hawariat G. W. Tilahun M. Mekbib T., (**2000)** , neonatal congenital hypothyroidism screening in Addis Ababa, Ethiopia, East African medical journal ISSN 0012-835X.

Fuse Y., Wakae E., Nemoto Y., Uga N., Tanaka M., Maeda M., (**1991**), Influence of perinatal factors and sampling methods on TSH and thyroid hormone levels in cord blood. Endocrinol Jpn; 38: 297-302.

Graham Jones and Antony Barker, **(2008),** Reference Intervals, Clin Biochem Rev Vol 29

Grant DB, Smith I, Fuggle PW, Tokar S, Chapple J, (**1992)**, Congenital hypothyroidism detected by neonatal screening: relationship between biochemical severity and early clinical features. Arch Dis Child, 67(1):87-90. in the clinical laboratory. Clin Chem 32:585–592

Grasberger H, Mimouni-Bloch A, Vantyghem MC, van Vliet G, Abramowicz M, Metzger DL, Abdullatif H, Rydlewski C, Macchia PE, Scherberg NH., **(2005),** Autosomal dominant resistance to thyrotropin as a distinct entity in five multigenerational kindreds: clinical characterization and exclusion of candidate loci. J Clin Endocrinol Metab, 90(7):4025-4034.

Grasberger H, Vaxillaire M, Pannain S, Beck JC, Mimouni-Bloch A, Vatin V, Vassart G, Froguel P, Refetoff S **(2005),** Identification of a locus for nongoitrous congenital hypothyroidism on chromosome 15q25.3-26.1. Hum Genet, 118(3-4):348-355.

Guyton & John E. Hall , (**2006),** Text Book of Medical Physiology, 11th Edition (931-942) ISBN 0-7216-0240-1

Herbstman J, Apelberg BJ, Witter FR, Panny S, Goldman LR. (**2008),** Maternal, infant, and delivery factors associated with neonatal thyroid hormone status. Thyroid;18(1):67-76.

Ingrida Mockute, Eimantas Švedas, Nijolė Raškauskienė, Narseta Mickuvien &, Robertas Bunevi čius, **(2010),** The newborn thyroid stimulating hormone levels in relation to maternal age and gestation at birth, rugsėjis, tomas XIII, Nr. 3

International atomic energy agency **(2005)** Screening of Newborns for Congenital Hypothyroidism, Guidance for Developing Programmes, vienna.

J. Kratzsch , G. Schubert , F. Pulzer , R. Pfaeffle , A. Koerner , A. Dietz , M. Rauh, W. Kiess & J. Thiery, **(2008),** Reference intervals for TSH and thyroid hormones are mainly affected by age, body mass index and number of blood leucocytes, but hardly by gender and

thyroid auto antibodies during the first decades of life, Clinical Biochemistry 41 1091–1098.

James C. Boyd **(2010**), Defining laboratory reference values and decision limits populations, intervals, and interpretations. Asian Journal of Andrology (12: 83–90)

Jinsong zhang and mitchell a. lazar **(2011),** The mechanism of action of thyroid hormones.

Kareena Schnabl, Man Khun Chan, Yanping Gong, Khosrow Adeli, **(2008),** Closing the Gaps in Paediatric Reference Intervals, The CALIPER Initiative Clin Biochem Rev Vol 29 November.

Klaudia Brix, (**2011),** Molecules important for thyroid hormone synthesis and action.

Klaus Kapelari, Christine Kirchlechner, Wolfgang Hogler, Katharina Schweitzer, Irene Virgolini and Roy Moncayo, **(2008)**, Pediatric reference intervals for thyroid hormone levels from birth to adulthood, 8:15 doi: 10.1186/1472-6823-8-15

LaFranchi S, **(1999)**, Congenital hypothyroidism: etiologies, diagnosis, and management. Pediatr Clin North Am, 26(1):33-51.

LaFranchi S.. **(2007**), Disorders of the thyroid gland. In: Kliegman RM, Behrman RE, Jenson Pediatr Clin North Am, 26(1):33-51.

LaFranchi SH:, **(1979)**, Hypothyroidism. Pediatr Clin North Am, 26(1):33-51.

Lago P & Garetti E, (**2009),** Guidelines for procedural pain in the newborn. Acta Paed.; 98(6):932-939.

Lania AG., Mantovani G. and Spada A., **(2006),** Mechanisms of disease: Mutations of G proteins and G-protein-coupled receptors in endocrine diseases. Nature clin pract endocrinol metab, 2(12):681-693

Mehmet Mutlu, Gulay Karaguzel, Yuksel Aliyazicioglu, Ilker Eyupoglu, Aysenur Okten and Yakup Aslan, **(2011),** Reference intervals for thyrotropin and thyroid hormones and ultrasonographic thyroid volume during the neonatal period in Turkey, The Journal of Maternal-Fetal and Neonatal Medicine, UK. Ltd, 10.3109/14767058..561894

Mandel SJ., Hermos RJ., Larson CA., Prigozhin AB., Rojas DA., Mitchell ML., **(2000)**, Atypical hypothyroidism and the very low birthweight infant. Thyroid 10(8):693-695.

Masanori Adachi1, Akiko Soneda, Yumi Asakura, Koji Muroya, Yuji Yamagami and Fumiki Hirahara, **(2012)**, Mass screening of newborns for congenital hypothyroidism of central

origin by free thyroxine measurement of blood samples on filter paper, European Journal of Endocrinology ,166 829–838

Maynika V Rastogi and Stephen H LaFranchi, (**2010)**,Congenital hypothyroidism, Rastogi and La Franchi Orphanet Journal of Rare Diseases 5:17 http://www.ojrd.com/content/5/1/17

McElduff A., McElduff P., Wiley V., Wilcken B., **(2005),** Neonatal thyrotropin as measured in a congenital hypothyroidism screening program: influence of mode of delivery. J Clin Endocrinol Metab 2005;90:6361–3

Norbert W.T, W.T.C., Esther F.F, John F.K, Howard M.R, **(1982**), Fundamentals of Clinical Chemistry. 2 ed., USA: W.B. Saunders company.

O¨ zo¨n ZA. (**2008)**, Congenital hypothyroidism monitorization programs, current status in Turkey Turkiye Klinikleri. J Endocrin Spec Top;1:1–7.

Offie P. Soldin, Megan Jang,Tiedong Guod & Steven J. Soldin, **(2009),** Pediatric Reference Intervals for Free Thyroxine and Free Triiodothyronine, Mary Ann Liebert, Inc. DOI: 10.1089=thy.0037.

Shiri B. Feingold and Rosalind S. Brown, (**2010),** Neonatal Thyroid Function, Neo Reviews; 11;e640-e646 Stanton BF, editors. Nelson textbook of pediatrics. 18th ed. Philadelphia: Sounders Elsevier;. pp 2316–2327.

Utah Governor Huntsman, (**2006),** Clinical Lab Products. Issue Stories. May 2006. http:// www.clpmag.com/issues/articles/2006-05_03.asp.

van Tijn DA., Vijlder JJ., Verbeeten B., Verkerk PH. And Vulsma T., (**2005),** Neonatal detection of congenital hypothyroidism of central origin. JClinEndocrinolMetab 90(6):3350-3359.

Wassie E., and Abdulkadir J., **(1990**), Normal thyroid function values in Ethiopians. Ethiopian medical journal, 28(3), 139.

Williams FL.,Ogston SA., and van Toor H., (**2005)**, Serum thyroid hormones in preterm infants: associations with postnatal illnesses and drug usage. J Clin Endocrinol Metab.; 90.

Yalemtsehay Mekonnen, Gizachew W. Hawariat, Bekele Chamiso and Friedhelm Raue, **(2004**), "Thyroid Stimulating Hormone values from cord blood in neonates." Ethiopian Journal of Health Development 17.2 .125-130.

Z. Karamizadeh and G.H. Amirhakimi, **(1997),** Thyroxine and Thyrotropin levels in cord blood sera from south of Iran Medical Journal of the Islamic Republic of Iran Volume 11.

ANNEX 1 QUESTIONNAIRE

INFORMATION FOR THE PARTICIPANT:

Dear participants of this study, we appreciate in advance for being part of this study and providing genuine information. This study is initiated by the department of Biochemistry, College of Health Sciences, AAU and Ethiopian Health Nutrition Research Institute (EHNRI).

Section 1-questionnaire identification

(1) Respondent's Identification code (to be created by yourself, this five digits number and/or alphabets will be used for you to retrieve your lab result in the future, thus please keep this number for future retrieving your result)_____

(2) Where is your Birth place? Region_____

(6) For how long have/had you been in your birth place ?_____years

(7) Where do you live currently?_____

(8)Age_____ (yrs)

9) How money births do you have before----------

10) Do you have a child with any type of disease? If Yes what kind?_____

Section 2. Clinical information

(11) Do you take any medication during pregnancy? YES----- NO----- If Yes what kind?_____

(12) Do you take any supplementation during your pregnancy? YES_____NO_____ If yes what type?_____

(13) Type of salt you use to prepare your food during pregnancy. Iodinated _____or Non iodinated_____

(14) Have you ever been sick for the last one year? Yes □ No □ ,

If yes, what was your illness? _____

Section –3 alcohol drinking habit during the pregnancy.

If you don't drink alcohol at all skip this section.

(15) How often do you drink alcohol? Daily □ Every weekend □ occasionally □

Section –4 chat chewing habit during the pregnancy

If you don't chew at all skip this section.

(16) How often do you chew chat?

Daily Every weekend Occasionally

Section –5 smoking habit

If you do not have smoking habit skip this section.

(17) How often do you smoke cigarettes? Chain smoker Occasional smoker

End of interview

Dear participant, Thank you very much for taking your time and due concern.

ANNEX .2. CONSENT FORM

2.1. ENGLISH VERSION

Participant's Code number_____

Addis Ababa University, Department of Biochemistry, in collaboration with Ethiopian Health Nutrition Research Institute (EHNRI) has designed a study to establish reference intervals cord blood for thyroid function test.

I have been informed about the study, which plans to establish reference interval of thyroid function tests. The objective and the application of the study were explained to me. I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and hospital access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the specimen from the umbilical cord of my new born infant and to give an interview for the mentioned study.

I also agreed that the specimen might be stored for further investigation to be done. Moreover I have had the opportunity to ask questions about the project and I have received clarifications to my satisfaction in a language I understand.

I was also told that results for the thyroid function tests would be reported timely to the nurse in charge of counseling and that I may ask my result if I want. The project will link me with nearby hospitals if my baby is in need of treatment.

I----------hereby give my consent for giving of the requested information and specimen for the purpose of the study.

Signature: Date_____

Participant:_____ _____

Nurse(counselor)_____ _____

2.2. AMHARIC VERSION (የአማሪኛ ቅጂ)

የዚህ ጥናት ተሳታፊወች የስምምነት ቅጽ፡

የተሳታፊ መለያ ቁጥር----------

በአዲስ አባባ ዩኒቨርሲቲ የህክምና ፋኩልቲ ባዮኬምስተሪ ትምህርት ክፍል ከኢትዮጵያ የጤናና ምግብ ምርምር ተቋም ጋር በመተባበር አዲስ ሰለሚወለዱ ህጻናት ጤንነት ሁኔታ ለማወቅ የሚያስችል ጥናት ለማከሄድ አቅደዋል፡፡

ይህ ጥናት ሲወለዱ የታሮይድ ንጥረ ነገር እጥረት ያለባቸውን ህጻናት ለመለየትና አስፈላጊውን ህክምና ለመስጠት የሚያስችል ማጣቀሻ ለማዘጋገጥ መሆኑ ተገልጾልኝ የኔም ሆነ የልጄ ሚስጥርም እነደሚጠበቅ ተነግሮኛል፡፡ ስለሆነም በዝህ ጥናት ያለመሳተፍም ሆነ ካለተመቸኝ የማቋረጥ ሙሉ መብት እንዳለኝ ተማምኜ÷ የምወሰዳቸው እርምጃዎች በማገኘው የህክምና አገልግሎት ላይ ምንም አይነት ተጽእኖ እንደማይፈጥሩብኝ ተነገሮኝ አዲስ ከወለድኩት/ዋት ልጀ እትብት ላይ የደም ናሙና ለመስጠትና እራሴም ቃለ መጠይቅ ለመስጠት ተስማምቻለሁ፡፡ ከልጀ እትብት የተወሰደው የደም ናሙና ከጥናቱ ብኋላም ለተጨማሪ ምርምር እንዲቀመጥ ተስማምቻለሁ፡፡ የምርምራ ውጤቱ ከፈለኩት በግዜው በአማካሪ ነርስ በኩል እንደሚደርሰኝና አስፈላጊ ሁኖ ከተገኘም አቅራቢያየ ወደሚገኝ የህክምና ማአእከል እነደሚላክልኝ ተገልጾልኛል፡፡

እኔ ---------- በዚህ ጥናት ለመሳተፍ መስማማቴን አረጋግጣለሁ፡፡

	ፊርማ	**ቀን**
የተሳታፊ	----------	----------
የአማካሪ(ነርስ)	----------	----------

ANNEX 3. STANDARD OPERATIONAL PROCEDURES

Ethiopian Health and Nutrition Research Institute
Infectious and Non-Infectious Diseases Research Directorate
Addis Ababa, Ethiopia

TITLE: Performing Free Thyroxine Hormone on the Elecsys 2010 Immunoassay System	**Origin Date: August 18/2010**
Document Number: CHEM.TP. 016	**Revision:** Current
Section: Clinical Chemistry	Page: **43** of **56**

Purpose

This procedure provides instructions for performing TSH on the Elecsys 2010 Immunoassay system.

Abbreviations

TSH-Thyroid Stimulating Hormone
EDTA-K_3 – Potassium Ethylene diamine tetra acetic acid
RT= Room Temperature
TT4= Total Thyroxine
Cal = Calibrator
ECLIA= Electrochemiluminescence Immunoassay

Materials

Reagents

TSH Reagent kits: Roche Cat., 117314591

- **M**-Streptavidine-coated microparticles, Streptavidin-Coated Microparticles, 0.72mg/ml, 470 biotin/mg microparticles (Transparent Cap)
- **R1** Anti-TSH-Ab-biotin, biotinylated monoclonal anti –TSH -antibody 2.0 mg/l phospha buffer 100mmol/l
- **R2** Anti-TSH -Ab~ $Ru(bpy)^{2+}_3$, 10 ml Monoclonal anti TSH antibody labeled with rutheniu complex 1.2 mg/l phosphate buffer 100mmol/l (Black Cap)

Reagents preparation: ready for use.
Reagents stability and storage:

- Unopened at 2-8°C up to the stated expiry date
- After opening at 2-8°C -12 weeks
- On board - 8 weeks

Note: Store reagent kit **upright** in order to ensure complete availability of the micro particles during automatic mixing prior to use.

Supplies

1.Assay tips, Roche, 11706799
2. Assay cups, Roche, 11706802
3. wash water additives, Roche, 11930346
4. Procell, Roche , 11662988
5. Clean cell, Roche ,11662970
6.Syswash, Roche, 11930346
7.Gauze
8.70% of alcohol
9.Applicator stick

Equipment

- Elecsys 2010 Analyzer
- Micro pipette(20-1000µl)
- Vortex
- Centrifuge
- Sample rack • Pipettes of Different Volume

Sample

Sample type	Amount required	Transport and Storage	Stability
Serum	0.3mL	• Transport whole blood at RT • Separate serum within 1 Hr. • store serum at 2-8°C or -20°C	• For seven days at 2-8°c, • One month at -20°c.
• **Plasma** treated with Heparin, EDTA-K_3, Sodium citrate or sodium fluoride/ Potassium oxalate	0.3mL	• Transport whole blood at RT • Separate serum within 1 Hr. • store serum at 2-8°C or -20°C	•For seven days at 2-8°c, •One month at -20°c.

Note: Serum should only be frozen once. Do not thaw and refreeze
Limitations: Gross hemolysis, lipemic and icterus specimen.
Sample retention: Specimens are discarded in accordance with EHNRI Specimen retention policy. This refers to both Specimens in the Primary and Secondary Containers.

Special Safety Precautions

- Use Universal safety precaution(wearing gloves lab coat and washing hands) when handling infectious materials
- Refer to the National Health and Safety Guidelines for standard safety procedures

Calibration

Calibrator	Level	Stability	Frequency	Preparation (y/n)
TSH Cal set, Roche, 11731483122	Cal1 Cal2	• Unopened up to expiry Date labeled on the Pack. •After opening •12 weeks at 2-8°C •Up to 5 hours on	• After 1 month (28 days) when using the same reagent lot • After Curative maintenance • When Quality control	No

			the analyzer at 20-25°C	results indicate need for recalibration	

Calibrator preparation:

Mix carefully, avoiding the formation of foam. Transfer into the empty labeled snap-cap bottles supplied. Due to Possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

Note:

- Calibrators must be at room temperature before use
- Lot reagent calibration has to be done within 24 hours after registering the new reagent kit on the analyzer
- Write the opening date on the bottles.

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
Precicontrol Universal, Roche, 11731416122	PC U1 PC U2	● Unopened up to expiry Date labeled on the Pack	● Daily	Yes

Control preparation:

Add 3.0mL of distilled water using a volumetric pipette. Let it sit for 30 minutes and mix carefully, avoiding the formation of foam.

- ● Stability: the prepared Quality Control is stable for 1 days at RT, 2-8 °C for 2 weeks and 1 month at -20°C.
- ●Write the reconstitution date on the bottles.

Note:

- Controls must be kept at room temperature before use
- Controls must be within range. If out of range, repeat the run. If still out of the range. Investigate for root cause. (Reagent, Calibration and QC Preparation....etc)
- Solve the Problem, Document and rerun the Quality controls and Patient samples.

Procedure

Refer to how to order tests of *Elecsys 2010 Immunoassay system* CHEM.TP.005

Result Interpretation

Step	Action
1	Values below detection limit are reported as < 0.005 µIU/mL.
2	Dilute values above the Analytical range (100 µIU/mL.) using on board diluent. The recommended dilution is 1:10 *If manual dilution, then multiply the result by the dilution factor*

Expected Values

Analyte	Reference Range		Analytical Range	Units
	Male	Female		
Adult	0.27- 4.2	0.27- 4.2	0.005-100	µIU/ml
Children 0-12 month 1-6 years 7-12 year	 1.36-8.8 0.85-6.5 0.28-4.3			

Principle	TSH is measured using Elecsys 2010 immunoassay analyzer which is a fully automatic run-oriented analyzer system for the determination of immunological tests using the electrochemiluminescence immunoassay "ECLIA"process. All components and reagents for routine analysis are integrated in or on the analyzer.
Clinical Utility	• first-line assay on suspicion of primary hyper or hypothyroidism • in combination with FT_4 & T_3 (FT_3). When Suspecting thyroid hormone resistance • in combination with FT_4 on suspicion of secondary hypothyroidism • Monitoring of T4 treatment in thyroid substitution or suppression therapy • Evaluation of hyperprolactinemia • Evaluation of hypercholesterolemia
Related Procedures and Documents	• Roche Elecsys 2010 Operator Manual • Elecsys 2010 Clinical Chemistry Analyzer Quality Control Running Procedure CHEM.TP.003 • Elecsys 2010 Clinical Chemistry Analyzer Calibration Procedure CHEM.TP.002 • Elecsys 2010 Clinical Chemistry Analyzer Procedures How to Order Test CHEM.TP.005 • Preventive Maintenance for Elecsys 2010 Clinical Chemistry Analyzer • CHEM.TP.004 • Elecsys 2010 Clinical Chemistry Analyzer Start up and Shutdown Procedure-CHEM.TP.001 • Specimens Collection and Processing Procedures • EHNRI Quality Manual
References	• Roche Elecsys 2010 Operator Manual • Roche Elecsys 2010 Package Inserts • Tietz NW, editor. Text book of Clinical Chemistry.3rd ed. Philadelphia: WB Saunders,2001 • Chernecky & Berger: Laboratory Tests and Diagnostic Procedures, 5th ed. 2008 • Lothar Thomas: Clinical Laboratory Diagnostics, use and assessment of Clinical Laboratory Results 1st ed.1998,

NB: Both TSH and fT3 also performed according the established standard operational principle of the institution which is the same content with that of fT4 (mentioned here for simplicity) except specific reagents were used.