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Hematological parameters of patients with thyroid dysfunction at S.t Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by, Kedir Mohammed, entitled: **Hematological parameters of patients with thyroid dysfunction at S.t Paul's Hospital Millennium Medical College (SPHMMC)**, **Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology specialty track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acronyms and Abbreviations

AAU	Addis Ababa University
BAS%	Basophil percentage
CBC	Complete blood count
CH	Congenital hypothyroidism
DLC	Differential leukocyte count
EDTA	Ethylene diamine tetra acetic acid
EOS%	Eosinophil percentage
EPHI	Ethiopian public health institute
EPO	Erythropoietin
Hb	Hemoglobin
HCT	Hematocrit
HT	Hashimoto's thyroiditis
LYM %	Lymphocyte percentage
MCH	mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MON%	Monocytes percentage
MPV	Mean platelet volume
MXD #	Mixed cells absolute number
NEU #	Neutrophil absolute number
NEU %	Neutrophil percentage
NRR	Normal reference range
PB	Peripheral blood
PCV	Packed cell volume

PDW	Platelet distribution width
PHT	Primary hypothyroid
PI	Principal Investigator
PLT	Platelet
RBC	Red blood cell
RDW	Red blood cell distribution width
SCH	Subclinical hypothyroidism
SOPS	Standard operating procedures
SPHMMC	St Paul's Hospital Millennium Medical College
SPSS	Statistical package for social sciences
T3	Triiodothyronine
T4	Tetraiodothyronine
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
TWBCs	Total white blood cells
WBC	White blood cell
WHO	World health organization

Abstract

Background: Thyroid hormones have a decisive role in metabolism and proliferation of blood cells and blood cells indices. Thyroid dysfunction leads different effects on blood cells such as anemia, leukopenia, thrombocytopenia, and in rare cases causes pancytopenia.

Objectives: To determine hematological parameters of patients with thyroid dysfunction at S.t Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia, from March-August, 2020.

Methods: A hospital based comparative cross sectional study was conducted from March to August, 2020, at SPHMMC, Addis, Ababa, Ethiopia. Convenient sampling technique was used for comprising of a total of 180. Participants' socio-demographic and clinical information was collected from hospital cards. Initially 10 ml of venous blood samples were collected in to two separated test tubes. The blood specimen in the plain tube used for measurement of TSH, T3 and T4 (Cobase e 411 immunoassay system). Sample in the EDTA tubes, used for CBC analysis (Beckman-coulter hematology analyzer). Descriptive statistics was used to express the socio demographic and clinical characteristics. Independent T test to compare mean value between the groups, binary and multiple logistic regressions were computed to assess association be-tween variables using SPSS version 20. P value less than 0.05 were taken as statistical significant.

Results: Of the 180 study participants, 74(41.1%) hypothyroidism and 49(27.2) hyperthyroidism and 57(31.8%) were apparently healthy controls. Of them 137 (71.1 %) were female, most in the age group 26-39 years (84, 46.7%). The result obtained showed a statistically significant decrease in RBC count, Hb, HCT, MCV, PLT counts and MPV in thyroid dysfunction patients when compared with apparently healthy controls (p value <0.05). MCHC, RDW, WBC and NEU% were statistically significant increased (p value <0.05). MCH, MON%, EOS% and BAS%, did not showed significant difference between the groups (p value >0.05).

Conclusion: Thyroid hormones have a significant influence on blood cell count and blood cell indices. This study showed that statistical significant difference in RBCs, Hb, HCT, MCV MCHC, total WBC count, neutrophils, PLT count, and MPV between patients with thyroid dysfunction and apparently healthy controls (p<0.05). But no difference showed MCH, monocytes, eosinophil and basophils between those groups (p- value > 0.05).

Keywords: Blood cell count, Blood cell indices, Hypothyroidism, Hyperthyroid

1. Introduction

1.1 Background

The thyroid gland is the largest endocrine gland in human body, which is located on the anterior side of the neck, right below the larynx. It has two lobes and is formed by many thin follicular cells with a type of epithelial tissue origin. These follicles store thyroid hormones in the form of thyroglobulin molecules until body needs them. Thyroid gland produces and secrets two major hormones known as triiodothyronine (T3) and tetraiodothyronine (T4). These two hormones are necessary for regulating metabolism rate [1]. Hormonal output from the thyroid is controlled by thyroid stimulating hormone (TSH) or thyrotropin secreted by anterior pituitary. TSH secretion itself is mediated by thyrotropin-releasing hormone (TRH), secreted by the hypothalamus [2].

Hypothyroidism is a condition in which the thyroid gland does not produce enough functionally active thyroid hormones. Primary hypothyroidism is marked by elevated TSH levels and reduced thyroid hormones (T3 andT4). Causes of primary hypothyroidism include: functional problems within the thyroid gland, infiltrative disease of the thyroid, silent or sub-acute thyroiditis, chronic autoimmune thyroiditis, radioiodine therapy, and postoperative hypothyroidism [3].
Secondary hypothyroidism caused due to anterior pituitary gland failures, it does not produce and release adequate amount of TSH that is needed by the body. Tertiary hypothyroidism is due to problems within the hypothalamus cause decreased synthesis of TRH and subsequent decreased stimulation of the pituitary gland [4].

Females are more affected thyroid dysfunction than male; reason for this is estrogen dominance in female. Estrogen breaks down by the liver, in order to remove from the body, the breakdown product of estrogen causes direct death of thyroid cells and it also causes increase immune markers responsible for autoimmune thyroid disease [5, 6].
Hematopoiesis is process of production of all of the cellular components of blood and blood plasma. Hematopoiesis occurs within the hematopoietic system, which includes organs and tissues such as the bone marrow, liver, and spleen. All blood components are derived from the hematopoietic stem cells which divided into three lineages [7]. Erythropoiesis, which results of the erythropoiesis, lymphopoiesis which produces T and B cell lymphocytes and myelopoiesis results granulocytes, megakaryocytes and macrophages. One of the influencing factors for hematopoiesis is thyroid hormones [8].

Thyroid hormones include involvement in hemoglobin production in adult and maturation of Hb in fetus (2- 4). They enhance erythropoiesis through hyper proliferation of immature erythroid progenitors and increase secretion of erythropoietin (EPO) by inducing erythropoietin gene expression. Thyroid hormones also augment repletion of hypoxia inducible factor1 (HIF-1) and then motivate growth of erythroid colonies (BFU-E, CFU-E).These hormones also intensify erythrocyte 2, 3 DPG compactness, which enhances the delivery of oxygen to tissues. T3 is as a precursor substance for normal B cell formation in bone marrow through its mediation of pro-B cell proliferation [9, 10].

Thyroid hormones may effect on megakaryocytes through modulation of bone marrow matrix proteins, such as fibronectin. Thyroid hormones increase the expression of fibronectin gene. Fibronectin appears to affect megakaryocyte maturation and thrombopoiesis through interaction with integrin α4β1 [11].

Hypothyroidism can alter platelet function, through changing adenozin diphosphate-induced aggregation which violates activation of platelets, slowing or stopping the adhesion and aggregation, increased disaggregation can lead to severe hemorrhage [12].

Hyperthyroidism affects hematopoiesis in many ways, but its pathogenesis is still unclear. Both thyrotoxicosis and the underlying autoimmunity of grave's disease may affect the production of blood cells. Therefore, thyroid disorders can induce different effects on various blood cell lineages] 13].

1.2 Statement of the problem

anemia, atrophic gastritis, celiac disease and autoimmune haemolytic syndrome [17, 18].

Hypothyroidism is one of the most common diseases of endocrine system, which affect all systems including hematopoietic system. Hypothyroidism causes decreases in total white blood cells (WBC) count, neutropenia, and thrombocytopenia. It also causes various forms of anemia (normochromic-normocytic, hypochromic-microcytic or macrocytic) [19, 20].

According to data of WHO, anemia prevalence is 24.8% throughout the world and it is more frequent in underdeveloped countries. Prevalence of anemia in subclinical and overt hypothyroid group was 26.6% and 73.2% respectively. Thus, frequency of anemia in The thyroid hormone has a very important role in the body metabolism in general including the hematopoiesis. Thyroid dysfunctions are frequently presented with the different hematological blood cell and blood cell indices abnormalities [14, 15].

Patients with thyroid abnormalities may have low iron levels which affect the hemoglobin levels, also they may have reduced levels of both folate and B12 which have been detected in up to 25% of patients, and this eventually affects the blood parameters including the hemoglobin and the red blood cells (RBCs) [16].

The erythrocytes appear to bear the brunt of thyroid dysfunction, with anemia being the most common finding in hypothyroidism and, to a certain extent, hyperthyroidism, even though erythroid hyperplasia can occur in the latter. In autoimmune thyroid disorders, anemia can be due to comorbid conditions like pernicious subclinical hypothyroidism is higher than that of general population [21].

Hyperthyroid cases also associated with hematological disorders, anemia is not frequently observed in this group, and erythrocytosis is seen more common. But relative reduction in the neutrophils and increase in eosinophils and mononuclear cells observed in shoes groups [22, 23].

Thyroid disorders are prevalent in Ethiopia, but the effects on hematological parameters levels have not been documented. Thus this study intends to determine hematological parameters of patients with thyroid dysfunction at S.t Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

1.3 Significance of the study

This study aims to compare hematological parameters between patients with thyroid dysfunction and apparently healthy controls. Which will help to establish appropriate timely screening, monitor prognosis and manage thyroid disorders before further complications. It will provide policy makers and planners the data for appropriate planning and expanding the service at least to the general hospitals which have appropriate technology. The study will also serve as a reference for researchers who are interested to similar study.

2. Literature review

The thyroid gland has a very important role in the body metabolism in general including the hematopoiesis. Blood disorders are frequently seen in patients with thyroid disorders because thyroid hormones have very crucial role in the proliferation and the metabolism of red blood cells and all other blood elements. A relationship between thyroid hormones and hematological abnormalities has been postulated by many authors [29].

A cross sectional study conducted by Ijaz et al in USA in 2018 showed serum TSH levels were inversely associated with platelet count; each one mIU/L was associated with a decrease in platelet count by 3,353 platelets/uL (95% confidence interval (CI) = 5,451-1,255; P = 0.002). But had no effect on MPV [30].

A prospective study done by MP Kawa, et al in Poland in 2010 showed that**,** compared with the controls' values; they noticed significant increase in the number of red blood cells (RBCs) in the patient's with hyperthyroidism. Hematocrit (HCT) and mean corpuscular volume (MCV) were increased in the hypo and hyperthyroid patients. However, they observed a well-defined population of 42% of hyperthyroid cases with decreased MCV values. The Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were significantly diminished in both groups of patients [31].

Retrospective study done by Olt S et al in turkey in 2016 revealed that 22 patients with hypothyroidism and 22 healthy subjects were enrolled.70% of Patients with hypothyroidism was female and 30% of patients were male. Mean age of healthy subjects was 31±14, 6. They compare hematological parameters consist of red cell distribution width (RDW), mean platelet volume (MPV), hemoglobin (Hb), platelet (PLT) count and white blood cells (WBC) between hypothyroid patients and healthy subjects. This study demonstrated that increased RDW values were associated with hypothyroidism (P=0.091) [32].

A prospective cross sectional study conducted by Aktas G. et al in Turkey (2014) a total of 165 subject's 102 patients with Hashimoto's thyroiditis and 63 as control subjects. RDW in study group (16.2 [14.9–17.8]) was significantly increased compared to control group (p = 0.015)There was no significant difference between study and control groups in terms of WBC, Hb, HCT, MCV, PLT, PDW and free triiodothyronine (FT3) levels.

However, free thyroxin (FT4) level was significantly lower and thyroid stimulating hormone (TSH) was significantly higher in study group compared to controls. RDW was significantly increased in study group compared to control group. They suggested that elevated RDW values in patients without iron deficiency anemia may require further evaluation for hashimoto's thyroiditis (HT) especially in female population [33].

A hospital based cross sectional study done by Mehmet et al in Turkey (2012) 100 patients with overt hypothyroid, 100 patients with, and 200 healthy controls were enrolled in this study. Anemia prevalence was 43% in the overt hypothyroid group, 39% in the SCH group, and 26% in the control group (p=0.0003 and p=0.021 respectively related to controls).Thus, the frequency of anemia in subclinical hypothyroidism (SCH) is as high as that in overt hypothyroidis. There was microcytic anemia in overt hypothyroid patients, SCH patients and normal subjects respectively 5%, 6%, 6% (p=0.933). There was macrocytic anemia in overt hypothyroid patients, SCH patients and normal subject respectively 10%, 11%, 5% (p=0.116).There was normocytic anemia in overt hypothyroid patients, subclinical hypothyroid patients and normal subject respectively 34%, 26%, 16.5% (p=0.02) [34].

The study done by Min Yu et al in Korea in 2014, shows that RDW was associated with TSH levels ($p < 0.00$). In the 4th group, RDW levels were more strongly associated with TSH levels than in the other control group groups ($p = 0.006$). They concluded, RDW levels are correlated with euthyroid and subclinical thyroid status. Notably, RDW is more correlated with subclinical hypothyroidism than the euthyroid status [35].

A prospective cross-sectional study conducted by Maheshwari, et al.in 2020 in India. 69 cases of hypothyroidism, 15 cases of hyperthyroidism and 6 cases of subclinical hypothyroidism. 99 control group comprising of healthy individuals without any thyroid dysfunction. Comparison between control and subclinical hypothyroid showed statistically significant difference in packed cell volume (PCV), Hb, MCHC, MCV and MCH. They concluded that investigating all the RBC indices in cases of thyroid disorders helps in the management of anemia associated with thyroid disorders which are refractory to treatment with iron supplementation [36]

A cross sectional study was conducted by Kamdar et al in 2019 India, the study includes 140 patients of hypothyroidism and 60 patients of hyperthyroidism. There was significant association of thyroid dysfunction on RBCs and red blood cell indices, like PCV, MCV, MCH and MCHC <0.001.

There was no any correlation found between WBC counts, RDW, PLT with thyroid dysfunction. They concluded that females are affected more from thyroid dysfunction than males. They recommended that patients with hypothyroidism and hyperthyroidism should be periodically evaluated for probable hematological changes and early treatment should start to prevent further progression of anemia as well as progression of thyroid dysfunction [37].

A comparative study conducted by Archana Shetty et al. in India in 2019.50 newly diagnosed cases of hyperthyroidism and hypothyroidism and 50 cases as controls. Hb was significantly lower with a mean of 11.4 g/dl indicating the prevalence of anemia in hypothyroid females.
cases. They concluded that all hematological indices are reduced in hypothyroid patients as compared to hyperthyroid cases and they recommended that evaluation of anemia in females must include screening for thyroid dysfunction [38].
Across sectional study done by Jacinta D.et al in India in 2019, Study of hematological parameters in hypothyroid patients in a tertiary care hospital, includes 130 patients among them 36 male hypothyroid patients, and of 94 female hypothyroid patients. Among 130 patients, the study showed 102 (78.46%) patients had PCV less than 40, 24 (18.46%) patient had PCV between 40 to 50 and 4(3.07%) patient had PCV more than 50.Among 130 study population, 88(67.69%) patients had RDW more than 14 and 42 (32.30%) patients had RDW less than 14. They found decreased levels of hematological parameters like Hb, PCV, MCV, MCH, MCHC and RDW in thyroid dysfunction patients. They concluded that abnormal levels of thyroid hormones might substantially influence size variability of circulating RBCs [39].

A cross-sectional study done by Singh P et al in India in 2016 thyroid hormones and hematological indices levels in thyroid disorder. Comparison of different parameters revealed that red cell indices including MCV and MCH have significant statistical difference (P value <0.05) but no difference was observed for Hb, RBC, Hct, RDW, PC, PDW and MCHC .Finally they suggested that all patients with hypothyroidism and hyperthyroidism should be periodically checked hematological parameters[40].

A prospective study conducted by Dorgalaleh A, et al in India (2013) shows, comparison between control group and two groups revealed statistically significant difference in RBC count, HCT, Hb, MCH, MCHC and RDW (P-value<0.05) parameters but no significant difference observed for MCV (P-value >0.05). Platelet and WBC counts in both patient groups compared

with the control group did not show significant differences. They concluded that, thyroid dysfunctions have a direct effect on most RBC indices and these changes should be considered by medical care provider [41].

A prospective study conducted by Geetha J P et al in India (2012). It is found to be significantly increased in both hypo and hyperthyroid patients. MCV values showed statistically significant difference among patients with abnormal thyroid function. MCV values were significantly decreased in hyperthyroidism and increased in hypothyroidism. Other parameters like Hb and Hct did not show any significant difference on comparison with euthyroidism status [42].

Another study by Ahmed S.S. and Mohammed A.A, et al.2020 Iran, showed a significant difference the RBC, HB, MCV, MCHC, RDW, and WBC (P values 0.000, 0.000, 0.001, 0.012.0.002, and0.027) respectively, while platelets showed no significant correlation (P value 0.08). The univariate analyses showed that RBC, the Hb, and the WBC were the most severely affected parameters (Sig. 0.000, 0.000, and0.005) respectively. They concluded that females are more affected by thyroid disorders than male and thyroid dysfunction affects all blood parameters except platelets. They recommended the follow up of patients with thyroid disorders should include the complete blood count and patients diagnosed with anemia should be evaluated for thyroid disorders before iron therapy [43].

Another prospective study done by F Saba et al in Kermanshah, Iran (2019) the relationship between severity of hypothyroidism and red blood cells indices. Data analysis between the two groups of patients revealed statistical difference in RBC counts, Hb and HCT. RBC counts in all patients in the two states including moderate and marked hypothyroid showed significant difference with 4.46 and 4.04 mil/L, respectively. Moreover, HCT were of statistically significant difference between the two groups (P-value<0.05). The Hb value of moderate hypothyroidism was 12.8 and 12.3 for patients with severe hypothyroidism. HCT was 39.8 and 38.0 in the patients with various severity hypothyroidisms [44].

A retrospective study conducted by H.Bashir, et al.in Iran (2012). In view of their study, they found increased levels of hematological parameters like Hb, RBC, MCV, HCT, RBC% and RDW in thyroid dysfunction patients of Kashmir valley, which suggests that abnormal levels of thyroid hormones might substantially influence the size variability of circulating RBCs, predisposing patient to normocytic anemia [45].

A retrospective observational study carried out by Erum S,et al in Pakistan 2020.Total number of recruited patients was 485, out of which 117 were labeled as hyperthyroid, 169 were hypothyroid and 199 were euthyroid Subjects for all three groups were between 20-60 years old. Comparison between hyperthyroid and hypothyroid groups revealed a statistically significant difference in the mean HB, and HCT and RBC. There was no statistical difference between hypothyroid and euthyroid patients for any of the hematological parameters. They proven that association between thyroid dysfunction and erythropoiesis, which caused hematological indices to fluctuate, therefore hematological parameters should be monitored in patients with thyroid diseases [46].

A cross-sectional study was conducted by Suhail, et al. Saudi Arabia 2020. Prevalence observed among female participants (60.27%) as compared to males (39.72%). Higher prevalence of anemia (60.27%) and iron deficiency (49.31%) was observed among hypothyroid group as compared to hyperthyroid and euthyroid group which was characterized by significantly lower values of erythrocyte indices (RBC count, hemoglobin, MCV, MCH) and iron parameters. They demonstrates that high prevalence of thyroid abnormalities particularly hypothyroidism, accompanied by increased prevalence of anemia [47].

A retrospective study conducted by Omar S. et al in Tunisia in 2010 erythrocyte abnormalities in thyroid dysfunction was carried out on 412 patients with peripheral thyroid disease hyperthyroidism (n=235) or hypothyroidism (n=177). The result shows, anemia was observed in 40.9% of patients with hyperthyroidism and 57.1% of patients with hypothyroidism. Among these, normocytic or macrocytic anemia was present in 46.3% of cases. Whereas, microcytosis, with or without anemia, was noted in 87.7% of, patients with hyperthyroidism. T4 was positively correlated with the number of RBCs and Hb and inversely correlated with MCV and MCH. According there study, after restoration of euthyroid state, most erythrocyte abnormalities were corrected [48].

Another retrospective study carried out by Iddah M. A. et al in Kenya (2013) thyroid hormones and hematological indices levels in thyroid disorders. The significance is seen in TSH levels and T3 only for those aged 30–39 years. WBCs, RBCs, HGB, and platelets among the immunological thyroid disease patients were WBC: 5.2 (4.1–7.0), RBC: 4.6 (4.4–4.8) Hb: 13.1 (11.6–13.9), and platelets: 292 (224–390), respectively [49].

3. Objectives

3.1 General objective

To determine hematological parameters of patients with thyroid dysfunction at S.t Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, from March to August, 2020

3.2 Specific objectives

- To compare RBC count and RBC indices between patients with thyroid dysfunction and apparently health controls at SPHMMC, Addis Ababa, Ethiopia
- To compare WBC count and WBC differential between patients with thyroid dysfunction and apparently health controls at SPHMMC, Addis Ababa, Ethiopia
- To compare platelet count and platelet indices between patients with thyroid dysfunction and apparently health controls at SPHMMC, Addis Ababa, Ethiopia

.

4. Hypothesis

There is no significant hematological parameter difference in patient with thyroid dysfunction and apparently health controls

5. Materials and Methods

5.1 Study area

The study was conducted at SPHMMC, Addis Ababa, Ethiopia. The Hospital was established in 1968 by the late Emperor Haile Selassie with the help of German evangelical church which is governed by a board under the Federal Ministry of Health. The college has more than 2800 clinical, academic, and administrative and support staffs that provide: medical specialty services to patients who are referred from all over the country. The hospital has more than 800 beds and gives diagnostic and treatment services for about 370,000-400,000 patients/year and gives full laboratory services including Clinical Chemistry, Hematology, Urinalysis, Parasitology, blood bank and microbiology [50].

5.2 Study design and period:

A hospital based comparative cross sectional study was conducted from March to August, 2020, at SPHMMC from, Addis Ababa, Ethiopia.

5.3 Populations

5.3.1 Source population

All patients with thyroid dysfunction who visited SPHMMC

5.3.2 Study Population

- Patients confirmed by laboratory for thyroid dysfunction at SPHMMC
- Apparently healthy volunteer controls were randomly selected from kolfe health center staffs and students

5.4 Inclusion and exclusion criteria:

5.4.1 Inclusion criteria for patients

- All age group
- All confirmed patients of thyroid hormones disorder at SPHMMC

- Patients willing to give consent.

5.4.2 Inclusion criteria for healthy controls

- All age group
- Individuals willing to give consent
- Apparently health subjects

5.4.3 Exclusion criteria for patients with thyroid dysfunction

- Patients taking any hormonal drugs which affects complete blood count (CBC)such as Non-steroidal anti-inflammatory drugs(NSAIDs),Penicillin and its derivatives, Phenazopyridine (pyridium), Quinidine.
- Individuals with comorbid disease (such us: TB, HIV,Heart disease and chronic kidney disease)
- Known cases of hypo or hyperthyroidism on treatment

5.4.4 Exclusion criteria for control group

- Clients not able to give consent
- Sign of illness
- Those who are taking medications

5.5 Study variables

5.5.1 Dependent variables

- Complete blood count (CBC) parameters

5.5.2 Independent variables

- Socio demographic characteristics
  - Sex
  - Marital status
  - Occupation
  - Education level
  - Residence

- ❖ Types of thyroid status
  - ➢ Thyroid dysfunction
    - ✓ Hypothyroidism
    - ✓ hyperthyroidism
  - ➢ Apparently healthy

5.6 Sample size calculation and sampling method

5.6.1 Sample size calculation:

Sample size was calculated based on the difference between two populations mean.

Required information:-

Anticipated values of the population means: μ1 & μ2

Standard deviation: s_1, s_2 n

Level of significance which is usually set to an α level of 0.05, respective Z value is 1.96

Power of the test: 100 (1-β) %, which is usually set to 80% which is equal to 0.84

Sample size can be estimated using the formula

$$n = \frac{2\sigma 2[z1 - \alpha/2 + z1 - \beta]\,2}{(\mu 1 - \mu 2)2}$$

Where ($\sigma^{2)}$ is pooled variance = s_1^2+$s_2^2{}_{/2}$

The anticipated mean of mean cell volume (MCV) level for hypothyroidism is 86.2 and for hyperthyroidism81.3with a standard deviation of 9.23 and 8.63 respectively. Since there was no study conducted in our country, the values are taken from study conducted in India [36].
i.e. $\sigma^2 = 9.23^2 + 8.63^2/2 = 79.83$

$n = 2(79.83)\,[1.96 + 0.84]^2/24.01$ n =52

52+ (10% of 52 for loss of participants) = 57

The sample size required for group1, n1= 57

The sample size required for group 2, n2 = n1 X allocation ratio, 55 x1 =57

N = n1 + n2 = 57+57=114

5.6.2. Sampling method:

Convenient sampling technique was used to enroll patients with thyroid dysfunction and apparently healthy controls in SPHMMC from March to August 2020.

5.7 Measurement and data collection

5.7.1 Data collection Procedure

After consent and assent was obtained study participants, socio- demographic and clinical information of the study participants was obtained using standardized and structured questionnaire by principal investigator (PI). Trained phlebotomist collected 10 ml of venous blood was aseptically collected using plain and ethyl diamine tetra acetic acid (EDTA) vacationer tubes (about 5 ml in each tube) for the determination of thyroid function and CBC. The blood specimen in the plain tube was centrifuged at 3000 RPM for 5 minutes to separate the serum and use for determination of thyroid function within one hour of separation. Thyroid function was assessed by measuring T3, T4 and TSH levels in patient's serum by using cobas e411 immunoassay system .The second tube that contains whole blood was used for the CBC determination (Beckman-coulter hematology analyzer).

5.7.2 Laboratory analysis

5.7.2.1 Thyroid function tests

Thyroid function tests was performed using cobas e411 (Roche Diagnostics GmbH, Mannheim, Germany) immunoassay system which is a fully automated, random access, software-controlled system for immunoassay analysis. It works based on electrochemiluminescence (ECL) assay principle. ECL is a process in which very reactive species are produced from stable forerunners at the surface of an electrode. These very reactive species react with one another based on the use of aruthenium chelate as the complex generating light. This is achieved by applying a voltage to the immunological complexes (Annex VIII).

Test Principles:

1. **Competitive principle:** for measurement of T3 and T4. This type of test is based on the competition between the test substance of interest and an enzyme conjugated version of the same test substance for a limited number of specific antibody binding sites.
2. The measurement is inversely proportional to the concentration of sample, high signal=low concentration and low signal = high concentration (figure 1).
3. **Sandwich principle:** for measurement of TSH based on two antibodies which sandwich the test substance between them. The measurement is directly proportional to the sample concentration-Low signal=low concentration; high signal=high concentration [51].

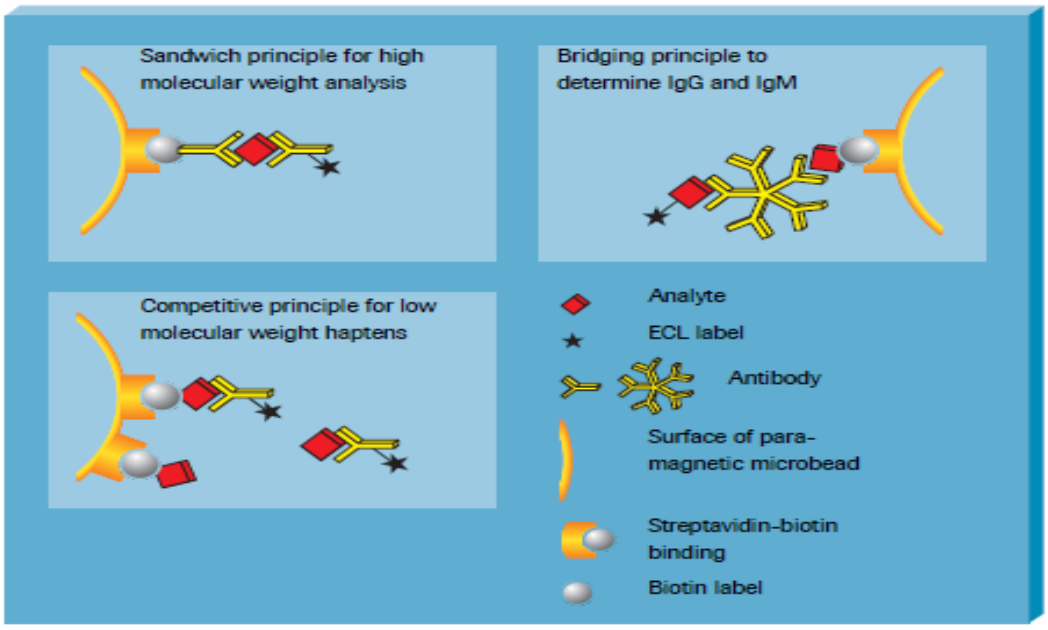

Figure 1: ECL assay principle (competitive principle: for measurement of T3 and T4, sandwich principle: for measurement of TSH)

5.7.2.2 Hematological analyses

The hematological analyses were performed by Beckman-coulter hematology analyzer (Beckman biotechnology & life science, Hamburg, Germany). It is fully automated five part WBC differential hematology analyzer which provides information on 29 parameters for the CBC, including WBC, RBC, Hct, MCV, MCH, MCHC, RDW, PLT, and MPV (Annex IX).

Test Principles:

1. **Impedance principle (electric resistance):** it is based on the detection and measurement of changes in electrical resistance produced by a particle suspended in a conductive liquid as it is drawn through a small aperture which allows to measure RBC, PLT, MPV, MCV. Hemoglobin is converted to SLS-hemoglobin and read photometrically. Hematocrit is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection
2. **Principles of light scatter (flow cytometry):** cells and particles are examined while they are flowing through a very narrow flow cell .when a single cell passes across a laser light beam, the light will be reflected and scattered.
3. The patterns of scatter are measured at various angles (forward scatter 180 degrees and right angle 90 degrees) which provides information about cell structure, shape and reflectivity. These characteristics used to differentiate the various types of WBCs and the reticulocyte count [52].

5.8 Data Quality Assurance

5.8.1 Pre-analytical

To assure the quality of the data, training was given to the data collectors and the questionnaire was pretested. Standard operating procedures (SOPs) were strictly followed during specimen collection and laboratory procedures.

5.8.2 Analytical

Commercially available low, normal and high quality control reagents were used to check the reliability (accuracy and precision) of the data generated by the hematology analyzer where as two level of quality control (preci control universal 1 and 2) were used to assure reliability of data generated by Cobas e411 immunoassay system. SOPs were strictly followed in each laboratory procedures. In addition to this, SPHMMC laboratory accredited by Ethiopia National accreditation office (ENAO).

5.8.3 Post analytical

The accuracy and completeness of the collected data were checked every day by the principal investigator. Data was cleaned, coded and entered correctly.

5.9 Data analysis and interpretation

All the data collected from the laboratory investigation and questionnaire were analyzed using statistical software for social sciences version 20 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was used to express the socio demographic and clinical characteristics. Binary and multiple logistic regressions were computed to assess association be-tween variables. Differences in mean values were determined by independent t test for patients with thyroid dysfunction and apparently healthy participants. P-values < 0.05 were taken as statistically significant.

5.10 Operational definitions

- **Blood cell count:** RBC, WBC and PLT count
- **Blood cell indices:** Hb, HCT, MCV, MCH, MCHC, RDW, NEU%, BAS%, EOS%, MON%, LYM% and MPV.
- **CBC:** RBC count , WBC count and PLT count, Hb, HCT, MCV, MCH, MCHC, RDW, NEU%, BAS%, EOS% MON%, LYM% and MPV.
- **Hematological parameters :** blood cell count and blood cell indices
- **Hyperthyroidism:** serum TSH value below normal range whereas the value of T3 and T4 were above normal range
- **Hypothyroidism:** serum TSH value above normal range whereas the value of T3 and T4 were below normal range
- **Subclinical hypothyroidism(SCH):** the value of T3 and T4 were at normal reference range but serum TSH levels are elevated above normal range
- **Thyroid dysfunction:** serum TSH level below or above normal range with normal or abnormal T3 and T4 value.
- **Thyroid function status:** study participants with normal thyroid function test result or abnormal thyroid function test result
- **Thyroid function test:** Measuring serum hormone concentrations of TSH, T4 and T3.

5.11 Ethical Consideration

Ethical clearance was obtained from departmental research and ethics review committee of department of medical laboratory sciences, Addis Ababa University. The proposal was also reviewed by research ethical board of SPHMMC. Written informed consent (signed or thumb print) was obtained from each participant. Confidential identifiers were used to code participant's identities. Any abnormal CBC results were timely reported to the clinicians for appropriate intervention for patients and for apparently healthy controls any abnormal results (CBC or thyroid function tests) were founded, linked to nearby health facility. Results with any information regarding patients were kept confidential during and after the completion of the research project by password protecting electronic and locking hard copy files.

5.12 Dissemination of results:

The results of the study will be submitted to department of medical laboratory sciences, Addis Ababa University and S.t Paul's Hospital Millennium Medical College. It will also be communicated and disseminated to stakeholders, public and concerned bodies through presentation in different professional association meetings and conferences. The final paper will be submitted to a national or international peer reviewed scientific journal for publication.

6. Result

6.1 Socio-demographic and clinical characteristics

A total of 180 participants of them 123 (32 males, 91 females) patients with thyroid dysfunction and 57(11 males, 46 females) apparently health controls were recruited for this study. In patients with thyroid dysfunction, 74(41.1%) were hypothyroidism and 49(27.2) were hyperthyroidism. minimum and maximum age was 10 and 88 years respectively.

Majority of study participants were females 137(71.1%). Majority of study participants in the age group 26-39 years (84, 46.7%). The mean and standard deviation age was 33.1 (12.7) years and there was statistical significant difference regarding to age between apparently healthy controls and thyroid dysfunction patients (p value =0.004).

Most of the respondents 42(73.7%) apparently healthy controls and 106(86.2%) patients were urban resident, and there was statistical significant difference in terms of residence (p=0.04). Majority of study participants 15(26.3%) normal controls and 62(50.4) abnormal thyroid function had primary education level. Analysis of educational status of the study participant showed significant difference at various educational level between normal and abnormal thyroid status (p=0.004). Regarding marital status the majority of the respondent 24(42.1%) normal and 66(53.7%) abnormal thyroid status and there was no statistical significant difference between apparently healthy and thyroid dysfunction patients (p>005) (Table 1 and Table 2).

Concerning hematological parameters, majority of study participants had hemoglobin (g/dl) value with in normal range hypothyroidism 56(31.1%), hyperthyroidism 36(20.0%) and apparently controls 37(28.7%) were and respectively. Majority of study participants also had normal MCHC value hypothyroidism 34(29.8%), hyperthyroidism and 48(42.1%) and apparently controls 32(28.1%) (Table 1).

Table1: Descriptive analysis of socio demographic and clinical characteristics of study participants at SPHMMC from March to August 2020

Variable	Ctegogy	Thyroid functional status		
		Normal 57(31.7%)	Hypothyroidism 74(41.1%)	Hyperthyroidism 49(27.2%)
Age/years	≤14	0(0%)	2(2.7%)	0(0%)
	15-25	19(33%)	15(20.3)	15(30.6%)
	26-39	32(56.1%)	32(43.2%)	20(40.8%)
	≥40	6(10.5%)	25(33.8%)	14(28.6%)
Sex	Male	11(25.6%)	20(46.5%)	12(27.9%)
	Female	46(33.6%)	54(39.4%)	37(27.0%)
Marital status	Married	24(26.7%)	34(37.8%)	32(35.6%)
	Single	33(37.1%)	39(43.8%)	17(19.1%)
	Divorced	0(0.0%)	1(100.0%)	0(0.0%)
Educational status	Illiterate	8(29.6%)	10(37.0%)	9(33.3%)
	Primary school	15(19.5%)	38(49.4%)	24(31.2%)
	High school	16(51.6%)	11(35.5%)	4(12.9%)
	Certificate and above	18(40.0%)	15(33.3%)	12(26.7%)
Residence	Rural	15(46.9%)	7(21.9%)	10(31.2%)
	Urban	42(28.4%)	67(45.3%)	39(26.4%)
Hb g/dl	11-16	37(28.7%)	56(75.6%)	36(73.4%)
	10.9 and below	0(0.0%)	10(43.4%)	8(27.9%)
	16.1 and above	19(59.4%)	8(55.6%)	5(44.4%)
RBC × 1012/L	4.31-5.9	1(5.9%)	7(41.2%)	9(52.9%)
	4.3 and below	54(36.0%)	60940.0%)	36(24.0%)
	5.91 and above	2(15.4%)	7(53.8%)	4(30.8%)
MCHC (g/dl)	32-36 g	34(29.8%)	48(42.1%)	32(28.1%)
	31.9 and below	20(66.7%)	7(23.3%)	3(10.0%)
	36.1 and above	3(8.3%)	19(52.8%)	14(38.9%)
MCV (fl)	80-100	54(94.4)	49(66.2%)	35(71.4%)
	≤ 79.9	0(0.0%)	22(29.7%)	10(20.4%)
	≥101.1	3(5.3%)	3(4.7%)	4(u8.2%)
WBCs count(cells/ µl)	4-10	48(84.2%)	74(100%)	48(98.0%)
	≤3.9	9(15.8)	0(0%)	0(0%)
	≥10.1	0(0%)	0(0%)	1(2%)
Neutrophil %	50-70	10(16.9%)	33(55.9%)	16(27.1%)
	49.9 and below	40(51.3%)	21(26.9%)	17(21.8%)
	70.1 and above	7(16.3%)	20(46.5%)	16(37.2%)
PLT count (K/mm3)	98-350	54(94.7)	67(y90.5%)	45(91.8%)
	≤97	0(0%)	7(9.5)	3(6.1%)
	≥351	3(5.3%)	0(0%)	1(2%)

6.2 Bivariate and multivariate logistic regression analysis

For each explanatory variable, bivariate analysis was done those variables fulfilled the minimum requirement of p-value < 0.05 significance level for further multivariate logistic analysis was done.

Multivariate logistic regression analysis shows study participants with educational level of at primary school were 3.4 times more likely affected by thyroid dysfunction when compared with study participants with educational level of certificate and above (AOR 3.420, 95% CI (1.349, 8.669) p value=0.004 and those whose residence rural were less likely associated with thyroid dysfunction,when compared with participates whose residence was urban AOR,95%(CI) 0.45(0.20,0.98)) p value=0.040 (Table 2)

Study participants with Hb value of 11.9 g/dl and below were more likely to be affected by thyroid dysfunction (AOR 4.394, 95% CI (1.83, 10.52)), p value=0.001) and whose MCHC value 36.1 g/dl and above were 7.13 times more likely to be affected by thyroid dysfunction (AOR 7.13 95% CI (1.921, 26.476)), p value=0.003 had significant association with thyroid dysfunction. Whereas those whose Neutrophil (%) of ≤49.9 were less likely affected by thyroid dysfunction (AOR 0.169, 95% CI (0.069, 0.413)), p value=0.001 when compared with those study participants whose Hb, MCHC, and Neutrophils are at normal level (Table 2)

Table 2. Bivariate and multivariate analysis outcome of apparently healthy participants and patients with thyroid dysfunction in SPHMMC from March to August, 2020, Addis Ababa, Ethiopia

Explanatory Variables		**Thyroid function status**		**COR,95%(CI)**	**AOR,95%(CI)**	**p-value**
		Normal	Abnormal			
Age /year	≤14	1(1.75%)	1(1.75%)	4.5(0.00)	1.886(0.00,)	1.00
	15-25	18(31.6%)	30(24.4)	0.243(0.086, 0.683)*****	0.207(.066, 0.073)	0.007
	26-39	32(56.1%)	52(42.3)	0.250(.095, 0.657)*****	.210(.073, 0.600**)**	0.004
	≥40	6(10.5%)	39(31.7)	1	1	
Sex	Male	11(19.3%)	32(26.0)	1	1	
	Female	46(80.7%)	91(74.0)	0.680(0.314,1.471)	0.674(.310, 1.466)	0.320
Marital status	Single	33(57.9%)	57(46.3)	0.625(0.248, 1.573)		
	Married	24(42.1%)	66(53.7)	0.617(.327, 1.164)	0.611(0.323, 1.156)	0.130
Education level	Illiterate	8(14.0%)	19(15.4%)	1.583(0.572,4.386)	2.60(0.85,8.01)	0.096
	Primary school	15(26.3%)	62(50.4)	2.756(1.213, 6.262)*	3.420(1.349, 8.669)*	0.004
	High school	16(18.1%)	15(12.2%)	0.625(0.248, 1.573)	0.76(0.296,1.97)	0.577
	Certificate and above	18(31.6%)	27(22.0)	1	1	
Residence	Urban	42(73.7%)	106(86.2)	1	1	
	Rural	15(26.3%)	17(13.8)	0.23(0.70,0.91)*	0.45(0.20,0.98)	0.040
Hb g/dl	11-16	37(66.1%)	92(74.8%)	1	1	
	≤10.9	0	18(14.6%)	3.63 (1.63,8.10)*	4.394(1.83,10.52)**	0. 001
	≥16.1	19(33.9%)	13(10.9%)	3.634(1.630,8.104)*	0.289(0.092,0 .903)	0.033
RBC ×1012/ L	4.31-5.9	1(1.8%)	16(13%)	1	1	
	≤4.3	2(3.6%)	11(8.9%)	0.111(.014, .861)	0.092(.011, .797)	0.03
	≥5.91	53(94.6%)	96(78.0%)	0.344(0.028, 4.273)	0.353(0.023, 5.443)	0.45
MCHC (g/dl)	32-36	34	80	1	1	
	≤31.9	20	10	0.212(0.090, 0.501)*	0.287 (0.107, 0.768)	0.01
	≥36.1	3	33	4.675(1.342, 16.3)*	7.131 (1.921, 26.476)	0.003
Neutrophil %	59-70	10	49	1	1	
	≤49.9	40	38	0.194(0.086,0.44)**	0.169(0.069,0.413)*	<0.001
	≥70.1	7	36	1.050(0.365,3.021)	.705(0.226,2.198)	0.547

* P < 0.05: statistically significant, 1 =Reference group, COR= Crude odd ration, AOR= Adjusted odd ration 95%C.I=95% confidence interval

6.3 Comparison of hematological parameters with thyroid dysfunction and apparently healthy controls

The result of this study showed that there was a statistically significant decrease in RBC count, Hb, HCT, MCV, WBC and PLT counts in thyroid dysfunction patients when compared with apparently healthy controls (p value <0.05). MCHC, RDW and Neutrophils were statistically significantly higher in thyroid dysfunction patients when compared with apparently healthy controls (p value <0.05). MCH, MON, EOS, BASO, showed no significant difference between the groups (p value >0.05) (Table 3, 4 and 5).

Table 3. Comparison of RBCs count and RBC indices between patients with hypothyroidism, hyperthyroidism and apparently healthy controls at SPHMMC from March to August 2020

Index	Thyroid status	No	Mean	Sd	P Value	95% CI
Hb (g/dl)	Hypothyroidism	74	14.0	2.65	<0.001	-2.25 to -.93
	Control	57	15.6	0.96		
	Hyperthyroidism	49	13.6	2.58	<0.001	-2.73 to -1.26
HCT (%)	Hypothyroidism	74	35.00	6.24	<0.001	-10.92to -5.78
	Control	57	43.34	8.03		
	Hyperthyroidism	49	35.89	11.66	<0.001	-11.28 to-3.60
RBC $\times 10^{12}$/L	Hypothyroidism	74	4.81	0.77	0.001	-0.64 to -0.18
	Control	57	5.23	0.45		
	Hyperthyroidism	49	4.64	0.94	<0.001	-0.18 to -0.30
MCV (fl)	Hypothyroidism	74	83.25	14.36	<0.001	-10.88 to -3.97
	Control	57	90.68	3.54		
	Hyperthyroidism	49	82.50	16.90	<0.002	-13.1 to 3.24
MCH (pg)	Hypothyroidism	74	29.42	3.43	0.467	-0.63 to 1.29
	Control	57	29.10	1.51		
	Hyperthyroidism	49	29.61	4.14	0.416	-0.73 to 1.76
MCHC (g/dl)	Hypothyroidism	74	34.54	2.58	<0.001	0.78 to 2.34
	Control	57	32.97	1.69		
	Hyperthyroidism	49	34.70	2.12	<0.001	0.99 to 2.46
RDW (%)	Hypothyroidism	74	13.84	2.38	0.014	0.16 to 1.37
	Control	57	13.70	0.97		
	Hyperthyroidism	49	14.51	3.63	0.009	0.36 to 2.51

Table 4: Comparison of Platelet count and MPV between patients with hypothyroidism, hyperthyroidism and apparently healthy controls at SPHMMC from March to August 2020

Index		No	Mean	Sd	P Value	95% CI
PLT count (K/mm3)	Hypothyroidism	74	170.12	90.08	<0.001	-118.7 to –55.5
	Control	**57**	257.26	96.2		
	Hyperthyroidism	49	186.63	91.18	<0.001	106.7 to 34.5
MPV (fl)	Hypothyroidism	74	9.42	2.51	<0.001	-2.39 to -1.06
	Control	57	11.14	1.24		
	Hyperthyroidism	49	9.36	1.98	<0.001	-2.43 to -1.13

Table 5: Comparison of WBC count and WBC differential between patients with hypothyroidism hyperthyroidism and apparently healthy controls at SPHMMC from March to August 2020

Index		No	Mean	Sd	P Value	95% CI
WBC Count (cells/ µl)	Hypothyroidism	74	7.11	1.26	0.021	0.17 to 1.41
	Control	57	6.31	2.29		
	Hyperthyroidism	49	7.74	1.22	<0.001	0.70 to 2.15
Lymph (%)	Hypothyroidism	74	31.24	13.13	0.03	-8.36 to -0.36
	Control	57	35.61	8.84		
	Hyperthyroidism	49	31.98	15.33	0.149	-8.57 to 1.32
Mono (%)	Hypothyroidism	74	7.07	6.01	0.24	-0.67 to 2.64
	Control	57	6.08	2.17		
	Hyperthyroidism	49	7.35	5.36	0.10	-0.28 to 2.80
Neut (%)	Hypothyroidism	74	58.46	16.56	<0.001	4.66 to 15.44
	Control	57	48.41	13.89	0.002	
	Hyperthyroidism	49	57.91	16.74		3.59 to 15.39
Eos (%)	Hypothyroidism	74	3.11	3.44	0.19	-1.78 to 0.36
	Control	57	3.82	2.53	3.44	
	Hyperthyroidism	49	3.68	3.8	0.81	-1.39 to 1.01
Baso (%)	Hypothyroidism	74	0.81	1.16	0.13	-0.07 to 0.54
	Control	57	0.58	0.26		
	Hyperthyroidism	49	0.82	1.15	0.12	-0.07 to 0.55

*** P< 0.05: statistically significan**

7. Discussion

Thyroid gland as the largest and the most important endocrine gland of human body with the secretion of two hormones, T3 and T4, has a major role in metabolism of cells and organs. Thyroid gland also has a crucial effect on hematopoiesis [24]. The most common thyroid dysfunctions, hypothyroidism and hyperthyroidism affect blood cells and cause anemia with different severity. These thyroid disorders also cause thrombocytopenia, leukopenia and even in rare cases cause pancytopenia. Other blood indices including MCV, MCH, MCHC, Hb also could change during thyroid dysfunction [25].

Thus, this study aimed to determine hematological parameters of patients with thyroid dysfunction. According to the result obtained independent T test analysis showed a statistically significant decrease in RBC count, Hb, HCT, MCV, PLT counts and MPV in thyroid dysfunction patients when compared with apparently healthy controls (p value <0.05). MCHC, RDW, WBC and Neutrophils were statistically significant increased in thyroid dysfunction patients when compared with apparently healthy controls (p value <0.05). MCH, monocyte, eosinophil and basophils, showed no significant difference between the groups (p value >0.05).

Multivariate logistic regression analysis showed that RBC count, Hb, MCHC, and Neutrophils had significant difference with thyroid dysfunction AOR, 95 %(CI), p-value: 0.092(.011, 0.797)0.03, 4.394(1.83, 10.52) 0.001, 7.131 (1.921, 26.476) 0.003, and 0.169(0.069, 0.413) <0.001 respectively. There was no significant association was observed in other hematological parameters.

7.1 Hypothyroidism and apparently healthy controls

Red blood cells and red blood cell indices

Red blood cells, Hemoglobin and Hematocrit

In this study in hypothyroid cases, the mean value of RBCs, Hb and HCT were significantly decreased when compared with apparently healthy participants (value <0.001). Our result is in agreement with the observation made by Suhail, et al in 2020 Saudi Arabia, Ahmed S.S. and Mohammed A.A ,et al (2020) Iran, F. Saba et al in 2019, in Iran, Dorgalaleh A, et al 2013 India, Kawa MP et al in 2010 in Poland, but the finding reported by H. Bashir, et al. 2012[45] showed RBCs,Hb and HCT values were significantly increased(p value <0.05). Singh P et al in 2016[40] showed no difference.The reason for this is most probably due to thyroid hormones regulates hemoglobin production in adult and maturation of Hb in fetus.

Thyroid hormones stimulate the proliferation of erythrocyte precursors directly and indirectly influence erythropoietin (EPO) production enhancement. EPO regulates survival, proliferation and differentiation of elytroid progenitor cells and the number of red blood cells in the peripheral blood [9].

MCV

According to the result obtained the mean value of MCV was significantly decreased in hypothyroid case when compared with apparently healthy participants (value <0.001).The finding was in line with the study by Jacinta D.et al and Suhail, et al. (2020) [39,47], Ahmed S.S. and Mohammed A.A ,et al (2020, Kamdar et al in 2019 India, Singh P et al in 2016,[40], Dorgalaleh A, et al 2013 and Geetha J P et al in 2012 India [42]. In contrast to our finding, Kawa MP et al in 2010[42] and Geetha J P et al in 2012[42] reported the mean value of MCV increases in hypothyroid case when compared with controls.

MCH

In this study MCH did not showed any difference between thyroid dysfunction and apparently healthy controls in respect to hypothyroidism (p value =0.467). Other previous studies by Suhail, et al. (2020) [39], Kamdar et al in 2019[37], Singh P et al in 2016[40] and Dorgalaleh A, et al 2013[41], showed that MCH value significantly decreases (p value < 0.001).

MCHC

MCHC was significantly increased in this study in respect hypothyroidism when compared with apparently healthy controls (p value <0.001). In contrast this finding, a study by Ahmed S.S. and Mohammed A.A, et al (2020)[43], Jacinta D.et al India and Suhail, et al. (2020) [39,47], Dorgalaleh A, et al 2013[41], Kawa MP et al in 2010[42], reported MCHC significantly decreases but a study by Maheshwari, et al. 2020[36] and Singh P et al in 2016[40], showed no difference between the groups.

RDW

When comparing the mean value of RDW between hypothyroid cases and apparently healthy controls showed significantly increased (p value 0.014). This findings also supported by studies done by Olt et al in turkey in 2016, Min Yu et al in 2014[35] Korea, Dorgalaleh A, et al 2013[41], Aktaş G at al and Geetha J P et al in 2012[42], but Kamdar et al in 2019[37] and Singh P et al in 2016[40] Ahmed S.S. and Mohammed A.A, et al reported that there was no difference between the groups. The reason for RDW increases may be due to thyroid hormone affects the regulation of volume status and vascular resistance. Hypothyroidism increases systemic vascular resistance as well as vascular resistance of afferent and efferent arterioles of the kidney.

This increased vascular resistance lowers the effective renal plasma flow and glomerular filtration rate (GFR) which causes increase in RDW.

Total WBC count and WBC differential

Total WBC count

Total WBC count was significantly increased in this study in respect hypothyroidism when compared with apparently healthy controls (p value = 0.021). Similar result reported by H. Bashir, et al. showed that there an increased WBC count in patients with hypothyroidism when compared with controls (p value=0.01). Other researchers Dorgalaleh A, et al and Kawa MP et al reported no difference in WBC count in those groups. The reason for WBCs count reduction the bone marrow is depressed and that thyroid hormones play an important role in the regulation of the human hematopoiesis in the bone marrow. With regard to WBCs, triodothyronine(T3) hormone has been proven to be a prerequisite for normal B-cell production in the bone marrow through its regulation of pro-B-cell proliferation [26].

Neutrophil, Lymphocyte, Monocyte, Eosinophil, and Basophils

In this study Neutrophils showed significantly increased (p value <0.001) and lymphocytes were decreased (p value 0.03) in hypothyroid cases when compared with apparently healthy controls. But monocyte, eosinophil, and basophils did not show any difference (p value >0.05).

Platelet count and mean platelet volume

Clinical studies have shown inconsistent associations between thyroid hormone levels and platelet count and mean platelet volume (MPV).According to the result obtained platelets count and MPV significantly decreased (p value <0.001) in hypothyroid cases when compared with apparently healthy controls. Huseyin. A et al 2018 [29], et al. and Coban et al [28] showed MPV levels increased in hypothyroid cases .On the other hand Ahmed S.S et al, Kamdar et al in 2019, ,Dorgalaleh A, et al Kawa MP et al, Erikci and Acta h et al [17], reported that no difference platelet count between the groups.

The reason for decreased platelet value that thyroid hormones modulate the production platelets which stimulate reticuloendothelial system (RES) will affect both platelet formation as well as prolong the survival of platelets, resulting in thrombocytopenia [17]. The other reason is thyroid hormones increase the expression of fibronectin gene, which appears to affect megakaryocyte maturation [11].

7.2 Hyperthyroidism and apparently healthy controls

Hyperthyroidism affects hematopoiesis in many ways, but its pathogenesis is still unclear. Both thyrotoxicosis and the underlying autoimmunity of thyroid disease may affect the production of blood cells. Imunological mechanisms are suggested to be involved, such as antineutrophil antibodies and antiplatelet antibodies, but the definitive ethiology remains uncertain [28].

Red blood cells and red blood cell indices

RBCs, Hb and HCT

In this study in hyperthyroid cases the mean value of RBCs, Hb and HCT were significantly decreased when compared with apparently healthy controls (value <0.001). This study in agreement with Jacinta D.et al and Suhail, et al. (2020) [39, 47]. F. Saba et al, Dorgalaleh A, et al but Kawa MP et al reported that RBC, Hb and Hct in patients with hyperthyroidism were significantly higher than in control group. Singh P et al in 2016[40] reported no difference in RBCs, Hb, and HCT in those groups. The effect of hyperthyroidism on the Hb is not clear cut and is undoubtedly complicated by concurrent changes in the plasma volume and red cell mass [27].

MCV, MCH, MCHC

In this study there was significantly decrease in MCV (pvalue=0.002) and significantly increase in MCHC (p value <0.001). But no difference shown in MCH (p value >0.05) of patients with hyperthyroidism when compared with apparently healthy controls. This study in agreement with other studies done by Maheshwari, 2020 in India, Dorgalaleh A, et, Kamdar et al, Singh P et al and Geetha J P et al in respect to MCV but and H. Bashir, et al.2012 Kawa MP et reported MCV values significantly increased (p value >0.05) but MCH and MCHC significantly decreases.

The reason is MCV is positively associated with serum levels of TSH, hypothesizing that the premature aging of erythrocytes in the circulation, the increased lipolytic potency of RBC's characteristic of of hyperthyroid patients or distribution of lipids in the erythrocyte membranes could play a role in determining this association

RDW

When comparing the mean value of RDW between hypothyroid cases and apparently healthy controls showed statistical significantly increased (p value 0.009). This findings also supported by previous studies done by Min Yu et al, Dorgalaleh A, et al, Aktaş G at al and Geetha J P et al in 2012[42], but Ahmed S.S. and Mohammed A.A ,et al, Kamdar et al and Singh P et al showed no difference observed between the groups.

Total WBC count and WBC differential

Total WBC count

Total WBC count showed significant difference in increased mean value of hyperthyroid patients when compared apparently healthy controls (p value <0.001). Other researchers Dorgalaleh A, et al and Kawa MP et al reported no difference in WBC count in those groups.

Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophils

In this study neutrophils showed significant difference in increased value (p value =0.002) but lymphocyte, monocyte, eosinophil, and basophils did not show any difference (p value 0.149, 0.10, 0.81 and 0.12 respectively) in hyperthyroid cases when compared with apparently healthy controls.

Platelet count and mean platelet volume (MPV)

Clinical studies have shown inconsistent associations between thyroid hormone levels and platelet count and MPV. According to the result obtained platelets count and MPV significantly decreased (p value <0.001) in hyperthyroid cases when compared with apparently healthy controls. Other previous studies in line with our finding were a study by Van Doormaal et al [26] and Sardar H; et al. stated that, hyperthyroidism results in low platelet count and MPV. On the other hand Ahmed S.S et al, Kamdar et al in 2019, ,Dorgalaleh A, et al, Kawa MP et al, and Acta h et al , reported that no difference in platelet count in between those groups.

8. Strength and Limitation

8.1 Strength

- To the best of our knowledge, this research is the first of its kind done in Ethiopia in providing basic awareness on relationship between hematological parameters and thyroid dysfunction.
- This study have control group
- All laboratory analysis was done in an accredited laboratory with competent personnel

8.2 Limitation

- Comparison of results of hematological parameters in patients with thyroid dysfunction needed before and after treatment
- Larger number of participants is required to increase the accuracy of the findings and some population based data are required to determine the normal geographical variation regarding the levels of the blood and thyroid test parameters.

9. Conclusion and Recommendation

9.1 Conclusion

Thyroid hormones (T3 and T4) have a significant influence on blood cell count and blood cell indices. This study showed that statistical significant difference in RBCs, Hb, HCT, MCV MCHC, total WBC count, neutrophils, PLT count, and MPV between patients with thyroid dysfunction and apparently healthy controls ($p<0.05$). However, there was no significant difference in MCH, monocytes, eosinophil and basophils between those groups (p- value > 0.05). It also indicated that there was a significant association between hematological parameters (Hb, RBCs, MCHC and neutrophils) patients with thyroid dysfunction.

9.2 Recommendation

Routine hematological tests particularly RBCs, Hb, RDW, MCHC, PLT, WBCs and differential count should be done for patients with thyroid dysfunction. So that complications could be detected and managed. In Ethiopia, data concerning hematological profile of thyroid dysfunction are absent so future longitudinal studies are needed to help health authorities to implement polices to improve health status of patients with thyroid dysfunction. Different trainings and workshops should be organized by the concerned bodies to revitalize and refresh the skills, knowledge and experiences of clinicians based on hematological parameters and thyroid dysfunction for better service provision in our country.

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11.Annexes

Annex I: Participant information sheet (English)

1. Study title:

Comparison hematological parameters of patients with thyroid dysfunction and apparently healthy controls at St. Paul's Hospital Millennium Medical College from March to August 2020, Addis Ababa Ethiopia

You have been invited to take part in this research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Thyroid disease is a medical condition that affects the function of the thyroid gland. The thyroid gland is located at the front of the neck and produces thyroid hormones. That travel through the blood to help regulate many other organs. These hormones normally act in the body to regulate energy use, infant development, and childhood development. The purpose of the study, I am evaluating the hematological parameters in patients with thyroid dysfunction.

Study coordinator and principal investigator:

Kedir Mohammed : 0909559470

SPHMMC research ethics committee: Tel:0112750125

Annex II: የጥናቱተሳታፊዎችመረጃቅጽአማርኛ

በጥናቱእንድሳተፉስለመጋበዝየጥናቱዓላማየጥናቱዓላማ፡በታይሮይድእጢ(እንቅርት)
ተጠርጣሪህመምተኞችላይየደምሴሎችመጠንላይየሚታዩለዉጦችንበቅዱስጳዉሎስሆስፒታልሚሊኒይምዲካልኮሌጅምርምርማድረግ፡፡በዚህጥና ትላይእንዲሳተፉእንጋብዘዎታለንነገርግንበጥናቱከመሳተፍዎበፊትየጥናቱንአላማናአስፈላጊነትበቅድሚያመረዳትያስፈልገዎታል፡፡ጐይተር፡ማለትየ ታይሮይድመጠንመጨመርእናትከክለኛዉንተግባሩንለማከናወንመቸገርምክንያትየአንገትወይምየጉሮሮማበጥእንዲከሰትየሚያደርግየጤናእክልነዉ ፡፡ከዚህምበተጨማሪምበሽታዉየታይሮይድሆርሞኖችበብዛትወይምከሚፈለገዉበታችመመረትበሚፈጥረዉችግርምክንያትየሚከሰትነዉ፡፡የዚህጥ ናትዉጤትስለበሽታውየተሻለእውቀትእንዲገኝእንዲሁምበሽታውከደምሴሎችጋርያለዉቁርኝትማወቅበጊዜለመለየትናለመከላከልበሚደለገዉጥረት ጉልህድርሻይኖረዋል፡፡

1. የጥናቱ አስተባባሪና ዋና ተመራማሪ ከድር መሀመድ፤ ስልክቁ.
2. 0909559470 ቅ/ጳዉሎስ ሆስፒታልቁ.0112750125

Annex III: Consent form (English version)

Consent form for adults (≥18 years)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study.To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy)

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy)Phone number:_____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy).

Consent form for parents/guardians

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study provided he/she gives assent. To collect her/his blood and be a participant in this study and understand that I have the right to withdraw my child from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy).

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)_____ ____/____/_____ (dd/mm/yy).

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy).

Annex V: የመረጃመጠየቂያቅፅአማርኛ ፡

መለያቁጥር _____

ተቁ	መጠይቆች	መልስ
ክፍል1፡ማህበራዊሁኔታ		
1.	ካሁንበፊትበእንቅርትበሽታታክመዉያዉቃሉ	1. አዎ 2. አላዉቅም
2.	ፆታ	1. ወንድ 2. ሴት
3.	እድሜ	----------
4.	የጋብቻሁኔታ	ሀ) ያገባ/ባች ለ) ያላገባ/ባች ሐ) የፈታ /ች መ) በሞትየተለየ ሠ) ሌላ …….
3.	የስራሁኔታ	1. አርሶአደር 2. የመንግስትሰራተኛ 3. የቤትእመቤት 4. የግል 5. የቀንሰራትኛ 6. ነጋዴ 7. ሌላ ……
8.	መኖሪያቦታ	ሀ) ገጠር ለ) ከተማ
9.	የትምህርትደረጃ	ሀ) ያልተማረ/ች ለ) አንደኛደረጃ ሐ) ሁለተኛደረጃ መ) ሰርትፍኬትእናከዚያበላይ
10.	ወርሀዊየግልገቢ (ብር)	ሀ) ከ 500 በታችለ) ከ 500-1000 ሐ) ከ1000-2500 መ) ከ2000 በላይ
11.	ሲጋራያጨሳሉ	ሀ)አዎ ለ)አላጨስም

12.	በቤትዉስጥአየ·ዲንጨዉይጠረቀማሉ	1. አዎ 2. አልጠቀምም
13.	በደምማነስበሽታታከመዉያዉ.ቃሉ	ሀ) አዎ ለ) አላዉቅም

ክፍል 2፡ህክምናነክ፡የታይሮይድህመምእናከደምሴልጋርተያያዥጥያቄዎች		
	ካሁንበፊትየእንቅርትበሽታታከመዉያዉ.ቃሉ	3. አዎ 4. አላዉቅም
	ከሆርሞንጋርየተያያዘሚወስዱትመድሀኒትአለእንድአንታይኢንፍላማቶሪ፣ፔንሲሊን፣ፌናዞፓይሪዲን፣ኩኒዲንየመሳሰሉት	ሀ)አዎ ለ)የለም
	ህመም አሎት	1. አዎ 2. የለኝም
	የህመም ምልክት አለዎት	1. አዎ 2. የለኝም
	በተ.ቁ 11 መልስዎአዎከሆነየህመሙአይነት	1. የሳንባበሽታ 2. ኤችአይቪ 3. የልብ በሽተ 4. የኩላሊት በሽተ
	ለሌላበሽታአጋላጭህመምአሎት	1. ሳምባመምች 2. ካነዲዲያሲስ 3. ሌላ ……..
	የታይሮይድህመሙአይነት	1. ሐይፖታይሮይዲዝም 2. ሐይፐርታይሮይዲዝም
	የታይሮይድምርመራአይነቶችበአዲስታካሚዎችላይ	ቲ3---------ቲ4--------- ቲኤስኤች----
ክፍል 3፡በታይሮይድእጢታማሚዎችየደምሴልአይነቶችመጠንዉጤትማሳያ		
	ነጭየደምሴል ---------10^3/ሚሜ^{3}ሊምፎሳይት --------% ኒዉተሮፋይል------% ኢኦሲኖፋይ-----% ሞኖሳ----% ቤዞፋ------%	
	ቀይየደምሴል ---------10^6/ሚሜ^{3}ሄሞግሎቢን ----------ሚግ/ዴሊሄማቶክሪት----------% ሬዲዊድዝ---------% ኤምሲቪ-------ማሜ^{3}ኤምሲች----------ኤምሲኤችሲ---------	
	ፕሌትሌት ----------10^3/ሚሜ^{3}ሜፕቮ--------ማሜ^{3}ፒሲቲ----------%ፕዲዊ----------%	

Annex IV. የስምምነትመጠየቂያቅጽ

የአዋቂዎችየስምምነትመጠየቂያቅጽ

ተሳታፊየሚፈርሙትየስምምነትቅጽ

የጥናቱንአላማናሂደትበዝርዝርከተረዱበኃላየሚከተለውንቅጽበጥንቃቄይፈርማሉ፡፡

- **የጥናቱተሳታፊእንድሆንበሙሉፈቃድወስጃለሁ**
- **ከዚህጋርየተያያዘውንየመግለጫቅጽበትክክልአንብቤተረድቻለሁ፡፡በእኔላይምስለሚደረግማንኛውምጥናትተገንዝቤአለሁ፡፡በተጨማሪም**
- **አስፈለቃጊውንገለጻናማብራሪያከላይበተጠቀሱትሰውተደርጎልኛል፡፡አንድየሾርባማንኪያየደምናሙናእንደሚወስዱበሚገባተረድቻለዉ፡፡**
- **አጥኚዎቹየደምምርመራውጤቴንወስደውይጠቀማሉበተጨማሪም**
- **ጥያቄየመጠየቅመብትየመወያየትእድልከላይከተጠቀሱትአጥኚዎችወይምከነሱተወካይጋርተሰጥቶኝበጥናቱላይበቂምክርናውይይትአድርጌ ያለሁ፡፡**
- **በተመራማሪዎቹየጥናቱውጤትይፋእንዲያደርጉእፈቅዳለሁ፡፡ነገርግንስምመጠቀስየለበትም፡**
- **ተመራማሪዎቹበጤናዬላይያለንችግርእንዲነግሩኝፈቅጄላቸኃለሁ፡፡**
- **በማንኛውምጊዜከጥናቱእራሴንማግለልእንደምችልአውቄያለሁ፡፡**
- **ከእኔየሚሰበሰበውማንኛውምመረጃበጥንቃቄናሚስጥራዊነቱበተጠበቀቦታእንደሚቀመጥአውቄያለሁ፡፡**

ስም_____ ፊርማ_____ ቀን_____

ይህበጥናቱየሚሳተፈውሰውመፈረምስለማይችልከላይየተዘረዘሩትመረጃዎችለተሳታፊውየተሰጡናተሳታፊውምለመሳተፍመስማማቱንገለልተኛታዛ ቢበመሆንአረጋግጣለሁ፡፡

_____ _____

የገለልተኛታዛቢስምፊርማቀን

ስለጥናቱዝርዝርመረጃስለመስጠቴአረጋግጣለሁ ……

የመተማመኛቅጹንከስምምነትቅጽጋርአያይዤሰጥቻለሁ

ፊርማ…… ስም……

ወላጆች/አሳዳጊዎችየስምምነትመጠየቂያቅጽ

የጥናቱርዕስ፡በታይሮይድእጢ (እንቅርት) ህመምታካሚዎችላይየደምሴሎችላይየሚታዩለዉጦችንማየት

እኔ----------የልጄ _____ወላጅ/ አሳዳጊስሆን፡በታይሮይድእጢ (እንቅርት) ህመምታካሚዎችላይየደምሴሎችላይየሚታዩለዉጦችንመመርመርጥናትላይልጄየጥናቱተሳታፊእንድትሆን/እንዲሆንበሙሉፈቃድወስጃለሁ

ከዚህጋርየተያያዘውንየመግለጫቅጽበትክክልአንብቤ/ተነቦልኝተረድቻለሁ፡፡ልጄላይስለሚደረግማንኛውምጥናትተገንዝቤአለሁ፡፡በተጨማሪምአስ ፈለቃጊውንገለጻናማብራሪያከላይበተጠቀሱትሰውተደርጎልኛል፡፡

- አጥኚዎቹለታይሮይድምርመራከሚወሰደዉደምበታጨማሪየልጇንአንድየሾርባማንኪያየደምናሙናከልጇከንድላይእንደሚወስዱበሚገባ ተረድቻለዉ።ነገርግንየደምናሙናበሚወሰድበትወቅትሊፈጠርየሚችለውንአነስተኛህመምናየደምመፍሰስለማስወገድልምድባላቸውናእና ስልጠናበተሰጣቸውባለሙያዎችእንደሚከናወንተረድቻለው በማንኛውምጊዜከጥናቱልጇንማግለልእንደምችልአውቄያለሁ።

- ከልጇየሚሰበሰበውማንኛውምመረጃበጥንቃቄናሚስጥራዊነቱበተጠበቀቦታእንደሚቀመጥአውቄያለሁ።
- ስለዚህምልጇንበጥናቱውስጥለማሳተፍበፍፁምፈቃደኝነትየስምምነትቃሌንመስጠቴንበፊርማዬአረጋግጠለሁ።

የተሳታፊው ሥም_____ፊርማ ----------ቀን /ወር/ዓ----------

ምስክር(ማንበብናመፃፍለማይችሉ)-_____ የምስክርፊርማ ------- ቀን/ወር/ዓ.ም -------

የተመራማሪውስም_____ ፊርማ ---------- ቀን /ወር/ዓ.ም- ----------

Annex VI:Questionaries (English version)

Code ……..

Part1. SOCIO DEMOGRAPHIC CHARACTERISTICS RELATED QUESTIONS.

SN	QUESTIONS	RESPONSE	SKIP TO
1.	Sex	1) Female 2) Male	
2.	Age (years)	_____	
3.	Marital status	1) Married 2) Single 3)Divorced 4) Widowed	
4.	Occupation	1) Civil servants 2) House wife 3) Private organization 4) Farmer 5) Daily laborer 6) Merchant	
5.	Residence	1.Rural 2.Urban	
6.	Educational status	1) Illiterate 2) Primary school 3) High school 4) Certificate and above	
7.	Monthly Personal Income (in Birr)	_____	

8.	Smoking status	1.smoker 2. Non smoker	
9.	Do you use Iodine based salt?	1.Yes 2.No	
10.	Ever treated with existing Anemia	1.yes 2.No	
PART2. Clinical, Thyroid dysfunction and hematological related questions			
11.	Have you ever treated for iodine-deficiency goiters	1.Yes 2.No	
12.	Do you use hormonal drugs like Non-steroidalanti-inflammatory drugs(NSAIDs),Penicillinand its derivatives,Phenazopyridine (pyridium),Quinidine and others	1.Yes 2.No	
13.	Do you have any illnesses now	1.Yes 2.No	
14.	Do you have sign of illness	1.Yes 2.No	
15.	If Yes, to above question what type of comorbidity	1.Tuberclosis 2.HIV 3.Heart disease 4. chronic kidney disease	
16.	opportunistic infections (OIs)	1.Chronic GE 2. Pneumonia 3.Candidiasis 4.Other Specify------	
17.	Type of Thyroid dysfunction	1. Hypothyroidism, 2. Hyperthyroidism	
205. Thyroid testing panel characteristics of newly Diagnosed with Thyroid Dysfunction			
18.	T3_____ T4_____ TSH_____		
PART 3.Hematological parameters results of patients with Thyroid dysfunction			

19.	WBC_____10^3/mm^3 LYM_____% NEU%_____ % GRAN _____%	
20.	RBC_____ 10^6/mm^3 Hb____g/dlHCT____ % MCV_____ μm^3 MCH_____pgMCHC_____g/dl RDW_____%	
21.	PLT_____10^3/mm^3 MPV_____μm^3 PCT_____ % PDW_____%	

Thank You

Annex VII. Venous Blood Collection

Supplies and Equipment

1. Alcohol (70%) and gauze square or alcohol wipes
2. Sterile disposable needles (double-pointed or syringe type)
3. Evacuated blood tubes (appropriate to the test ordered) and a needle holder or a syringe (in special cases)

Procedure

1. Identify the patient
2. . Assemble all necessary equipment
3. . Visually inspect both arms. Choose the arm that has not been repeatedly used for veinpunctures and one that is free of bruises, abrasions, and sites of infection. In the arm, threeveins are commonly used for vein puncture: the cephalic, basilic, and median cubital.
4. Applying the tourniquet
5. Using a cotton ball saturated with 70% alcohol or an alcohol pad saturated with 70% alcohol cleanse the skin in the area of the vein puncture site. Using a circular motion, clean the area from the center and move outward. Do not go back over an area once it has been cleansed. Allow the site to dry.
6. Use one hand to hold the evacuated tube assembly or syringe. Use one or more fingers of the other hand to secure the skin area of the forearm below the intended vein puncture site. This will tighten the skin and secure the vein. Position the patient arm in a slightly downward position.
7. Hold the needle with attached syringe or evacuated tube about 1 to 2 inches below and in astraight line with the intended vein puncture site. Position the blood drawing unit at an angleof about 20°. The bevel of the needle should be upward.
8. Gently insert the needle through the skin and into the vein. The insertion motion should be smooth.
9. The tourniquet may be released as soon as the blood begins to flow into the evacuated tube or syringe or immediately before the final amount of blood is drawn.
10. Ask the patient to open the hand. After the desired amount of blood has been drawn, place a gauze pad over the vein puncture site.
11. Withdraw the blood collecting unit with one hand and immediately press down on the gauze pad with the other hand.
12. Mix tubes with anticoagulant by inverting the tubes several times. If a syringe was used, carefully remove the needle before dispensing the blood into a test tube. Blood should never be forced back

through the needle, and the syringe plunger should be slowly depressed. Discard the used needle into an appropriate safety container.

13. Label all test tubes as required by the laboratory.
14. Clean up supplies from the work area, remove gloves, and wash hands. Note: If the patient is an outpatient, wait a few minutes after the vein puncture is complete, and check to be sure that the patient does not feel dizzy or nauseated before discharge. Discard all contaminated supplies in a biohazard disposal bag.

Cause for rejection

1. Hemolysis
2. clotted specimen
3. Tube not filled with minimum volume
4. Improperly labeled specimen

Annex VIII. Thyroid analysis

Standard Operating Procedures (SOP) for Performing TSH

Purpose: This procedure provides instructions for performing TSH on the Cobas e411.

Abbreviations:

Cal = Calibrator

ECLIA= Electrochemiluminescence Immunoassay

EDTA-K_3 == Potassium Ethylene diamine tetra acetic acid

RT= Room Temperature

TT4= Total Thyroxine

TSH-Thyroid Stimulating Hormone

Clinical Utility

Thyroid-stimulating hormone (TSH): is a glycoprotein having a molecular weight of approx. 30000 daltons and consisting of two subunits. The beta-subunit carries the TSH-specific immunological and biological information, whereas the alpha- chain carries species-specific information and has an identical amino acid sequence to the alpha-chains of LH, FSH and HCG. TSH is formed in specific basophil cells of the anterior pituitary and is subject to a circadian secretion sequence. The hypophyseal release of TSH (thyrotropic hormone) is the central regulating mechanism for the biological action of thyroid hormones. TSH has a stimulating action in all stages of thyroid hormone formation and secretio; it also has a proliferative effect. The determination of TSH serves as the initial test in thyroid diagnostics. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level.

Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypothalamus.

Principle:

Sandwich principle and the total duration of test were 18 minutes.

- In the first step, patient sample is combined with a reagent containing biotinylated TSH antibody and a ruthenium- - labelled TSH – specific antibody in an assay cup. During a nine – minute incubated step, antibodies capture the TSH present in the sample.

- In the second step, streptavidin – coated paramagnetic particles are added. During a second nine- minute incubation, the biotinylated antibody attaches to the streptavidin – coated surface of micro particles.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by the system buffer.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of TSH present in the sample.

Equipment

- Cobas e411
- Micro pipette(20-1000µl
- Vortex
- Centrifuge
- Sample rack
- Pipettes of Different Volume

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

- Evaluation and calculation of the concentration of the antigen are carried out by means of calibration curve that was established using standards of known antigen concentration.

Reagents

- **R2** Anti-TSH -Ab~ $Ru(bpy)^{2+}_{3,}$ 10 ml Monoclonal anti TSH antibody labeled with ruthenium complex 1.2 mg/l phosphate buffer 100mmol/l (Black Cap)
- **TSH Reagent kits: Roche Cat.,** 117314591
- Streptavidine-coatedmicroparticles,Streptavidin-Coated Microparticles,0.72mg/ml; binding Capacity: 470ng biotin/mg microparticles (Transparent Cap)
- **R1** Anti-TSH-Ab~ biotin, biotinylated monoclonal anti –TSH -antibody 2.0 mg/l phosphate buffer 100mmol/l

Calibration

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:

Calibrator	**Level**	**Stability**	**Frequency**	**Preparation (y/n)**
	Cal1 Cal2	● Unopened up to expiry Date labeled on the Pack. ●After opening ● 12 weeks at 2-8ºC ● Up to 5 hours on the analyzer at 20-25ºC	● After 1 month (28 days) when using the same reagent lot ● After Curative maintenance • When Quality control results indicate need for recalibration	No

- 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.

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 ºC fo 2 weeks and 1 month at -20ºC.
- 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

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

Quality Control

Solve the Problem, Document an rerun the Quality controls and Patient samples

Expected Values	Analyte	Reference Range	Analytical Range	Units
	TSH	0.28-4.30	0.005-100	μIU/ml

Standard Operating Procedure (SOP) for Performing Thyroxin (T4) and Triiodothyronine (T3)

Purpose This procedure provides instructions for performing in vitro test for the quantitative determination of T4=Thyroxin and Triiodothyronine (T3) in human serum/ plasma on Cobas e411 systems

Abbreviations

Cal = Calibrator

ECLIA=Electrochemiluminescence Immunoassay

EDTA-K_3 = Potassium Ethyl Diamine Tetra Acetic Acid

RT= Room Temperature

Clinical utility

Triiodothyronine (T3): is the hormone principally responsible for the development of the effects of the thyroid hormones on the various target organs. T3 (3,5,3' Triiodothyronine) is mainly formed extrathyroidally, particularly in the liver, by enzymatic 5' deiodination of T4. Accordingly, the T3 concentration in serum is more a reflection of the functional state of the peripheral tissue than the secretory performance of the thyroid gland. A reduction in the conversion of T4 to T3 results in a decrease in the T3 concentration. It occurs under the influence of medicaments such as propranolol, glucocorticoids or amiodarone and in severe non-thyroidal illness (NTI), and is referred to as "low T3 syndrome".

As with T4, over 99 % of T3 is bound to transport proteins. However, the affinity of T3 to them is around 10 fold lower.

The determination of T3 is utilized in the diagnosis of T3 hyperthyroidism, the detection of early stages of hyperthyroidism and for indicating a diagnosis of thyrotoxicosis factitia.

Thyroxin (T4): is the main product secreted by the thyroid gland and is an integral component of the hypothalamus-anterior pituitary thyroid regulating system. It has the function of anabolically influencing metabolism. Thyroxine is formed in a coupling reaction from two DIT molecules (3,5-diiodotyrosine) in the thyroid gland. It is stored bound to thyroglobulin in the lumina of the thyroid follicles and is secreted as required under the influence of TSH. The major part (> 99 %) of total thyroxine (T4) in serum is present in protein bound form. As the concentrations of the transport proteins in serum are subject to exogenous and endogenous effects, the status of the binding proteins must also be taken into account in the assessment of the thyroid hormone concentration in serum. The determination of T4 can be utilized for the following indications: the detection of hyperthyroidism, the detection of primary and secondary hypothyroidism, and the monitoring of TSH-suppression therapy.

Reagents	
1. T3 Cassettes	Catalog No. 11731360 122
2. T4 Cassettes	Catalog No. 11731360 122
3. ProCell,	Catalog No. 11662988122(cobas e 411)
4. CleanCell,	Catalog No.. 11662970122(cobas e 411)
5. SysWash	Catalog No.11930346122(cobas e 411

Supplies	Equipment
• Assay tips, Roche, 11706799	• Cobas e 411 analyzer
• Assay cups, Roche, 11706802	• Micro pipette(20-1000µl)
• Syswash, Roche, 11930346	• Vortex
• Procell, Roche , 11662988	• Centrifuge
• Clean cell, Roche ,11662970	• Sample rack
• wash water additives, Roche, 11930346	

Materials

Reagents preparation:

Ready for use.

Reagents stability and storage:

Unopened at 2-8ºC up to the stated expiry date

After opening at 2-8ºC -12 weeks On board -8 weeks

Note: Store reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to us.

Calibration

Calibrator	Level	Stability	Frequency	Preparation (y/n)
T3 Cal set, 11731548122	Cal1 Cal2	➢ Unopened up to expiry Date labeled on the Pack. ➢ After reconstitution ➢ 8 weeks at 2-8ºC ➢ Up to 5 hours on the analyzer at 20-25ºC	➢ After 1 month (28 days) when using the same reagent lot ➢ after 7 days (when using the same reagent kit on the analyzer) ➢ After Curative maintenance ➢ When Quality control results indicate need for recalibration	T3 Cal set, 11731548122

Calibrator preparation:

Add 1.0mL of distilled water using a volumetric pipette. Let it sit for 15 minutes and mix care/fully, 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.

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	❖ When the test performed.	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 day at RT, 2-8 ºC for
- ❖ Weeks and 1 month at -20ºC.
- ❖ Write the reconstitution date on the bottles
- ❖ Controls must be kept at room temperature before use

no	Activity
1. CALIBRATOR	
	❖ Two set of two levels is provided in separate kit. ❖ Dissolve carefully the contents of one bottle by adding exactly 1.0 ml of distilled water and allow standing closed for 15 minutes to reconstitute. ❖ Mix carefully, avoiding the formation of foam. ❖ Aliquot calibrators (250 uL aliquot volume) separately into microvials and keep frozen at minus 20 or below for future use. ❖ e. Keep frozen at minute 20 or below for future use.

2. CONTROLS (Lyphochek Immunoassay Plus Control Levels 1, 2 and 3)	
	❖ Reconstitute each vial with 5.0 mL of distilled or deionized water. ❖ Allow the control to stand for 15 minutes. ❖ Aliquot controls (250 uL aliquot volume) separately into Hitachi cup for use.
3. DAILY START-UP	
	❖ Check status of reagents in System Overview. ❖ Check levels of PC2/CC2 & PC1/CC1. ❖ Empty liquid waste. Check solid waste less than 1100. ❖ Fill up water reservoir (1 L dH20: 10 mL Syswash). ❖ Go to Maintenance > Finalization Maintenance
4. ORDER CONTROL	
	With barcoded controls ❖ Place the relevant barcoded controls in the sample rack. ❖ Press START. ❖ Go QC > Control. Select control, and then click Position Assignment. ❖ Select control, select rack position. Press Assign > OK ❖ Click Save
5. ORDER SAMPLE	
	❖ Go to Workplace > Test Selection ❖ Enter rack number, position and sample ID. ❖ Select test/profile. Click Save ❖ Press START

Expected Value

Analyte	Reference Range	Analytical Range	Units
T3	0.84-2.02	0.195-6.51	nmol/L
T4	5.13-14.06	0.420-24.86	nmol/L

Annex IX. Hematological analysis

Standard operating Procedure (SOP) for Examination on

Beckman Coulter DxH 800 Hematology Analyzer

Purpose:

The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer for in vitro diagnostic use in screening patient populations found in clinical laboratories.

The UniCel® DxH 800Analyzer provides a:

- Complete Blood Count (CBC),
- Leukocyte 5 Part Differential (Diff),
- Reticulocyte (Retic) and
- Nucleated Red Blood Cell (NRBC) on whole blood
- Total Nucleated Count (TNC) and Red Cell Count (RBC) on Body Fluids (cerebrospinal, serous and synovial) (BF)
-

Abbreviations:

CBC	complete blood count	CSF	Colony-Stimulating Factor
fl	femtoliters	QC	Quality Control
pg	picogram	SRV	Sample Rotor Valve
RT	Room temperature	SAM	Sample Aspirating Module
µl	Microliter	SPM	Specimen Transporting Module
STM	Specimen Transporting Module	AMTC	Air Mixing Temperature Control
BSV	Blood Supply Valve	DV	Distribution Valve
MTM	Multi-transducer Module	PSM	Pneumatic Supply Module
SM	System Manager	NE	Neutrophil percent
UWBC	Uncorrected White Blood Cell count		

Principle

Coulter Method (impedance)

Accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluents) acts as an insulator.

As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

VCS Technology

The COULTER VCS established WBC differential technology using three measurements: individual cell volume, high-frequency conductivity, and laser-light scatter. The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the SPM into data plots.

Volume Analysis

Electronic Leukocyte Volume Analysis using low-frequency current has been used since 1967.Ithas been evaluated as a possible adjunct to the differential white cell count.

Conductivity Analysis

Cell walls act as conductors to high-frequency current. The current, while passing through the cell walls and through each cell interior, detects differences in the insulating properties of the cell components. The current characterizes the nuclear and granular constituents and the chemical composition of the cell interior.

Light Scatter Analysis

Coulter's experience in flow cytometer dates back decades to Fulwyler's pioneering use of light scatter for cell analysis. Loken et al. and Jovin et al. discuss the relationship of particle size and refractivity to the angle of light scattered from a laser beam

Reticulocyte Analysis

Reticulocytes are immature, non-nucleated erythrocytes retaining a small network of basophilic organelles, consisting of RNA and protoporphyrin. The enumeration of reticulocytes provides a simple, effective means to determine red cell production and regeneration.

The most common means of measuring reticulocytes is to use supra vital dyes, such as New

Methylene Blue or Brilliant Cresyl Blue. These dyes precipitate and aggregate the basophilic substances within the reticulocyte, resulting in a granular, staining pattern easily seen with light Microscopy. Reticulocyte immaturity is related to cell volume and light scatter. Since more immature reticulocytes are larger, contain more RNA and cause increased light scatter, the cell volume and light scatter will increase with the immaturity of the cell.

Clinical utility:

A complete blood count (CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. A CBC helps to check any symptoms, such as weakness, fatigue, or bruising. It also helps to diagnose conditions, such as anemia, infection, and many other disorders. In general, the complete blood count can be done as part of routine health examination and general screening.

Reagent	**consumable supplies**	
UniCel® DxH 800 Diluent (10L)	Distilled water	container for a bleach solution
UniCel® DxH 800 Cell lyse (5 L)	Ethanol (70%)	container for DI water
UniCel® DxH 800 diff pack (3L)	Alcohol resistant marker	5 to 6% solution of sodium hypochlorite
UniCel® DxH 800 Retic pack (3L)	Plastic dispensing bottles	soft cloth or tissue, lint-free swab or tissue
UniCel® DxH 800 clean (5 L)	Gauze	

Reagent stability and storage:

- ✓ Stable up to expiry date and, up to 60 days after opening and store at room temperature except for cleaner it's stable up to 90 days after opening.

Equipment:

- ✓ UniCel® DxH 800 haematology Analyzer
- ✓ Printer
- ✓ Test tube cassette
- ✓ LIS computer
- ✓ Barcode reader

Sample and container type

Whole Blood

Collect whole blood in EDTA according to tube manufacturer's instructions and procedures :

• CLSI publication H4-A5 (for capillary)

• CLSI publication H3-A6 (for venipuncture)

Beckman Coulter recommends using K2 or K3 EDTA.

Body Fluids

To reduce body fluid sample viscosity, use hyaluronidase to treat synovial fluids prior to analysis according to your laboratory standards.

Add in the ratio of 1 mL of synovial fluid to 5 mg of

- ✓ hyaluronidase. Mix for 5 minutes.
- ✓ **NB:** Store patient sample at RT and do not analyze after 8 hours of collection

Safety precautions

Read all product safety data sheet and don't attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter representative.

Alerts for Warning and Caution

WARNING indicates a potentially hazardous situation, which, if not avoided, could result in death or serious injury. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

Risk of operator injury if:

- ❖ All doors covers and panels are not closed and secured in place prior to and during instrument operation.
- ❖ The integrity of safety interlocks and sensors is compromised.
- ❖ Instrument alarms and error messages are not acknowledged and acted upon.
- ❖ You contact moving parts.
- ❖ You mishandle broken parts.
- ❖ Doors, covers, and panels are not opened, closed, removed and/or replaced with care.
- ❖ Improper tools are used for troubleshooting.

WARNING

To avoid injury:

- Keep doors, covers and panels closed and secured in place while the instrument.
- Take full advantage of the safety features of the instrument.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter representative.
- Open/remove and close/replace doors, covers, and panels with care.
- Use the proper tools when troubleshooting.

Calibration:

- The UniCel® DxH 800 haematology Analyzer is calibrated biannually by the service engineer or application specialist.

Quality control:

- 3 level commercial control which is called **Coulter 6C cell control**, **Coulter Retic-X cell control**, and **Coulter Body fluid control** are used as a quality control material
- Whenever the commercial control is stock out validated in-house control is used as a replacement.
- The quality control is run every working morning and whenever it is needed i.e.
    - If the instrument is calibrated
    - After major instrument service maintenance.
    - When a new lot of reagent opened.

Daily Checks (Menu > QA > Daily Checks)

Daily Checks ensure that your DxH 800 System is running correctly.

Log on to the System Manager

Daily Checks are initiated by the System Manager.

1 Type your user name.

2 Type your password. If you forget your password, contact your laboratory administrator to

- Reset your password.

Run Daily Checks

1 Select the **Daily Checks** button from the top of any screen to display the Daily Checks-Summary

- Screen with results of the most recent Daily Checks.

2 Select the **Daily Checks** button at the bottom of the screen. A DxH 800 dialog box displays

the following:

3 Select **OK** to run Daily Checks,

Print Daily Checks

Select the **Print** icon at the top of the Daily Checks screen to manually print Daily Checks Summary or Detail reports.

Quality Control

Quality Control is the routine monitoring of performance and service using commercial or Patient controls. Controls have known characteristics when running on a given system and are analyzed periodically in the same manner that patient specimens are analyzed. The results of analyzed controls are then compared to the known characteristics using statistical methods.

Analyze Commercial Controls

Note: Before running the control, the lab personnel should be feed all control data from inserted leaflet by barcode scanner or manually.

⇨ **Menu >QA >QC >QC Setup**

To view control files on the Quality Control (Data View) screen :**(Menu > QA > QC)**

1 Select the **QC Status** icon at the top of any screen to display the Quality Control screen.

Select a Control

1 From the Quality Control (Data View) screen or the Quality Control (Graph View) screen, select the **Select Control** button on the Local Navigation Bar to display the QC Select Controls dialog box.

2. Select-control and select the **OK** button

3. Select the **Print** button to print the report after review.

When a Commercial Control is outside its Expected Range

1. Ensure the control:

- ❖ The material was mixed properly. If not, mix it according to the package insert.
- ❖ The identification information was entered correctly. If using a bar-code reader, ensure the barcode labels are clean and positioned correctly. If entering the ID manually, ensure that you typed the correct information.
- ❖ Setup information (assigned values and expected ranges) matches either the control package insert or your lab's established values. If they do not, change the control’s information to match.

2. If any of the problems mentioned in item 1 above existed, rerun the control; otherwise, proceed to the next step.

3. Rerun the control to ensure the problem was not a statistical outlier.

4. Ensure the control material was not contaminated by running another vial or level of control.

5. Ensure there are no errors during the cycle. If necessary, call your Beckman Coulter Representative.

Calibration

The calibration procedure consists of comparing instrument measurements to known values for

WBC, RBC, HGB, MCV, PLT, and MPV. Calibration assures that an instrument's data output

Accurately reflects sample input. Calibration is performed using materials based on or traceable to known reference preparations or materials. In general, the procedure may indicate that the Instrument requires standardization, by first determining the deviation from 'calibrator reference.

For best performance, verify and calibrate all the CBC parameters. The WBC differential, NRBC, and Retic parameters are calibrated by an authorized Beckman Coulter Representative in your laboratory. The VCSn parameters do not require calibration in the laboratory.

NOTE Ensure your SPM is properly maintained and the apertures are clean prior to calibration.

You should verify the calibration of your instrument:

- As dictated by your laboratory procedures, local or national regulations
- When controls begin to show evidence of unusual trends
- When controls exceed the manufacturer’s defined acceptable limits
- If the average ambient room temperature changes more than 10°F or 12° C from the calibrating temperature.
- At installation

Calibrate with COULTER S-CAL Calibrator (Menu > QA > CBC Calibration)

NOTE Before you can start or restart the calibration process, the SPM must be offline.

1. Select **Calibration Setup** on the CBC Calibration Screen to display the CBC Calibration Setup dialog box.
2. Type "10" in the **Number of an Aspirations** text box.
3. Select **Cassette Presentation Mode** from the drop-down list.
4. Select BCI from the **Calibrator Type** drop-down list.
5. Select **Upload** and use the handheld bar-code scanner to scan the 2D bar code
6. Insert or type/select the following information:
   - **Lot #**
   - **Expiration Date**
   - **Reference Values**
7. Select **OK** and follow the screen prompts.
8. Place the calibrator in a cassette.
9. Place the cassette in the input buffer and select **OK**.
10. Review the calibration results.
11. Select the **Finish** button at the bottom of the screen.
12. Verify your calibration with the controls.

When all results are acceptable, the **Edit System Recommendations** button at the bottom right-hand corner of the screen is enabled. This button allows modifying the calibration recommended by the system by selecting or deselecting checkboxes.

Calibrating with Whole Blood

NOTE Before you can start or restart the calibration process, the SPM must be offline.

Sample Requirements

For whole blood calibration, use a donor who:

- is not receiving medication
- has normal hematologic parameters
- has normal erythrocyte, leukocyte, and platelet morphology

You must draw into and store specimens in the proper amount of EDTA. If you use vacuum collection tubes, ensure they are filled to correct capacity.

Calibrating with Whole Blood

1. Select Calibration Setup on the CBC Calibration Screen to display the CBC Calibration Setup dialog box.

2. Type “3” in the **Number of the Aspirations** text box.
3. Select the **Cassette Presentation** from the drop-down list.
4. Select **Whole Blood** from the **Calibrator Type** drop-down list.
5. Select **OK** to close the **Calibration Setup** dialog box.
6. Place the 20 samples in cassettes. Place the cassettes in the input buffer and select **OK**.
7. When the calibration procedure is complete, the CBC Calibration (Summary) Screen displays.
8. Review the calibration results.
9. Select the **Finish** button at the bottom of the screen.
10. Verify your calibration with the controls.

Repeatability (Menu > QA > Repeatability > Repeatability)

Sample Requirements

For Repeatability studies, ensure the patient has normal erythrocyte, leukocyte, and platelet morphology.

NOTE Before you can start Repeatability process, the SPM must be offline.

Repeatability

1. Ensure you have enough normal whole blood from a single donor for a minimum of 10 cycles.
2. Select **Repeatability Setup** to display the Repeatability Setup dialog box.

Repeatability in Cassette Presentation

1. From the Repeatability Setup dialog box, select a test panel from the **Test Panel** drop-down list.

NOTE The Repeatability Setup dialog box defaults to **Cassette Presentation**.

2. Type ‘10’ in the **Number of the Aspirations** text box.
3. Select **OK** and follow the screen prompts.
4. Select **OK** to start the Repeatability test.
5. Separate the well-mixed normal specimen into two tubes.
6. Place the tubes into consecutive positions in a cassette and place the cassette in the input buffer.
7. Select **OK** on the DxH dialog box to start the cycle.
8. When the cycle has completed review the results on the Repeatability screen. Use the

scroll bar to review results that do not appear in the results panel.

9. Verify that the CV (Coefficient of Variation) does not exceed the established repeatability Limits.
10. Verify the results and if satisfied, select **Finish**.

NOTE The Finish button is active only when each parameter has two or more valid results.

Repeatability in Single-tube Presentation

Follow the instructions for Cassette Presentation Repeatability, using the Single-tube Presentation station to present each specimen tube.

Repeatability Run Details

When the Repeatability procedure is complete, the results display on the Repeatability Run Details screen.

Carryover (Menu > QA > Carryover)

NOTE Before you can start or restart the Carryover process, the instrument must be offline.

NOTE The Blood 1, Blood 2, and Blood 3-row headings on the carryover screen represent 3 aspirations of a single blood specimen.

1. Select **Carryover Setup** to display the Carryover Setup dialog box.
2. Select a test panel from the **Test Panel** drop-down list. Select **OK** and follow the screen prompts.
3. Select **OK** to start a **Carryover** procedure.

4. Place a cassette in the input buffer with one blood tube followed by three diluent tubes and select **OK** to start Carryover.
5. When Carryover is complete, review the results on the Carryover screen.

When performing a Carryover procedure, the calculated % Carryover and/or Background for each parameter is compared to the Carryover and Background limits for acceptability.

The status of each parameter is based on the following criteria:

a. The status of the parameter is Pass for carryover, if

- Diluents 1,2, and 3 are within the Background limits as defined in the Performance section of the System Overview chapter of this manual for WBC, RBC, HGB, PLT, Diff, Retic, or NRBC, as applicable to the panel.
- The calculated % Carryover for the WBC, RBC, HGB, and PLT parameters and the total events for Diluents 1 and 2 for Diff, Retic, and NRBC are within carryover limits. And Diluent 3 sample results for WBC, RBC, HGB, PLT, Diff, Retic, and NRBC are within

background limits.

b. The status of the parameter is to FAIL for carryover if the criteria described above are not met.

Exporting Quality Assurance Data

Select the Export button on the Navigation bar at the bottom of the CBC Calibration, Repeatability and CBC Calibration screens to export Quality Assurance data.

1. Select the type of file from the drop-down list.
2. Select a Destination and select Start.

Run Samples

Cassette Presentation

1. Ensure the SPM is set up for the appropriate test for your workflow.
2. Ensure your specimens have been collected and stored properly.
3. Load the specimens into the cassettes.
4. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes.
5. After the SPM cycles the samples, review the sample results at the System Manager.

Single-tube Presentation

1. Ensure your specimens have been collected and stored properly.
2. Select the Single-tube Presentation icon at the top of any screen to display the Single-tube
3. Place the specimen on the bar-code reader platform of the Single-tube Presentation Station with the bar code facing the SPM to allow the Single-Tube Presentation Bar-code Reader to scan the specimen label, if no barcode please write patient ID manually.
4. Verify the **Specimen Identifier** and **Test** request. Acknowledging the ID that displays on the System Manager screen indicates that you accept the bar-code label read or manual entry.
5. Mix the specimen according to your laboratory standards.
6. Place the specimen into the correct Single-tube position.

Clearing an Exception from the Not Processed Tab

1. From the Work list - Not Processed tab, select or the Exceptions you want to clear.
2. Select the **Clear** button to display the Clear Exceptions dialog box.
3. Select from the following options:

- Selected Exceptions
- All Exceptions in Current Filter

4. Select **OK** to clear the selected exceptions.

How to Review Patient Results

To access the Patient Results screen, do one of the following:

- Select **Menu > Patient Results**.
- Select a result, then select the **Details** button on the Work list screen.
- **Tap** a result twice on the Work list screen.
- Results are highlighted with a yellow background if action limits are exceeded and results are highlighted with a red background if critical limits are exceeded.
- Flags are contained in a column next to the results.
- Non-numeric codes replace results.
- The Panels Tab is the default tab when there is only one Run Order for a patient's results.
- A History tab will be available on the Patient Results screen if there are one or more released specimens associated with the patient.
- A Rerun tab will display if a Rerun has been done.

Release Results

Patient Results can be released from the Panels Tab or the Rerun Tab (for the selected) when the "Release" button is selected. There is no Review Tab for the Patient Results screen.

1. Select the **Release** button on the Patient Results - Review tab to release the results.
2. Select **Yes** to release the panels,

Reject Results

Patient Results can be rejected from the Panels tab or the Rerun tab (for the selected) when the

Reject button is selected.

1. Select the **Reject** button on the Patient Results screen to reject the results. A DxH dialog box displays the following message:
2. **Are you sure you want to reject these selected results?**
3. Select **Yes** to reject the panels,

Released Tab (Menu >Work list> Released Tab)

The Work list - Released tab displays the released results according to the filter that you select.

The Filter Name drop-down list at the top right of the Released Tab allows you to filter by the following:

Transmit Released Results to the LIS

1. From the Work list - Release Results screen, select the **Transmit** button to display the Transmit dialog box.
2. Select from the following options in the Transmit option box:
   - ❖ Selected Results,All Results in Current Filte, Select **OK** to transmit the results.

Parameter	Units	Range	Limit
WBC	x103/L	4.000–10.000	≤5.0% CV
RBC	x106/µL	4.3 to 5.9	≤1.5% CV
HGB	g/dL	12 to 16 11 to 15	≤1.5% CV
HCT	%	36-48 33-45	≤1.5% CV
MCV	fL	80 to 100	≤1.0% CV
RDW	%	11.6 to 16.8	≤2.5% CV
RDW-SD	fL	33.00 to48.00	≤2.5% CV
PLT	x103 /µL	98 to 350	≤12.0% CV
MPV	fL	8 to 10	≤2.5% CV
NE	%	50 to 70	≤3.5% CV
LY	%	20to 40	≤5% CV
MO	%	3 to 12	≤10.0% CV
EO	%	0.5 to 5	SD ≤ 0.5 or≤13.5% CV
BA	%	0 to 1	SD ≤ 0.5

Prediluted Blood (N=10)

WBC	x103/µL	5.000–10.000	≤6.0% CV
RBC	x106/µL	4.5 to 5.5	≤3.0% CV
HGB	g/dL	14 to 16	≤3.0% CV
PLT	x103/µL	200 to 400	≤7.0% CV

Carryover results should not exceed the following limits:

Parameter	Limit
WBC	≤0.5%
RBC	≤0.5%
HGB	≤1.0%
PLT	≤1.0%
NRBC	≤75 events
DIFF	≤ 200 events
RET	≤600 events

Background - Daily Checks

Parameter	Limit
WBC	≤0.05 x 103/µL
RBC	≤0.005 x 106/µL
HGB	≤0.1 g/dL
PLT	≤3 x 103/µL
NRBC Region	≤10 events
NRBC Total	≤60 events
DIFF	≤100 events
RET	≤600 events

Result interpretation:

A low haemoglobin level indicates anaemia. However, haemoglobin findings are even more dependent upon the total number of RBC's. In other words, for the diagnosis of anemia, the number of RBC's is as important as the haemoglobin level. In response to an acute infection, trauma, or inflammation, white blood cells release a substance called colony-stimulating factor (CSF).CSF stimulates the bone marrow to increase white blood cell production.

Limitations

All Specimens Misleading results can occur if the specimen is not:

- ❖ properly collected,
- ❖ Stored or transported.
- ❖ Contain clots.
- ❖ Is not properly mixed.

For preventing the error Always use good laboratory practices for inspecting specimens for

clots, ensure specimens are appropriately mixed and verifying results. Do not bypass or circumvent the automated mixing process used on the DxH 800.

Panic/critical values:

- White Blood Count (WBC): ≤1.0 x10^3/mm^3 or ≥50.0 x10^3/mm^3
- Hemoglobin: ≤6 g/dl or ≥22 g/dl (adult)
- Hematocrit: ≤20% or ≥70% (adult)
- Platelet count: ≤ 10x103/mm^3 or ≥ 1,000 x10^3/mm
- **Preventive maintenance procedure.**

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Kedir Mohammed (B.Sc.)

Signature: _____

Date of submission: 29 March 2021

This thesis has been submitted with our approval as advisors.

Advisors: MikyasNegash (MSC, Assistant Professor of Hematology and Immunohematology)

Signature: _____

Date: _____

Melatwork Tibebu (MSC, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.