Sudan University of Science and Technology
College of Graduate Studies

# Determination of Complete Blood Cell Count of Sudanese Patients with Thyroid Dysfunctions at Khartoum State

# تحديد تعداد الدم الكامل للمرضى السودانيين المصابين بإضطرابات الغدة الدرقية في ولاية الخرطوم

A Dissertation submitted in partial fulfillment of the Requirements for the Degree of Master in Medical Laboratory Science
Hematology and Immunohematology

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**بسم الله الرحمن الرحيم**

الآيـــــــة

قال الله تعالى :

الّذِي لَهُ مُلْكُ السّمَاوَاتِ وَالأَرْضِ وَلَمْ يَتّخِذْ وَلَدًا وَلَمْ يَكُن لّهُ شَرِيكٌ فِي الْمُلْكِ وَخَلَقَ كُلّ شَيْءٍ فَقَدّرَهُ تَقْدِيرًا

صدق الله العظيم

سورة الفرقان الآية 2

## Dedication

I dedicate this study to

My Parents, my everything

My Husband, my soul mate

My Sisters and My Brother

My Husband's Family

A special dedication to my supervisor,

Dr. Khalda Mirghani Hamza

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First of all, Thanks to Allah as suitable for the grace of his face and the greatness of his supreme authority.

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## Abstract

This is a case control study attempted to measure complete blood cell count in Sudanese patients of thyroid dysfunctions in Khartoum state at Sharq Alneil Hospital, Alzarra Hospital and Antalia Medical Centre. The study period extended from March to April 2015.

The study included 52 hyperthyroid patients (age ranged between 22-60 years) and 58 hypothyroid patients (age ranged between 18-58 years) and 65 healthy subjects as control (age ranged between 18-53 years).

Test group were selected according to Thyroid Stimulating Hormone (TSH) levels. Two ml of EDTA blood sample was collected and a complete blood cell count was automatically determined by DIURI BCC-3000B Auto Hematology Analyzer. Finally, obtained results were analyzed by SPSS software version 20 using One Way ANOVA test and multiple comparison table describing each parameter .

Results revealed that Hb, MCH and MCHC increased in hyperthyroidism patients compared to control with *P* value of 0.005, 0.02, 0.00 respectively. Platelets count decreased significantly in hyper-thyroidism patients (mean $2.69\pm0.54x10^9$/L) compared to control (mean $3.15\pm0.55x10^9$/L). RBCs and PCV decreased significantly in hypothyroidism group (mean $4.67\pm0.37x10^{12}$/L, $39.5\pm2.93$ L/L, respectively) with increase in MCH and MCHC compared to control.

Significant difference between hyperthyroidism and hypothyroidism patients in HB, RBCs and PCV with *P* value of 0.002, 0.000, 0.038 respectively. The Platelet count decreased in hyperthyroid patients compared to hypothyroid patients and MCV increased in hypothyroid patients (mean $84.7\pm5.22$ fL) , but no significant difference in WBC and RDW.

In conclusion, Hemoglobin, RBCs, PCV, red cell indices MCV, MCH, MCHC and Platelets count significantly different between groups

of thyroid patients and compared to control. No significant difference in both of thyroid groups in WBCs and RDW when compared to control.

## المستخلص

اُجرِيَت هذه الدراسة بمقارنة العينات القياسية مع عينات المرضى لتقييم تأثير قصور وفرط نشاط الغدة الدرقية على عدد كامل خلايا الدم في السودانيين في ولاية الخرطوم بمستشفى شرق النيل ومستشفى الذرة ومركز انطاليا الطبي. فترة الدراسة امتدت من مارس إلى أبريل 2015م.

تم جمع العينات من اثنين وخمسون مريضا بفرط نشاط الغدة الدرقية تتراوح أعمارهم بين( 22-60 عاما) , وثمان وخمسون مريضا مصابون بقصور في نشاط الغدة الدرقية تتراوح اعمارهم بين (18-58 عاما) بالإضافة الى خمسة وستون عينة قياسية كمجموعة حيث تتراوح اعمارهم بين (18 -53 عاما).

تم اختيار مجموعة الاختباربناءاً على مستويات الهرمون المنشط للغدة الدرقيةTSH وتم جمع 2 مل من الدم في علب بلاستيكية مع مانع التجلط EDTA وإجراء تعداد كامل للدم بواسطة محلل الدم الاوتوماتيكي DUIRI BCC-3000B. تم تحليل النتائج بواسطة برنامج الحزم الاحصائية للعلوم الاجتماعية SPSS الاصدار 20 باستخدام اختبار Independent T Test.

أشارت نتائج الدراسة إلى وجود فرق ذو دلالة معنوية في متوسط خضاب الدم ومتوسط تركيز خضاب الدم في الخلية الواحدة ومتوسط تركيز خضاب الدم في 100 مل من الدم في مرضى زيادة نشاط الغدة الدرقية بالمقارنة مع العينات القياسية حيث ثابت التغير $P$ value كان 0.00 ,0.02 ,0.01 على التوالي. كما وجد لديهم نقص ذو دلالة معنوية في عدد الصفائح الدموية (mean2.69$\pm$0.54x$10^9$/L) بالمقارنة مع العينات القياسية (mean3.15$\pm$0.55x$10^9$/L) . بينما لدى مرضى نقص نشاط الغدة الدرقية بالمقارنة مع العينات القياسية نقص عدد خلايا الدم الحمراء والدم المكدس نقصا معنويا (mean 4.67$\pm$0.37x$10^{12}$/L, 39.5$\pm$2.93 L/L) على التوالي مع زيادة في متوسط تركيز خضاب الدم في الخلية الواحدة ومتوسط تركيز خضاب الدم في 100 مل من الدم بالمقارنة مع العينات القياسية.

كما نستنتج وجود فرق ذو دلالة معنوية بين مرضى فرط وقصور نشاط الغدة الدرقية في عدد خلايا الدم الحمراء ومتوسط خضاب الدم و الدم المكدس حيث ثابت الاختلاف 0.038 ,0.00 ,0.001 على التوالي. كما نقص عدد الصفائح الدموية نقصا معنويا لدى مرضى فرط نشاط الغدة الدرقية و زاد متوسط حجم الخلايا الحمراء لدى المرضى

بقصـور نشـاط الغـدة الدرقيـة زيـادة معنويـة (mean84.7±5.22fL) بينمـا لا يوجـد فـرق إحصائي بالنسبة لعدد الخلايا البيضاء ومعدل انتشار الخلايا الحمراء.

فـي الخلاصـة, يوجـد فـرق ذو دلالـة معنويـة فـي تركيـز خضـاب الـدم و عـدد خلايـا الـدم الحمـراء والـدم المكـدس بالاضـافة الـى متوسـطات ابعـاد الخلايـا الحمـراء MCV,MCH,MCHC والصـفائح الدمويـة بـين مرضـى الغـدة الدرقيـة والعينـات القياسـية بينمـا لـم يوجـد فـرق فـي عـدد خلايـا الـدم البيضـاء ومعـدل انتشـار الخلايـا الحمـراء بالمقارنة بين مرضى الغدة الدرقية و مع العينات القياسية.

## Abbreviations

**BFU-E.** Burst Forming Unit- Erythroid

**CBC.** Complete Blood Count

**CFU-E.** Colony Forming Unit- Erythroid

**CFU-Eo**. Colony Forming Unit-Eosinophil

**CFU-S**. Colony Forming Unit-Spleen

**EDTA.** Ethylene Diamine Tetra Acetic Acid

**EPO.** Erythropoietin

**FT4.** Free Thyroxine

**FTI.** Free Thyroxine Index

**G-CSF.** Granulocyte- Colony Stimulating Factor

**GM-CSF.** Granulocyte Monocyte - Colony Stimulating Factor

**HB.** Hemoglobin

**HCT.** Hematocrit

**IDD.** Iodine Deficiency

**MCH.** Mean Cell Hemoglobin

**MCHC.** Mean Cell Hemoglobin Concentration

**MCV.** Mean Cell Volume

**Meg-CSF**. Megakaryocyte- Colony Stimulating Factor

**PCV.** Packed Cell Volume

**PHA.** Phyto Hemagglutinie

**Plts.** Platelets

**RBCs.** Red Blood Cells

**RDW**. Red cell Distribution Width

**SCF**. Stem Cell Factor

**T3.** Tri Iodo Thyronine

**T4.** Thyroxine

**THSC.** Pluripotential or Totipotential Stem Cell

**TRa.** Thyroid Receptor a

**TRb.** Thyroid Receptor b

**TRH.** Thyrotropin Releasing Hormone

**TSH.** Thyroid Stimulating Hormone

**WBCs.** White Blood Cells

## List of contents


| No. | Subject | Page |
|---|---|---|
| 1 | الآيـــــــــة | I |
| 2 | Dedication | II |
| 3 | Acknowledgements | III |
| 4 | English Abstract | IV |
| 5 | المستخلص | VI |
| 6 | Abbreviations | VIII |
| 7 | List of contents | X |
| 8 | List of Tables | XIII |
| 9 | List of figures | XIV |
| **CHAPTER ONE**<br>**Introduction and Literature Review** | | |
| 1-1 | Introduction | 2 |
| 1-2 | Literature Review | 4 |
| 1-2-1 | Blood Cell Production | 4 |
| 1-2-1-1 | Granulopoiesis | 6 |
| 1-2-1-2 | Erythropoiesis | 7 |
| 1-2-1-3 | Megakaryopoiesis | 8 |
| 1-2-2 | CBC tests | 9 |
| 1-2-2-1 | Red Blood Cell Count | 9 |
| 1-2-2-2 | White Blood Cell Count | 10 |
| 1-2-2-3 | Platelets Count | 10 |
| 1-2-3 | Thyroid Gland Structure and Function | 10 |
| 1-2-3-1 | Thyroid Disorders | 11 |
| 1-2-3-1-1 | Hyperthyroidism | 12 |
| 1-2-3-1-2 | Hypothyroidism | 12 |

| | | |
|---|---|---|
| 1-2-3-1-3 | Graves Disease | 12 |
| 1-2-3-1-4 | Thyroiditis | 13 |
| 1-2-3-2 | Thyroid Function Tests | 14 |
| 1-2-3-2-1 | TSH Tests | 14 |
| 1-2-3-2-2 | $T_4$ Tests | 14 |
| 1-2-3-2-3 | $T_3$ Tests | 15 |
| 1-2-3-2-4 | Thyroid Antibody Test | 15 |
| 1-2-3-2-5 | Thyroglobulin | 16 |
| 1-2-3-2-6 | Radioactive Iodine Uptake | 16 |
| 1-2-4 | Effect of Thyroid Dysfunctions on blood cells | 17 |
| 1-2-5 | Previous Studies | 18 |
| 1-2-5-1 | Prevalence of Goiter in Sudan | 18 |
| 1-2-5-2 | Previous Studies | 19 |
| 1-3 | Rationale | 21 |
| 1-4 | Objectives | 22 |
| **CHAPTER TWO**<br>**Materials and Methods** | | |
| 2-1 | Study Design and Study Area | 23 |
| 2-2 | Study Population | 23 |
| 2-2-1 | Inclusion Criteria | 23 |
| 2-2-2 | Exclusion Criteria | 23 |
| 2-3 | Sample Size | 23 |
| 2-4 | Sampling Procedure | 23 |
| 2-5 | Method and Principle of Automated Tech-nique | 24 |
| 2-6 | Statistical Analysis | 24 |
| 2-7 | Ethical Consideration | 24 |
| | | |

| | | |
|---|---|---|
| **CHAPTER THREE**<br>**RESULTS** | | |
| 3-1 | Results | 25 |
| **CHAPTER FOUR**<br>**Discussion, Conclusion and Recommendations** | | |
| 4-1 | Discussion | 30 |
| 4-2 | Conclusion | 33 |
| 4-3 | Recommendation | 34 |
| **References** | | 35 |


## List of Tables


| | | |
|---|---|---|
| Table 1-1 | Hematology reference ranges | 40 |
| Table 1-2 | Leukocyte values in humans | 40 |
| Table 3-1 | Charactaristics of patients with hyper and hypothyroidism | 26 |
| Table 3-2 | Gender and age frequency according to thyroid status of study population | 27 |
| Table 3-3 | Comparison of study groups using ANOVA | 27 |

## List of Figures


| | | |
|---|---|---|
| Figure 2-1 | DIURI BCC-3000B Auto Hematology Analyzer | 39 |


## Appendix


| | |
|---|---|
| Appendix I | 39 |
| Appendix II | 40 |
| Appendix III | 41 |
| Appendix IV | 42 |

# Chapter One

# Introduction
# and
# Literature Review

# Chapter One

## Introduction and Literature review

### 1-1 Introduction:

About forty five percent of blood is cellular components. Because mature blood cells are predominantly short lived, stem cells are required throughout life to replenish multilineage progenitors and the precursors committed to individual hematopoietic lineages. Hematopoietic stem cells reside as rare cells in the bone marrow in adult mammals and sit atop a hierarchy of progenitors that become progressively restricted to several or single lineages. These progenitors yield blood precursors devoted to un-ilineage differentiation and production of mature blood cells, including red blood cells, megakaryocytes, myeloid cells (monocyte/macrophage and neutrophil), and lymphocytes ( Stuart and Leonard , 2008).

As with all other stem cells, Hematopoietic stem cells are capable of self-renewal—the production of additional Hematopoietic stem cells — and differentiation, specifically to all blood cell lineages ( Stuart and Leonard , 2008).

Blood cell production system is influenced by different factors and Thyroid hormones have a crucial role in metabolism and proliferation of hematopoietic cells. Thyroid dysfunctions might be responsible for pro-found disturbances in functions of the hematopoietic system and thus might affect various hematological parameters of circulating blood due to the fact that they modulate hematopoietic cell production in the bone marrow (Dorgalaleh *et al.,* 2013).

Thyroid dysfunction induces different effects on blood cells such as anemia, erythrocytosis leukopenia, thrombocytopenia, and in rare cases causes' pancytopenia. It also alter RBC indices include MCV, MCH, MCHC and RDW (Dorgalaleh *et al.,* 2013).

This study is done to assess a complete blood count in thyroid dysfunction Sudanese patients and this could yield valuable results concerning the hematological parameters which are affected by thyroid dysfunctions. Hence these parameters could be added to thyroid dysfunction diagnosis.

## 1-2 Literature Review:

### 1-2- 1 Blood Cell Production (Hematopoiesis):

Blood cell production (hematopoiesis) encompasses cellular proliferation, differentiation, morphogenesis, functional maturation, and death (Anne *et al*., 1998).

Hematopoiesis begins during embryonic development in the blood islands of the yolk sac at approximately 19 to 20 days of gestation, and their appearance marks the beginning of the mesoblastic period of Hematopoiesis. These blood islands develop from the mesodermal extraembryonic layer of the yolk sac and remain active only through the 8th to 12th week of gestation and are primarily responsible for red cell production (erythropoiesis). Immature red cells or erythroblasts produced by the yolk sac are unique in their morphology as well as the type of hemoglobin they produce. Erythroblasts of the later definitive series are smaller than the primitive series but still larger than the cells found in adults (Anne *et al*., 1998).

Definitive morphologic hematopoiesis begins in the liver during the fifth to sixth week of gestation and marks the beginning of hepatic hematopoiesis. The liver is the primary site of blood cell development between 10th and 30th weeks of gestation and remains active until the first or second week after birth. The fetal liver produces red cells containing fetal hemoglobin (two alpha and two gamma globin chains). Because

liver hematopoiesis is intravascular, infants normally have a few circulating nucleated red cells. The production of granulocytes and lymphocytes is minimal in the fetal liver. During the hepatic period, the spleen, thymus, and lymph nodes also become active in blood cell production. These other sites, except for the thymus, continue to produce lymphocytes throughout life (Anne *et al*., 1998).

Hepatic as well as splenic hematopoiesis may occur in the adult and that referred to as extramedullary hematopoiesis(i.e., blood cell production outside the bone marrow). During the fifth month of gestation, bone cavities begin to form and this marks the beginning of the myeloid period. During this time hemoglobin A1, consisting of two alpha and two beta globin chains, begins to appear and gradually increases in concentration (Anne *et al*., 1998).

After the first three weeks postpartum, the bone marrow becomes the only normal site of blood cell production and remains throughout life. The volume of bone marrow increases from 1.5% of body weight at birth to about 4.5% in the adult. Blood volume, on the other hand, decreases from 8% of total body weight at birth to 7% in the adult (Anne *et al*., 1998).

Replenishment of blood cells is dependent on the presence of undifferentiated hematopoietic cells, termed Stem Cells. Such cells have a high degree of proliferative capability. All hematopoietic cells originate from a common cell termed the Pluripotential or Totipotential Stem Cell( THSC) that gives rise to partially committed progenitor cells of both a myeloid ( CFU-S or CFU-GEMM) and a lymphoid nature(CFU-L). Stem cell environment mediates their developmental fate, either self-renew or differentiate (Anne *et al*., 1998).

The bone marrow microenvironment ( surrounding structures and stromal cells) contributes significantly to hematopoiesis through the pres-

entation of an extracellular matrix constituting of collagens, proteoglycans, and various cytokines known as growth factors. These growth factors interact with specific cell surface receptors, causing activation of intracellular pathways and resulting in changes in cell proliferation. Many of growth factors have very little lineage specificity, they are frequently synergistic in their action (Anne *et al*., 1998).

**1-2-1-1 Granulopoiesis :**

The blood granulocytes and monocytes are formed in the bone marrow from a common precursor cell. In the granulopoietic series progenitor cells, myeloblasts, promyelocyte and myelocytes form a proliferative or mitotic pool of cells while the metamyelocytes, band and segmented granulocytes make up a post mitotic maturation compartment. Large numbers of band and segmented neutrophils are held in the marrow as a 'reserve pool'. The bone marrow normally contains more myeloid cells than erythroid cells in the ratio of 2:1 to 12:1. In the stable or normal state, the bone marrow storage compartment contains 10 to 15 times the number of granulocyte found in the peripheral blood. Following their release from the bone marrow, granulocytes spend only 6-10 h in the circulation before moving into tissues where they perform their phagocytic function (Anne *et al.*, 1998).

The earliest recognizable precursor is the myeloblast which represent up to 4% of normal bone marrow. Myeloblasts give rise by cell division to promyelocyte which are slightly larger cells and have developed primary granules in the cytoplasm. These cells then produce myelocytes which have specific or secondary granules. The nuclear chromatin is now more condensed and nucleoli are not visible. Separate myelocytes of the neutrophils, Eosinophil, and basophil series can be identified. The myelocytes give rise by cell division to metamyelocytes, non dividing cells,

which have an indented nucleus and a cytoplasm filled with primary and secondary granules. Neutrophil forms between the metamyelocyte and fully mature Neutrophil are termed 'band' or 'stab'. These cells may occur in normal peripheral blood. They do not contain the clear, fine filamentous distinction between nuclear lobes that is seen in mature neutrophils (Anne *et al*., 1998).

Regulation of Granulopoiesis in vivo is mediated by Colony Stimulating Factors (CSF) that act on Granulocyte-monocyte/macrophage progenitors CFU-GM. Macrophages are an important source of CSFs. Eosinophils, and possibly basophils / mast cells, are derived in Granulopoiesis from a committed stem cell(CFU-Eo) that appears to be regulated by interleukin-5 (Anne *et al*., 1998).

**1-2-1-2 Erythropoiesis:**

Erythropoiesis passes from the stem cell through the progenitor cells colony-forming unit granulocyte, erythroid, monocyte and megakaryocyte(CFU-GEMM) , burst-forming unit erythroid (BFU-E) and erythroid (CFU-E) to the first recognizable erythrocyte precursor in the bone marrow, the Pronormoblast. This is a large cell with dark blue cytoplasm, a central nucleus with nucleoli and slightly clumped chromatin. The Pronormoblast gives rise to smaller normoblasts which contain more hemoglobin (which stains pink) in the cytoplasm; the cytoplasm stains paler blue as it loses its RNA and protein synthetic apparatus while nuclear chromatin becomes more condensed. The nucleus is finally extruded from the late normoblast within the marrow and a reticulocyte stage results which still contains some ribosomal RNA and is still able to synthesize hemoglobin. This cell is slightly larger than a mature red cell, spends 1-2 days in the marrow and also circulates in the peripheral blood for 1-2 days before maturing, mainly in the spleen, when RNA is completely lost. A completely pink staining mature erythrocyte results which

is a non-nucleated biconcave disc. A single Pronormoblast usually gives rise to 16 mature red cells. Nucleated red cells (normoblasts) are not present in normal human peripheral blood. They appear in the blood if extramedullary erythropoiesis occur and also with some marrow diseases (Anne *et al*., 1998).

Erythropoiesis is regulated by the glycoprotein hormone Erythropoietin (EPO), which is present in normal serum in concentrations that vary according to the oxygen-carrying capacity of the blood. It augments erythropoiesis by stimulating the production of new erythroblastic cells synthesizing hemoglobin, whether in vivo or in vitro. These cells are unable to produce hemoglobin without erythropoietin.

In addition to EPO, interleukin-3 (IL-3) and stem cell factor also affect erythrocyte progenitors differentiation (Anne *et al*., 1998).

**1-2-1-3 Megakaryocytopoiesis:**

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes , one of the largest cells in the body. The precursor of the megakaryocyte – the megakaryoblast – arises by process of differentiation from the hematopoietic stem cell. The megakaryocyte matures by endomitotic synchronous replication ( i.e., DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Approximately each megakaryocyte giving rise to 1000 to 5000 platelets.

It appears that platelet production is regulated by humoral factors such as IL-3, stem cell factor and Thrombopoietin which is constitutively produced by the liver and kidneys(Anne, 1998). It increases the number and rate of maturation of megakaryocytes via c-Mpl receptor(Hoffbrand *et al.* 2006).

A factor that increases CFU-Meg colony formation, megakaryocyte-colony-stimulating factor (Meg-CSF), responds to the number of bone

marrow megakaryocytes rather than the peripheral blood platelet count (Anne *et al*., 1998).

**1-2-2 CBC tests:**

**1-2-2-1 Red blood cells count**:

The evaluation of erythrocytes is an important part of the complete blood count (CBC), which is performed routinely in most clinical laboratories. For all CBC parameters a whole blood specimen, anticoagulated with EDTA and less than 24 hours old, is required (Anne *et al*., 1998).

RBCs Count is the number of red blood cells in a micro liter of blood. Normal ranges of RBCs are as follows: Adult males 4.6 million to 6.2 million cells per micro liter. Adult females 4.2 million to 5.4 million cells per micro liter (Moini, 2012).

Mean cell volume (MCV):

MCV indicates the average volume of a single erythrocyte in a given blood sample. It is expressed in SI units as femtoliters (Fl; 1 fL = $10^{-15}$L). Reference Range at various ages are in (Table 1-1) (Anne *et al*., 1998).

Mean cell Hemoglobin (MCH):

MCH indicates the mean weight of hemoglobin per erythrocyte, expressed in SI units as pictograms (pg; 1 pg = $10^{-12}$g). Reference Range at various ages are in (Table1-1) (Anne *et al*., 1998).

Mean cell Hemoglobin Concentration(MCHC):

MCHC indicates the average concentration of Hemoglobin in the erythrocytes in a specimen. It is expressed in SI units as g/dl. Reference range at various ages are in (Table1-1) (Anne *et al*., 1998).

Red cell Distribution Width (RDW):

RDW is a size distribution measurement generated from a red cell histogram. It functions as an index of red cell population heterogeneity and can reflect anisocytosis on the peripheral blood film. It is expressed as the ratio of standerd diviation (width of histogram) to the MCV or the

coefficient of variation(CV) of red cell size within a given red cell population. The RDW is said to identify minor populations of microcytic and macrocytic cells that are not apparent from the MCV. Reference range for RDW as(CV): 12.8±1.2% (Bates and Bain, 2001).

**1-2-2-2 White blood cells count**:

Using either manual or automated procedures, we are aiming to calculate cellular elements in a blood sample. Reference Ranges for total WBCs count and differential count of neutrophils, lymphocytes, Eosinophils, monocytes and basophils are listed in (Table 1-2) ( Bates and Bain ,2001).

**1-2-2-3 Platelet count:**

Normal hemostatic function requires peripheral blood platelets that are normal in number (approximate reference range 150 – 400 X $10^9$/L) and function (Anne *et al.*, 1998).

**1-2-3 Thyroid Gland Structure and Function:**

Thyroid gland , the largest of the endocrine glands, is a bilobed structure connected by an isthmus which crosses the second and third tracheal rings in the lower part of the neck. It normally weighs about 20 gm, but may increase 20 to 30 times normal size. The parathyroid glands are in close proximity,as their name implies and are usually located at the upper and lower poles of each lobe. The normal thyroid histology is follicular in structure with cuboidal cells surrounding a pink staining material known as colloid, which is largely thyroglobulin. The follicles of the thyroid under control of (Thyroid Stimulating Hormone) TSH make two hormones, Tetraiodothyronine or Thyroxine ($T_4$) and Triiodothyronine ($T_3$) which differ only in the absence of one iodine atom on $T_3$. Although the thyroid secrets both $T_3$ and $T_4$ ,$T_4$ is also converted to $T_3$ in peripheral tissues. Thyroxine is also converted to reverse $T_3$(an inactive product) under conditions of stress or starvation. This appears to be the major

means of reducing the catabolic effect of circulating thyroid hormone. The thyroid is unique among the endocrine glands in storing large amount of hormone within the gland . Genetic defects may occur at any step of thyroxine synthesis, resulting in very large goiters and often juvenile hypothyroidism (Ryan,1980).

The thyroid gland usually makes more $T_4$ than $T_3$. Once these hormones are released into the blood stream, they are bound fairly specifically to serum proteins called thyroid- binding globulin (TBG) and thyroid binding prealbumin and non specifically to serum albumin. A larger percentage of $T_3$ is found free in the blood stream than of $T_4$. Then they are transported to tissues where their prime function appears to be regulation of the metabolic rate (how quickly the body uses and stores energy) (Ryan,1980). These molecules have also critical roles in early brain development, somatic growth, bone maturation, protein synthesis and regulating production of red blood cells. All these functions are regulated by attachment of the active form of thyroid hormone $T_3$ to specific members of the nuclear receptors family (TRa and $TR_B$). Other effects of thyroid hormones include involvement in hemoglobin production in adult and maturation of Hb in fetus (Dorgalaleh *et al.*, 2013).

Hormonal output from the thyroid is mediated by thyroid stimulating hormone (also known as TSH or thyrotropin) secreted by anterior pituitary. The secretion of thyrotropin itself is mediated by thyrotropin-releasing hormone (TRH) secreted by the hypothalamus (Dorgalaleh *et al.*, 2013).

**1-2-3-1 Thyroid Disorders :**

The most common disorders of the thyroid gland include hyperthyroidism, hypothyroidism and thyroid nodules, which are generally benign thyroid neoplasm but may change to thyroid cancer (Dorgalaleh *et al.*, 2013).

**1-2-3-1-1 Hyperthyroidism (Thyrotoxicosis):**

Any condition resulting in excessive delivery of thyroid hormones to tissue may result in the hyper metabolic state known as thyrotoxicosis. Most commonly, this is due to excessive release of thyroid hormones from a goiter or hyper functioning nodule. Rarely, ectopic thyroid tissue in the ovary (Struma ovarii) may cause thyrotoxicosis. An occasional cause of thyrotoxicosis is ingestion of excessive amounts of thyroid hormone by the patient . Often this condition is seen in paramedical personnel who are depressed and take the medication in the mistaken idea that it will give them more energy (Ryan,1980).

**1-2-3-1-2 Hypothyroidism:**

Hypothyroidism may result from any factor interfering with Thyroxine synthesis or resulting in varying degrees of destruction of the gland. It may range from a condition of insidious onset to mild as to be in apparent to the patient and undetectable clinically except by laboratory examination to florid myxedema resulting eventually in coma and death. The most common cause currently is iatrogenic (following radioactive iodine or surgical treatment of hyperthyroidism or cancer). The various forms of thyroiditis (particularly Hashimoto's) constitute the second most common cause, and idiopathic hypothyroidism is occasionally seen. Iodide deficiency causing goiters and hypothyroidism is seen endemically in some areas of the world ( Ryan,1980).

**1-2-3-1-3 Graves Disease:**

Graves' disease is an autoimmune condition affecting the thyroid gland that results in abnormally high levels of thyroid hormone to be released into the body causing hyperthyroidism. There is no cure for Graves disease but progression of the disease can be halted by removing the thyroid gland. Graves disease is the leading cause of hyperthyroidism. Complications can include hypertension, cardiac arrhythmias, and

thyroid eye disease but treatments are available. Graves disease is 7 times more common in women than in men. Graves patients are among the 190 million people worldwide who experience goiter but in the case of Graves's the goiter is "Toxic" ( Toxic Diffuse Goiter) , meaning it is causing abnormally high thyroid hormone levels or "Thyrotoxicity" (Lowrance, 2010).

**1-2-3-1-4 Thyroiditis:**

Thyroiditis is an inflammation (not an infection) of the thyroid gland. Several types of thyroiditis exist, and the treatment is different for each (Lowrance,2010).

**Hashimoto's Thyroiditis:**

With Hashimoto's thyroiditis, auto antibodies including the "Anti-thyroidperoxidase" and the "Anti-thyroidglobulin" are created by the immune system to attack the thyroid gland. For reasons yet to be understood by medical research, the immune system will at times target a natural part of the body and will relentlessly attack it. As the killer cell called "Thyroid Antibodies" begin to destroy natural thyroid gland protein cells, it begins to cause death to the gland at a gradual rate. As the damage occurs, the gland becomes less capable of producing thyroid hormone to regulate the metabolism of the body. This result in condition called 'Hypothyroidism" (Lowrance ,2010).

**1-2-3-2 Thyroid function Tests:**

**1-2-3-2-1 TSH Tests**:

The best way to initially test thyroid function is to measure the TSH level in a blood sample. The synthesis and release of TSH are controlled by the circulatory level of thyroid hormones; triiodothyronine ($T_3$) and Thyroxine ($T_4$) and by the hypothalamic Thyrotropin-Releasing Hormone. Thyroid hormones regulate the secretion of TSH by a negative feed-back mechanism.(Turbo TSH [$I^{125}$] IRMA KIT, 2014). If the pituitary gland senses that there is not enough thyroid hormone being released (Hypothyroidism) due to the gland struggling or being hindered by a disease process, it will send an excess of TSH to further stimulate thyroid hormone production. This is the point at which lab values of blood tested TSH levels will usually be flagged high and will continue to rise as the hypothyroidism worsens. The hormone level will be sensitive enough, so that even mild, subclinical cases of hypothyroidism will be detected.

The opposite effect will occur when the thyroid gland is producing too much thyroid hormone (hyperthyroidism). The pituitary gland will back-off and send less of the stimulating hormone when the thyroid is overactive (Lowrance, 2013).

**1-2-3-2-2 $T_4$ Tests**:

$T_4$ circulates in the blood in two forms:

1): $T_4$ bound to proteins that prevent the $T_4$ from entering the various tissues that need thyroid hormone.

2): Free $T_4$, which does enter the various target tissues to exert its effects. The free $T_4$ fraction is the most important to determine how the thyroid is functioning, and tests to measure this are called the Free $T_4$ ($FT_4$) and the Free $T_4$ Index ($FT_4I$ or FTI). Individuals who have hyperthyroidism will have an elevated $FT_4$ or FTI, whereas patients with hypothyroidism will

have a low level of $FT_4$ or FTI. Combining the TSH test with the $FT_4$ or FTI accurately determines how the thyroid gland is functioning.

The finding of an elevated TSH and low $FT_4$ or FTI indicates primary hypothyroidism due to disease in the thyroid gland. A low TSH and low $FT_4$ or FTI indicates hypothyroidism due to a problem involving the pituitary gland. A low TSH with an elevated $FT_4$ or FTI is found in individuals who have hyperthyroidism (American Thyroid Association, http://www.thyroid.org/ , 2015).

**1-2-3-2-3 $T_3$ Tests**:

$T_3$ tests are often useful to diagnosis hyperthyroidism or to determine the severity of the hyperthyroidism. Patients who are hyperthyroid will have an elevated $T_3$ level. In some individuals with a low TSH, only the $T_3$ is elevated and the $FT_4$ or FTI is normal. $T_3$ testing rarely is helpful in the hypothyroid patient, since it is the last test to become abnormal. Patients can be severely hypothyroid with a high TSH and low $FT_4$ or FTI, but have a normal $T_3$. In some situations, such as during pregnancy or while taking birth control pills, high levels of total $T_4$ and $T_3$ can exist. This is because the estrogens increase the level of the binding proteins. In these situations, it is better to ask both for TSH and free $T_4$ for thyroid evaluation (American Thyroid Association, http://www.thyroid.org/, 2015).

**1-2-3-2-4 Thyroid Antibody Tests:**

The immune system of the body normally protects us from foreign invaders such as bacteria and viruses by destroying these invaders with substances called antibodies produced by blood cells known as lymphocytes. In many patients with hypothyroidism or hyperthyroidism, lymphocytes make antibodies against their thyroid that either stimulate or damage the gland. Two common antibodies that cause thyroid problems

are directed against thyroid cell proteins: thyroid peroxidase and thyroglobulin. Measuring levels of thyroid antibodies may help diagnose the cause of the thyroid problems. For example, positive anti-thyroid peroxidase and/or anti-thyroglobulin antibodies in a patient with hypothyroidism make a diagnosis of Hashimoto's thyroiditis. If the antibodies are positive in a hyperthyroid patient, the most likely diagnosis is autoimmune thyroid disease (American Thyroid Association, http://www.thyroid.org/ , 2015).

**1-2-3-2-5 Thyroglobulin**:

Thyroglobulin (Tg) is a protein produced by normal thyroid cells and also thyroid cancer cells. It is not a measure of thyroid function and it does not diagnose thyroid cancer when the thyroid gland is still present. It is used most often in patients who have had surgery for thyroid cancer in order to monitor them after treatment. Tg is included in this brochure of thyroid function tests to communicate that, although measured frequently in certain scenarios and individuals, Tg is not a primary measure of thyroid hormone function (American Thyroid Association, http://www.thyroid .org / , 2015).

**1-2-3-2-6 Radioactive Iodine Uptake**:

Because $T_4$ contains much iodine, the thyroid gland must pull a large amount of iodine out from the blood stream in order for the gland to make an appropriate amount of $T_4$. The thyroid has developed a very active mechanism for doing this. Therefore, this activity can be measured by having an individual swallow a small amount of iodine, which is radioactive. The radioactivity allows the doctor to track where the iodine molecules go. By measuring the amount of radioactivity that is taken up by the thyroid gland (radioactive iodine uptake, RAIU), doctors may determine whether the gland is functioning normally. A very high RAIU is seen in individuals whose thyroid gland is overactive (hyperthyroidism), while a

low RAIU is seen when the thyroid gland is underactive (hypothyroidism). In addition to the radioactive iodine uptake, a thyroid scan may be obtained, which shows a picture of the thyroid gland (American Thyroid Association, http://www.thyroid.org/ , 2015).

**1-2-4 Effect of Thyroid Dysfunctions on Blood Cells:**

Thyroid disorders are frequently accompanied by red blood cell abnormalities. Thyroid hormones often have important effect on erythropoiesis. 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. Generally it seems that hypothyroidism causes hypoplasia in all myeloid cell lineages and hyperthyroidism result in hyperplasia. Hypothyroidism can cause various forms of anemia (normochromic -normocytic, hypochromic -microcytic or macrocytic) through reducing the oxygen metabolism. (Dorgalaleh *et al*., 2013).

On the other hand, anemia frequently is not seen in patients with hyperthyroidism, while there were erythrocytosis in this situation, but when anemia present, may be morphologically similar to that observed in hypothyroidism. Patients with hypothyroidism have a decreased erythrocyte mass due to reduction of plasma volume and may undetectable by routine measurement such as hemoglobin concentration, whereas an increased erythrocyte mass is observed in most hyperthyroid patients . Alteration in other hematological parameters such as hemoglobin (HG), hematocrit (HCT), mean corpuscular volume (MCV) ,mean corpuscular hemoglobin (MCH), white blood cell (WBC) count and platelet count is associated with thyroid dysfunction is observed as well, but all changes

return to normal if an euthyroid (normal) state is obtained. Immunological mechanisms have been offered for decline of the life-span of erythrocytes and platelets (Dorgalaleh *et al.* ,2013).

**1-2-5 Previous Studies:**

**1-2-5-1 Prevalence of Goiter in Sudan:**

According to World Health Organization website April 2015,

In Sudan, the total prevalence of goiter reported in studies in the period from the early 1980s to the mid 1990s ranged from 13% in the eastern city of Port Sudan and 17% in Khartoum state, to 78% in the central region and 87% in Darfur, in the west. According to a national study conducted in 1997, the overall prevalence of all types of goiter was 22,8% and prevalence figures ranged from 5% in the city of Khartoum to 42% in the Upper Nile region. The prevalence of all types of goiter in 15 December 2009 was found to be 38.8% overall and ranged from 12.2% in Omdurman to 77.7% in Kosti city. In the studied cities, the prevalence of endemic goiter ranged from mild to severe (World Health Organization, http://www.who.int/bulletin/volumes/89/2/09-075002/en , 2015).

Iodine deficiency is probably the most important but not the only factor leading to the high prevalence of goitre in Sudan.

Despite the fact that IDD control programmes were initiated in the Sudan more than 25 years ago, IDDs continue to be an important public health problem in the country. Although imported iodized salt is commercially available, no data on its consumption exist (World Health Organization, http://www.who.int/bulletin/volumes /89/2 /09-075002/en , 2015).

The exceptionally high median urinary iodine concentration (46.40 µg/dl) in Port Sudan is well above the level (30 µg/dl) indicative of a high individual risk of iodine-induced hyperthyroidism and, in genetically susceptible individuals, of autoimmune thyroid disease from excess iodine

intake (World Health Organization, http://www.who.int/bulletin/volumes /89/2 /09-075002/en , 2015).

**1-2-5-2 Previous Studies:**

Stimulation of erythropoiesis by thryoid hormones appears to be mediated through erythropoietin as Das, Mukherjee , Sarkar , Dash, Rastogi have concluded in their study while measuring erythropoietin and erythropoiesis in hypo and hyperthyroidism (Das *et al.*, 2013).

In the absence of thyroid hormones, anemia frequently develops and may be normocytic, hypochromic-microcytic, or macrocytic. According to a review by Fein and Rivlin in North America , Complete correction of anemia often requires restoration of thyroid function as well as specific hematinic therapy. Continued attention to hematologic status is essential in the management of patients with thyroid diseases (Fein and Rivlin, 1975).

In a study by Geetha and Srikrishna in India , red blood cell indices were compared in patients with hypothyroidism and hyperthyroidism and revealed that RDW and MCV in these two groups of patients in comparison to euthyroid individuals have statistically significant difference but other RBC parameters including HB and HCT did not show any significant difference in comparison with euthyroid status (Geetha and Srikrishna, 2012)**.**

Bashir and *et al.* in Iran reported that the hematological parameters including Hb, RBC, MCV, HCT, RDW,RBC% were significantly increased in untreated subclinical hypothyroidism and untreated primary hypothyroidsm, whereas, in treated subclinical hypothyroidism and Primary Hypothyroid, results were insignificant (Bashir *et al.*, 2012).

Kawa and *et al.* in Poland reported that RBC, HB and HCT in patients with hyperthyroidism were significantly higher than control groups while RBC and HB were decreased in hypothyroidism, while HCT increased. They also showed that MCH and MCHC were lower in both groups in comparison with control group and MCV was increased in two groups of hypothyroidism and hyperthyroidism (Kawa *et al*., 2010).

Dorgalaleh *et al.* in Iran found a statistically significant difference between the two groups of patients with hypo and hyperthyroidism in RBCs, MCH, MCHC, RDW, HB and HCT (Dorgalaleh *et al.*, 2013).

## 1-3 Rationale:

Thyroid dysfunctions are frequently associated with hematological parameters abnormalities. Thyroid hormones has an important role in metabolism and proliferation of hematopoietic cells. Their levels affect blood cells counts and red blood cells indices including MCV, MCH ,MCHC and RDW (Dorgalaleh *et al*., 2013).

Because of high prevalence of thyroid dysfunctions in Sudanese population (WHO records, 2015), And few published data concerning the alteration of hematological parameters of Sudanese patients with thyroid dysfunctions. The present study attempt to evaluate the effect of hypo and hyperthyroidism on blood cell counts in Sudanese patients.

## 1-4 Objectives

### 1-4-1 General Objective:

Determination of CBC of Sudanese patients with thyroid dysfunctions in Khartoum state.

### 1-4-2 Specific Objectives:

**1-** Measurement of Hb, RBCs, WBCs, Platelets count, MCV, MCH, MCHC, RDW, PCV in patients with hypothyroidism and hyperthyroidism compared to control.

**2-** To compare the CBC of patients with hypo and hyperthyroidism according to gender and age.

# CHAPTER TWO

## Materials and Methods

### 2-1 Study Design and Study Area:

This is a case-control study aimed to determined CBC of patients with thyroid dysfunctions. The study included Sharq Alneil Hospital, AL Zarra Hospital and Antalia Center during the period of March to April 2015.

### 2-2 Study Population:

Sudanese patients previously diagnosed with Thyroid dysfunctions .

### 2-2-1 Inclusion Criteria:

All patients who referred to laboratory from March to April 2015 with hyperthyroidism and hypothyroidism indicated by TSH levels, were enrolled in study. Both sexes was included at different age groups.

### 2-2-2 Exclusion Criteria:

Patients with hematological abnormalities such as bleeding or congenital anemic diseases were excluded .

### 2-3 Sample Size:

One hundred and Ten patients with Thyroid dysfunctions were selected for the study and 65 samples with normal thyroid profile as control group with matched ages.

### 2-4 Sampling Procedure:

- Two ml of Ethylene Diamine Tetra Acetic acid (EDTA) anti coagulated whole blood samples were collected from participants for complete blood count.
- The collected whole blood is then analyzed using Auto Hematology Analyzer BCC-3000B for CBC. (Figure 2-1)
- Thyroid Stimulating Hormone TSH was considered to test the function of Thyroid gland. TSH is produced by the pituitary gland. It tells the thyroid gland to make and release thyroid hormones into the blood.

- Normal values range from 0.27- 3.75 milli-international units per liter (mIU/L) (TSH [$I^{125}$] IRMA KIT,2014).
- It has been used to group the patients into Hypothyroid (TSH >3.75 mlU/L) and Hyperthyroid (TSH <0.27mlU/L).

**2-5 Method and Principle of Automated Technique:**

1. An Auto Hematology Analyzer BCC-3000B was used. The sample number was entered before each sample.
2. On whole blood mode, a well-mixed anticoagulated sample was set to the sample probe, and the start switch was pressed till the aspirating process was finished. The sample volume is 13 microleters.
3. The aspirated sample was then automatically suspended into the different detector blocks and different parameters were measured.
4. The results of parameters were then viewed on the screen and subsequently printed out (DIRUI Co.LTD, 2011).

**2-6 Statistical Analysis:**

The collected data was analyzed using Statistical Package for Social Science (SPSS) computer programme. Statistical One Way ANOVA test was used to evaluate the significance of differences between two groups of patients and the control group. P-value < 0 .05 was considered as significantly different.

**2-7 Ethical Consideration:**

It was considered that all information which obtained from patients was kept as highly secure data and specimens or results were not permitted.

The participators were provided with information about the study and informed consents were verbally obtained from participants before collecting samples.

Permission was obtained from the hospital Laboratory and Training department for collected samples.

## Chapter Three

## Results

This is a case control study included 175 subjects aged between 18 – 60 years who were categorized as 52 patients with hyperthyroidism, 58 patients with hypothyroidism and 65 controls based on TSH levels for each group. The mean ages were 46 years, 40 years and 39 years $\pm$ SD, respectively (Table 3-1).

The male participants in this study were 37 representing 21.1% of study population and females were 138 participants representing 78.8%. Male : Female ratio was 1: 3.7 . Hyperthyroid group included 14 males and 38 females while hypothyroid group included 18 males and 40 females mainly in the ages between 41-50 years. The control group were 5 males and 60 females. Gender and age frequency according to thyroid status of study population were shown in (Table 3-2).

Comparing Patients with hyperthyroidism to control, there was a significant increase in Hb ,which increased from 13.2 $\pm$ 1.63 g/dl in control to 13.9 $\pm$ 1.46 g/dl in hyperthyroidism patients. MCH, MCHC were also significantly increased with *P* values of 0.020 and 0.000, respectively. RBCs, WBCs counts did not show any significant difference with *P* values of 0.835 and 0.375, respectively. Platelets count was significantly decreased from 3.15 $x10^9$plt/L in control to 2.69 $x10^9$/L in hyperthyroidism group (*P* value 0.003).

RBCs count decreased significantly in hypothyroid patients compared to control (5.02 x $10^{12}$cell /L in control group became 4.67 $x10^{12}$cell/L in hypothyroidism group) with *P* value 0.000. PCV decreased significantly from 41.5 L/L in control to 39.5 L/L in hypothyroidism group. MCH, MCHC increased significantly in hypothyroidism group compared to con-

trol with *P* values of 0.002 and 0.004, respectively as illustrated in (Table 3-3).

Hb, RBCs count and PCV were significantly different between patients with hyper and hypothyroidism with *P* values of 0.002, 0.000 and 0.038, respectively ( Table 3-3). They increased in hyperthyroidism and decreased in hypothyroidism.

Also, comparing hyper to hypothyroidism patients, MCV showed significant increase in hypothyroidism patients ( mean MCV $\pm$ SD was 84.7$\pm$ 5.22 fL) and the Platelet count decreased in hyperthyroidism ( mean Plt $\pm$ SD was 2.69$\pm$ 0.54 x $10^9$ Plt /L).

MCH and MCHC did not show significant difference between patients with hyper and hypothyroidism with *P* values of 0.536 and 0.218, respectively.

WBCs count and RDW did not show any difference between thyroid patients and compared to control.

(Table 3-3 ) showed the Comparison of study groups using ANOVA multiple comparison table.

**(Table 3-1):**

**Charactaristics of patients with hyper and hypothyroidism:**

| Study Population | NO. of patients | Age (year) (mean$\pm$SD) | TSH(mIU/L) (mean$\pm$SD) | $T_3$(mIU/L) (mean$\pm$SD) | $T_4$(mIU/L) (mean$\pm$SD) |
|---|---|---|---|---|---|
| Hyperthyroid | 52 | 46$\pm$10.3 | 0.087$\pm$0.09 | 5.2$\pm$2.5 | 219.9$\pm$60.8 |
| Hypothyroid | 58 | 40$\pm$10.6 | 24.0$\pm$30.7 | 1.5$\pm$0.7 | 79.2$\pm$40.5 |
| Control | 65 | 39$\pm$10.1 | 1.6 $\pm$ 0.86 | 1.9$\pm$0.3 | 108.4$\pm$25.9 |

**(Table 3-2) Gender and age frequency according to thyroid status of study population**:

| Group | | Age Group | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Sex | 1-20 years | 21-30 years | 31-40 years | 41-50 years | 51-60 years | |
| **Hyperthyroid** | Male | 0 | 3 | 0 | 6 | 5 | 14(26.9%) |
| | Female | 0 | 3 | 3 | 17 | 15 | 38(73.1%) |
| | Total | 0 | 6 | 3 | 23 | 20 | 52 |
| **Control** | Male | 0 | 0 | 3 | 2 | 0 | 5(7.7%) |
| | Female | 3 | 10 | 20 | 20 | 7 | 60(92.3%) |
| | Total | 3 | 10 | 23 | 22 | 7 | 65 |
| **Hypothyroid** | Male | 0 | 3 | 0 | 9 | 6 | 18(31%) |
| | Female | 3 | 11 | 3 | 20 | 3 | 40(69%) |
| | Total | 3 | 14 | 3 | 29 | 9 | 58 |

**Table 3-3 :** Comparison of study groups using ANOVA**:**

| Variable | Mean $\pm$ SD | (I) group | (J) group | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|---|
| RBC | Hyper 5.04 $\pm$ 0.44 | hyper | Control | .01796 | .08626 | .835 |
| | | | Hypo | .36662 | .08854 | .000 |
| | Control 5.02 $\pm$ 0.55 | Control | Hyper | -.01796 | .08626 | .835 |
| | | | Hypo | .34866 | .08375 | .000 |
| | Hypo 4.67 $\pm$ 0.37 | Hypo | Hyper | -.36662 | .08854 | .000 |
| | | | Control | -.34866 | .08375 | .000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hemoglo-bin | Hyper 13.9 ± 1.46 | Hyper | Control | .7765 | .2748 | .005 |
| | | | Hypo | .8717 | .2821 | .002 |
| | Control 13.2 ± 1.63 | Control | Hyper | -.7765 | .2748 | .005 |
| | | | Hypo | .0951 | .2668 | .722 |
| | Hypo 13.1 ± 1.30 | Hypo | Hyper | -.8717 | .2821 | .002 |
| | | | Control | -.0951 | .2668 | .722 |
| PCV | Hyper 40.9±4.08 | Hyper | Control | -.5954 | .7357 | .419 |
| | | | Hypo | 1.4088 | .7552 | .038 |
| | Control 41.5 ± 4.59 | Control | Hyper | .5954 | .7357 | .419 |
| | | | Hypo | 2.0041 | .7142 | .006 |
| | Hypo 39.5 ± 2.93 | Hypo | Hyper | -1.4088 | .7552 | .038 |
| | | | Control | -2.0041 | .7142 | .006 |
| MCV | 82.2 ± 4.73 | Hyper | Control | -.5077 | .9977 | .611 |
| | | | Hypo | -2.4512 | 1.0241 | .018 |
| | Control 82.8 ± 5.92 | Control | Hyper | .5077 | .9977 | .611 |
| | | | Hypo | -1.9435 | .9686 | .046 |
| | Hypo 84.7 ± 5.22 | Hypo | Hyper | 2.4512 | 1.0241 | .018 |
| | | | Control | 1.9435 | .9686 | .046 |
| MCH | Hyper 27.8 ± 2.83 | Hyper | Control | 1.4000 | .5938 | .020 |
| | | | Hypo | -.3782 | .6096 | .536 |
| | Control 26.4 ±3.41 | Control | Hyper | -1.4000 | .5938 | .020 |
| | | | Hypo | -1.7782 | .5765 | .002 |
| | Hypo 28.2 ± 3.24 | Hypo | Hyper | .3782 | .6096 | .536 |
| | | | Control | 1.7782 | .5765 | .002 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MCHC | Hyper 33.8 ± 2.11 | Hyper | Control | 1.8908 | .4586 | .000 |
| | | | Hypo | .5824 | .4707 | .218 |
| | Control 31.9 ± 2.67 | Control | Hyper | -1.8908 | .4586 | .000 |
| | | | Hypo | -1.3084 | .4452 | .004 |
| | Hypo 33.2 ± 2.52 | Hypo | Hyper | -.5824 | .4707 | .218 |
| | | | Control | 1.3084 | .4452 | .004 |
| Platelets | Hyper 2.69 ±0.54 | Hyper | Control | -.45415 | .15022 | .003 |
| | | | Hypo | -.45028 | .15419 | .004 |
| | Control 3.15 ± 0.55 | Control | Hyper | .45415 | .15022 | .003 |
| | | | Hypo | .00388 | .14584 | .979 |
| | Hypo 3.14 ± 0.93 | Hypo | Hyper | .45028 | .15419 | .004 |
| | | | Control | -.00388 | .14584 | .979 |
| WBC | Hyper 6.91 ± 2.67 | Hyper | Control | .3842 | .4316 | .375 |
| | | | Hypo | -.3676 | .4430 | .408 |
| | Control 6.53 ± 1.96 | Control | Hyper | -.3842 | .4316 | .375 |
| | | | Hypo | -.7518 | .4190 | .075 |
| | Hypo 7.28 ± 2.34 | Hypo | Hyper | .3676 | .4430 | .408 |
| | | | Control | .7518 | .4190 | .075 |
| RDW | Hyper 13.9 ± 1.22 | Hyper | Control | -.3104 | .1907 | .105 |
| | | | Hypo | -.1166 | .1957 | .552 |
| | Control 14.1 ± 1.03 | Control | Hyper | .3104 | .1907 | .105 |
| | | | Hypo | .1937 | .1851 | .297 |
| | Hypo 13.9 ± 0.80 | Hypo | Hyper | .1166 | .1957 | .552 |
| | | | Control | -.1937 | .1851 | .297 |

*****The mean difference is significant at the 0.05 level.

## Chapter Four

### 4 -1 Discussion:

This study included 175 participants. There ages ranged from 18 – 60 years and the most frequent age group among thyroid patients was 41 – 50 years and the least frequent age group was 1-20 years ,suggesting that thyroid dysfunctions prevailed more in the adult group. Khattak *et al* ( 2001) agreed with this as their study included 25237 thyroid patients with increased prevalence among adults and females. Morganti *et al*, (2005) showed that epidemiological data from the aging population confirms that men are less affected by thyroid disease than women . Objective Autoimmune thyroid disease (AITD) is a common disorder especially in women, and both genetic and environmental factors are involved in its pathogenesis. Estrogen use is associated with a lower risk, and pregnancy with a higher risk for developing hyperthyroidism (Strieder *et al*., 2003).

The study results for patients with hyperthyroidism – compared to control- increased significantly in Hb ,MCH, MCHC and that can be understood as the thyroid hormones known to have a crucial role on erythropoiesis by induction of erythropoietin secretion and proliferation of erythroid progenitors (Dorgalaleh *et al*., 2013). RBCs count did not change significantly, although erythrokinetic studies also provided evidences of increased erythropoietic activity in the bone marrow in patients with hyperthyroidism ( Das *et al.*, 2013). Failure of erythrocytosis to occur here may be due to deficiency of hemopoietic nutrients such as iron, vitamin $B_{12}$ and folate ( Das *et al*., 2013). Another reason for that can be the hypervolemia which has been brought about by the increased oxygen requirement of this disease state (Gibson and Harris, 1939). The Platelets

count significantly decreased in hyperthyroid patients (P-value 0.003) due to the accelerated platelets turnover and their increased destruction by an activated reticuloendothelial system referred to the action of thyroid hormones which accelerated the metabolic rate of the entire body (Hofbauer and Heufelder , 1997).

Hypothyroid patients results showed significant decrease in RBCs and PCV and increase in MCH, MCHC compared to control . According to Das *et al.*( 2013) , majority of patients with the hypothyroid state had significant reduction in red blood cell mass per kg of body weight. The presence of anemia in many of these patients was not evident from hemoglobin values due to concomitant reduction of plasma volume. These changes in erythropoiesis in the hypothyroid state appear to be a part of physiological adjustment to the reduced oxygen requirement of the tissues due to diminished basal metabolic rate. The current study resulted in no significant change in WBCs count in patients with hypothyroidism agreed with Athens,(1993) Granulocyte and lymphocyte counts are described as being normal in overt hypothyroidism, with the exception of isolated reports of basophilia in circulating blood cells. However, a more recent study found no difference in basophil counts between normal subjects and patients with overt hypothyroidism (Petrasch *et al.*, 1993).

The results of the current study showed that RBCs count, Hemoglobin and PCV were significantly different between hyper and hypothyroid patients and similar results were obtained by ( Dorgalaleh *et al*, 2013 ) in Iran. These results may be due to the fact that thyroid hormones mediate erythropoiesis through erythropoietin as( Das *et al*, 2013) have concluded in their study.

MCV increased in the current study hypothyroid patients compared to hyperthyroid patients. Macrocytic anemia is caused by malabsorption of

vitamin B12, folic acid, pernicious anemia and inadequate nutrition. Pernicious anemia occurs 20 times more frequently in patients with hypothyroidism than generally. Macrocytosis is found in up to 55% patients with hypothyroidism(Antonijević *et al*., 1999). Other studies have shown that hypothyroidism causes anemia or hyper proliferation of immature erythroid progenitors, and the anemia is usually macrocytic hypochromic anemia (Horton *et al*,1976). Kawa *et al,* (2010) in Poland also showed significant difference in RBC, HB and PCV comparing patients to control. The difference in red blood cell parameters and indices although the positive relation between thyroid hormones and erythropoiesis may attributed to the possibility of the developed anemia in hypothyroid case to be normocytic, hypochromic microcytic or macrocytic anemia according to the review of ( Fein and Rivlin, 1975) in North America.

## 4-2 Conclusion:

1- The mean age of hyperthyroid group is 46 years, hypothyroid group mean age is 40 years and control group mean age is 39 years.
2- Females constituted 78.9% of study population. Females are more common than males.
3- Hb, MCH, MCHC increased significantly in hyperthyroidism patients compared to control. Platelets count decreased significantly.
4- In hypothyroidism patients, RBCs and PCV decreased significantly compared to control but MCH and MCHC increased significantly.
5- Comparing hyperthyroid and hypothyroid patients, Hemoglobin, RBCs and PCV increased significantly in hyperthyroid patients but decreased in hypothyroid patients. MCV increased in hypothyroid patients and PLT decreased in hyperthyroid patients.

**4-3 Recommendations:**

1- Hematological parameters Hb, RBCs, PCV should be included in the routine investigation of thyroid dysfunctions.
2- Furthers studies are needed to cover larger population and offer a potential for the future development of diagnosis of thyroid diseases.

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**Appendix I**

**Figure 2-1**

**DIURI BCC-3000B**

**Auto Hematology Analyzer**

## Appendix II

**Table 1-1:Hematology reference ranges (Mean± 2SD)**

| Age (Years) | RBCs Count ($*10^{12}$/L) | MCV (f L) | MCH (pg) | MCHC (g/dl) | HB (g/dl) |
|---|---|---|---|---|---|
| Birth(cord blood) | 3.9-5.5 | 98-118 | 31-37 | 30-36 | 13.5-19.5 |
| 1 to 3 days | 4.0-6.6 | 95-121 | 31-37 | 29-37 | 14.5-22.5 |
| 0.5 to 2 | 3.7-5.3 | 70-86 | 23-31 | 30-36 | 10.5-13.5 |
| 2 to 6 | 3.9-5.3 | 75-87 | 24-30 | 31-37 | 11.5-13.5 |
| 6 to 12 | 4.0-5.2 | 77-95 | 25-33 | 31-37 | 11.5-15.5 |
| 12 to 18 (male) | 4.5-5.3 | 78-98 | 25-35 | 31-37 | 13.0-16.0 |
| 12 to 18 (female) | 4.1-5.1 | 78-102 | 25-35 | 31-37 | 12.0-16.0 |
| 18 to 49 (male) | 4.5-5.9 | 80-100 | 26-34 | 31-37 | 13.5-17.5 |
| 18 to 49 (female) | 4.0-5.2 | 80-100 | 26-34 | 31-37 | 12.0-16.0 |

(Anne *et al*., 1998).

**Table 1- 2:Leukocytes Values in Humans** (Anne *et al*., 1998).

| Age | Total WBCs ($*10^9$/L) | Neutro-phils | Lympho-cytes | Mono-cytes | Eosinophils | Basophils |
|---|---|---|---|---|---|---|
| 12 hr | 13.0-38.0 | 6.0-28.0<br>68% | 2.0-11.0<br>31% | 0.40-3.6<br>5.3% | 0.02-0.95<br>2.0% | 0-0.5<br>0.4% |
| 1 wk | 5.0-12.0 | 1.5-10.0<br>45% | 2.0-17.0<br>41% | 0.30-2.7<br>4.8% | 0.07-1.10<br>4.1% | 0-0.25<br>0.4% |
| 12mo | 6.0-17.5 | 1.5-8.5<br>31% | 4.0-10.5<br>61% | 0.05-1.1<br>4.8% | 0.05-0.70<br>2.6% | 0-0.02<br>0.4% |
| 6 yr | 5.0-14.5 | 1.5-8.0<br>51% | 1.5-7.0<br>42% | 0.0-0.8<br>4.7% | 0-0.65<br>2.7% | 0-0.2<br>0.6% |
| 14 yr | 4.5-13.0 | 1.8-8.0<br>56% | 1.2-5.8<br>37% | 0.0-0.8<br>4.7% | 0-0.50<br>2.5% | 0-0.2<br>0.5% |
| 21 yr | 4.5-11.0 | 1.8-7.7<br>59% | 1.0-4.8<br>34% | 0.0-0.8<br>4.0% | 0-0.45<br>2.7% | 0-0.2<br>0.5% |

## Appendix III

**Sudan University for Science and Technology**

**Collage of Graduate studies**

**Questionnaire to measure CBC IN Patients who were requested for Thyroid Profile**

Date : / / 2015.

No(___).

Name:_____ .

Age:_____ years.

Gender: _____ .

Hospital :_____ .

Presence of Anemia or hematological diseases: Yes__ No __ .

Previous use of Thyroid medications : Yes__ No __ .

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براءة أخلاقية

**Informed Consent**

**جامعة السودان للعلوم والتكنولوجيا**

**كلية الدراسات العليا**

**ماجستير مختبرات طبية**

**تخصص علم أمراض الدم ومبحث المناعة الدموية**

الاسم:_____

سوف يتم أخذ عينة من الدم (2 مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة العينة بواسطة مطهر . كل الادوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية.

أوافق أنا المذكور أعلاه على أخذ عينة لإجراء الدراسة.

الإمضاء :_____
التاريخ :_____