بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate Studies

Evaluation of the Effect of Thyroid Dysfunction on Complete Blood Cell Count on Sudanese Patients in Khartoum State

تقويم تأثير الخلل فى الغدة الدرقية في تعداد خلايا الدم الكاملة فى المرضى السودانيين فى ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of M.Sc. degree

In Medical laboratory Science (Hematology and Immunohematology)

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2015

الآية

بسم الله الرحمن الرحيم

قالى تعالى :

﴿ وَقُلِ اعْمَلُواْ فَسَيَرَى اللّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ﴿105﴾ ﴾

صدق الله العظيم

التوبة 105

DEDICATION

To those who always believe on my efforts….

My Mother,

Who the words fail to describe, and can't say more than She is my life and my oxygen…

My Father,

Who supported me encouraged me at every step in my life ….

To my Brothers and sisters for their understanding support..

To all these who help me to proceed ahead….

ACKNOWLEDGMENT

First of all I would like to thank god for making this work possible and for giving me the will and strength to keep going on .

I express my deep gratitude to my Supervisor Dr. Nadia Madani , for all help , support , device and useful guidance.

Ithanks with great appreciation to Military Hospital staff in Omdurman for all help valuable advice and guidance.

Abstract

A descriptive cross- sectional hospital –based study was conducted from (January 2015 to April 2015).100 blood sample were taken from Sudanese thyroid disease patients (35 patients were selected 35 with hyperthyroidism 35 with hypothyroidism) in Military hospital addition to 30 apparently healthy person were selected as controlgroup. The study group were a selected with age range from 20 -60 years. 3ml of venous blood was withdrawn, From each participant , placed in a test tube containing EDTA. Complete blood count were determined using an automated cell counter (Sysmx Kx.N21).The results were analyzed by Stastiscal Package of Social Science (SPSS) version16.The result Showed that percent of female with hyperthyroidism and hypothyroidism higher than percent of male .Significant decrease in Hemoglobin, Hematocrit , Mean cell Hemoglobin in patients with hyperthyroidism & hypothyroidism compared to control group (P.value<0.05). Red Blood Cell showed significant decrease in hypothyroidism patients when compared with control group(P.Value<0.05).Significant decrease in Mean Cell Volume in hyperthyroidism group when compared with control(P.value< 0.05).No significant difference were noted for White Blood Cell count , platelets count and Mean cell Hemoglobin Concentration in patients with hyperthyroidism and hypothyroidism (P.Value>0.05).

From obtained dataThyroid dysfunctions have direct effect on most red blood cells indices and this change should be considered by medical care provider. These finding suggest that thyroid hormone has effect on red cell count and red cell indices which can cause different type of anemia.

المستخلص

أجريت هذه الدراسةالوصفيـــه المقطعيــه في الفتره مابين شهر يناير الى أبريل 2015.تم أخذ عينـــات دم من مائــــه مريض بالغده الدرقيه (35 مريض يعاني من زيادة هرمونات الغدةالدرقية و 35مريض بانخفاض هرمونات الغدةالدرقية) بالمستشفى العسكري بالاضافه إلى 30 عينه لمتطوعين كمجموعة ضابطة.تراوحت أعمار مجموعه الدراسة مابيـن 20- 60 سنــــة.تـــم اخذ 3 مل من الدم الوريدي من كل مشارك . وضعت في أنبوب اختبار يحتوي على (ETDA) وذلك لحساب الدم الكامل باستخدام عداد الخلايا الالي(Sysmex kx21N) وتم تحليل النتائج بواسطة برنامج الحزم الاحصائيه للعلوم الاجتماعية نسخه 16.أظهرت الدراسة أن نسبه الإناث المصابات بزيادة ونقصان هرمونات الغدةالدرقية أعلى من نسبه الذكور. هناك انخفاض ذو دلاله احصائيه في متوسط الهيموغلوبين ومتوسط حجم الكريه في المرضى المصابين بانخفاض او زياده هرمونات الغدة الدرقية.أظهرت خلايا الدم الحمراء انخفاضاً كبيراً فى المرضى الذين يعانون قصورالغدة الدرقية مقارنة مع مجموعة الضابطة القيمة المعنوية اصغر من (0.05).هناك ايضا انخفاض ذو دلالة إحصائية في متوسط حجم الكرية في المرضى المصابين بزياده في هرمونات الغده الدرقيه مقارنه مع المجموعه الضابطه القيمه المعنويه اصغر من (0.05). لم يلاحظ وجود فروق ذات دلاله احصائيه لمجموع كريات الدم لبيضاء والصفائح الدمويه و متوسط هيموغلوبين الكريه بين المرضى المصابين بانخفاض و زياده هرمون الغده الدرقيه مقارنه بالمجموعه الضابطه (القيمه المعنويه اكبر من (0.05).

من البيانات التي تم الحصول عليها ان اختلالات الغدة الدرقية لها تأثيرمباشر على معظم خلايا الدم الحمراء ومؤشراتها وينبغي النظر فى هذا التغيير من قبل مقدمي الرعاية الطبية.

هذه النتائج تشير إلى أن هرمونا لغدة الدرقية له تأثيرعلى عدد الخلايا الحمراء ومؤشرات الخلايا الحمراء التي يمكن أن تسبب في انواع مختلفة من فقرالدم .

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List of Abbreviations

Abbreviations	Full Term
MCV	Mean Cell Volume
MCH	Mean Cell Heamoglobin
MCHC	Mean Cell Hemoglobin Concentration
RBCs	Red Blood Cells
WBCs	White Blood Cells
CBC	Complete Blood Count
TSH	Thyroid Stimulating Hormone
T3	Triiodothyonin
T4	Thyroxin
TC II	Transcoblamine II
K3-EDTA	Potassium3- EthleneDiamine Tetra Acetate
Hb	Hemoglobin
HCT	Heamtocrit
PCV	Packed cell volume
Plts	Platelet
SPSS	Stastiscal Package of Social Science

CHAPTER ONE

Introduction & Literature review

Chapter one

1.0 Introduction and literature review

1.1 General introduction:

Thyroid gland as largest and most important endocrine gland of human body with the secretion of two hormones, T3 , T4. Thyroid hormones involves in many metabolic processes, increasing oxygen consumption, essential for normal growth, mental development and sexual maturation .They also affect the cardiovascular and central nervous s systems. Thyroid gland also has crucial effect on erythropoiesis by induction of erythropoietin secretion and also proliferation of erythoid progenitor (1,8and 11) (Dorgalaleh , *et al* 2013).

Anemia is a decrease in number of Red blood cells (RBCs) or less than the normal quantity of haemoglobin in the blood . However, it can include decreased oxygen binding ability of eash Heamoglobin molecule due to deformity or lack in numerical development as in some other types of haemoglobin deficiency
(Hoffbrand,et al., 2004 and Barbara, 1993).

1.2 **Literature review:**

A study that came out of tertiary care center in eastern India in Dec 2012 that Normocytic, normochromic anemia was present in 31 patients (51.6%) followed by microcytic anemia in 26 patients (43.3%). Six patients (10%) had megaloblastic anemia with vitamin B12 deficiency including 3 cases of pernicious anemia. Two patients had combined deficiency of iron and vitamin B12 .In another cross-sectional survey was conducted from January 2006 through April 2006 in Iran showed a high rate of association between Macrocytic anemia and hypothyroid in Iranian population which completed by MitraKazemi-jahromi.Also in Tehran university of Medical sciences, 2011 in hematology- oncology department, RasualAkram hospital, found the anemia is outcome of low function of thyroid gland.

1.2.1 Anatomy of thyroid gland :

Thyroid gland is found in the neck , below the thyroid cartilage (which forms the laryngeal prominence, or “adam’s apple”). the isthmus (the bridge between the two lobes of the thyroid) is located inferior to the cartilage. the thyroid gland controls how quickly the body uses energy, makes proteins, and controls how sensitive the body is to other hormones . it participates in these processes by producing thyroid hormones, the principal ones being tri-iodothyronine (T3) and thyoxine which can sometimes be referred to as tetraiodothyronine (T4). These hormones regulate the rate of metabolism and affect the growth and rate of function of many other systems in the body. T3 and T4 are synthesized from both iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homestasis. Hormonal output from the thyroid is regulated by thyroid – stimulating hormone (TSH) produced by the anterior pitutitary, which itself is regulated by thyrotropin- releasing hormone(TRH)producedby hypothalamus. The thyroid gets its name from the Greek adjective, duo to the shape of the related thyroid cartilage .the most common problems of thyroid gland consist of an overactive thyroid gland , referred to hyperthyrodim, and an underactive thyroid gland , referred to hypothyroidism.

Thyroid and parathyroid glands

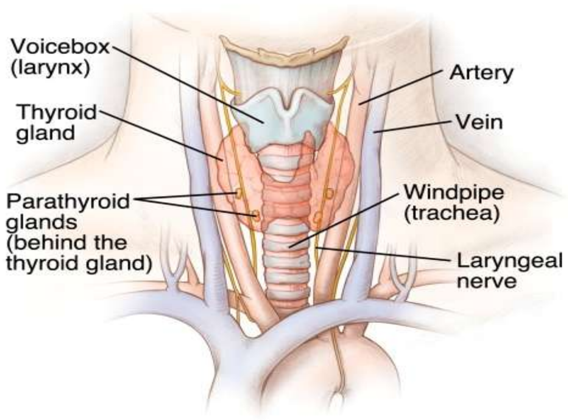

Fig:1.1 Anatomy of thyroid and parathyroid gland

1.2.2 Physiology of thyroid hormone:

Thyriod hormones are synthesized in the thyroid gland by iodination and coupling of two molecules of the amino acid tyrosine, a process that is dependent on an adequate supply of iodide (Levi *et al* ,2007).Iodide in the diet is a absorbed rapidly from the small intestine. Most foods contain adequate amounts. In areas where the iodide content of the siol is very low there used to be a high incidence of enlargement of the thyroid gland (goiter), but the general use of artificially iodized salt has made this a less common occurrence. Sea foods have a high iodide content. Fish and iodized salt are the main dietary sources of the elements. Normally about a third of dietary iodide is taken up by the thyroid gland (Kester*et al*, 2004)

1.2.3 Synthesis of thyroid hormones:

Iodide is actively taken up by the thyroid gland under the control of thyroid stimulating hormone (TSH).Up take is blocked by thiocyanate and per chlorate. The concentration of iodide in the gland is at least times that in plsma and may exceed it by a hundred times on more (Jansen *et al*,2005).Iodide is rapidly converted to iodide, within the thyroid gland.Iodination of thyroglobulin residues in alarge glycoprotein, thyroglobulin, takes place from mono-and Diiodotyrosine (MIT and DIT). This step is inhibited by carimazolecropylthiouracil. diiodotyrosine are coupled from thyroxine (T4) (DITand (DIT) and triiodothyrosine (MIT and DIT) (Bianco *et al*,2002). Normally much more T4 than T3 is synthesized but if there is an inadequate supply of iodide the ratio of T3 to T4 in the gland increases. The thyroid hormones, still incorporated in thyroglobulin is taken up by the follicular cells and T4 and T3 are released by proteolytic enzymes into the bloodstream. The thyroid hormones are immediately bound to plasma protein. Mono- and diiodotyrosine, released at the same time, are deiodinated and the iodine is reused. Eash step is controlled by specific enzymes and congenital deficiency of any of these enzymes can lead to enlargement of the thyroid gland (goiter)and , if severe, hypothyroidism. The uptake of iodide, as well as the synthesis and secretion of thyroid hormones, is regulated by TSH, secret from The anterior pituitarygland (Martinez *et al*, 2004).

1.2.4 Action of thyroid hormones:

- Thyroid hormones affect many metabolic processes, increasing oxgen consumption binding to specific receptors in cell nuclei, they change the Expression of some genes.
- Thyroid hormones are essential for normal growth , mental development and maturation.
- They also increase the sensitivity of the cardiovascular and central nerves systems to catecholamine's and is influence cardiac output and heart rate (Stephen and saffron 2001).

1.2.5 Disorders of the thyroid gland :

The most common presenting clinical features of thyroid disease are the result of : - Hyperthyroidism, duo to excessive thyroid hormone secretion.-hypothyroidism , duo to deficient thyroid hormone secretion (Merck and Dohme , 2010)

1.2.5.1 Hyperthyroidism (thyrotoxicosis):

Hyperthyroidism, produced by sustained high plasma concentration of thyroid hormone, may be easy to diagnose clinically or may remain unsuspected for a long time.

Path physiology:

Plasma T4 and T3 concentration are usually both increased in hyperthyroidism. Mush of the T3 is secreted directly the thyroid gland and the increase in plasma T3level is greater, and usually evident earlier, than that of T4 (Hidaka *et al* ,1993).

1.2.5.2 Hypothyroidism:

Hypothyroidism is caused by suboptimal circulating concentration of thyroid hormones. The presenting signs and symptoms depend to some extent on the a etiology of the disorders.

Path physiology:

As primary hypothyroidism develops TSH secretion from anterior pituitary gland increase as negative feedback, associated with the falling plasmaT4 concentration, decreases. initially, the plasma T4 concentration

may be within population reference range , although abnormally low for the individual . for this reason the plasma TSHconcentration is most sensitive index of early disease .
T3 assay are of no help in making the diagnosis as plasma T3 concentration are often normal .secondary hypothyroidism may be caused by impaired TSH secretion, duo either to a disorder to pituitary gland itself, or to former case there is a subnormal rise in plasma TSH concentrations after administration of exogenous TSH (Medicine net, 2010).

1.2.6 Investigation of thyroid function:

The only validated test to diagnose primary hypothyroidism, is to measure thyroid stimulating hormone (TSH) and free thyroxin (T4).(Medicine net,2010) However, the levels can be effected by non-thyroidal illness. High levels of TSH indicate that thyroid is not producing sufficient levels of thyroid hormone (mainly as thyroxin (T4) smaller amounts of triiodthyronine (T3)).However, measuring just TSH fails to diagnosis secondary and tertiary hypothyroidism, thus leading to the following suggested testing if the TSH is normal and hypothyroidism is still suspected :

- Free triiodothyronine (fT3).

-Free thyroxin (fT4) .-Total T4

- Total T3.Additionally, the following measurement may be needed:

-Free T3 from 24-hoururine catch.

- Anti thyroid antibodies

-for evidence of autoimmune diseases that may be damaging the thyroid gland .

-Serum cholesterol

–which may be elevated in hypothyroidism.

-Prolactin – as a widely available test of pituitary function.

-Testing for anemia , including ferritin.

-Basal body temperature (Medicine net .2010)

1.2.7 Anemia:

1.2.7.1 General introduction:

Anemia can be classified in a variety of ways, based on the morphology of RBCs, underlying etiologic mechanisms, and discerible clinical spectra, to mention a few. The three main classes of anemiainclude excessive blood loss (acutely such as a hemorrhage or chronicallythrough low –volume loss), excessive blood celldestruction (hemolysis) or deficient red blood cell production (ineffective hematopoiesis) (Pallister,1993).

Anemia is typically diagnosed on a complete blood count. Apart from reporting the number of red blood cells and the hemoglobinlevel , the automatic counters also measure the size of red blood cells by flow cytometers, which is an important tool in distinguishing between the cause of Anemia. Examination of a stained blood smear using a microscope canalso be helpful, and is sometimes a necessity in regions of the world where automated analysis is less accessible. In modern counter, four parameters (RBC count ,Hemoglobin concentration, MCV and RDW) are measured, allowing others (hematocrit, MCH and MCHC) to be calculated , and compared to values adjusted for age and sex. Some counters estimate hematocrit from direct measurement.

1.2.7.2 Definition of Anemia :

Anemia is defined as reduction in the hemoglobin concentration of the blood. Although normal values vary between laboratoriestypical value would be less than 13.5g/dl in adult males and less than 11.5 g/dl in adult females (Hoffbrand *et al* ., 2001).

Anemia signifies a decreased amount of Hemoglobin in the blood and, therefore, decreased amount of oxygen reaching the tissues and organs of the bloody.

1.2.8 Classification of Anemia

1.2.8.1 Etiological classification :

The etiological classification approach focuses on the principle underlying pathologic mechanisms.

1. Relative anemia occur when there is a fluid shift from the extracellular to the intravascular compartment, expanding plasma volume and diluting the red cell mass (RCM), usually found in pregnancy, hyperproteinaemia and intravenous fluids.
2. Anemia associated with defective hemoglobin synthesis such as anemias associated with iron deficiency or iron utilization defective, anemia of and thalassemia .
3. Anemia associated with vitamin b12 or folate deficiencies that result from inhibition of DNA synthesis in red blood cell production .
4. Anemia associated with impaired bone marrow or stem cell production duo to bone marrow injury, bone marrow replacement abnormal cells ,inffective hemopoiesis , decrease marrow stimulation that result from reduce secretion of erythropoietin and paroxysmal nocturnal hemoglobinuria which caused by mutation in a single bone marrow precursor that results in an abnormal clone of bone marrow stem cell affecting erythrocytes ,leukocyte and platelets.
5. Anaemias associated with decreased red cell survival and increased red cell destruction such as hereditary spherocytosis, G6PD deficiency ,haemoglobinopathies, thalasseamias, hemolytic disease of new born , autoimmune haemolytic anemia, microangiopathic hemolytic anemia and hemolytic anemia associated with infections and toxic agents.
6. Anemia secondary to acute and blood loss (Stiene-Martin *et al.,*1998) .

1.2.8.2 The Morphological classification :

Anaemia can be established using red cell indices and direct examination of their morphology. The indices include :

Mean Corpuscular Volume (**MCV**) which indicates the average volume of asingle erythrocyte in a given blood sample ,it is expressed in SI units as femtoliters.

Mean corpuscular Hemoglobin (**MCH**) which indicates the mean weight of HB per erythrocyte, expressed in SI units as pictogram.

Mean Corpuscular Hemoglobin Concentration (**MCHC**) which indicates the average concentration of HB in the erythrocytes in a specimen .It is Expressed in SI units a g/dl. Formerly, MCHC was expressed in percent (Lewis, *et al,*2001).

Three main types of anemia are recognized on the basis of the red cell indices: Microcytic Hypochromic Anaemias : the MCV is subnormal , as is the MCH and MCHC,corresponding to microcytosis and hypochromia of the red cells in the blood film. This particular type of anomaly can be viewed as the result of a defect in red cell information in which hemoglobin synthesis is impaired to a greater extent than of other cellular components. The most important examples are iron deficiency, in which there is inadequate iron for formation of the heam component of hemoglobin, and thalassemia's, in which the formation of the globin component of hemoglobin is defective.

NormocyticNormochromicAnemia's: the MCV,MCH and MCHC are within the normal range, corresponding the normal size and haemoglobinization of red cells in the blood film. It can occur following loss of substantial volume of blood, or in haemolysis. It also occurs when red cell production is impaired by bone marrow failure , when bone marrow is replaced by infiltrating neoplastic tissue, and as result of the effects of renal failure and chronic inflammation or infection. Macrocytic Anaemia can be duo to a number of underlying disorder. The most important f which megaloblastosis of the bone marrow duo to vitamin B12 or folic acid deficiency. A significant elevation in the proportion of reticulocytes, which are larger than mature red cells , can also produce elevation of MCV (Firki*et al .,1996)*

Dimorphic when two causes of anemia act simultaneously, e.g., macrocytic
hypochromic, duo to hookworm infestation leading to deficiency of both iron and vitamin B12 or folic acid or following a blood transfusion more than abnormality of red cell indices may be seen (Hoffbrand *et al.,*2005).

1.2.8.3 The physiological classification :-

Based on the ability of the bone marrow to respond to anemia with increased erythropoiesis, it involves assessing erythrocyte production using the reticulocyte count and calculated RPI. When anaemia occurs, if the bone marrow is capable of responding increased numbers of young , nonnucleated red cells enter circulation .

These young poly chromatophilic red cells ,released prematurely from the bone marrow because of erythropoietin stimulation , are called shift reticulocyte , a term reflecting their premature shift from bone marrow to peripheral blood .Thus, reticulocyte may be significantly increased in circulation without n actual increase in marrow red cell production (Stiene Martin*et al,.*1998). In healthy patient, the reticulocyte percentage is essentially stable. When the Number of RBCs and hematocrit decreased, the percentage of reticulocytes may appear increased compared to the overall number of RBCS. In order to get a more accurate assessment of bone marrow function, the calculate reticulocyte percentage (%) is often corrected with a calculation called a corrected reticulocyte count or reticulocyte index (RI).This calculation compares the patient's hematocrit with a normal hematocrit value. An additional calculation called the reticulocyte production index (RPI) is calculated to correct for the degree of reticulocyte immaturity-reflecting how quickly the reticulocyte were released from the bone marrow ad how long it will take them to mature in the bloodstream.The RPI and maturation time vary with hematocrit as following :

1.0 day when the PCV is 0.45l/l,

1.5 days when the PCV is 0.35l/l ,

2.0 days when the PCV is 0.25l/l,

2.5 days when the PCV is 0.15l/l

(Chernechy and bergr , 2008).

An RPI higher than 3.0 indicates an effective bone marrow response, whereas an RPI lower than 2.0 suggests an infective bone marrow bone response . Each group can be subdivided into two categories. An ineffective response is associated with hypo proliferative anemia and anemia resulting from maturation disorders.Aneffective response is characteristic of hemolytic anemia and anemia’s secondary to blood loss (Stiene-Martin *et al.,1*998).

1.2.9 Signs and symptoms of Anemia:-

Anemia goes undetermined un many people , and symptoms can be minor or vague. The signs and symptoms can be related to the anemia itself , or the underlying cause. Most commonly, people with anemia report non- specific symptoms of a feeling of weakness, or fatigue , general malaise and sometimes poor concentration. They may also report dyspnea (shortness of breath) on exertion. In very severe anemia , the body may compensate for the lack oxygen-carrying capability of the blood by increasing cardiac output. The patient may have symptoms related to this , such as palpitations , angina (if preexisting heart disease is present), intermittent claudication of the legs, and symptoms of failure. On examination, the signs exhibited may include pallor (pale skin, mucosal lining and nail beds) but this is not a reliable sign. There may be signs of specific causes of anemia, e.g. Koilonychias (in iron deficiency), jaundice (when anemiaresults from abnormal break down of red blood cell in hemolytic anemia), bone deformities (found in thalassemia major) or leg ulcer. In severe anemia, there may be signs of a hyper dynamic circulation : a fast heart rate (tachycardia), bounding pulse , flow murmurs, and cardiac ventricular hypertrophy (enlargement).There may be signs of failure. Pica , The consumption of non – food based items such as dirt , paper , wax, grass, ice and hair, may be a symptoms of iron deficiency, although it occurs often in those who have normal levels of haemoglobin. chroinc anemia may result in behavioral disturbances in children asa direct result of impaired neurological development in infants, and reduced scholastic performance in children of school age, Restless is more common in those with iron-deficiency anemia. Less common symptoms may include swelling of the legs or arms, chronic heartburn, vague bruises, vomiting , increased sweating, and blood I stool (Bain and lewis*.* , 2001).

1.2.10 Diagnosis of anemia:-

Physical examination and medical history play a crucial role in diagnosis causes of anemia.

Some of the important features in medical history cover question about family history, previous personal history of anemia or other chronic condition,
medication, color of stool and urine, bleeding problems , and occupation and social habits (such as alcohol intake).While performing a complete physical examination, the physician may particularly focus on general appearance (Signs of fatigue, paleness), jaundice (yellow skin and eyes), paleness of the nil beds , enlarged spleen (splenomegaly) or liver (hepatomegaly), heart sounds , and lymph nodes. Lab tests for anemia may include the following:

- **Complete blood count (CBC):** Determines the severity and type of anemia (microcytic anemia, normocytic anemia, or macrocytic anemia) and is typically the first test ordered. Information about other blood cells (White cells and platelets) is also included in the CBC report.
- **Stool hemoglobin test:** Tests for blood in stool which may detect bleeding from the stomach or the intestines (stool occult blood test).
- **Peripheral blood smear:** Looks at the red blood cells under a microscope to determine the size, shape, number, and color as well as evaluate other cells in blood.
- **Iron level:** An iron level may tell the doctor whether anemia may be related to iron deficiency or not. This test is usually accompanied by their tests that measure the body's iron storage capacity, such as transferring level and ferritin level.
- **Transferrin level:** Evaluates a protein that carries iron around the body.
- **Ferritin:** Evaluates at the total iron available in the body.
- **Folate :** A vitamin needed to produce red blood cells, which is low in people with poor eating habits.

- **Vitamin B12 :** A vitamin needed to produce red blood cells , low in people withpoor eating habits or in pernicious anemia .
- **Bilirubin:** Useful to determine if the red blood cells are being destroyed with the body which may be a sign of hemolytic anemia.
- **Lead level:** lead toxicity used to be one of the more common causes of anemia in children.
- **Hemoglobin electrophoresis:** Sometimes used when a person has a family history of anemia,this test providesinformation on sickle cell anemia or thalassemia.
- **Reticulocyte count:** A measure of new red blood cells produced by the bone marrow.
- **Liver function tests:** A common test to determine how the liver is working, which may give a clue to other underlying disease causing anemia .
- **Kidney function test :** A test that is very routine and can help determine whether kidney dysfunction exits.
- **Bone marrow biopsy:** Evaluates production of red blood cells and may be done when a bone marrow problem is suspected (Fauci*et al.,* 2008).

1.2.10.1 Complete Blood count Values

The CBC provides information on the following :

- Erythrocyte , Or Red Blood Cell (RBC) ,Count.
- Measure Of Hemoglobin (Hgb).
- Hematocrit (Hct) (percentage).
- Mean Corpuscular Hemoglobin (MCH) Measurement.
- Mean corpuscular Hemoglobin Concentration (MCHC).
- Mean Corpuscular Hemoglobin Volume (MCV)
  Explanation of cell morphology.

1.2.10.2 Factor which effect on CBC values:

Several factors can affect CBC values, postnatal fluid shifts can alter the hemoglobin and hematocrit levels, and late clamping of the umbilical cord may result in an elevated hematocrit and transitory polycythemia.

Values can vary between sample sites. For example, capillary samples have approximately an 82 present correlation with venous samples and approximately a 77 percent correlation with arterial samples , with the capillary site having a higher hemoglobin concentration and hematocrit value duo to the slugging of RBCs in the low-flow capillaries and transudation of plasma, the sample site must be taken into consideration when the practitioner reviews the CBC because it can impact the intervention. For example, a capillary sample may reveal an elevated hemoglobin level and hematocrit percentage, an indicator of polycythemia. in this situation an arterial or venous sample would give a more accurate value.

1.2.10.3 Hemoglobin:

Hemoglobin, also spelled hemoglobin and abbreviated Hb or Hgb , is the iron-containing oxygen-transport, loprotein in the red blood cells of all vertebrates. As well as the tissues of some invertebrates. Hemoglobin in the blood carries oxygen from respiratory organs (lungs or gills) to the rest of the body (i.e the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of organism, and collects the resultant carbon dioxide to bring it back to respiratory organs to be dispensed from the organism, hemoglobin is main component of red cell it is concentration with the red cell is approximately 34 g/dl. It is a red pigment with molecular weight 64.5 kd .protein (globin's) synthesized and conjugated iron particles (heme) to form hemoglobin. Hemoglobin estimation is measurement of red dye which is main consistent of red cell and gives its color.(Maton*et al.* , 1993).

1.2.10.4 The RBCs count:

The RBCs count measures the number of circulating erythrocytes. A mature RBC is nucleated, biconcave disc, surrounded by a flexible membrane. Fetal (and neonatal) differ from adult RBCs in that they are lager in size, have a shorter life span, altered and deformability, and they contain a high fetal hemoglobin concentration.

1.2.10.5 Hematocrit :

Hematocrit is a measure of how much space red blood cells take up in your blood. A high hematocrit level might mean you're dehydrated. A low hematocrit level might mean you have anemia. Abnormal hematocrit levels also may be a sign of a blood or bone marrow disorder

1.2.10.6 Red blood cell indices:

There are other indices that can provide estimates of the average size of the of the erythrocytes and the average concentration and quantity of hemoglobin in the erythrocytes. These indices can be measured directly or calculated electronically using modern hematology analyzes. They can be useful in further classifying anemia according to the hemoglobin quantity in the RBCs or the size of the RBCs or in identifying the pathologic process causing the anemia. The erythrocyte indices include the MCV, The MCHC, AND THE MCH .The MCV measures the average size ofcirculating erythrocytes. It can help to quantify anemia as microcytic (small cells) or Macrocytic (large cells). An elevated MCV is seen with hyper viscosity/polycythemia and also in anemia caused by folate or vitamin B12 deficiency. The MCHC measures the hemoglobin concentration in a given volume of red blood cells. The RBCs can be described as normochromic ,hypochromic or hyperchromic,depending on their color, which is determined by the amount of hemoglobin present in the RBC.The MCH measures the average amount of hemoglobin per RBC in a sample of blood. (Hoff brand *et al*., 2005)

1.3 Rationale:

Thyroid hormones have a crucial role in metabolism and proliferation of blood cells. Thyroid dysfunction induces different effect on blood cells.

(Dorgalaleh , et, al 2013). Some studies about these effect was conduct worldwide to determine the complete blood count of patients with hyperthyroidism and hypothyroidism .The aim of this study is to correlate the complete blood count of study group with hyperthyroidism and hypothyrdism this may be give a good chance to follow up patients with hyperthyroidism and hypothyroidism to avoid serious hematological complication .

1.4 Objective:

1.4.1 General Objective:

To determine the blood cell count and red blood cell indices among Sudanese patient with thyroid disease.

1.4.2 Specific Objective:

- To verify if there any change in blood cell count (specifically Red cell indices).

- To determine the incidence of thyroid disease induced anemia.

CHAPTER TWO

Materials and Methods

Chapter Two

2.0 Materials and methods

2.1 Study design:

This is description cross-sectional study was conducted to study the effect of thyroid disease on blood cells counts and RBCs indices.

2.2 Study population:

70 Sudanese patients with thyroid disease (35 patients were hypothyroidism and 35 patients were hyperthyroidism , their ages ranged 20 – 60 years old .Apparently healthy subjects 30 were enrolled as controls group.

2.3 Study area/period:

The study was carried out in Military Hospital – Khartoum state- during period of January 2015 to April 2015 among Sudanese out-patients with thyroid disease.

2.4 Inclusion criteria:

-20 -60 years old

-Either male or female.

–untreated patients.

2.5 Exclusion criteria:-

Presence of other disease.

- Thyroxin treatment.

2.6 Ethical consideration:

This study was approved by the board of the faculty of medical laboratory science of Sudan university. All enrolled subject were informed about the objectives, procedures and benefit of the study and informed consent has been taken verbally.

2.7 Data Collection :

Demographic data were obtained by direct interview and clinical data were taken from patient medical record.

2.8.1 Material:

2.8.1.1 Equipment:

A. Plastic K3 EDTA vacationer tubes.

B. Holder with disposable needle .

C. 70% alcohol (ethanol).

D. Cotton.

E. Automated Hematological analyzer(Sysmex KX-N21)(Appendix 4).

Principle:

Measurement of blood cells (RBCs, WBCs, &plats) ,Hb concentration obtained by aspiration of small volume of well mixed k3EDTA blood . By sample probe and mix with isotonic diluents in neubulizer.diluted mixture aspiration delivered of RBCs aperture bath for providing information about RBCs and PLATS based on cell sizes, particles of 2-20 Fl counted as platelet, above 36FL counted as red cells. Some portion of aspired mixture induced into WBC bath in which hematological reagent (stomatolyzer) was added automatically to measure Hb concentration in build calorimeter, based on cyanomethemaglobin method (HiCN).Blood cell counted and size information generated in triplicate pukes according electronic conductivity, and translated into digital number using in build calculator programmed and designed for that RBCs , WBCs counts. Hence three values were directly measured (RBCs, WBcS ,Hb) and displayed on (LCD0.Other value of red cell indices , plts count, leukocyte differential, and absolute count calculated from given information and automated constructed histogram . the result printed out according to setting mood.

Standard operating Procedure For SysmexKx21N :

1\ Inspection before turning on power :

i\ inspection of reagent.

ii\ inspection of instrument.

iii\ inspection of waste.

iv\ inspection of printer paper.

2\Turning on the power and self-check.

3\ confirming the ready.

4\ Collecting and preparing sample.(Collecting in EDTA anticoagulant blood sample).If refrigerator sample leave at room temperature for 30 min until equilibrated.

5\Selecting Whole blood mood:

i\Confirm the ready status.

ii\press mode key to display the change mode.

iii\ select WB (whole blood).

Iv\ press (enter) key to change over the analysis mode and return to analysis mood. 6\ In put sample number:

i\press (sample No.) key.

ii \The cursor appear under sample NO input sample NO.

iii \press(ENTER) key.

7\ Analyzing sample :

i\ mix the sample sufficiently.

ii \ remove the plug while taking care not to allow blood scatter.

iii \ set the tube to the sample probe and in the condition, press the start switch.

8\ the buzzer sound two times and while LCD screen display (analyzing remove the tube.

9\ Automatic analysis and display the result on LCD screen and become rady for analysis for next sample.

2.8.1.2 Reagents :

Commercial close system reagents were provided by sysmex KX-N21 operator and consist of :

A. Cell pack (Stromatolyzer).
B. Cell Clean (Detergent).

2.8.2 Methods:

2.8.2.1 Collection of blood sample :

Venous blood was collected under aseptic condition after cleaning the area around the vein with 70% alcohol .3 ml of blood was collected in EDTA container and it was mixed well before processing.

2.9 Data analysis :

The collected data was analyzed to obtained the mean , standard deviation and the probability (P.value) between patients and controls using SPSS.

CHAPTER THREE

Results

Chapter three

3.0 The result

3.1 Distribution of age among study group:

The age of the study group ranged between 20 and 60 Years, with a mean of (42.5) years. Table (3.1) showed 20% of hypothyroidism patients with age ranged between 31-40 years.

Table (3.1) Distribution of test group according to age.

Age group years	Hypothyroidism		Hyperthyroidism		Total
	Frequency	Percent %	Frequency	Percent%	
20 – 30	5	7.1%	8	11.4%	13 (18.6%)
31 – 40	14	20%	5	7.1%	19 (27.1%)
41 - 50	9	12.9%	13	18.6%	22 (31.4%)
51 -60	7	10%	9	12.9%	16 (22.9%)
Total	35	50 %	35	50%	70 (100%)

3.2 Distribution of age among control group:

Table (3.2) showed that Age of control group ranged between 20-60 years, with mean (41.3) years.(33.3%) of control group were between the age 41- 50 years.

Table (3.2) Distribution of control group according to age .

Age **group years**	Frequency	Percent %
20 -30	**7**	**23.3%**
31 – 40	**6**	**20.0%**
41 – 50	**10**	**33.3%**
51 – 60	**7**	**23.3%**
Total	**30**	**100%**

3.3 Distribution of study group according gender.

Majority of these patients were females 58 (82%) and 12 (17.1) males .Table (3.2) Showed that 44% of patients with hyperthyroidism and 38.6% of patients with hypothyroidism were females .

Table(3.3) distribution of test group according gender.

Sex	Hyperthyroidism		Hypothyroidism		Total
	Frequency	Percent%	Frequency	Percent%	
Male	4	5.7%	8	11.4 %	12 (17.1%)
Femal e	31	44.3%	27	38.6%	58 (82.9%)
Total	35	50%	35	50%	70 (100%)

Table (3.4) showed Distribution of gender of control group.

Sex	Frequency	Percent
Male	5	16.7%
Female	25	83.3%
Total	30	100%

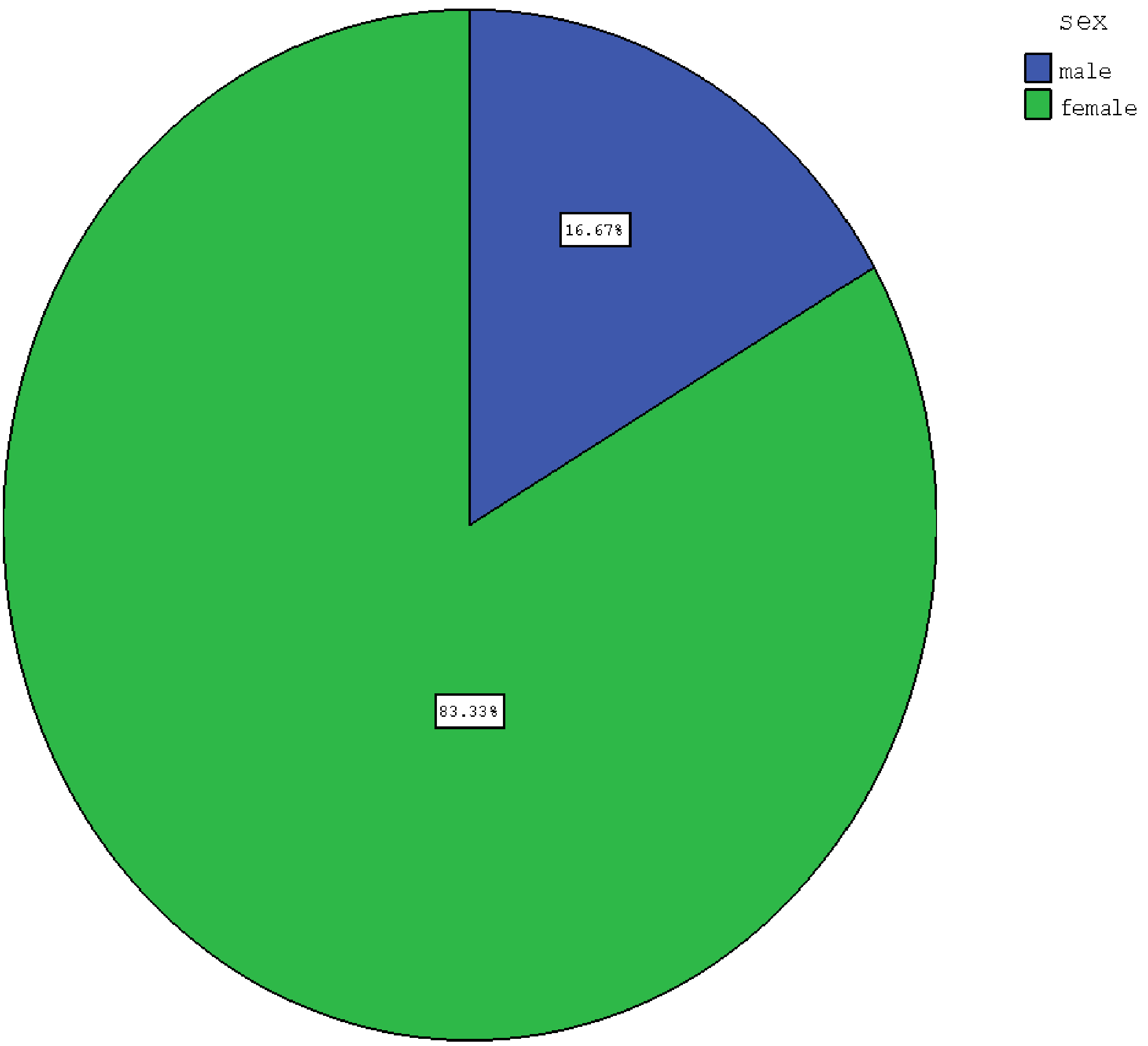

Fig (3.1) : Frequency of gender among the studied control group .

According to table (3.5) – (3.6) Hb, Hct MCH, of Hyperthyroidism and Hypothyroidism Significant decrease when compared to control group.RBC showed significant decrease in hypothyroids patients .MCV showed significant decrease in hyperthyroidism.MCHC show not significant decrease when compare with control group.

Table (3.5) Comparison of Hb , RBC and RBC related indices of Hyperthyroidism patients compared to control

Index	Group study	Mean ± SD	P.value
Hb g/dl	Hyperthyroidism Control	12.2±1.3 13.0±1.5	0.028
RBC ×10*6/µl	Hyperthyroidism Control	4.7±0.3 4.6±0.4	0.975
Hct %	Hyperthyroidism Control	39.4±3.4 42.5±5.3	0.006
MCV Fl	Hyperthyroidism Control	82.5±10.2 88.2±5.0	0.007
MCH pg	Hyperthyroidism Control	26.1±3.5 27.8±2.4	0.037
MCHC g/dl	Hyperthyroidism Control	31.2±1.5 31.1±1.7	0.758

Table (3.6) Comparison of Hb , RBC and RBC related indices of Hypothyroidism patients compared to control

Index	Group study	Mean ± SD	P.value
Hb g/dl	Hypothyroidism Control	11.3±1.8 13.0±1.5	0.000
RBC ×10*6/µl	Hypothyroidism Control	4.4±0.5 4.6±0.4	0.020
Hct %	Hypothyroidism Control	37.4±4.7 42.5±5.3	0.000
MCV Fl	Hypothyroidism Control	84.8±9.7 88.2±5.0	0.090
MCH pg	Hypothyroidism Control	25.4±4.1 27.8±2.4	0.008
MCHC g/dl	Hypothyroidism Control	30.1±2.5 31.1±1.7	0.064

According to table (3.7) there was no significant difference were noted for WBC count and platelets count in both group

Table (3.7)Comparison of WBC count and PLT count of patients compared to control.

Index		Mean ± SD	P. value
WBC×10*3/µL	Hyperthyroidism	5.7±1.1	0.457
	Control	6.1±2.4	
	Hypothyroidism	5.9±1.7	0.767
platlete×10*3/µl	Hyperthyroidism	289.8±74.1	0.485
	Control	303±76.2	
	Hypothyroidism	307.7±93.1	0.824

CHAPTER FOUR

Discussion

Conclusion

Recommendation

Chapter four

4.0 Discussion

This study was carried out to determine complete blood count (CBC) of patients with thyroid disease (Hypothyroidism and hyperthyroidism in Khartoum state during period from January 2015 April 2015. Age of study population Ranged between 20- 60 years old with mean of 42.5 years .Both sexes were included in the study group, Female were (n=58) or 82.9% and male were (n=12) or 17.1% from study group .While control groups male were account were (n=5) or 16.7% and female (n= 25) or 83.3%.The study show the majority of patients were female (82%)& 20% of hypothyrodisam patients with age ranged between 31-40 years. The result showed that for patient comparison with control that there was highly significant decrease in mean of Hb (12.2±1.3)p.value (0.02),(11.3±1.8)p. value (0.000) of Hyperthyroidism &hypothyroidism Respectively, Mean of PCV Hyperthyroidism patients(39.4±3.4) p. value (0.006) , Hypothyroidism mean PCV(37.4±4.7) p.vaule (0.000) this result agrees with the study done by (Dorgalaleh,et al 2013) . Mean of RBCs ×10^6/µl of Hypothyrodisam patients (4.4±0.5) p.value (0.02).Mean of MCH (26.1±3.5) p.value (0.03), (25.4±4.1) p.value (0.008) of Hyperthyrodisam&Hypothyrodisam patients. MCV showed significant decrease in Hyperthyroidam Mean (82.5±10.2) p. value (0.007). In contrast there is no difference in MCHC , WBCs and plts between study group and control group which disagreed with result obtained by Kawa MP IN 2010 reported MCHC is lower in both group when comparison with control group and MCV is increase in both group Hyperthyroidism and hypothyroidism.

According to obtained data we suggested that all patients in both group should be periodically evaluated for probably hematological change & Thyroid dysfunction have direct effect on most red blood cell indices that agree with study done by Dogalaleh which found thyroid hormone has effect on blood cell count and red blood cell indices which can cause different type of anemia.

4.1 .Conclusion:

The study concludethat :

- The majority of patients suffer from thyroid disease(Hyperthyroidism &Hypothyrodism) were female 58 (82.9%) and male 12(17.1%) . The peak age between 41- 50 years .
- Majority of patients were anaima(lowHb , PCV , RBCs) were decrease in compare to control groups.
- MCV and MCH were effected MCHC are not effected with thyroid disease.
- WBCS and Plts were not unaffected thyroid disease .

4.2 Recommendation ;

- In order to get more in formation data ,sample size should be increase in realted subsequent researches .
- Regular measurement for RBcs indices in patient with hypothyroidism and hyperthyroidism to avoid severe anemia.
- Also recommend to more detailed study on patient with thyroid disease measure serum iron profile ,Vitamin B12 , folic acid , antipartial cell antibody , bone marrow study , and stool for occult blood , coomb's test , and complete hemogram with reticulocyte count .

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APPENDIX

Appendix (1)

Questionnaire

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Faculty of Graduate Studies

Hematology Department

Questionnaire

Date: / /2015

Patient No: …………………….

Name: ……………………………………………………………………….

Telephone No: …………………………………………

Gender: ..... Male ….. Female

Age: ……………………..

Clinical Information:

General Health State: ……………………...

Beginning of disease: …………………………………………………….

Family History:………………………………………………………………….

Other Disease: …………………………………………………………

Type of Drug(s): ………………………..

Appendix (2)

بسم الله الرحمن الرحيم

اقرار

التاريخ : ....

.

أقر أنا .... وأنا بكامل قواي العقليه أن أكون جزء من هذا البحث وذلك بعد أن تم شرح وتوضيح كل الجوانب المتعلقه بهذا البحث.

.

التوقيع :...

Appendix (3)

Reference range of hematological Values

Red Blood Cell Count	
Men	5.0±0.5 ×10^12/ L
Women	4.3 ± 0.5 ×10^ 12 / L
Hemoglobin	
Men	150 ± 20 g/l
Women	135 ± 15 g/l
Hematocrit (Hct) Or PCV	
Men	0.45 ± 0.05 (L/L)
Women	0.41 ± 0.05 (L/L)
Mean Cell Volume (MCV)	
Men and Women	92±9 fL
Mean Cell Hemoglobin (MCH)	
Men and Women	29.5 ± 2.5 pg
Mean Cell Hemoglobin Concentration (MCHC)	

Reference range of hematological Values

White blood cell count
Men and women (3,500 to 10,500 cells/mcL)
Platelet count
Men and women (150,000 to 450,000/mcL)

Appendix (4)

Reference range of Thyroid function test

Test	Abbreviation	Normal Range
Serum Thyroid-Stimulating Hormone	TSH	0.3-3.0µU/ml
Serum Thyroxin	T4	4.6-12ug/dl
Free thyroxin	FT4	0.7-1.9 ng/dl
Radio Active Iodine UpTake	RAIU	10-30%
Serum Tiriodothryonine	T3	80-180ng/dl
Free Triiodothryonine	FT3	230-619 pg/dl
Free Thyroxine Fraction	FT4F	0.03-0.005%

Appendix (6)

Figure of Sysmex KX21N.