

**Sudan University of Science and Technology**

**College of Graduate Studies**

**TSH and T3 Levels among Sudanese  
Children With Sickle cell Anemia in  
Heglig City**

**مستوى الهرمون المنبه للغدة الدرقية وثالث يود الثيرونين عند الأطفال  
المرضى بالأنيميا المنجلية فى مدينة هجليج**

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## ﴿ بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ ﴾

قال تعالى :

لَا يُكَلِّفُ اللّٰهُ نَفْسًا وِجْرًا إِلَّا وُسْعَهَا ۗ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا  
تُؤَاخِذْنَا إِن نَّسِينَا أَوْ أَخْطَأْنَا ۗ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِكْرًا كَمَا حَمَلْتَهُ عَلَى  
الَّذِينَ مِنْ قَبْلِنَا ۗ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۗ وَاعْفُ عَنَّا وَارْحَمْنَا  
وَأَرْحَمْنَاهُ ۗ أَنْتَ مَوْلَانَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ

صدق الله العظيم

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# **DeDication**

**To my parents.....**

**To my sister.....**

**To my brothers.....**

**To my daughter.....**

**To my friends.....**

**To my colleagues.....**

**With my best wishes to all.....**

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## Abstract

Endocrine dysfunction in hematological disease like SCD can be encountered commonly and at

early ages, ischemia and inflammatory mediators are proposed reasons for endocrine dysfunction, the etiology of thyroid dysfunction in SCD is not clear.

This is case control study conducted during the period from April to December 2016 in Heglig cityHeglig hospital to determine the levels of TSH and T3 among Sudanese children with sickle cell anemia, the levels of TSH and T3 was estimated by using mind ray instrument.

The study includes 80 subjects, 43 of them were sickle cell anemia children, 7 of them were sickle cell trait, and 30 of them were control group.

Statistical analysis was done by using SPSS ,and the results revealed a significant decreased in means of TSH level in sickle cell anemia children  $(0.87 \pm 0.79)$  when compared to trait group  $(4.0 \pm 3.8)$  with  $(p. \text{value} = 0.000)$ . The results also revealed ,The mean of TSH level was insignificant difference in sickle cell anemia children  $(0.87 \pm 0.79)$  when compared to control group  $(0.81 \pm 0.66)$  with  $(p. \text{value} = 0.6)$ . The study results also revealed a significant increased of TSH level in sickle cell trait  $(4.0 \pm 3.8)$  when it compared to control group  $(0.81 \pm 0.66)$  with  $(p. \text{value} = 0.000)$ . The study results also revealed insignificant difference in mean of T3 level in sickle cell anemia children  $(0.64 \pm 0.2)$  when compared to trait group  $(0.8 \pm 0.3)$  with  $(p. \text{value} = 0.1)$ . The study results also revealed insignificant difference in mean of T3 level in sickle cell anemia children  $(0.64 \pm 0.2)$  when it compared to control group  $(0.66 \pm 0.24)$  with  $(p. \text{value} = 0.7)$ . and The results revealed insignificant difference in mean of T3 in sickle cell trait,  $(0.8 \pm 0.3)$  when it compared to control group  $(0.66 \pm 0.24)$  with  $(p. \text{value} = 0.1)$ . The study results also revealed, in significant difference in mean of TSH Level in sickle cell anemia children male  $(0.87 \pm 0.7)$  in compared to female children group  $(0.82 \pm 0.84)$  with  $(p. \text{value} = 0.8)$ , the results revealed insignificant difference in mean of T3 level in sickle cell anemia male  $(0.59 \pm 0.2)$  when it compared to female group  $(0.82 \pm 0.89)$  with  $(p. \text{value} = 0.3)$ . The study results revealed insignificant positive correlation  $(r = 0.038, p. \text{value} = 0.8)$  between duration of sickle cell anemia and TSH among Test group.

The study results also revealed insignificant negative correlation ( $r=-0.17$ , p.value.0.2) between duration of sickle cell anemia and T3 among test group.

The study observed sickle cell anemia had no affect on the levels of serum TSH and T3, also there were no affect on the levels of TSH and T3 according to gender, also duration of sickle cell anemia increase the level of TSH and decrease level of T3.

## المستخلص

ديسفونشن الغدد الصماء في أمراض الدم مثل مرض الأنيميا المنجلية يمكن أن تصادف عادةً وفي سن مبكرة ، الأوكسيميا ووسطاء الإلتهابات هي الأسباب المقترحة للغدد الصماءديسفونشن مسبباتديسفونشن الغدة الدرقية في مرض الأنيميا المنجلية ليست واضحة.

تمت الدراسة في الفترة من شهر أبريل إلى شهر ديسمبر 2016م في مدينة هجليج مستشفى هجليج لتحديد مستوى الهرمون المنبه للغدة الدرقية وثالث يود الثيرونين في اطفال الأنيميا المنجلية بالسودان، مستوى الهرمون المنبه للغدة الدرقية وثالث يودالثيرونين تم تقديره بواسطة جهاز مندرى شملت الدراسة على 80 طفل، 43 منهم مصابين بداء الأنيميا المنجلية و 7 منهم بداء الأنيمياالحميدة و 30 يبدون أصحاء كمجموعة ضابطة تم عمل تحليل إحصائي بواسطة برنامج التحليل الاحصائي وقد أوضحت النتائج أن هنالك نقصان ذي دلالة إحصائية في متوسط الهرمون المنبه للغدة الدرقية في أطفال الأنيميا المنجلية مقارنة مع المجموعة الحميدة القيمة المعنوية المطلقة 0.000 والمتوسط الحسابي  $\pm$  الانحراف المعياري (0.79 $\pm$ 0.87) ( 3.8  $\pm$  4.0 ) وان هنالك فرقليس ذي دلالة إحصائية في متوسط الهرمون المنبه للغدة الدرقية في أطفال الأنيميا المنجلية مقارنة مع المجموعة الضابطة القيمة المعنوية المطلقة 0.6 والمتوسط الحسابي  $\pm$  الانحراف المعياري (0.79  $\pm$  0.87) (0.66 $\pm$ 0.81).

كذلك أوضحت الدراسة أن هنالك زيادة ذات دلالة إحصائية في متوسط الهرمون المنبه للغدة الدرقية في أطفال الأنيميا الحميدة مقارنة مع المجموعة الضابطة القيمة المعنوية المطلقة 0.000 والمتوسط الحسابي  $\pm$  الانحراف المعياري (3.8  $\pm$  0.4) (0.66  $\pm$  0.81). وكذلك أوضحت الدراسة أن هناك فرق ليس ذي دلالة إحصائية في متوسط ثالث يود الثيرونين في أطفال الأنيميا المنجلية مقارنة مع المجموعة الحميدة القيمة المعنوية المطلقة 0.1 والمتوسط الحسابي  $\pm$  الانحراف المعياري (0.64  $\pm$  0.64).

0.2) (0.3± 0.8) وان هنالك فرق ليس ذي دلالة إحصائية في متوسط ثالث يود الثيرونين في أطفال الأنيميا المنجلية مقارنة مع المجموعة الضابطة القيمة المعنوية المطلقة 0.7 المتوسط الحسابي ± الانحراف المعياري (0.2 ± 0.64) (0.24± 0.66) كذلك كشفت النتائج أن هناك فرق ليس ذي دلالة إحصائية في متوسط ثالث يود الثيرونين في أطفال الأنيميا الحميدة مقارنة مع المجموعة الضابطة القيمة المعنوية المطلقة 0.1 والمتوسط الحسابي ± الانحراف المعياري (0.8 ± 0.24) (0.3) (0.24 ± 0.66).

كذلك كشفت النتائج ان هنالك فرق ليس ذي دلالة إحصائية في متوسط الهرمون المنبه للغدة الدرقية في مجموعة الأطفال الذكور مقارنة مع مجموعة الأطفال الإناث المصابين بالأنيميا المنجلية القيمة المعنوية المطلقة 0.8 والمتوسط الحسابي ± الانحراف المعياري (0.7 ± 0.87) (0.82 ± 0.84).

كذلك أوضحت النتائج أن هنالك فرق ليس ذي دلالة إحصائية في متوسط هرمون ثالث يود الثيرونين في مجموعة الأطفال الذكور مقارنة مع الإطفال الإناث المصابين بالأنيميا المنجلية القيمة المعنوية المطلقة 0.3 والمتوسط الحسابي ± الانحراف المعياري (0.2±0.59) (0.89±0.82). كشفت النتائج أيضاً أن هنالك علاقة إيجابية ليست ذات دلالة إحصائية بين الهرمون المنبه للغدة الدرقية ومدة المرض القيمة المعنوية المطلقة 0.8 الإيجابية 0.038 كشفت النتائج أيضاً أن هناك علاقة سلبية ليست ذات دلالة إحصائية بين ثالث يود الثيرونين ومدة المرض القيمة المعنوية المطلقة 0.2 معامل بيرسون للإرتباط 0.17.

أظهرت الدراسة أن مرض الأنيميا المنجلية ليس له تأثير على معدل الهرمون المنبه للغدة الدرقية وثالث يود الثيرونين وكذلك ليس له تأثير على معدل الهرمون المنبه للغدة الدرقية وثالث يود الثيرونين على حسب العمر وأن زيادة مدة المرض تزيد من معدل الهرمون المنبه للغدة الدرقية وأن زيادة مدة المرض تؤدي إلي نقصان معدل هرمون ثالث يود الثيرونين.



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# **Chapter One**

**Introduction and literature review**

# **1. Introduction and literature review**

## **1.1 Introduction**

Sickle cell disease (SCD) is a hemolytic anemia, characterized by abnormal hemoglobin production of autosomal recessive inheritance. SCD may lead to many organ dysfunction due to intermittent small vascular obstructions (Ress et al., 2010).

While studies related to malnutrition, growth retardation and pubertal development retardation were more frequently reported in pediatric patients with SCD (Smiley et al., 2008).

Studies in gonadal insufficiency, thyroidal disorders and bone metabolism were conducted with adult hood and endocrine organ dysfunctions more frequently reported in SCD patients (Ress et al., 2010).

On other hand, as observed in other chronic disease, malnutrition, which may negatively affect growth and development of childhood, is commonly encountered in SCD (AL-Saqladi et al., 2008).

Sickle cell disease may lead to various acute and chronic complications several of which have a high mortality rate (yawn et al., 2014).

Endocrine dysfunction has been reported as the most common and earliest toxic effect seen in iron overload subjects (Chatterjee and Katz, 2000).

One of the most endocrine disorders in patients with sickle cell disease hypothyroidism, the reports of thyroid assessment in patient with (SCD) have been consistent.

Abnormal thyroid function studies have been reported in patient with (SCD) (Phillips et al., 1992).

Abnormal thyroid function studies have been reported in patients with SCD were suggestive primary thyroidal failure (parshad et al., 1989).

Endocrine and metabolic disorders reported in patients with SCD include hypogonadism diabetes, pubertal development, carbohydrate intolerance and primary hypothyroidism.

## **1.2. Literature reviews**

### **1.2.1. Sickle cell anemia**

Sickle cell anemia runs an extremely variable clinical course, at one end of the spectrum it is characterized by crippling hemolytic anemia.

In some studies children have tended to be short for their age while post adolescent are usually tall, in equalities between upper and lower segment. As stressed in early literature is unusual. The only other physical sign that is frequently present in chronic leg ulceration (Bunn, 1997).

(SCD) may lead to acute and chronic tissue ischemia and many organ dysfunctions due to intermittent small vascular obstruction (Rees et al., 2010).

While studies related to malnutrition, growth retardation and pubertal development retardation were more frequently reported in pediatric patients with (SCD) (Smiley et al., 2008).

Studies in gonad insufficiency, thyroidal disorder, and bone metabolism were conducted with adult hood and endocrine organ dysfunction more frequently reported in (SCD) patient, especially in studies performed at adult hood (Rees et al., 2008).

The physiopathology of metabolic and endocrine disorders in these patients is not clear yet, Investigators propose that endocrine organ dysfunctions in (SCD) patients may be caused by iron overload due to recurrent blood transfusion or disruptions of tissue vitalization during vaso-occlusive crisis and inflammatory mediators (Rees et al., 2010).

#### **1.2.1.1. Sickle cell crisis**

The term sickle cell crisis may be used to describe several independent acute conditions occurring in patients with (SCD) result in anemia and crisis could be several types including the vaso-occlusive crisis, a aplastic crisis, sequestration crisis, haemolytic crisis and others (Best et al., 2010).

Although infection, dehydration, and acidosis can act as triggers, in most instances, no predisposing cause is identified (Kumar et al., 2009).

#### **A aplastic crisis**

Producing pale appearance, fast heart rate, and fatigue. This crisis is normally triggered by parvovirus B19 which directly affects production of red blood cell precursors and multiplying in a destroying them (Kumar et al., 2009).

## **Hemolytic crisis**

hemolytic crisis are acute accelerated drops in hemoglobin level .the red blood cells break down at a fasterrate. This particularly common in patients with coexistentG6PD (Balgir, 2012).

## **vaso-occlusive crisis**

The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischemia,pain, necrosis and often organ damage. Painful crisis and treated with hydration,analgesics,and blood transfusion (Olujohungbe and Burnett,2013).

Vaso-occlusive crisis involving organs such as hepaticor lungs, are considered an emergency and treated with red blood transfusion, incentive spirometry, atechnique to encourage deep breathing to minimize the development of atelectasis is recommended (Glassberg, 2011).

## **Spleen sequestration crisis**

Because of its narrow vessels and function in clearing defective red blood cells the spleen is frequently affectedit is usually infractedbefore the end of childhood in individuals suffering from sickle cell anemia. The spleen damage increases the risk of infection from encapsulated organisms (Pearson,1977).

## **Other types of sickle cell crisis**

One of the earliest clinical manifestations is dactylitis, presenting as early as six months of age, and may occur in children with sickle cell trait(Jadavjiandprober, 1985).

Another recognizedtype of sickle cell crisis, acute chest syndrome. Given the pneumonia and sick ling in the lung can those produce these symptoms; the patient is treated for both conditions (Miller,2011).

### **1.2.1.2. Patho physiology of sickle cell anemia**

The anemia from which sickle cell(SS) anemia derives its name is broadly categorized as an uncompensated hemolytic anemia ,in which a markedly shortened over all red cell(RBC)survival (increased rate of RBC destruction )is insufficiently balanced by increase in production (erythropoiesis)to maintain normal levels of total RBCS and hemoglobin (Hb) concentrations .Although reduced RBC survival is usually considered primary in SS ,both the intrinsic features of Hb Sand its secondary effects result in sub maximal erythropoietic response ,thereby contributing to the anemia (Robert,1996).



### **1.2.1.3. Complications of sickle cell anemia**

(SCD) can lead to various complications, including:

Increased risk of severe bacterial infections due to loss of functioning spleen tissue these infections are typically caused by encapsulated organisms such as streptococcus pneumonia (Kavanag et al., 2011).

#### **Silent stroke**

Causes no immediate symptoms, but is associated with damage to the brain, about 10-15 children with (SCD) suffer strokes, with silent stroke predominating in younger patients (Adams et al., 2001).

#### **Cholestithiasis**

(Gall stones) and Chloecystitis may result from excessive bilirubin production and precipitation due to prolonged haemolyses.

**A vascular necrosis** (aseptic bone necrosis) of hip and other joints may occur as result of ischemia (Marti-carvajal et al., 2004).

**Pianism** and infarction of penis (Chrouser et al., 2011).

**Osteomyetitis** (bacterial bone infection), the most common cause of osteomyetitis in (SCD) is salmonella (ALmeida and Roberts, 2005).

In eyes, background retinopathy, proliferative retinopathy, and retinal detachment can result in blindness (Elagouz et al., 2010).

#### **Chronic pain**

Even in the absence of acute vaso-occlusive pain, many patients have UN reported chronic pain (Smith et al., 2008).

#### **Pulmonary hypertension**

21% of children and 30% of adults have evidence of pulmonary hypertension when tested. This is associated with reduced walking distance and increased mortality (Caughey et al., 2015).

### **1.2.1.4 Sickle cell trait**

Sickle cell trait (sickle cell anemia) describes condition in which a person who has two copies of the allele (homozygous), those who are heterozygous for the sickle cell allele produce both normal and abnormal hemoglobin the two alleles are co dominant with respect to actual concentration of hemoglobin in circulating cells.

Sickle cell trait is a hemoglobin genotype AS and is generally regarded as benign condition (Roach, 2005).

However, individuals with sickle cell trait may have rare complications, suggested, probable complications include hyphema, fetal loss, neonatal death, acute chest syndrome and anemia in pregnancy (Tsaras et al., 2009).

## **1.2.2. Thyroid gland**

The thyroid is one of the largest endocrine glands in the body, and consists of two connected lobes, it is found in the anterior neck, below the larynx prominence (Adam's apple), the thyroid gland controls the body's sensitivity to other hormones regulate the growth and rate of function of many other systems in the body  $T_3$  and  $T_4$  are synthesized from iodine and tyrosine, the thyroid also produce calcitonin, which play role in calcium homeostasis.

Hormonal output from the thyroid is regulated by (TSH) produced by anterior pituitary which itself is regulated by (TRH) produced by hypothalamus (Baron and Boulapep, 2012).

The thyroid may be affected by some frequent thyroid diseases ,hyperthyroidism occurs when the gland produce excessive amounts of thyroid hormones ,the most common cause being graves'disease an auto immune disorder. Incontrast, hypothyroidism is state of insufficient thyroid hormones production .worldwide, the most common cause is iodine deficiency, thyroid hormones are important for development, and hypothyroidism secondary to iodine deficiency remains the leading cause of preventable intellectual disability (Longo et al.,2012).

The primary function of thyroid is production of hormones  $T_3$ ,  $T_4$  and calcitonin.up to 80% of the  $T_4$  is converted to  $T_3$  by organs such as liver, kidney,spleen. $T_3$  is several times more powerful than  $T_4$ ,which is largely a prohormone perhaps four or even ten times more active (Stephennussey &Saffron,2001).

### **1.2.2.1.Thyroid hormones**

#### **Actions of thyroid hormones**

Thyroid hormones are essential for normal growth and development and have many effects on metabolic processes thus stimulating the synthesis of poly peptides including hormones and enzymes, the effect of thyroid hormones is to increase net catabolism; weight loss and muscle wasting are typical features of excessive secretion of thyroid hormones, thyroid hormone also increase the sensitivity of cardio vascular and nervous systems to catecholamine's ,the former leading to increases in heart rate and cardiac output, and latter to increased arousal (Marshall,2012).

#### **Thyroid hormones in the blood ( $T_3$ , $T_4$ )**

The normal plasma concentration of  $T_3$  is 1.0-2.9nmole/l the hormone is protein bound some 99.66% is bound principally to specific thyroxin -binding globulin TBG is approximately one-third saturated at normal concentrations of thyroid hormone, it is generally accepted that only the free non protein-bound thyroid hormone are physiologically active .although the total  $T_4$  concentration is normally 50 times that of  $T_3$  ,the difference extends to which these hormones are bound to protein mean that the free  $T_4$  concentration is only 2-3 times that of free  $T_3$  reference range is 9.0 pmol/l for free  $T_3$  .

The precise physiological function of TBG is unknown. Individuals who have genetically determined deficiency of the protein show no clinical abnormality.

Total (free bound) thyroid hormone concentrations in plasma are dependent not only on thyroid function but also on the concentrations of binding protein. Binding would stimulate TSH release and this would restore the free hormone concentration to normal, if binding protein concentration were to fall, the reverse would occur, in either situation, there would be a change in the concentration of total hormone, but the free hormone concentrations would remain normal (Marshall, 2012).

### **Thyroid hormone regulation (T3, T4)**

The production of thyroxin and triiodotyrosine is primarily regulated by (TSH) released by the anterior pituitary, the thyrotropes in the anterior pituitary, from a negative feedback loop.

TSH production is suppressed when the free T4 levels are high the negative feedback occurs on both the hypothalamus and the pituitary, but it is of particular importance at the level of the pituitary (Johannes et al., 2002).

The TSH production itself is modulated by (TRH), which is produced by the hypothalamus. As the release of (TSH) from pituitary is controlled through negative feedback by thyroid hormones, measurement of (TSH) can be used as an index of thyroid function (Marshall, 2012).

If primary thyroid disease is suspected and plasma (TSH) concentration is normal, it can be safely inferred that patient is euthyroid, in overt primary hypothyroidism (TSH) concentrations are greatly increased, often to ten or more times the upper limit of normal. Smaller increases are seen in borderline cases, but (TSH) measurement is more sensitive than T4 under these circumstances.

(TSH) concentrations are suppressed to very low values in hyperthyroidism, but low concentrations can also occur in individuals with subclinical hyperthyroidism and euthyroid patients with non-thyroid illness, while slightly elevated concentration is frequently due to recovery from illness or incipient hypothyroidism (Marshall, 2012).

### **1.2.2.2. Clinical significance of thyroid disorder**

#### **Hyperthyroidism**

is defined as an overproduction of the thyroid hormones T3 and T4. This condition is most commonly caused by the development of Graves' disease, an autoimmune disease in which autoantibodies stimulate the thyroid to secrete excessive quantities of thyroid hormones (Siegenthaler, 2007).

## **Hypothyroidism**

An under active thyroid gland results in hypothyroidism ,typical symptoms are abnormal weight gain, tiredness,constipation,heavy menstrual bleeding, baldness, cold intolerance and slow heart rate, hypothyroid disorders may occur as result of autoimmune disease such as Hashimotos thyroiditis, iodine deficiency as result of medical treatments such as surgical removal or radio ablation of the thyroid (Davidson's ,2010)

## **Thyroiditis**

Thyroiditis is a group of inflammatory thyroid disorders .patients with chronic lymphocytic thyroiditis (also referred to as Hashimotos thyroiditis )present with hypothyroidism,goiter,or both .measurement of serum thyroid autoantibodies and thyroglobulin confirms the diagnosis .sub acute granulomatous thyroiditis (sometimes referred to as de Quervains disease) is self limited but painful disorder of the thyroid. Physical examination, elevated erythrocyte sedimentation rate, elevated thyroglobulin level and depressed radioactive iodine uptake (RAIU) confirm the diagnosis .sub acute lymphocytic thyroiditis(silentthyroiditis) is considered autoimmune in origin and commonly occurs in the postpartum period. Symptoms of hyperthyroidism and depressed. Acute (suppurative) thyroiditis is a rare, infectiousthyroid disorder caused by bacteria and other microbes .The rare, invasive fibrous thyroiditis (Riedels thyroiditis)presents with slowly enlarging anterior neck mass that is sometimes confused with malignancy (Slatosky et al., 2000).

### **1.3. Rationale**

Fifty percent of patients with sickle cell anemia survived beyond the fifth decade (Orah,1994).

A number of researches indicated that sickle cell disease has numbersimmediate health effects on thyroid,brain,chest,heart (Chatterjee etal., 2000).

Only a single publish study was conducted in anemicSudanese to evaluate biochemical changes in westernSudan patients (Khalid etal., 2012).

but no study were found in evaluation of Sudanesechildren with sickle cell anemiathat's why we attempted to evaluate the levels of TSH,T3 in Sudanesechildren with sickle cell anemia.

## **1.4. Objectives**

### **General objective**

To study the levels of TSH and T3 in Sudanese children with sickle cell anemia.

### **Specific objectives**

1. To estimate and compare between serum TSH and T3 levels in sickle cell anemia children, trait and control.
2. To correlate between levels of TSH and T3 in sickle cell anemia, gender, and duration of disease.

# **Chapter Two**

## **Material and Methods**

## **2. Material and Methods**

### **2.1. Materials**

**2.1.1. Study design:** This is a case control study.

**2.1.2. Study area and duration:** The study was conducted in Heglig hospital in western Kordofan state from April to December 2016.

**2.1.3. Study population:** 43 children with sickle cell anemia, 7 of them were trait and 30 apparently healthy children to serve as control group were enrolled in this study, and age was matched between two groups of test and control from (1-18) years.

**Inclusion criteria:** Sickle cell anemia children were included and age was matched (1-13) years.

**Exclusion criteria:** sickle cell anemia children with goiter, thyroid disease, and certain medication (glucocorticoids, dopamine, somatostatin) that may influence the levels of TSH and T3.

**2.1.4. Samples:** About 5ml of venous blood were collected from each patient at random state, in plain containers for TSH and T3. The samples were collected under aseptic conditions, after clotting centrifuged for 3 minutes at 3000 RPM to obtain serum, and analyzed.

**2.1.5. Ethical consideration:** patients who voluntarily accepted to participate in the study were included, and data collected by questionnaire (Appendix I).

**2.1.6. Data analysis:** Data was analyzed by using the SPSS computer program. One-way ANOVA was used for analysis and comparison between means of serum TSH and T3 in case, trait and control group, and also T-test was used for analysis and comparison between mean of serum TSH, T3 in sickle cell anemia children according to gender.

**2.1.7. Quality control:** pathological and normal control sera were used to insure the reliability of results in the patches.



## **2.2. Methodology**

### **2.2.1. Estimation of Total T3 level using Elisa technique (Appendix II)**

#### **Principle Elisa method**

Competitive Enzyme immunoassay, the essential reagents required for a solid phase enzyme immunoassay include immobilized anti body, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme antigen conjugate and serum containing the native antigen, a competition reaction results between the native antigen and the enzyme antigen conjugate for a limited number of insolubilized binding sites. After equilibrium is obtained, the antibody-bound fraction is separated from UN bound antigen by decantation or aspiration, the enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration.

### **2.2.2. Estimation of TSH level using immune enzyme metric assays (Appendix III)**

#### **Principle immune enzyme metric assays**

The essential reagents required for an immune enzyme metric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized) with different and distinct epitope recognition, in excess and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a micro plate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody, upon mixing monoclonal biotinylated antibody the enzyme labeled antibody and a serum containing the native antigen, reaction result between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

Point on the curve, and read the concentration in  $\mu\text{g/ml}$ , from horizontal axis of the graph.

Each laboratory should assay controls at levels in the low, normal, and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed.

# **Chapter Three**

## **Results**

### 3.Results

80 samples were collected (43 from sickle cell anemia children, 7 from sickle cell trait and 30 from control) to evaluate the level of TSH and T3 analysis was done by ELISA method (mindry instrument).

SPSS computer program was used for data analysis and the results were as follow:

**Table 3.1** Shows comparison between means of level of serum TSH in case, trait and control groups.

Case versus trait, significant decrease (mean±SD:0.87±0.79, 4.0±3.8 p.value0.000), case versus control, insignificant difference (mean±SD:0.87±0.79, 0.81±0.66 p.value 0.6), trait versus control, significant increase (mean±SD:4.0±3.8, 0.81±0.66 p.value0.000).

**Table 3.2** shows comparison between means of level of T3 in case, trait and control groups.

Case versus trait, insignificant difference (mean±SD:0.64±0.2, 0.8±0.3 p.value 0.1) case versus control, insignificant difference (mean±SD:0.64±0.2, 0.66±0.24 p.value 0.7) trait versus control, insignificant difference (mean±SD:0.8±0.3, 0.66±0.24 p.value0.1).

**Table 3.3** shows comparison between means of levels of TSH and T3 in sickle cell anemia children according to gender.

TSH insignificant difference (mean±SD:0.87±0.7, 0.85±0.84 p.value0.8) T3 insignificant difference (mean±SD:0.59±0.2, 0.66±0.2 p.value 0.3)

**Figure 3.1a** scatter plot shows insignificant positive correlation (r=0.038 .p.value0.8) between TSH and duration of disease among test group.

**Figure 3.2a** scatter plot shows insignificant negative correlation (r=-0.17 .p.value0.2) between T3 and duration of disease among test group.

**Table 3.1: comparison between mean of serum TSH in case, trait and control group.**

<b>Variable</b>	<b>Mean±SD</b>	<b>P. value</b>
TSH(miu/ml) Case N = 42	0.87 ± 0.79	0.000
Trait N = 7	4.0±3.8	
TSH(miu/ml) Case N = 42	0.87±0.79	0.6
Control N = 30	0.81±0.66	
TSH(miu/ml) Trait N = 7	4.0±3.8	0.000
Control N = 30	0.81±0.66	

On way an ova was used for analysis

P. value ≤ 0.05 is considered to be significant

**Table 3.2 comparison between mean of serum T3 in case, trait and control group.**

<b>Variable</b>	<b>Mean <math>\pm</math>SD</b>	<b>p.value</b>
T3 (ng/ml) Case N = 42	0.64 $\pm$ 0.2	0.1
Trait N = 7	0.8 $\pm$ 0.3	
T3(ng/ml) Case N = 42	0.64 $\pm$ 0.2	0.7
Control N = 30	0.66 $\pm$ 0.24	
T3(ng/ml) Trait N = 7	0.8 $\pm$ 0.3	0.1
Control N = 30	0.66 $\pm$ 0.24	

One way an ova was used for analysis

P. value $\leq$ 0.05 is considered to be significant

**Table 3.3: comparison between mean of serum TSH, T3 in sickle cell anemiachildren according to gender.**

<b>Variable</b>	<b>mean±SD</b>	<b>p. value</b>
TSH (miu/ml) Male N = 13	0.87±0.7	0.8
Female N = 30	0.82±0.84	
T3 (ng/ml) Male N = 13	0.59±0.2	0.3
Female N = 30	0.82±0.89	

T. test was used for analysis

P. value  $\leq 0.05$  is considered to be significant

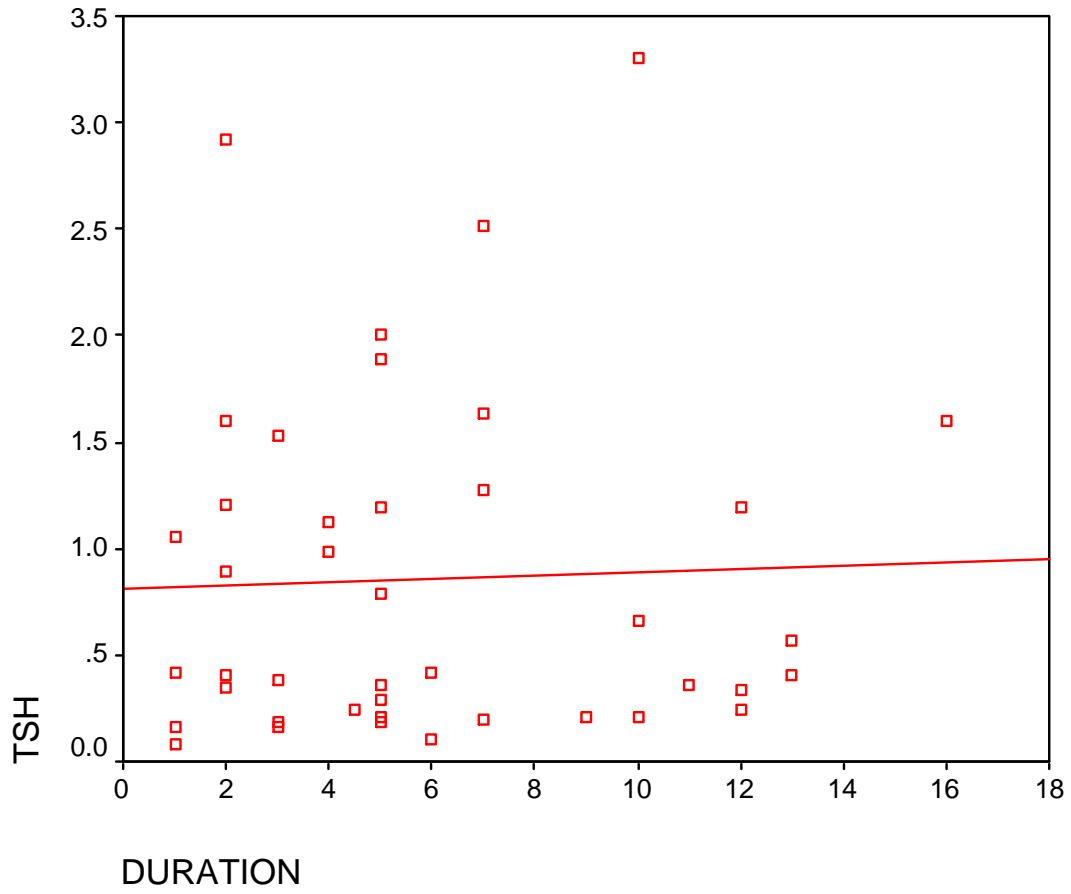


Figure 3.1: correlation between duration of sickle cell anemia and TSH among test group.

$r=0.038$  p.value 0.8

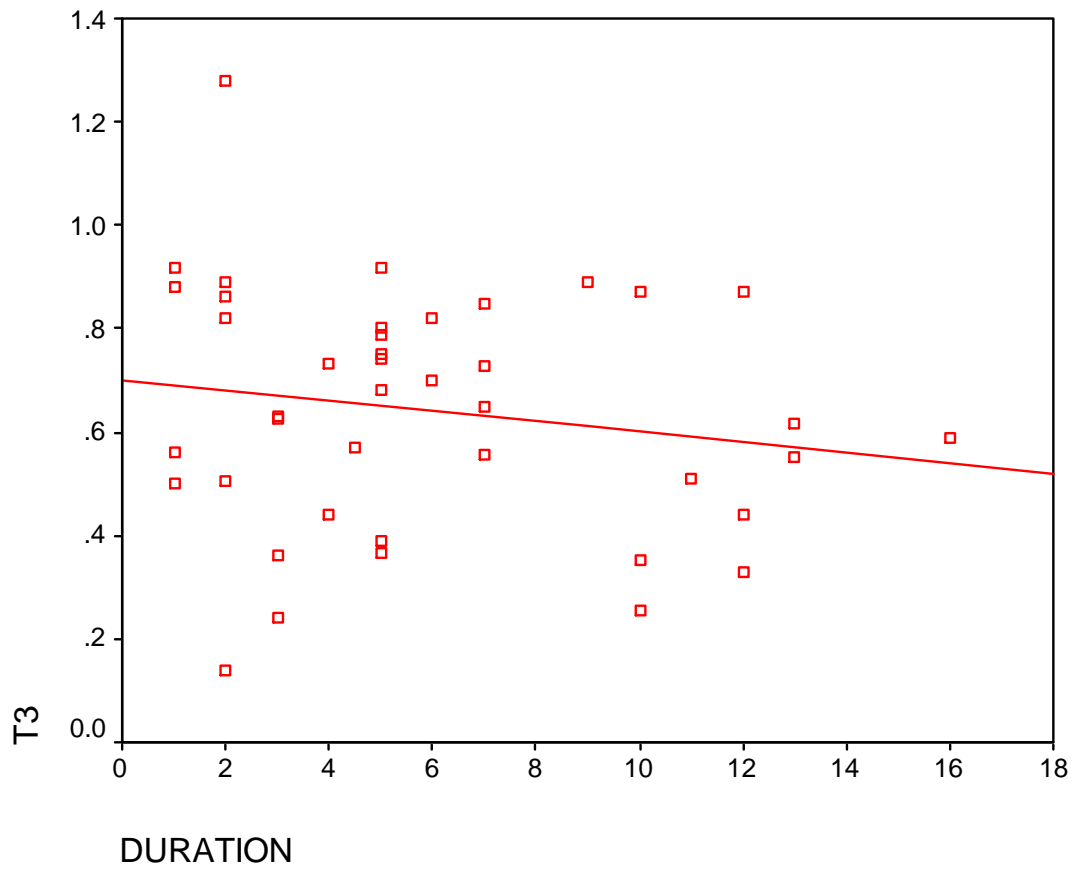


Figure 3.2: correlation between duration of sickle cell anemia and T3 among test group.

$r=-0.17$  p.value0.2



# **Chapter four**

**Discussion, conclusion and Recommendation**

## 4. Discussion, conclusion and Recommendation

### 4.1. Discussion

The etiology of thyroid dysfunction in SCD is not clear however, most affected patients have received multiple transfusions consistent with severe iron overload, autopsy reports in some patients have shown significant iron deposition in thyroid gland, suggesting that the etiology of the primary thyroid failure might will be transfusion hemosiderosis and subsequent cellular damage to the thyroid gland (Ohene-frempong and Steinberg, 2001).

The present study is a case control study aimed to determine the levels of serum TSH, T3 in children with sickle cell anemia, the study included 80 children (43 of them were sickle cell anemia children, 7 of them were trait and 30 of them control group).

The finding revealed that there was a significant decrease of TSH between case and trait groups (means  $\pm$  SD:  $0.87 \pm 0.79$ ,  $4.0 \pm 3.8$  respectively), at level of  $p$ -value = 0.000. there were no previous study.

The finding revealed that there was insignificant difference of TSH between case and control group (means  $\pm$  SD:  $0.87 \pm 0.79$ ,  $0.81 \pm 0.66$  respectively), at level of  $p$ -value = 0.6 this findings were disagreed with study done by II Olatunji and his team, they were found a significant increase of TSH (mean  $\pm$  SD:  $3.90 \pm 0.32$ ,  $2.68 \pm 0.32$  respectively), at level of  $p$ -value = 0.015 (II Olatunji et al., 2002).

Also statistical analysis revealed a significant increase of TSH in trait compared to control group (mean  $\pm$  SD:  $4.0 \pm 3.8$ ,  $0.81 \pm 0.66$  respectively), at level of  $p$ -value = 0.000, also there were no previous study.

The statistical analysis revealed that there was insignificant difference of T3 in case compared to trait group (mean  $\pm$  SD:  $0.64 \pm 0.2$ ,  $0.8 \pm 0.3$  respectively), at level of  $p$ -value = 0.1, there were no previous study.

The results of this study revealed insignificant difference of T3 in case in compared to control group (mean  $\pm$  SD:  $0.64 \pm 0.2$ ,  $0.66 \pm 0.24$  respectively) at level of  $p$ -value 0.1, this findings were disagreed with study done by II Olatunji and his team, they were found insignificant decrease in T3 in case compared to control group and (mean  $\pm$  SD:  $1.22 \pm 0.18$ ,  $1.68 \pm 0.41$  respectively,  $p$ -value = 0.059). (II Olatunji et al., 2002).

And the study results revealed that insignificant difference of T3 in trait in compared to control group (mean  $\pm$  SD:  $0.8 \pm 0.3$ ,  $0.66 \pm 0.24$  respectively), at level of  $p$ -value = 0.7, also no previous study.

The statistical analysis revealed that there was insignificant difference of TSH in male when compared to female group (mean  $\pm$  SD:  $0.87 \pm 0.7$ ,  $0.82 \pm 0.84$  respectively), at level of  $p$ -value = 0.8, this findings were disagreed with study done by Mashwa and his team, Boston in Cairo university, they were found insignificant increase of TSH in male compared to female group (mean  $\pm$  SD:  $2.46 \pm 1.6$ ,  $1.4 \pm 0.7$  respectively). at level of  $p$ -value = 0.7 (Mashwa et al., 2009).

The statistical analysis revealed that there was insignificant difference of T3 in male compared to female group (mean  $\pm$  SD:  $0.59 \pm 0.2$ ,  $0.82 \pm 0.89$  respectively), at level of

p.value= 0.3, this findings disagreed with results of study done by Mashwa and his team Boston in Cairouniversity, they were found insignificant decrease in male compared to female group and (mean± SD: 12.1± 5.2 vs. 12.4 ± 5.4 respectively), at p.value=0.1 (Mashwaetal., 2009).

The statistical analysisreveled that there wasinsignificant positive correlation between TSH and duration of disease.(P.value 0.8 r=0.038).

The statistical analysis reveled that there wasinsignificant negative correlation between T3 and duration of sickle cell anemia.(p.value 0.2 r=-0.17) also no previous study.

## **4.2. Conclusions**

1. It is concluded that serum TSH level had insignificant difference and T3 level had insignificant difference in sickle cell anemia children.
2. There were a significant increase in TSH level, insignificant difference in T3 level in sickle cell trait.
3. There were insignificant difference in TSH level, insignificant difference in T3 level and this directly proportional with gender.
4. The TSH level had insignificant positive correlation, T3 level had insignificant negative correlation and this directly proportional with duration of sickle cell anemia in children.

### **4.3.Recommendations**

- 1.Further studies with measurements of unoccupied protein binding site for T4andT3 in the serum, are recommended to be done.
- 2.Further studies with measurements of or free T3 and T4 in the serum are recommended to be done to get accurate results.

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# **Appendices**

## Appendix 1

**Sudan University of Science & Technology**

**College of Graduate Studies**

**Clinical chemistry**

### **Questionnaire**

Name: .....

No of Sample: .....

Age: ..... Gender: .....

Tribe: .....

Exclusion Criteria: .....

History of other disease: .....

Result: .....

TSH: .....

T3: .....