



بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

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**Serum Level of Vitamin D among Females with Sickle
Cell Anemic Patients in Khartoum State**

**مستوى فيتامين د في مصل الدم لدى الإناث المصابات بالانيميا المنجلية
في ولاية الخرطوم**

*A dissertation submitted for the partial fulfillment for the requirement of
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قال تعالى:

هُوَ الَّذِي خَلَقَكُمْ مِنْ تُرَابٍ ثُمَّ مِنْ نُطْفَةٍ ثُمَّ مِنْ عَلَقَةٍ ثُمَّ يُخْرِجُكُمْ طِفْلًا
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صدق الله العظيم
سورة غافر (الآية 67)

Dedication

To my precious mother

To my brother and sisters

To my beloved husband

To my teachers

To any person who helped me to fulfil this research

Acknowledgment

Thanks you my God, for giving me the ability and courage to bring this research to light.

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Abstract

Vitamin D deficiency is now recognized as one of the most common nutritional conditions among persons with Sickle Cell Disease (SCD). The motivation for better understanding the magnitude of vitamin D deficiency among populations with SCD.

This is case control study conducted during the period from February to June 2016, patients are chosen from Gaafer Bin Aoufto determine the level of vitamin D among Sudanese female with sickle cell disease, the level of vitamin D was estimated by using ELISA.

The study include 80 subject, 50 sickle cell anemic patients, (40 malignant + 10 benign) and 30 of them were apparently healthy individuals as control group.

Statistical analysis was done by using SPSS. And the result showed a significant decrease in means of vitamin D in sickle cell anemic patients (27.07 ± 15.95) when compared to control group (35.18 ± 12.09) with p-value 0.020. The study has revealed a significant negative correlation between vitamin D level and age ($r = -0.445$, p-value = 0.005). The result has revealed there was no correlation between vitamin D level and duration of treatment ($r = 0.107$, p-value 0.694).

It is concluded that the serum level of vitamin D is decreased in patients with sickle cell disease, there were no relationship between the level of vitamin D and duration of treatment and the increasing of age of sickle cell anemic patients has associated with decreasing of vitamin D level.

المستخلص

يعد نقص فيتامين د من أهم العوامل الغذائية ذات التأثير البالغ على مرضى الانيميا المنجلية والذي كان دافعا اساسيا لقياس مقدار نقص فيتامين د لدى مرضى الانيميا المنجلية واجريت هذه الدراسة للمقارنة بين مجموعتي المرضى والاصحاء في الفترة من فبراير الي يونيو 2016 تم اختيارهم من مستشفى جعفر بن عوف لتحديد مستوي فيتامين د لدى المرضى الاناث . تم قياس مستوي فيتامين د باستخدام المقايسة الامتصاصية المناعية المرتبطة بالانزيم ELISA.

شملت هذه الدراسة 80 شخص 50 منهم يعانون من مرض الانيميا المنجلية (40 حالة خبيثة + 10 حالات حميدة) و30 اصحاء ظاهريا كمجموعة تحكم . تم اجراء التحليل الاحصائي باستخدام برنامج SPSS واطهرت النتائج انخفاض بشكل ملحوظ في متوسط مستوي فيتامين د لدي المرضى (27.07±15.95) عند مقارنتها بمجموعة التحكم (35.18±12.09) مع القيمة المعنوية 0.020 اظهرت الدراسة ان هنالك علاقة عكسية ملحوظة بين مستوي فيتامين د والعمر (r=-0.445) والقيمة المعنوية 0.005. اظهرت الدراسة انه لا توجد علاقة ملحوظة بين فيتامين د وفترة العلاج (r=0.107) والقيمة المعنوية 0.694.

خلصت الدراسة الي ان هناك علاقة ملحوظة بين انخفاض مستوي فيتامين د في مصل الدم ومرض الانيميا المنجلية كذلك لا توجد علاقة بين مستوي فيتامين د وفترة العلاج , اخيرا لوحظ ان التقدم في العمر يؤدي الي انخفاض مستوي فيتامين د لدى مرضى الانيميا المنجلية .

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List of abbreviations:

ACS	Acute Chest Syndrome
DVT	Deep Vein Thrombosis
E6V	Glu6val position
ELISA	Enzyme Linked Immune Sorbent Assay
FDA	Food and Drug Association
G6PD	Glucose 6 Phosphate Dehydrogenase
HBB gene	Beta globin gene
HbF	Hemoglobin F
HbS	Hemoglobin S
HbSS	Hemoglobin SS
IOM	Institute of Medicine
IU	International Unit
IUPAC	International Union of Pure and Applied Chemists
PCOS	Poly cystic Ovary Syndrome
PMS	Premenstrual Syndrome
RBCs	Red Blood Cells
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SD	Standard deviation
SNP	Single Nucleotide polymorphism
TMB	Tetramethylbenzidine
UL	Unit per Liter

Chapter one

Introduction and literature review

1 Introduction and Literature review

1.1 Introduction

Sickle cell disease is caused by a mutation resulting from an exchange of nitrogenous bases in the sixth codon of the beta-globulin hemoglobin gene, generating an abnormal hemoglobin called hemoglobin S (HbS) (Zago, 2002). The manifestations of sickle-cell disease are due to the presence of HbS, of which molecules are organized into polymeric beams when deoxygenated and give the RBC an elongated and rigid conformation, called a “sickle-shaped red blood cell” (Zago,2007). After the sickling process, the red blood cells begin to show changes in membrane proteins and increased expression of adhesion molecules that, consequently, lead to red blood cell adhesion to the endothelium.

This process triggers an inflammatory phenomenon, activation of coagulation, hypoxia, ischemia and local infarction, in addition to reduced RBC survival (Zago, 2007). Sickle-cell disease is one of the most common genetic diseases in the world. The chronic hemolysis, anemia, and vaso-occlusive phenomena that occur in patients with the disease are triggers of an accelerated metabolism, and this process leads to a basal metabolic rate 20% higher in patient with sickle-cell anemia than in the normal population (Zago,2002),(Carneiro and Murad,2002). However, there are no records of methodologies and equations to estimate the energy expenditure in children with sickle-cell anemia, (Damiao andSiqueira, 2008).

Children with sickle cell anemia have a higher risk of developing nutritional deficiencies due to reduced appetite (Mitchell *etal.*, 2004).poor dietary intake of nutrients (Kawchak*etal.*, 2007). And infectious complications, which demands greater attention from health professionals. Among the

vitamins, vitamin D must be carefully evaluated in children with sickle-cell anemia. This is due to the high concentration of melanin in the skin, low levels of physical activity (Buchowski*et al.*, 2002) and low food intake. (Kawchak*et al.*, 2007), (Buison*et al.*, 2004) Children with sickle-cell anemia are more likely to develop vitamin D deficiency when compared to the healthy controls (Rovner*et al.*, 2008).

Calcium and vitamin D are important for bone metabolism, and the low calcium intake leads to a reduction in the ideal bone mass peak in children and adolescents with sickle cell anemia, which determines growth failure. Vitamin D deficiency, in turn, is associated with increased respiratory infections, muscle weakness and increased risk of falls and microlesions (Holick, 2012). Additionally, in children with sickle-cell anemia, whose bones are affected by infarction, osteoporosis and osteonecrosis, vitamin D deficiency may worsen bone condition (Serarslan*et al.*, 2010). Considering these facts, this study aimed to carry out an integrative literature review to analyze the frequency of vitamin D deficiency and its consequences in children and adolescents with sickle-cell anemia.

1.2 Literature review

1.2.1 Sickle cell Disease

Sickle-cell disease (SCD), also known as sickle-cell anemia (SCA), is a group of genetically passed down blood disorders. It results in an abnormality in the oxygen-carrying protein haemoglobin found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle-cell crisis"), anemia, bacterial infections, and stroke. Long term pain may

develop as people get older. The average life expectancy in the developed world is 50 years. (Yawn BP *et al.*, 2010).

Sickle-cell disease occurs when a person inherits two abnormal copies of the haemoglobin gene, one from each parent. Several subtypes exist, depending on the exact mutation in each haemoglobin gene. An attack can be set off by temperature changes, stress, dehydration, and high altitude. A person with a single abnormal copy does not usually have symptoms and is said to have sickle-cell trait. Such people are also referred to as carriers. Diagnosis is by a blood test and some countries test all babies at birth for the disease. Testing is also possible during pregnancy (Kumar *et al.*, 2009).

The complications of sickle-cell disease can be managed to a large extent with vaccination, preventive antibiotics, high fluid intake, folic acid supplementation, and pain medication. Other measures may include blood transfusion, and the medication hydroxycarbamide (hydroxyurea). A small proportion of people can be cured by a transplant of bone marrow cells (Kumar *et al.*, 2009).

1.2.1.1 Signs and symptoms of Sickle cell Disease

Sickle-cell disease may lead to various acute and chronic complications, several of which have a high mortality rate. (Yawn BP *et al.*, 2010).

1.2.1.2 Sickle-cell crisis

The terms "sickle-cell crisis" or "sickling crisis" may be used to describe several independent acute conditions occurring in patients with SCD. SCD results in anemia and crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, haemolytic crisis, and others. Most episodes of sickle-cell crises last between five and seven days. "Although infection, dehydration, and acidosis (all of which favor sickling)

can act as triggers, in most instances, no predisposing cause is identified (Kumar *et al.*, 2009)."

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 ischaemia, pain, necrosis, and often organ damage. The frequency, severity, and duration of these crises vary considerably. Painful crises are treated with hydration, analgesics, and blood transfusion; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manages on NSAIDs (such as diclofenac or naproxen). For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia devices are commonly used in this setting. Vaso-occlusive crisis involving organs such as the penis (Olujohungbe and Burnett, 2013) or lungs are considered an emergency and treated with red-blood cell transfusions. Incentive spirometry, a technique to encourage deep breathing to minimize the development of atelectasis, is recommended (Glassberg, 2011)

Splenic sequestration crisis

Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected (Anie and Green, 2012). It is usually infarcted before the end of childhood in individuals suffering from sickle-cell anemia. This spleen damage increases the risk of infection from encapsulated organisms (Pearson HA , 1977) ,(Wong *et al.*, 1993). Preventive antibiotics and vaccinations are recommended for those lacking proper spleen function.

Splenic sequestration crises are acute, painful enlargements of the spleen, caused by intrasplenic trapping of red cells and resulting in a precipitous fall

in hemoglobin levels with the potential for hypovolemic shock. Sequestration crises are considered an emergency. If not treated, patients may die within 1–2 hours due to circulatory failure. Management is supportive, sometimes with blood transfusion. These crises are transient, they continue for 3–4 hours and may last for one day (Khatib *et al.*, 2009).

Acute chest syndrome

Acute chest syndrome (ACS) is defined by at least two of the following signs or symptoms: chest pain, fever, pulmonary infiltrate or focal abnormality, respiratory symptoms, or hypoxemia (Glassberg, 2011). It is the second-most common complication and it accounts for about 25% of deaths in patients with SCD, majority of cases present with vaso-occlusive crises then they develop ACS (Mekontso *et al.*, 2008), (Paul *et al.*, 2011). Nevertheless, about 80% of patients have vaso-occlusive crises during ACS.

Aplastic crisis

Aplastic crises are acute worsening of the patient's baseline anemia, producing pale appearance, fast heart rate, and fatigue. This crisis is normally triggered by parvovirus B19, which directly affects production of red blood cells by invading the red cell precursors and multiplying in and destroying them (Kumar *et al.*, 2009). Parvovirus infection almost completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of SCD patients' results in an abrupt, life-threatening situation. Reticulocyte counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to the drop in haemoglobin. This crisis takes 4 days to one week to disappear. Most patients can be managed supportively; some need blood transfusion (Slavov *et al.*, 2011).

Haemolytic crisis

Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with coexistent G6PD deficiency (Balgir, 2012). Management is supportive, sometimes with blood transfusions (Glassberg, 2011).

Other

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 (Jadavji, 1985). The crisis can last up to a month (Worrall and Butera, 1976). Another recognized type of sickle crisis, acute chest syndrome, is characterized by fever, chest pain, difficulty breathing, and pulmonary infiltrate on a chest X-ray. Given that pneumonia and sickling in the lung can both produce these symptoms; the patient is treated for both conditions (Miller, 2011). It can be triggered by painful crisis, respiratory infection, bone-marrow embolization, or possibly by atelectasis, opiate administration, or surgery.

1.2.1.3 Genetics of Sickle cell Disease

Normally, humans have haemoglobin A, which consists of two alpha and two beta chains, haemoglobin A₂, which consists of two alpha and two delta chains, and haemoglobin F, consisting of two alpha and two gamma chains in their bodies. Of these, haemoglobin F dominates until about 6 weeks of age. Afterwards, haemoglobin A dominates throughout life.

Sickle-cell conditions have an autosomal recessive pattern of inheritance from parents. The types of haemoglobin a person makes in the red blood cells depend on what haemoglobin genes are inherited from her or his parents. If one parent has sickle-cell anemia and the other has sickle-cell

trait, then the child has a 50% chance of having sickle-cell disease and a 50% chance of having sickle-cell trait. When both parents have sickle-cell trait, a child has a 25% chance of sickle-cell disease, 25% do not carry any sickle-cell alleles, and 50% have the heterozygous condition.

Sickle-cell gene mutation probably arose spontaneously in different geographic areas, as suggested by restriction endonuclease analysis. These variants are known as Cameroon, Senegal, Benin, Bantu, and Saudi-Asian. Their clinical importance is because some are associated with higher HbF levels, e.g., Senegal and Saudi-Asian variants, and tend to have milder disease (green, 1993).

In people heterozygous for HgbS (carriers of sickling haemoglobin), the polymerisation problems are minor, because the normal allele is able to produce over 50% of the haemoglobin. In people homozygous for HgbS, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated. The sickle-cell disease occurs when the sixth amino acid, glutamic acid, is replaced by valine to change its structure and function; as such, sickle-cell anemia is also known as E6V. Valine is hydrophobic, causing the haemoglobin to collapse on itself occasionally. The structure is not changed otherwise. When enough haemoglobin collapses on itself the red blood cells become sickle-shaped(Allison, 2009).

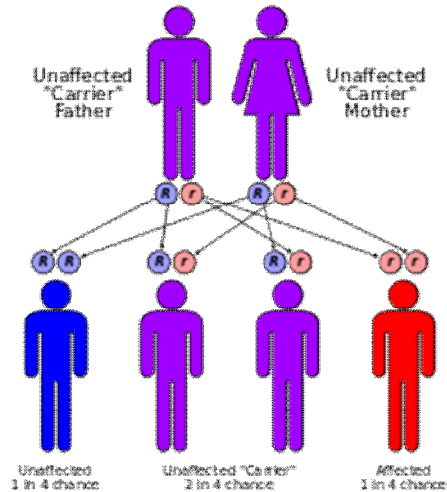


Figure 1.1: Sickle-cell disease is inherited in the autosomal recessive pattern. The gene defect is a known mutation of a single nucleotide (see single-nucleotide polymorphism - SNP) (A to T) of the β -globin gene, which results in glutamic acid (E/Glu) being substituted by valine (V/Val) at position 6. Note, historic numbering put this glutamic acid residue at position 6 due to skipping the methionine (M/Met) start codon in protein amino acid position numbering. Current nomenclature calls for counting the methionine as the first amino acid, resulting in the glutamic acid residue falling at position 7. Many references still refer to position 6 and both should likely be referenced for clarity. Haemoglobin S with this mutation is referred to as HbS, as opposed to the normal adult HbA. The genetic disorder is due to the mutation of a single nucleotide, from a GAG to GTG codon on the coding strand, which is transcribed from the template strand into a GUG codon. Based on genetic code, GAG codon translates to glutamic acid (E/Glu) while GUG codon translates to valine (V/Val) amino acid at position 6. This is normally a benign mutation, causing no apparent effects on the secondary, tertiary, or quaternary structures of haemoglobin in conditions of normal oxygen concentration. What it does allow for, under conditions of

low oxygen concentration, is the polymerization of the HbS itself. The deoxy form of haemoglobin exposes a hydrophobic patch on the protein between the E and F helices. The hydrophobic side chain of the valine residue at position 6 of the beta chain in haemoglobin is able to associate with the hydrophobic patch, causing haemoglobin S molecules to aggregate and form fibrous precipitates.(Allison, 2009).

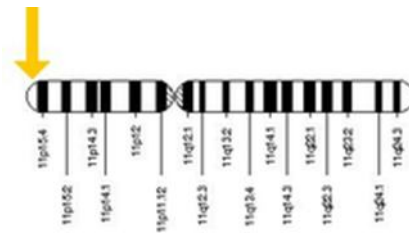


Figure .1.2: HBB gene (responsible for sickle-cell anaemia) is located on the short (p) arm of chromosome 11 at position 15.5

The allele responsible for sickle-cell anaemia can be found on the short arm of chromosome 11, more specifically 11p15.5. A person who receives the defective gene from both father and mother develops the disease; a person who receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier or heterozygote. Heterozygotes are still able to contract malaria, but their symptoms are generally less severe (Allison, 2009).

Due to the adaptive advantage of the heterozygote, the disease is still prevalent, especially among people with recent ancestry in malaria-stricken areas, such as Africa, the Mediterranean, India, and the Middle East (Kwiatkowski, 2005). Malaria was historically endemic to southern Europe,

but it was declared eradicated in the mid-20th century, with the exception of rare sporadic cases (Ponçon, 2007).

1.2.1.4 Pathophysiology of sickle cell disease

The loss of red blood cell elasticity is central to the pathophysiology of sickle-cell disease. Normal red blood cells are quite elastic, which allows the cells to deform to pass through capillaries. In sickle-cell disease, low oxygen tension promotes red blood cell sickling and repeated episodes of sickling damage the cell membrane and decrease the cell's elasticity. These cells fail to return to normal shape when normal oxygen tension is restored. As a consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischaemia (Clarke and Higgins, 2000).

The actual anaemia of the illness is caused by haemolysis, the destruction of the red cells, because of their shape. Although the bone marrow attempts to compensate by creating new red cells, it does not match the rate of destruction. Healthy red blood cells typically function for 90–120 days, but sickled cells only last 10–20 days (Clarke and Higgins, 2000).

1.2.1.5 Diagnosis of Sickle cell Disease

In HbSS, the complete blood count reveals haemoglobin levels in the range of 6–8 g/dl with a high reticulocyte count (as the bone marrow compensates for the destruction of sickled cells by producing more red blood cells). In other forms of sickle-cell disease, Hb levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies).

Sickling of the red blood cells, on a blood film, can be induced by the addition of sodium metabisulfite. The presence of sickle haemoglobin can also be demonstrated with the "sickle solubility test". A mixture of haemoglobin S (Hb S) in a reducing solution (such as sodium dithionite)

gives a turbid appearance, whereas normal Hb gives a clear solution.(Clarke and Higgins, 2000).

Abnormal haemoglobin forms can be detected on haemoglobin electrophoresis, a form of gel electrophoresis on which the various types of haemoglobin move at varying speeds. Sickle-cell haemoglobin (HgbS) and haemoglobin C with sickling (HgbSC)—the two most common forms—can be identified from there. The diagnosis can be confirmed with high-performance liquid chromatography. Genetic testing is rarely performed, as other investigations are highly specific for HbS and HbC. An acute sickle-cell crisis is often precipitated by infection. Therefore, a urinalysis to detect an occult urinary tract infection, and chest X-ray to look for occult pneumonia, should be routinely performed (Clarke and Higgins, 2000).

People who are known carriers of the disease often undergo genetic counseling before they have a child. A test to see if an unborn child has the disease takes either a blood sample from the fetus or a sample of amniotic fluid. Since taking a blood sample from a fetus has greater risks, the latter test is usually used. Neonatal screening provides not only a method of early detection for individuals with sickle-cell disease, but also allows for identification of the groups of people that carry the sickle cell trait(Lee *etal.*, 2000).

1.2.1.6 ComplicationsofSickle cell Disease

Sickle-cell anaemia can lead to various complications, including:

Increased risk of severe bacterial infections due to loss of functioning spleen tissue (and comparable to the risk of infections after having the spleen removed surgically). These infections are typically caused by encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Daily penicillin prophylaxis is the most commonly used treatment during

childhood, with some haematologists continuing treatment indefinitely. Patients benefit today from routine vaccination for *S. pneumoniae* (Kavanagh *et al.*, 2011).

Stroke, which can result from a progressive narrowing of blood vessels, prevents oxygen from reaching the brain. Cerebral infarction occurs in children and cerebral haemorrhage in adults.

Silent stroke causes no immediate symptoms, but is associated with damage to the brain. Silent stroke is probably five times as common as symptomatic stroke. About 10–15% of children with SCD suffer strokes, with silent strokes predominating in the younger patients (Adams *et al.*, 2001),(Adams ,2007).

Cholelithiasis (gallstones) and cholecystitis may result from excessive bilirubin production and precipitation due to prolonged haemolysis.

Avascular necrosis (aseptic bone necrosis) of the hip and other major joints may occur as a result of ischaemia(Marti, 2004).

Decreased immune reactions due to hyposplenism (malfunctioning of the spleen),(Kenny ,1980).

Priapism and infarction of the penis (Chrouser,2011).

Osteomyelitis (bacterial bone infection), the most common cause of osteomyelitis in SCD is *Salmonella* (especially the atypical serotypes *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella choleraesuis* and *Salmonella paratyphi B*), followed by *Staphylococcus aureus* and Gram-negative enteric bacilli perhaps because intravascular sickling of the bowel leads to patchy ischaemic infarction (Almeida andRoberts,2005).

Opioid tolerance can occur as a normal, physiologic response to the therapeutic use of opiates. Addiction to opiates occurs no more commonly

among individuals with sickle-cell disease than among other individuals treated with opiates for other reasons.

Acute papillary necrosis in the kidneys and Leg ulcers (Rudge,1991).

In eyes, background retinopathy, proliferative retinopathy, vitreous haemorrhages, and retinal detachments can result in blindness Regular annual eye checks are recommended(Elagouz *etal.*, 2010).

During pregnancy, intrauterine growth retardation, spontaneous abortion, and pre-eclampsia and Chronic pain: Even in the absence of acute vaso-occlusive pain, many patients have unreported chronic pain (Smith *et al.*, 2008).

Pulmonary hypertension (increased pressure on the pulmonary artery) can lead to strain on the right ventricle and a risk of heart failure; typical symptoms are shortness of breath, decreased exercise tolerance, and episodes of syncope.[citation needed] 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 *etal.*, 2015).

Chronic kidney failure due to sickle-cell nephropathy manifests itself with hypertension, protein loss in the urine, loss of red blood cells in urine and worsened anaemia. If it progresses to end-stage renal failure, it carries a poor prognosis (Powars *etal.*,1991).

1.2.1.7 Epidemiology of Sickle cell Disease

The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, tribal regions of India and the Middle-East.(Weatherall and Clegg, 2001) Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries

sickle-cell disease has now overtaken more familiar genetic conditions such as haemophilia and cystic fibrosis (Roberts and Montalembert,2007). In 2013 it resulted in 176,000 deaths due to SCD up from 113,000 deaths in 1990.

Sickle-cell disease occurs more commonly among people whose ancestors lived in tropical and sub-tropical sub-Saharan regions where malaria is or was common. Where malaria is common, carrying a single sickle-cell allele (trait) confers a selective advantage in other words, being a heterozygote is advantageous. Specifically, humans with one of the two alleles of sickle-cell disease show less severe symptoms when infected with malaria (Wellems *etal.*, 2009).

1.2.2 Sickle cell trait (or sickle cell anemia)

Sickle cell trait means having one gene for a condition called sickle cell disease (SCD). This in itself does not normally cause problems and sickle cell trait is not considered as a disease. It is extremely rare for it to cause problems or complications, which mainly occur under conditions of severe physical stress (explained below) (Eichner,2007).

Sickle cell trait is important because your children can inherit the sickle cell gene. If BOTH parents have sickle cell trait, their children could get a double dose of the sickle cell gene, which would give them the serious condition called SCD (Roach,2005).

Sickle cell disease is a blood disorder in which there is a single amino acid substitution in the hemoglobin protein of the red blood cells, which causes these cells to assume a sickle shape, especially when under low oxygen tension. Sickle cell disease also confer some resistance to malaria parasitization of red blood cells, so that individuals with sickle-cell trait (heterozygotes) have a Sickle cell trait is a hemoglobin

genotype AS and is generally regarded as a benign condition. However, individuals with sickle cell trait may have rare complications. There is a selective advantage in environments where malaria is present (Eichner, 2007).

1.2.2.1 symptoms and complications of Sickle cell trait

Sickle cell trait is a hemoglobin genotype AS and is generally regarded as a benign condition. However, individuals with sickle cell trait may have rare complications. In some cases, athletes with sickle cell trait do not achieve the same level of performance as elite athletes with normal hemoglobin AA. Athletes with sickle cell trait and their instructors must be aware of the dangers of the condition during anaerobic exertion especially in hot and dehydrated conditions. In rare cases, exercise-induced dehydration or exhaustion may cause healthy red blood cells to turn sickle-shaped, which can cause death during sporting activities (Tripette *et al.*, 2010).

The complications are extremely uncommon for people with sickle cell trait to have any symptoms. Most of the complications which do occur are due to extreme physical conditions, and so are usually preventable. The possible complications can occur in conditions where oxygen is extremely low - such as flight in an unpressurised aircraft, very high altitude or problems during a general anaesthetic. Also, if you become severely lacking in fluid in the body (dehydrated) (Tripette *et al.*, 2010).

In these situations, sickle cells can form and can block small blood vessels. This may cause episodes of pain in bones, muscles or the spleen. If in the spleen, it is called splenic infarction and may cause pain in the tummy (abdomen) or chest. These can be treated by oxygen and rehydration to bring its level to normal. Complications of severe physical exercise can particularly occur, under very hot conditions, if they are unused to the

training, or if they do not drink enough fluid. Anyone can become ill under these conditions, but people with sickle cell trait are probably at higher risk. In these conditions, people with sickle cell trait may get pain episodes or pain in the spleen (as explained above) (Tripette *et al.*, 2010).

Other complications can also be found. Blood in the urine (haematuria) - this may be noticeable, or in a tiny quantity found only on urine tests. It may affect about 1 in 50 people with sickle cell trait. If you have blood in the urine, it should be investigated (tests done) to see if there is any particular cause. Treatment is needed in some cases. As well as bladder or kidney infections (urinary tract infections). Farther more a blood clot in the leg or lung (deep vein thrombosis (DVT) or pulmonary embolus) (Roach, 2005).

1.2.3 Vitamin D

Vitamin D refers to a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. In humans, the most important compounds in this group are vitamin D₃ (also known as cholecalciferol) and vitamin D₂ (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. A more accurate description of vitamin D is that it is a prohormone and thus, vitamin D is metabolized to a biologically active form that functions as a steroid hormone (Zemleniet *al.*, 2007).

1.2.3.1 Vitamin D structure

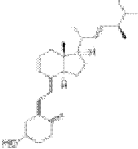
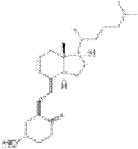
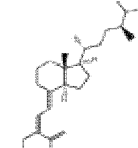
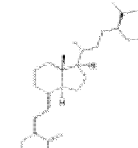
Vitamin D refers to a family of structurally related compounds that display antirachitic activity. Members of the D-family are derived from the cyclopentanoperhydrophenanthrene ring system, which is common to other steroids, such as cholesterol, vitamin D has only three intact rings; the B ring has undergone fission of the 9, 10-carbon bond resulting in the conjugated triene system that is present in all the vitamins (Zemleniet *al.*, 2007).

1.2.3.2 Vitamin D nomenclature

Vitamin D is named according to the new revised rules of the International Union of Pure and Applied Chemists (IUPAC). Vitamin D is designated seco because its B ring has undergone fission. Asymmetric centers are named using R, S notation and Cahn's rules of priority. The configuration of the double bonds is notated E, Z; E for Trans, Z for cis. The formal name for

vitamin D₃ is 9,10-seco(5Z,7E)-5,7,10(19)-cholestatriene- 3b-ol and for vitamin D₂ it is 9,10-seco (5Z,7E)-5,7,10(19), 21-ergostatetraene-3b-ol (Zemplenet *al.*, 2007).

Table1.1: Vitamin D types

Name	Chemical composition	Structure
Vitamin D ₁	Mixture of molecular compounds of <u>ergocalciferol</u> with <u>lumisterol</u> , 1:1	
Vitamin D ₂	<u>ergocalciferol</u> (made from <u>ergosterol</u>)	
Vitamin D ₃	<u>Cholecalciferol</u> (made from <u>7-dehydrocholesterol</u> in the skin).	
Vitamin D ₄	<u>22-dihydroergocalciferol</u>	
Vitamin D ₅	<u>sitocalciferol</u> (made from <u>7-dehydrositosterol</u>)	

Several forms (vitamers) of vitamin D exist. The two major forms are vitamin D₂ or ergocalciferol, and vitamin D₃ or cholecalciferol; vitamin D without a subscript refers to either D₂ or D₃ or both. These are known collectively as calciferol. Vitamin D₂ was chemically characterized in 1931. In 1935, the chemical structure of vitamin D₃ was established and proven to

result from the ultraviolet irradiation of 7-dehydrocholesterol. (Eriksen&Glerup,2002).

Chemically, the various forms of vitamin D are secosteroids, i.e., steroids in which one of the bonds in the steroid rings is broken. The structural difference between vitamin D2 and vitamin D3 is the side chain of D2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24.

1.2.3.3 Vitamin D sources

Vitamin D is found in few dietary sources (Holick, 2006; Norman, 2008; Holick, 2007; Brown,2008). Sunlight exposure is the primary source of vitamin D for the majority of people, other than supplements (Calvo *et al*, 2005). While some studies have found that vitamin D3 raises 25(OH)D blood levels faster and remains active in the body longer, (Tripkovic, (2013) ; Alshahrani *et al*,2013) others contend that vitamin D2 sources are equally bioavailable and effective as D3 for raising and sustaining 25(OH)D (Keegan *et al.*, 2013; Biancuzzo *et al.*, 2013; Borelet *et al.*,2015).

The richest source with vitamin D2 is mushrooms which are a good dietary source. They contain high concentrations of ergosterol (provitamin D2). The richest foods with vitamin D3 are Fish liver oils, such as cod liver oil, Fatty fish species, such as; Salmon, pink, cooked, dry heat. Mackerel, Pacific and jack, mixed species, cooked, dry heat. Tuna, canned in oil. Sardines, canned in oil, drained. Cooked egg yolk. Beef liver, cooked, braised (Keegan *et al.*, 2013; Biancuzzo *et al.*, 2013; Borelet *et al.*,2015).

1.2.3.4 Absorption of vitamin D

Vitamin D can be obtained from the diet, in which case it is absorbed in the small intestine with the aid of bile salts, the specific mode of vitamin D absorption is via the lymphatic system and its associated chylomicrons, only

about 50% of a dose of vitamin D is absorbed. However, considering that sufficient amounts of vitamin D can be produced daily by exposure to sunlight, it is not surprising that the body has not evolved a more efficient mechanism for vitamin D absorption from the diet (Zemleniet *al.*, 2007).

1.2.3.5 Production and Metabolism of Vitamin D

Vitamin D is normally produced in skin through a robust photolytic process acting on a derivative of cholesterol (ie, 7-dehydrocholesterol) to produce previtamin D, which is then slowly isomerized to vitamin D₃ (Brehmet *al.*, 2010). Vitamin D₃ is the natural form of vitamin D produced in skin, and vitamin D₂ is derived from irradiation of ergosterol, which occurs to some degree in plankton under natural conditions and is used to produce vitamin D₂ from the mold ergot (which contains as much as 2% ergosterol). The concept that vitamin D is a vitamin. Another important fact is that vitamin d is required throughout life. It not only is needed for the formation of bone but also likely plays an important role in several other physiologic systems. Its use may well prevent several degenerative diseases, and it may also play a role as an anticancer agent (Levin *et al.*, 2011; Glover *et al.*, 2012).

Vitamin D₃ itself is biologically inert, as clearly indicated by genetic defects that result in the disease rickets despite normal intakes of vitamin D (Gupta *et al.*, 2011). By 1967, the concept that vitamin D is converted to an active form had appeared (Levin *et al.*, 2011; Glover *et al.*, 2012). By 1969, the circulating form of vitamin D had been isolated, chemically identified, and synthesized This compound, 25-hydroxyvitamin D₃ [25(OH) D₃], is now currently monitored in serum to indicate the vitamin D status of patients.(kimet *al*, 2013; McBeth *et al*, 2010).

1.2.3.6 Vitamin D Mechanism of action

Vitamin D is carried in the bloodstream to the liver, where it is converted into the prohormone calcidiol. Circulating calcidiol may then be converted into calcitriol, the biologically active form of vitamin D, in the kidneys, following the final converting step in the kidney, calcitriol is released into the circulation. By binding to vitamin D-binding protein, a carrier protein in the plasma, calcitriol is transported to various target organs (Glover *et al*, 2012). In addition to the kidneys, calcitriol is also synthesized by monocyte-macrophages in the immune system. When synthesized by monocyte-macrophages, calcitriol acts locally as a cytokine, defending the body against microbial invaders by stimulating the innate immune system (Adams *et al.*, 2010). Whether it is made in the skin or ingested, cholecalciferol is hydroxylated in the liver at position 25 (upper right of the molecule) to form 25-hydroxycholecalciferol (calcidiol or 25(OH) D). This reaction is catalyzed by the microsomal enzyme vitamin D 25-hydroxylase, (Cheng *et al.*, 2004) which is produced by hepatocytes. Once made, the product is released into the plasma, where it is bound to a α -globulin, vitamin D-binding protein (Laing and Cooke 2004).

Calcidiol is transported to the proximal tubules of the kidneys, where it is hydroxylated at the 1- α position (lower right of the molecule) to form calcitriol (1, 25-dihydroxycholecalciferol and abbreviated to 1, 25(OH) 2D). This product is a potent ligand of the vitamin D receptor, which mediates most of the physiological actions of the vitamin. The conversion of calcidiol to calcitriol is catalyzed by the enzyme 25-hydroxyvitamin D3 1-alpha-hydroxylase, the levels of which are increased by parathyroid hormone (and additionally by low calcium or phosphate) (Laing and Cooke 2004).

1.2.3.7 Deficiency of vitamin D

A diet deficient in vitamin D in conjunction with inadequate sun exposure causes osteomalacia (or rickets when it occurs in children), which is a softening of the bones in the developed world, this is a rare disease. However, vitamin D deficiency has become a worldwide issue in the elderly and remains common in children and adults (Eriksen and Glerup,2002; Holick, 2007). Low blood calcidiol (25-hydroxy-vitamin D) can result from avoiding the sun.[19] Deficiency results in impaired bone mineralization and bone damage which leads to bone-softening diseases (Grant and Holick, 2005; Brown, 2008).

Rickets

Rickets, a childhood disease, is characterized by impeded growth and soft, weak, deformed long bones that bend and bow under their weight as children start to walk. This condition is characterized by bow legs, (Grant and Holick ,2005)which can be caused by calcium or phosphorus deficiency, as well as a lack of vitamin D; today, it is largely found in low-income countries in Africa, Asia, or the Middle East (Brown, 2008) and in those with genetic disorders such as pseudovitamin D deficiency rickets(Zargaret *al.*,2000). Rickets was first described in 1650 by Francis Glisson, who said it had first appeared about 30 years previously in the counties of Dorset and Somerset (Gibbs,1994).

Vitamin D deficiency remains the main cause of rickets among young infants in most countries, because breast milk is low in vitamin D and social customs and climatic conditions can prevent adequate sun exposure. In sunny countries such as Nigeria, South Africa, and Bangladesh, where the disease occurs among older toddlers and children, it has been attributed to low dietary calcium intakes, which are characteristic of cereal-based diets with limited access to dairy products (Dunniganet *al.*, 2007).

Osteomalacia

Osteomalacia is a disease in adults that results from vitamin D deficiency. Characteristics of this disease are softening of the bones, leading to bending of the spine, bowing of the legs, proximal muscle weakness, bone fragility, and increased risk for fractures (Inselet *et al.*, 2006). Osteomalacia reduces calcium absorption and increases calcium loss from bone, which increases the risk for bone fractures. Osteomalacia is usually present when 25-hydroxyvitamin D levels are less than about 10 ng/mL. The effects of osteomalacia are thought to contribute to chronic musculoskeletal pain (Holick, 2003; Stewart and Leavitt, 2009). There is no persuasive evidence of lower vitamin D levels in chronic pain sufferers (Straube *et al.*, 2009).

Influence of skin pigmentation

Some research shows dark-skinned people living in temperate climates have lower vitamin D levels (Azmina, 2010; Ford *et al.*, 2010). Dark-skinned people may be less efficient at making vitamin D because melanin in the skin hinders vitamin D synthesis; however, a recent study has found novel evidence that low vitamin D levels among Africans may be due to other reasons (Signorello *et al.*, 2010). Recent evidence implicates parathyroid hormone in adverse cardiovascular outcomes. Black women have an increase in serum parathyroid hormone at a lower 25(OH) D level than white women (Aloia *et al.*, 2010). A large-scale association study of the genetic determinants of vitamin D insufficiency in Caucasians found no links to pigmentation (Wang *et al.*, 2010; Bouillon, 2010).

Vitamin D deficiency and infertility

It is a fat soluble vitamin that is present in a variety of forms, although most of them are considered to be inactive inside the body and actually have limited functionality. However, the reason it is so essential to fertility and

getting pregnant because it is needed to help the body create sex hormones. The correct amount of sex hormones in your body is vital to your overall well-being, otherwise you may suffer PMS, PCOS, and sadly, infertility (Pal *et al.*, 2012).

Some study found that nearly 40% of the women who had ovulatory dysfunction also had a clinical deficiency in Vitamin D. Therefore, it is easy to understand how important Vitamin D is to a woman's fertility (Hollis and Wagner, 2004).

1.2.3.8 Excess of Vitamin D

Vitamin D toxicity is rare (Holick, 2007). It is caused by supplementing with high doses of vitamin D rather than sunlight. The threshold for vitamin D toxicity has not been established; however, the tolerable upper intake level (UL), according to some research, is 4,000 IU/day for ages 9–71 (Ross *et al.*, 2011). Whereas another research concludes that in healthy adults, sustained intake of more than 1250 µg/day (50,000 IU) can produce overt toxicity after several months and can increase serum 25-hydroxyvitamin D levels to 150 ng/ml and greater; (Holick, 2007), those with certain medical conditions, such as primary hyperparathyroidism (Vieth, 1999). Are far more sensitive to vitamin D and develop hypercalcemia in response to any increase in vitamin D nutrition, while maternal hypercalcemia during pregnancy may increase fetal sensitivity to effects of vitamin D and lead to a syndrome of mental retardation and facial deformities (Vieth, 1999).

Hypercalcemia is a strong indication of vitamin D toxicity, noted with an increase in urination and thirst. If hypercalcemia is not treated, it results in excess deposits of calcium in soft tissues and organs such as the kidneys, liver, and heart, resulting in pain and organ damage (Holick, 2007; Brown,

2008), (Inselet *al.*, 2006). Pregnant or breastfeeding women should consult a doctor before taking a vitamin D supplement. The FDA advised manufacturers of liquid vitamin D supplements that droppers accompanying these products should be clearly and accurately marked for 400 international units (IU). In addition, for products intended for infants, the FDA recommends the dropper hold no more than 400 IU (DeLancey, 2010). For infants (birth to 12 months), the tolerable upper limit (maximum amount that can be tolerated without harm) is set at 25 µg/day (1,000 IU). One thousand micrograms per day in infants has produced toxicity within one month. After being commissioned by the Canadian and American governments, the Institute of Medicine (IOM) as of 30 November 2010, has increased the tolerable upper limit (UL) to 2,500 IU per day for ages 1–3 years, 3,000 IU per day for ages 4–8 years and 4,000 IU per day for ages 9–71+ years (including pregnant or lactating women) (Ross *et al.*, 2011).

Symptoms of vitamin D toxicity

Vitamin D overdose causes hypercalcemia, and the main symptoms of vitamin D overdose are those of hypercalcemia: anorexia, nausea, and vomiting can occur, frequently followed by polyuria, polydipsia, weakness, insomnia, nervousness, pruritus, and, ultimately, renal failure. Proteinuria, urinary casts, azotemia, and metastatic calcification (especially in the kidneys) may develop. Other symptoms of vitamin D toxicity include mental retardation in young children, abnormal bone growth and formation, diarrhea, irritability, weight loss, and severe depression (Holick, 2007; Inselet *al.*, 2006).

Vitamin D toxicity treatment

Vitamin D toxicity is treated by discontinuing vitamin D supplementation and restricting calcium intake. Kidney damage may be irreversible.

Exposure to sunlight for extended periods of time does not normally cause vitamin D toxicity. The concentrations of vitamin D precursors produced in the skin reach an equilibrium, and any further vitamin D produced is degraded (Vieth,1999).

Published cases of toxicity involving hypercalcemia in which the vitamin D dose and the 25-hydroxy-vitamin D levels are known all involve an intake of $\geq 40,000$ IU (1,000 μg) per day (Vieth,1999).

1.3 Rationale

Vitamin D (25-hydroxyvitamin D) deficiency has emerged as a public health focus in recent years for its contribution to adverse skeletal and extra-skeletal manifestations (Holick,2007).Race, age, body mass index (BMI), latitude, diet, sunlight exposure, and skin pigmentation are all factors influencing vitamin D status (Mitha*et al.*,2009). In addition to its effects on bone health (Sadat *et al.*, 2011), vitamin D deficiency has been linked to multiple health conditions including cardiovascular disease (Bjelakovic*et al.*, 2011). Asthma, nephropathy, and chronic pain. Patients with SCD are susceptible to all of these complications, although it is unclear to what extent vitamin D deficiency is a contributing causal factor(Bjelakovic*et al.*, 2011).

Vitamin D deficiency is now recognized as one of the most common nutritional conditions among persons with SCD (Buison*et al.*, 2004).The motivation for better understanding the magnitude of vitamin D deficiency among populations with SCD, and whether the prevalence is higher among those with SCD compared with similar persons without SCD, is that vitamin D deficiency can be reliably and inexpensively treated, making it a prime intervention to potentially improve health outcomes. Accordingly we conduct this study which is aimed to carry out an integrative literature

review to analyze the frequency of vitamin D deficiency and its consequences in children and adolescents with sickle-cell anemia(Buisonet *al.*, 2004).

1.4 Objectives

1.4.1 General objective

To study the level of vitamin D among Sudanese females with Sickle Cell Disease SCD.

1.4.2 Specific objective

- To estimate and compare Vitamin D level among study groups.
- To compare level of vitamin D between adult and children Sickles.
- To correlate between vitamin D level and study variable (Age, duration of treatment).

Chapter two

Materials and methods

2. Materials and Methods

2.1 Materials

2.1.1 Study design

This is a descriptive analytical cross-sectional study carried out during the period from the January 2016 to September 2016.

2.1.2 Study area

The study was conducted in Khartoum state in Gaffer Bin woof hospital.

2.1.3 Study population

Eighty subjects were enrolled in the present study, and then classified as 50 patients as case group and 30 healthy apparently as control group.

2.1.4 Including Criteria

Female Sickle cell anemic patient were included.

2.1.5 Excluding Criteria

Sickle cell anemic patient with kidney disease, gastrointestinal disease and liver disease were excluded.

2.1.6 Sampling

Vein puncture Blood (5ml) was collected by standard procedure, from the study groups, into sterile containers without anticoagulant and preserved at – 20 degree centigrade.

2.1.7 Ethical consideration:

Volunteers who accept to participate in research were included.

2.2 Methods

2.2.1 Estimation of vitamin D

Vitamin D was estimated by ELISA.

Principle of method for vitamin D:

- I. This ELISA test kit is designed for in vitro determination of 25-OH vitamin D in human serum. In the first analysis step, the calibrator and patients samples are diluted with biotin-labelled 25-OH vitamin D and added to microplate wells coated with monoclonal anti -25-OH vitamin D antibodies. During the incubation unknown amount of 25-OH vitamin D in the patient samples and known amount of biotin-labelled 25-OH vitamin D compete for the antibody binding sites in microplate wells plate. Unbound 25-OH vitamin D is removed by washing. For the detection of bound biotin-labelled 25-OH vitamin D, a second incubation is performed using peroxidase labeled streptavidin. In the third incubation using peroxidase substrate tetramethylbenzidine (TMB) The bound peroxidase promotes a color

reaction. The color intensity is inversely proportional to the 25-OH vitamin D concentration in the sample. Result for the sample can be calculated directly using standard curve(Appendix)

2.3 Quality control

For quality control, calibrators are used, the reagents must be mixed before use, The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

2.4 Statistical analysis

Mean and standard deviation were used to describe the groups, independent T test was employed to compare mean concentration of study parameter, person correlation was used to compare between study parameter and variables, significant difference consider as $p \text{ value} \leq \text{ or } = 0.05$, all this was analyzed by using the computer (SPSS) programmer.

Chapter three

Results

3. Results

Eighty randomly samples were collected to evaluate the level of vitamin D among study groups, then classified as 30 healthy apparently as control group and 40 sickle cell anemic case, 10 cases were trait. Children females account 15 (30%) with average age (5.00 ± 2.85) years, and adults account female 25 (50%) with average age (19.0 ± 7.04) years, and participants average age is (12 ± 8) years.

Table (3.1): Shows mean concentration of vitamin D in sickle cell anemic case (27.07 ± 15.95) versus control group (35.18 ± 12.09), with p-value=0.020

Table (3.2): Shows mean concentration of vitamin D in children females (23.1 ± 8.25) versus control group for children (35.96 ± 15.07), with P-value = 0.010

Table (3.3): shows mean concentration of vitamin D in adult female (22.99 ± 13.11) versus control group (21.96 ± 8.58), with P-value =0.756.

Table (3.4): shows mean concentration of vitamin D in traits (21.84 ± 13.58) versus control (21.96 ± 8.58), with P-value = 0.978

Figure (3.1) Shows negative correlation results between vitamin D and age with r-value -0.445 and P-value 0.005

Figure (3.2) shows no correlation results between vitamin D and duration of treatment with r-value- 0.107 and P-value 0.694.

Table 3.1 Comparison between mean of vitamin D in case and control:

Variable		Mean±SD	P-value
Vitamin D (ng/ml)	Case	27.07±15.95	0.020
	Control	35.18±12.09	

Independent sample T.Test, p-value ≤ 0.05 considered significant

Table 3.2 Comparison between means of vitamin D level in case and control for children

Variable		Mean \pm SD	P-value
Vitamin D (ng/ml)	Case	23.1 \pm 8.25	0.010
	Control	35.96 \pm 15.07	

Independent sample T.Test ,p-value \leq 0.05 considered significant

Table 3.3 Comparison between means of vitamin D level among adult female:

Variable		Mean \pm SD	P-value
Vitamin D (ng/ml)	Case	22.99 \pm 13.11	0.756
	control	21.96 \pm 8.58	

Independent sample T.Test, p-value \leq 0.05 considered in significant

Table 3.4 Comparison between means of vitamin D level among traits female:

Variable		Mean ± SD	P-value
Vitamin D (ng/ml)	Case	21.84 ±13.58	0.978
	control	21.96 ± 8.58	

Independent sample T.Test, p-value ≤ 0.05 considered in significant

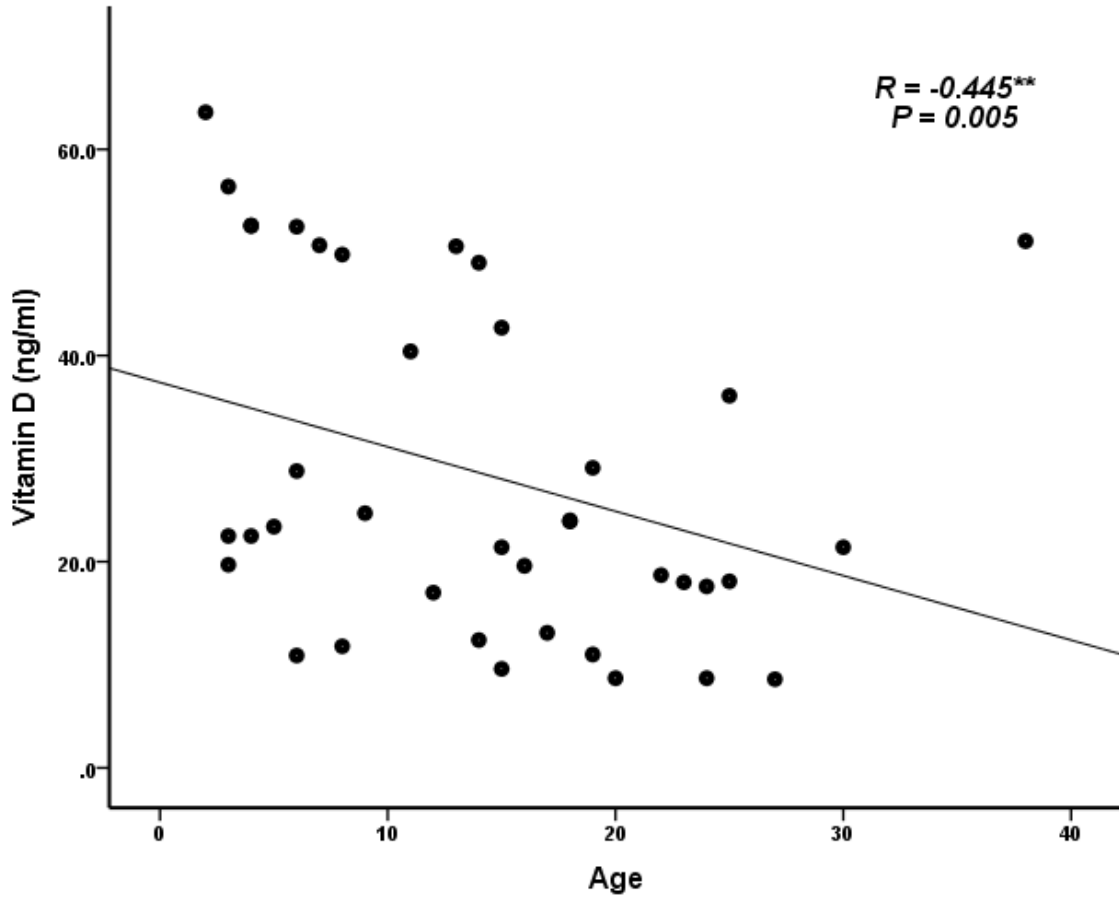


Figure (3.1): Correlation between vitamin D and age among study group.

r- value -0.445. P-value 0.005

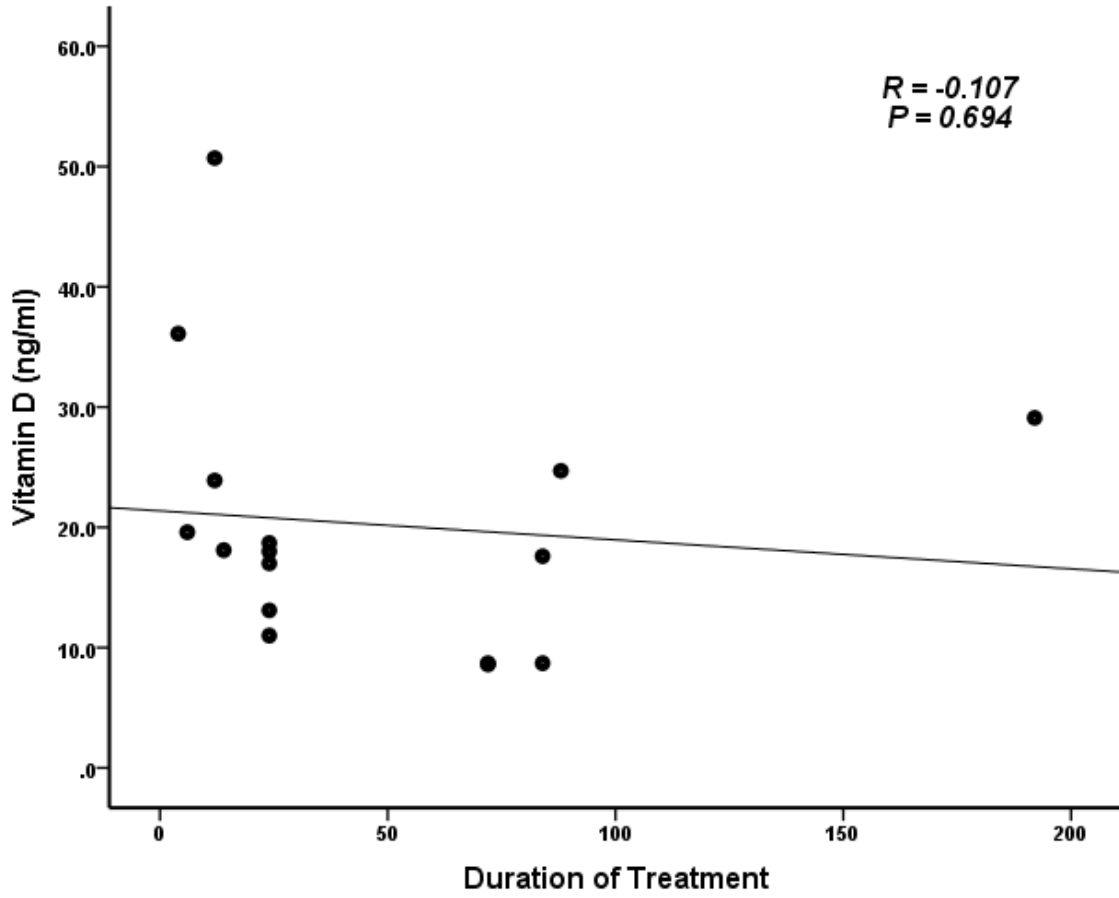


Figure (3.2) Correlation between vitamin D and duration of treatment.
r-value- 0.107. P-value 0.694

Chapter four

Discussion, conclusion and recommendation

4.1 Discussion

Vitamin D deficiency is one of the most common nutritional conditions among persons with SCD (Buison *et al.*, 2004) and there are characteristics specific to SCD that may contribute to this phenomenon including decreased appetite (Ibido and Akinyanju, 2000), inability to absorb nutrients due to damage to the intestinal mucosa (Julka *et al.*, 2008) as well as an increased basal metabolic rate and higher nutritional demands to sustain normal physiologic functioning (Barden *et al.*, 2000). Vitamin D deficiency has been associated with bone health cardiovascular disease, asthma, nephropathy, and chronic pain and individuals with SCD are susceptible to all of these complications. While the role of vitamin D deficiency as a contributing factor in these complications is unclear, vitamin D deficiency can be reliably and inexpensively treated, making it a prime intervention to potentially improve health outcomes among those with SCD (Sadat *et al.*, 2011).

This study conducted to evaluate the level of vitamin D in female with sickle cell disease. Preliminary investigated and findings obtained from specially designed questionnaire revealed that children females account 15 (30%) with average age (5 ± 2.85) years, and adults account female 25 (50%) with average age (19 ± 7.04) years, and participants average age is (12 ± 8) years.

From the findings of this study it appears that serum level of vitamin D in children female (23.1 ± 8.25 versus control subjects 35.96 ± 15.07) was significantly decreased at (p -value = 0.010) in the Sudanese children females with sickle cell anemia versus control subjects and serum level of vitamin D in adult female with sickle cell anemia (22.99 ± 13.11 versus 21.96 ± 8.58) was insignificantly different at (p -value = 0.756) and serum level of vitamin D in adult female trait (21.84 ± 13.58 versus 21.96 ± 8.58) was insignificantly different at (p -value = 0.978). This result agreed with another result of study carried by (Buison *et al.*, 2004; Rover *et al.*, 2008) demonstrated that the frequency of low serum levels of vitamin D in the group with sickle-cell anemia exceeded the frequency found in the healthy group.

In this study there were no significant difference between the level of vitamin D compared to duration of treatment (hydroxyurea) and there is significantly decrease level of vitamin D compare to age. This was agreed with another study which was conducted in USA mainly in North America

in which the level of active form vitamin D was declined through aging by 50%.

The level of vitamin D during the aging process was also demonstrated by (Garrido *et al.*, 2012) who reported low serum levels of vitamin D in children younger than 5 years, but, in children older than 5 years, this deficiency was even higher. No child older than 5 years showed acceptable levels of vitamin D. These results are in parallel with another study, which showed an inverse correlation between the levels of vitamin D and age that is, the older the age, the lower the levels of vitamin D (Garrido *et al.*, 2012).

4.2. Conclusion

According to the result of this study it is concluded that:

1-Vitamin D decreased in patients with sickle cell disease.

2- Vitamin D level decreased with age.

4-no association between vitamin D level and duration of treatment.

4.3. Recommendations

-From the findings of this study it is recommended that:

-Vitamin D must be carefully evaluated in children and adolescent with sickle-cell anemia to avoid complication of diseases also the study recommended vitamin D protocol supplements for sickle cell anemia patients.

- Further studies should be done to evaluate levels of Parathyroid hormone, Gonads hormones and Bone marker which may be disturbed according to disturbance of vitamin D level.

-Must be estimate vitamin D2.

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