Sudan University of Sciences and Technology
College of Graduate Studies

Detection of ٢٥-Hydroxy Vitamin D٣ as Modifier of Sickle Cell Complication Among Saudi Children with Sickle Cell Disease in Dammam, Kingdom of Saudi Arabia.

الكشف عن ٢٥ – هيدروكسي فيتامين د ٣ معدلات لمضاعفات الأطفال المصابين بمرض الأنيميا المنجلية في الدم – المملكة العربية السعودية

A Dissertation Submitted for the Partial Fulfillment of the Requirements ofM.Sc. Degree in Medical Laboratory Science –Hematology and Immunoheamatology

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June ٢٠٢٢

الآيـــــة

بسم الله الرحمن الرحيم

قال تعالي:

﴿ وقَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ﴾

صدق الله العظيم

سوره النمل الآية (١٩)

Dedication

To who was pushing me forward my dear father: **Ibrahim Hussein**.

To the symbol of love, her calls to me were successful, followed me step by step in my work :**Hanan Al-Tigani .**

To the candles of the house my brothers and my sister: **Walaa, Mohammed and Hussein**

My partner in the journey of a lifetime my dear husband: **Mohamed Ata**

To my friends who brought pleasure in my heart **Sarah Elshaikh** and **Ghadeer Al-Dossari**.

To my colleagues and dear friends

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Abstract

Sickle cell disease refers to a collection of autosomal recessive genetic disorders characterized by the HbS variant of the β-globin gene. One nutrient of concern for individuals with SCD is ٢٥-OH vitaminWhich plays an important role in cell growth and differentiation, cardiovascular health, immunity, and bone health.

This is case-control study aimed to estimate and comparethe level of vitamin D between children with SCD (as case) and children without SCD (as control), and to correlate between ٢٥-OHvitamin D level and study variables (Age, duration of treatment, gender, history of disease, and number of blood transfusions).

The study carried out during the period from the November ٢٠١٨ to December ٢٠١٩.The study was conducted in Dammam Kingdom of Saudi Arabia. Eighty-eight subjects were enrolled in this study classified as ٤٤ Patients with SCD as case group and ٤٤ apparently healthy as control one withmean age is ٦ ±٤.Blood was collected in K٢EDTA, blood sample for full blood count and analyzed by CELL-DYN Emerald. An erythrocyte sedimentation rate (ESR) per halfhrs collected in tri-sodium citrate. Measured of ٢٥-OH Vitamin D by Enzyme Linked Immunosorbant Asaay. Data analysis using SPSScomputer programmed version ٢٤ by *P.value,*T- test and one – way anova test were used.

There was statistically significant differences of vitamin D level in case compared to control groups with mean Std. Deviation (١٢±٣ ng/ml and ٢٧±٩ ng/ml) respectively *p.value* (٠,٠٠١). There was statistically significant differences of vitamin D level in group HbSS with mean ٩,٤±٢,٥ compare with other HbAS and HbSC with mean ١٤,١±١,٨ and ١٣,٨±١,٠*p.value* (٠,٠٠). Correlation of ESR among study group (case and control) and Hb differentiation showed significant elevation .The

Mean values of full blood counts in SCD patients and control ,showed average WBC count among cases ١٣٧٠٥±٢٨١٥ cell/mm^{٣}was significantly raised than controls ٧٤٣٩±٢٥٩٢ cell/mm^{٣} , the RBCs, Hb ,PCV,MCV and MCH were lower significantly (٣,٠٠±٠,٧٦ mill/mm^{٣}, ٧,١±.٢g/dl,٢١,١±٣,٧٪ ,٧٤,٧±١٣,٧ fl,٢٤,٤±٤,١ g/dl respectively).In cases compared with controls (٤,٦٧±٠,٥١mill/mm^{٣}, ١٣,١±١,٤ g/dl,٤٠,٥±٣,٨٪, ٨٦,٥±, ٤,٣ fl,٢٧,٧±٢,٥ g/dl, respectively). There was highly significant elevation in platelets counts in cases (٥٢٧±١٣١ × ١٠^{٣}/µl) compared to control (٢٧٣±٤١ × ١٠^{٣}/µl).*p.value* (٠,٠٠).

مستخلص الدراسة

يشير مرض الخلايا المنجلية إلى أنه مجموعة من الإضطرابات الوراثية الجسدية المتنحية التي تتميز بمتغير بيتا غلوبين مما يؤدي الى البلمرة وتبلور جزيْ الهيموجلوبين الذي بسبب شكل المنجل في خلية الدم الحمراء تحت ظروف معينة . يعتبر ٢٥-هيدروكسي فيتامين د أحد العناصر الغذائية المثيرة للقلق للأفراد اللذين يعانون من الأنيميا المنجلية.فهو يلعب دورا مهما في نمو الخلايا والتمايز و صحة القلب والأوعية الدموية والمناعة و صحة العظام .

هذه دراسة حالة و تحكموالتي تهدفلقياس و مقارنة مستوى فيتامين د - او اتش في الاطفال السعوديين اللذين يعانون من فقر الدم المنجلي كمجموعة (الحالة) والأطفال الأصحاء كمجموعة (تحكم) و للربط بين مستوى فيتامين (د) ومتغيرات الدراسة (العمر ، مدة العلاج ، الجنس وتاريخ المرض و عدد مرات نقل الدم) .

أجريت هذه الدراسة خلال الفترة من نوفمبر ٢٠١٨ إلى ديسمبر ٢٠١٩ في الدمامالمملكة العربية السعودية . تم تسجيل ثمانية وثمانين فرد في هذه الدراسة ، ثم صنفت على أنها ٤٤ فرداً يعانون من فقر الدم المنجلي كمجموعة (الحالة) و ٤٤ فرداً أصحاء كمجموعة (تحكم). و كان متوسط الأعمار٦ ±٤.تم جمع عينات الدم في حاويه تحتوي علي الثيامين ديامين تترا حمض الخليك المضادة للتخثرلتعداد الدم الكامل وتحليلها بواسط جهاز تحليل الدم العام . وتم ايضا قياس معدل ترسيب كرات الدم الحمراء بوساطة وضع الدم في سترات الصوديوم المضادة للتخثر لمده نصف ساعه . تم تحليل مصل فيتامين (د) بواسطة جهاز كشف الاجسام المضاده بواسطه الانزيم المرتبط وتم تحليل النتائج بواسطه برنامج التحليل الإحصائي (اس بي اس اس) النسخه ٢٤ باستخدام القيمة الاحتمالية اختبار تي و تحليل التباين في اتجاه واحد.

وجدت الدراسه بأن فيتامين د ٢٥-هيدروكسي في المصل في الحالات المصابه بفقر الدم المنجلي أقل بشكل كبير من الحالات الغير مصابة بفقر الدم المنجلي (٣±١٢ مقابل ٩±٢٧ نانوغرام / مل في مجموعه التحكم) على التوالي حيث وجد فرقا احصائيا بينهم (٠،٠٠١) .

وجدت الدراسه بأن فيتامين د ٢٥-هيدروكسي في مجموعات الرحلان الكهربائي HbSS مقارنة بالمجموعات الاخرى . ارتفاع معدل ترسيب كرات الدم الحمراء بين مجموعتي الدراسة (المرضي والأصحاء) ومتوسط تركيز الهيموقلوبين للخليه، ارتفاع ايضا متوسط عدد كرات الدم البيضاء بين الاطفال المصابين بفقر الدم المنجلي ١٣٧٠٥ ± ٢٨١٥ خلية / مم ٣ مقارنة مع متوسط عدد كريات الدم البيضاء في الأطفال الأصحاء ٧٤٣٩ ± ٢٥٩٢ خلية / مم^{٣} ،المرضى الذين يعانون من مرض فقر الدم المنجليلديهم قيم أقل بشكل ملحوظ في تركيز الهيموغلوبين ،

تعداد خلايا الدم الحمراء ، متوسط حجم الخلية معبأة حجم الخلية و متوسط الهيموغلوبين الخلية (٣,٠٠ ± ٠,٧٦ مل / م^{٣} ، ٧,١ ± .٢جرام ديسلتر ، ٣,٧ ± ٢١,١٪ ، ١٣,٧ ± ٧٤,٧ فمتولترات ، ٤,١ ± ٢٤,٤ جم / دل على التوالي) في الاطفال المصابين بالأنيمياء المنجليه مقارنة مع الأطفال الأصحاء (٤,٦٧ ± ٠,٥١مل / م^{٣}، ١,٤ ١٣,١ جم / دل ، ٤٠,٥ ± ٣,٨ ٪ ، ٨٦,٥ ± ، ٤,٣ فمتولترات ، ٢٧,٧ ± ٢,٥ جم / دل ، على التوالي). كان هناك ايضا ارتفاع كبير للغاية في تعداد الصفائح الدموية في الأطفال المصابين بفقر الدم المنجلي (٥٢٧ ± ١٣١ ×١٠^{٣} / ميكرولتر) مقارنة مع الأطفال الأصحاء (٢٧٣ ± ٤١ × ١٠^{٣} / ميكرولتر) بحيث وجد فرقا احصائيا (٠,٠٠).

استخلصت أن الأطفال السعوديين الذين يعانون من مرض فقر الدم المنجلي كان قياس مستوى فيتامين د – او اتش منخفض انخفاضا كبيراً.كما كانت لديهم قيم أقل لتركيز الهيموغلوبين وعدد خلايا الدم الحمراء و متوسط حجم الخلية المعبأة بالخلايا و هيموغلوبين الخلية المتوسط ؛ لكن كانت لديهم القيم الأعلى لتركيز الهيموغلوبين في الخلايا و عدد خلايا الدم البيضاء و عدد الصفائح الدموية ومعدل ترسيب كرات الدم الحمراء مقارنة بمجموعات التحكم .

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List of abbreviations

Abbreviation	Full Name
ACS	Acute chest syndrome
ALT	Alanine aminotransferase
AST	Aspirate aminotransferase
ATPase	Adenyltriphospstase
AV	Arterio venous
BMD	Bone mineral density
Ca^{٢}	Calcium
CBC	Complete blood count
CNS	Central nervous system
CO^{٢}	Carbon dioxide
CRP	C - reactive protein
CSSCD	Cooperative study of sickle cell disease
DBP	D-binding protein
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immune sorbent asaay
ESR	Erythrocyte sedimentation rate
ESRD	**:** End-stage renal disease
FDA	Food and drug association
FACS	Fluorescent-activated cell sorting
FGF-^{٢٣}	Fibroblast growth factor-^{٢٣}
FSGS	Focal segmental glomerulosclerosis
GFR	Glomerular filtration rate
Hb	Hemoglobin
Hb AS	Sickle cell trait
HBB	Hemoglobin beta globin gene
HBC	Hemoglobin C disease
HbF	Hemoglobin F
HbS	Hemoglobin S
HBSC	Hemoglobin S-C disease
HbSS	Hemoglobin of **s**ickle cell disease
HCT	Hematocrit
HPLC	High performance liquid chromatography

IEF	IsoElectric focusing
IQs	Intelligence quotients
K^+	Potassium
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MiA	Microalbuminuria
MPV	Mean platelet volume
MRI	Magnetic resonance imaging
Na^+	Sodium
O٢	Oxygen
OM	Osteomyelitis
OSC	Orbital compression syndrome
PBM	Peak bone mass
PCR	**:** Polymerase chain reaction
PCV	Packed cell volume
PD	Parkinson's disease
PI	Isoelectric points
PTH	Parathyroid hormones
Plts	Platelets
RBCs	Red blood cells
RDW	Red Distribution Width
RFLP	Restriction fragment length polymorphism
SCA	Sickle cell anemia
SCD	Sickle cell disease
SPSS	Statistical package for social sciences
STD	Standard deviation
TCD	Trans cranial doppler
TMB	Tetra methyl benzidine
UCD	Urine concentrating defect
UK	United kingdom
US	United state
UV-B	Ultraviolet-B
VD	Vitamin D
VDD	Vitamin D deficiency
VDR	Vitamin D receptor
WBCs	White blood cells

Chapter I
Introduction

١,٠ Introduction

Sickle cell disease (SCD) is an inherited disorder.(Soe, *ethic* ٢٠١٧) caused by a variant (rs٣٣٤) in the -globin gene encoding hemoglobin. It is one of the most common and severe monogenic disorders worldwide, mutation of the rs٣٣٤ nucleotide from a thymine to an adenine base pair produces a hydrophobic motif, which, when deoxygenated, leads to polymerization and crystallization of the hemoglobin molecule, causing a sickle shape (Serarslan *ethic* ٢٠١٠).Role of blood transfusion and hydroxylcarbamide for prevention of the complications is starting to be understood(Rees, *ethic* ٢٠١٠). Recurrent episodes of vaso-occlusion and inflammation result in progressive damage to most organs, including the brain, kidneys, lungs, bones, and cardiovascular system, which become apparent with increasing age , children with sickle-cell anemia are more likely to develop vitamin D deficiency when compared to thehealthy controls (Rees, *ethic* ٢٠١٠).Vitamin D are important for bone metabolism,which determines growth failure, it is associated with increased respiratoryinfections, muscle weakness and increased risk of falls and microlesions. (Holick, ٢٠١٢) .Bones of children with SCD are affected by infarcts, osteoporosis, osteomyelitis, or osteonecrosis, all associated with an increased risk of fractures and bone deformities (Serarslan *ethic* ٢٠١٠). Thus, low levels of vitamin D could further impair the bone comorbidities frequently associated to children with SCD (Gharrido*ethic* ٢٠١٢).Studies on the relationship between vitamin D and cytokines are scanty, and none has been conducted among SCD patients. Since SCD has been described as a chronic inflammatory condition, this study aims to determine the serum levels of pro- and anti-inflammatory cytokines in children with SCA and compare with haemoglobin AA matched controls. In addition, because of the postulated anti-inflammatory property of vitamin D, we examined the relationship that exists between serum cytokine levels and ٢٥-hydroxyvitamin D (٢٥-OHD) in the patients and the effects of daily vitamin D supplementation on these cytokines.(Samuel Ademola, et al, ٢٠١٧)

١,١ Rationale:

The sickle-cell disease is most prevalent in sub-Saharan Africa, where ٢٧٪ of Saudi Arabia's population is carrier-positive, for instance. discovered four causes for the rise in prevalence: a high proportion of congenital marriages; big families; a lack of efficient illness prevention and awareness programs; and a dearth of patient advocacy groups. Patients with SCD are typically kept in their homes, given emergency room care, or admitted to the hospital when a crisis occurs. These elements alter a patient's appetite, reduce their dietary intake, and limit their exposure to sunshine, all of which contribute to vitamin D insufficiency.The aim of this study is to detect ٢٥-hydroxyvitamin D٣ as a modifier of sickle cell complications among Saudi children in Dammam, Kingdom of Saudi Arabia.

١,٢ Objectives

١,٢,١ General objective:

To Detection of ٢٥-hydroxyvitamin D٣ as modifier of sickle cell complication among Saudi childreninDammam, Kingdom of Saudi Arabia.

١,٢,٢ Specific objectives:

١. To estimate level of ٢٥-OH vitamin D in children with SCD (as case) and children without sickles anemia (as control) using Enzyme Linked Immunosorbant Asaay(ELISA).

٢. To correlate between level of ٢٥-OH vitamin D and hemoglobin electrophoresis in children with SCD.

٣. To determine mean vitamin D ٢٥-OH level and CBC parameteramong study volunteers.

٤. To comparison between vitamin D ٢٥-OH level and study variables (Age, gender, type of treatment, history of disease and number of blood transfusion and history of crisis).

Chapter II

Literature Review

٢,٠ Introduction (SCD)

Sickle cell disease (SCD) was first reported by Herrick in ١٩١٠ even though reports suggest prior description of the disorder it is the result of homozygous and compound heterozygote inheritance of a mutation in the β-globin gene (Atagaethic ٢٠١٠). A single base-pair point mutation (GAG to GTG) results in the substitution of the amino acid glutamic acid (hydrophilic) to Valine (hydrophobic) in the ٦th position of the β-chain of hemoglobin referred to as hemoglobin S (HbS) **(**Hobanethic٢٠١٦). Phenotypic variation in clinical presentation is a unique feature of disease despite a well-defined Mendelian inheritance, the first to be molecularly characterized as described by Pauling and confirmed to be due to a single amino acid substitution by Ingram almost ٧٠ years ago. Disease is a multi-organ or multi-system disorder with both acute and chronic complications presenting when foetalhemoglobin (HbF) drops towards the adult level by five to six months of age **(**Hobanethic ٢٠١٦**).**

٢,١ History of Sickle Cell Disease

The first modern report of sickle cell disease has been in ١٨٤٦, where the autopsy of an executed runaway slave was discussed; the key finding was the absence of the spleen. Reportedly, African slaves in the United States exhibited resistance to malaria, but were prone to leg ulcers (Basit, ٢٠١٣). The abnormal characteristics of the red blood cells, which later lent their name to the condition, was first described by Ernest E. Irons (١٨٧٧–١٩٥٩), intern to Chicago cardiologist and professor of medicine James B. Herrick (١٨٦١–١٩٥٤), in ١٩١٠ (Serjeant,٢٠١٠).Irons saw "peculiar elongated and sickle-shaped" cells in the blood of a man named Walter Clement Noel, a ٢٠-year-old first-year dental student from Grenada.Noel had been admitted to the Chicago Presbyterian Hospital in December ١٩٠٤ suffering from anaemia(Serjeant, ٢٠١٠). Noel was readmitted several times over the next three years for "muscular rheumatism" and "bilious attacks" but completed his studies and returned to the capital of Grenada (St. George's) to practice dentistry (Ballasethic ٢٠١٢). He died of pneumonia in ١٩١٦ and is buried in the Catholic cemetery at Sauteurs in the north of Grenada. Shortly after the report by Herrick, another case appeared in the Virginia Medical Semi-Monthly with the same title, "Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe anemia (Deverethic٢٠١٦).Memphis physician Lemuel Diggs, a prolific researcher into sickle cell disease, first introduced the distinction between sickle

cell disease and trait in ١٩٣٣, although until ١٩٤٩, the genetic characteristics had not been elucidated by James V. Neel and E.A. Beet ١٩٤٩ was the year when Linus Pauling described the unusual chemical behavior of hemoglobin S, and attributed this to an abnormality in the molecule itself (Ballas*ethic* ٢٠١٢),the actual molecular change in HbS was described in the late ١٩٥٠s by Vernon Ingram. The late ١٩٤٠s and early ١٩٥٠s saw further understanding in the link between malaria and sickle cell disease. In ١٩٥٤, the introduction of hemoglobin electrophoresis allowed the discovery of particular subtypes, such as HbSC disease (Dever*ethic* ٢٠١٦).

٢,٢ Prevalence of Sickle Cell Disease

The gene frequency is highest in West African countries with ١ in ٤ to ٣ (٢٥–٣٠٪) being carriers of HbS compared to ١/٤٠٠ African Americans and is variable in European populations(Lobitz *ethic* ٢٠١٨). The prevalence of SCD in developed countries is increasing partly due to migration from high prevalent countries(Lobitz *ethic* ٢٠١٨). It is estimated that over ١٤,٠٠٠ people live with SCD in the UK, similar to France, while countries like Italy, Germany have seen increasing numbers from Africa (Dormandy*ethic* ٢٠١٧).With increasing survival, the age distribution of SCD is changing from a childhood disorder pattern that patients now survive into adulthood and old age(Grosse,*ethic* ٢٠١١) It is now reported that over ٩٤٪ of those born with SCD now survive into adulthood in the US, France and UK in contrast to the high mortality in SSA where ٥٠–٩٠٪ may die in the first five years of life (Quinn *ethic* ٢٠١٠**).** In low resource settings and countries where newborn screening is not yet standard care, patients may die young even before diagnosis is confirmed among the common causes of death in the absence of early diagnosis followed by education and preventive therapies such as penicillin prophylaxis and regular surveillance include infections, severe anemia (acute splenic sequestration, aplastic anemia) and multi-organ failure (Chakravorty*ethic*٢٠١٥) .It is essential therefore that newborn and early infant diagnosis is given the priority it deserves by those countries where SCD is a public health problem (Kuznik*ethic*٢٠١٥).The implementation of early infant diagnosis remains out of reach for the majority of countries in SSA despite multiple declarations by international organizations and public statements by politicians to honor such commitments (Weatherall, ٢٠١١). The benefits for screening can only become meaningful when such practice is embraced by policy-makers across the continent and India where the majority of SCD are born and live (Weatherall, ٢٠١١). The prevalence of SCD in Saudi Arabia varies significantly in different parts of the country, with the highest prevalence is in the Eastern province,

followed by the southwestern provinces. The reported prevalence for sickle-cell trait ranges from ٢٪ to ٢٧٪, and up to ٢,٦٪ will have SCD in some areas (Jastaniah ٢٠١١).

٢,٣ Pathophysiology of Sickle Cell Disease

Red blood cells (RBCs) that contain HbS or HbS in combination with other abnormal β alleles, when exposed to deoxygenated environment undergo polymerization and become rigid (Ballas*ethic* ٢٠١٢).The rigid RBC's are liable to hemolysis, density may affect blood flow and endothelial vessel wall integrity (Lindenau *ethic* ٢٠١٦).The dense rigid RBC's lead to vaso-occlusion, tissue ischemia, infarction as well as hemolysis (Lindenau *ethic*٢٠١٦).The consequence of hemolysis is a complex cascade of events including nitric oxide consumption; hemolysis linked nitric oxide dysregulation and endothelial dysfunction which underlie complications such as leg ulceration, stroke, pulmonary hypertension and priapism (Hebbel,٢٠١٤). Unlike normal RBC's with half-life of approximately ١٢٠ days, sickle RBC's (sRBC) may survive just ١٠–٢٠ days due to increased hemolysis during deoxygenation; healthy hemoglobin rearranges itself into a different conformation, enabling binding with carbon dioxide molecules which reverts to normal when released (Heeney *ethic* ٢٠١٦). During acute sickling, intravascular hemolysis results in free hemoglobin in the serum, while RBC's gaining Na^{+}, $Ca^{٢+}$ with corresponding loss of K^{+}. Increase in the concentration of $Ca^{٢+}$leads to dysfunction in the calcium pump (Makani *ethic* ٢٠١٥). Calcium depends on ATPase but it is unclear what role calcium plays in membrane rigidity attributed to cytoskeletal membrane interactions. Furthermore, hypoxia also inhibits the production of nitric oxide, thereby causing the adhesion of sickle cells to the vascular endothelium (Grosse*ethic* ٢٠١٦). Lysis of erythrocytes leads to increase in extracellular hemoglobin, thus increasing affinity and binding to available nitric oxide or precursors of nitric oxide; thereby reducing its levels and further contributing to vasoconstriction (Quinn *ethic* ٢٠١٠).

٢,٤ Complications of Sickle Cell Disease

٢,٤,١ Anemia

Associated with sickle cell disease is chronic and caused by intravascular hemolysis resulting in a reduced lifespan of the abnormal red blood cells (١٠–٢٠ days compared with ١٠٠–١٢٠ days in a healthy adult) (Hsu *ethic* ٢٠١٦). Children with sickle cell disease may compensate fromanemia with an increasing heart rate and stroke volume, but often suffer from reduced stamina when taking part in physical exercise at school. It is will worsen during a vaso-occlusive crisis and presence of Parvovirus B١٩ infection as the hemolytic rate increases. Anemia can also worsen in and if the blood pools in the liver or spleen, known as sequestration (Hsu *ethic* ٢٠١٦).

٢.٤,٢ Acute Splenic Sequestration

Variations in size of spleen during childhood in SCD. It may be initially enlarged in children with SCD but may become dysfunctional as early as in first year of life (Gyang *ethic*٢٠١١).More than ٩٠٪ of children with sickle cell anemia (SCA) may have total loss of functional splenic tissue by early childhood .Children with SCA who have not yet gone through auto splenectomy, as well as SC disease and sickle beta thalassemia, may be at risk for developing splenic sequestration (Newaskar *ethic*٢٠١١).Acute splenic sequestration in SCD results from the trapping of red cells in the splenic sinuses which leads to a sudden rapid enlargement of the spleen which could be massive. These episodes are generally associated with viral or bacterial infections (Gyang *ethic*٢٠١١). Patients present with pallor, tachycardia, tachypnea, weakness, abdominal pain and distension, and shock due to hypovolemia and acute decline in hemoglobin level. Mild thrombocytopenia may also be present (Terpos and Voskaridou,٢٠١٠). Immediate treatment of acute splenic sequestration includes the correction of hypovolemia to avoid hypovolemic shock and the transfusion of packed red cells to maintain oxygen-carrying capacity. Once the cardiovascular status is restored, the patient improves rapidly and the spleen shrinks in few days releasing the trapped red cell back into circulation (Newaskar *ethic* ٢٠١١).

٢,٤,٣ Gastrointestinal/Hepatobiliary Complications

Sickle cell disease affects the hepatobiliary system in different ways at different ages. Intrinsic disease results from recurrent ischemia and bilirubin stones. These result from the vascular obstruction and red cell hemolysis of sickle cell (Ghugre and Wood, ٢٠١١). Biliary sludge is a common finding that is often clinically unimportant. Viral infections that affect the liver may be independent of or secondary to red cell transfusions (Tsay *ethic* ٢٠١٠). The iron overload that accompanies red cell transfusions can lead to liver dysfunction and fibrosis (Ghugre and Wood, ٢٠١١). Many medications taken by sickle cell patients may cause or worsen hepatobiliary disease. The dysfunction of the liver can affect the lungs, kidneys, and coagulation systems. Treatment is directed at the etiology of the dysfunction as well as the underlying sickle cell disease (Guggenbuhl *ethic* ٢٠١١). Chronic cholecystitis may be related to persistent gallstones or persistent biliary sludge. Recurrent symptoms consistent with colic warrant screening with blood work and imaging. If the blood work shows increases in conjugated (direct) bilirubin during the attacks, and there are ultrasonographic signs of a thickened gallbladder wall, then cholecystectomy may decrease these symptoms. However, just as in chronic cholecystitis in the general population, the symptoms may recur several months after surgery (King *ethic* ٢٠١١).

٢,٤,٤ Osteomyelitis/Septic Arthritis

Bacterial infections involving the cortical bone (osteomyelitis) and joint space (septic arthritis) have been commonly reported in SCD, particularly in association with avascular necrosis and bone infarcts (Hernigou *ethic*٢٠١٠).The prevalence of OM is lower in individuals with the Bantu haplotype, and it may occur as a complication of severe leg ulcers .The most common etiologic organism in sickle OM is salmonella followed by Staphylococcus aureus and enteric gram-negative bacilli .The femur, tibia, and humerus are the most commonly affected sites (Minniti *ethic* ٢٠١٠).The presence of pain, swelling, and immobility around a joint is usually assumed to be from a typical vaso-occlusive episode. Persistence of symptoms of pain and swelling with or without fevers should prompt imaging studies and further laboratory workup, serum CRP should be obtained and if elevated should raise suspicion for septic arthritis. The CRP is typically the first marker to be elevated and the first to respond to treatment. Delayed diagnosis is associated with rapid joint deterioration and collapse (Enninful *ethic* ٢٠١٠).

٢.٤,٥ Neurological Complications

Ischemic stroke data from the cooperative study of sickle cell disease (CSSCD) revealed that stroke occurred in ١١٪ of children with hemoglobin SS (HbSS) below the age of ٢٠ years (Hinesethic ٢٠١١). However, the use of Trans Cranial Doppler ultrasonography (TCD) in the past two decades to identify persons at high risk for ischemic stroke and the prophylactic management of those patients with chronic transfusion has dramatically reduced the incidence of childhood stroke to approximately ٢-٣٪. Consistent with previous CSSCD findings, a recent retrospective study confirmed that high systolic blood pressure, leukocytosis, and severe anemia were correlated with MRI-documented brain injury in children with sickle cell anemia (Vichinsky *ethic* ٢٠١٠). Seizure, sensory, and motor events were associated with the highest risk for brain injury, while the less specific problems of headache and poor school performance were not correlated with increased risk (Kavanagh *ethic* ٢٠١١).

٢,٤,٥.١ Ophthalmologic Complications

Vaso-occlusion can affect any vascular bed in the eye, including the conjunctiva, anterior segment, retina, choroid, or optic nerve with potentially blinding consequences (Elagouz, *ethic* ٢٠١٠).

٢,٤,٥,٢ Orbital Involvement

Sphenoid bone infarction led to a subperiosteal hematoma and an inflammatory response that resulted in acute proptosis, periorbital pain, restricted motility, and compressive optic neuropathy (Cho and Kiss, ٢٠١١). Paton reported that these comma signs were more common in SS than in SC disease and were uncommon in patients with high HbF levels. The results of a study investigating the influence of clinical, laboratory, and genetic features on conjunctival and retinal vessel alterations indicated that low levels of Hb and hematocrit may be risk factors for conjunctival alteration; abnormalities were more evident in patients with SS disease (Amtmann *ethic* ٢٠١٠).

٢,٤,٥,٣ Proliferative Sickle Retinopathy (PSR)

Arteriolar occlusion and loss of capillary perfusion in the peripheral retina are the most striking features of sickle cell retinopathy. They are generally more prominent in the temporal peripheral retina, especially super temporally (Rajagopal and Apte, ٢٠١٠). Ischemic areas caused by these occlusions release substances that can stimulate angiogenesis, the initial vascular remodeling at the junction between the perfused central and no perfused peripheral retina includes the creation of arterio venous (AV) anastomoses and hairpin loops (Rajagopal and Apte, ٢٠١٠).

٢,٤,٦ Pulmonary Complications

٢,٤,٦,١ Acute Chest Syndrome

The clinical manifestations of acute chest syndrome (ACS) complicating SCD include chest pain, tachypnea, fever, hypoxia, dyspnea, cough, leukocytosis, decreasing Hb level, and new infiltrates on chest X-ray (Alhashimi ,٢٠١٠). Not all these signs and symptoms occur in all cases of ACS with the exception of the new pulmonary infiltrates which are considered the sine qua non for the diagnosis (Head *ethic* ٢٠١٠). About ٥٠٪ of ACS episodes occur after hospital admission for acute painful crises Moreover, ACS seems to be the most common cause of death among patients and the second most common cause of hospitalization of patients with SCD (Alhashimi ,٢٠١٠).

٢,٤,٧ Renal/Genitourinary Complications

Infants with SCD have hyposthenuria/urine concentrating defect (UCD), supranormal glomerular filtration rate (GFR) and proximal tubular function, and an impaired ability to acidify urine or excrete potassium reviewed in references (Ware *et al* ٢٠١٠). A majority of patients with SCD have evidence of microscopic hematuria and may even develop gross hematuria from renal papillary necrosis, older individuals have been found to have glomerulopathy, that manifests as microalbuminuria (MiA, defined as urine albumin of ٣٠–

٣٠٠ mg/g urine creatinine), macroalbuminuria (MaA, defined as urine albumin >٣٠٠ mg/g urine creatinine), or end-stage renal disease (ESRD) (Ware *ethic*٢٠١٠).

٢,٤,٧,١ Hematuria

Vascular obstruction from sickled RBCs leads to microscopic-to-gross painless hematuria, occurring from medullary congestion and renal papillary necrosis, the hypoxic (pO٢ ٣٥–٤٠ mm Hg), hyperosmolar medullary environment promotes sickling (Sundaram *ethic* ٢٠١١). The vasa recta become congested, tortuous, occluded, and hemorrhage, resulting in painless hematuria (Sundaram *ethic* ٢٠١١).

٢,٤,٧,٢ Tubular Defects

Urine-concentrating defect the first manifestation of distal tubular defect is impaired urine concentrating ability, which is almost universal in patients with SCD and occurs in children, even infants.UCD is transiently reversible before ١٠–١٥ years of age with RBC transfusions but becomes irreversible thereafter (Ataga *ethic*٢٠١٠).UCD in SCD has been attributed to polymerization of Hb S in the hyperosmolar, acidic and relatively hypoxic renal medulla, resulting in sludging of blood flow in the vasa recta, loss of medullary osmolar gradient, and eventual destruction of the vasa recta from vaso-occlusions/thrombosis . UCD is associated with increased tendency to dehydration and sickling, enuresis and nocturia (Ataga *ethic* ٢٠١٠).

٢,٤,٧,٣ Glomerulopathy

Glomeruli, especially the juxtamedullary glomeruli in young SCA patients, are enlarged and congested, reaching a size that is ٦٠–٨٠٪ larger than normal glomeruli (Drasar *ethic* ٢٠١١). Glomerulopathy is associated with albuminuria, with MiA present in ٢٥٪ of children <١٠ years of age, and ٤٥٪ of adults. MaA develops later with progression to FSGS. Glomerular lesions are typically FSGS, mesangial proliferation, endothelial damage and sclerosis from hyperfiltration, immune-complex nephritis from autoantigens released from damaged tubules, and deposition of iron protein complexes in the kidney. A small proportion of patients have membranoproliferative glomerulonephritis with or without immune deposits (Kato and Taylor, ٢٠١٠).

٢,٤,٨ Priapism

Is a condition in which a penis remains erect for hours in the absence of stimulation or after stimulation have ended,there were three types: ischemic (low-flow), nonischemic (high-flow), and recurrent ischemic (intermittent).Most cases are ischemicIschemic priapism is generally painful while nonischemic priapism (Brunetta *ethic*٢٠١١).In ischemic priapism, most of the penis is hard; however, the glans penis is not.Innonischemic priapism, the entire penis is only

somewhat hard Very rarely, clitoral priapism occurs in women.(Sickle cell disease is the most common cause of ischemic priapism.Other causes include medications such as antipsychotics, SSRIs, and blood thinners, as well as drugs such as cocaine and cannabis(Fernandes *ethic* ٢٠١٨).

٢,٥ Diagnosis of Sickle Cell Disease

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. (Kavanagh *ethic* ٢٠١١).People who are known carriers of the disease often undergo genetic counseling before they have children (Ribeil *ethic* ٢٠١٧). 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 who carry the sickle cell trait, additional tests are needed to confirm which form of SCD the patient has. There are four tests that are commonly used: hemoglobin electrophoresis, isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and DNA analysis. (Greene *ethic* ٢٠١٥) 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. (Ribeil*ethic* ٢٠١٧).

٢,٦ Laboratory Finding of Sickle Cell Disease

٢,٦,١ CBC and Blood Film

Homozygous SS and heterozygous S/β° patients typically have lower RBC count, hemoglobin, and hematocrit due to the hemolytic anemia. On the other hand, WBC count, platelet count, and reticulocyte count are elevated. (Howard and Oteng, ٢٠١٢) Furthermore, RDW is elevated in SCD patients due to increased heterogeneity within RBC subpopulations. (Howard and Oteng, ٢٠١٢). Hemoglobin levels in the range of ٦-٨ 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 were seen (Dixit*ethic* ٢٠١٨).

٢,٦,٢ Sickling test

The principle of sickling test was based on microscopical observation of sickling of red blood cells when exposed to a low oxygen tension. The proportion of the number of red blood cells

that were sickled was then expressed as percentage and results were considered positive when more than ٢٥٪ of the red blood cells sickled (Okwi *ethic*٢٠١٠).

٢,٦,٣ Sickle solubility test

The presence of sickle hemoglobin can also be demonstrated with the "sickle solubility test"A mixture of hemoglobin S (HbS) in a reducing solution(such as sodium dithionite) gives a turbid appearance, whereas normal Hb gives a clear solution.(Dixit *ethic* ٢٠١٨).

٢.٦,٤ Hemoglobin electrophoresis

Abnormal hemoglobin forms can be detected on hemoglobin electrophoresis, a form of gel electrophoresis on which the various types of hemoglobin move at varying speeds, sickle cell hemoglobin (HgbS) and hemoglobin C with sickling (HgbSC) the two most common forms can be identified from there (Gladwin *ethic* ٢٠١١).

٢,٦,٥ High Performance Liquid Chromatography (HPLC)

HPLC separates a fluid into its components based on molecular size and charge using cation exchange chromatography to identify the various hemoglobin types in a blood sample; it utilizes absorbent materials such as granular silica or other polymers as a sieving medium. (Makani *ethic*٢٠١٣).A pressure pump drives the fluid through the material, and a computer detects the separation. Unique aspects of this test are full automation and accurate quantification of the hemoglobin levels (Makani *ethic* ٢٠١٣).

٢,٦,٦ Isoelectric focusin IEF

IEF exploits the fact that the net charge of a protein varies with the pH of the surrounding medium. Utilizing this variation, proteins are separated based on their isoelectric points (pI), which can be defined as the point at which a protein possesses zero net charge. The technique uses an applied electrical field across a gel medium with a fixed pH gradient, in which each Hb type becomes immobilized once it reaches its pI. IEF exhibits higher resolution than Hb electrophoresis, thus it is capable of distinguishing between a larger number of Hb variants (Bain *ethic*٢٠١٣).

٢,٦,٧ Molecular Methods

Molecular testing for hemoglobinopathies generally uses three techniques. These are Restriction Fragment Length Polymorphism (RFLP), Allelic Discrimination using Real Time PCR end point data and DNA Sequencing. DNA extraction of whole blood in the DBS filter paper matrix is needed for all PCR-based assays. Methods for DNA extraction include crude boiling preparations, alkaline lysis preparations and commercial methods. (Cordovado, ٢٠١٣).

٢,٦,٨ Flow cytometry

Flow cytometry Surface characteristics of blood cells are typically measured with conventional techniques, such as fluorescent-activated cell sorting (FACS), immunohistochemistry, or microscopic imaging methods. In FACS, cells of interest are isolated, extensively processed, incubated with a fluorescent-labeled antibody raised against a cellular protein (e.g. integrin, receptor, adhesion molecule), and sorted by optical recognition (Manwani *ethic* ٢٠١٥). Measurement by flow cytometry of aberrant surface molecule expression or activation has served as a surrogate for directly measuring abnormal adhesion in humans with SCD (Picot *ethic*٢٠١٤).

٢,٦,٩ Additional Test

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. (Ribeil*ethic* ٢٠١٧).

٢.٧Follow up of SCD in children

٢,٧,١ Liver function Test

The wide range of hepatic dysfunctions that occur in SCD patients are not only a result of the sickling process but also a result of multiple blood transfusions that these patients undergo in their lifetimes (Simon*ethic*٢٠١٦). Chronic hemolysis leads to elevated bilirubin levels (mainly unconjugated) which correlated with lactate dehydrogenase levels, elevated plasma aspartate transaminase (AST) and plasma alanine transaminase (ALT), and altered liver function test results (Ebert *ethic*٢٠١٠).

٢.٧,٢ Kidney function test

Serum potassium, phosphate, and uric acid were higher while sodium, chloride, bicarbonate, calcium, and eGFR were lower in SCA patient(Rasheed*ethic*٢٠١٧)

٢.٨Treatment for SCD

SCD is a disease that worsens over time. Treatments are available that can prevent complications and lengthen the lives of those who have this condition. These treatment options can be different for each person depending on the symptoms and severity. Hydroxyurea is a medicine that can decrease several complications of SCD (Gardner, ٢٠١٨). This treatment is very safe when given by medical specialists experienced caring for patients with SCD. However, the side effects of taking hydroxyurea during pregnancy or for a long time are not completely known (Niihara *ethic*٢٠١٨). The Food and Drug Administration has also approved a new medicine to reduce the number of sickle cell crises in adults and children older than age five; it is called Endari (L-glutamine oral powder) (Niihara *ethic*٢٠١٨).

Another treatment, which can actually cure SCD, is a stem cell transplant (also called a bone marrow transplant); this procedure infuses healthy cells, called stem cells, into the body to replace damaged or diseased bone marrow (bone marrow is the center of the bone where blood cells are made) (Wiebking *ethic* ٢٠١٧). Although transplants of bone marrow or blood from healthy donors are increasingly being used to successfully cure SCD, they require a matched donor (a person with similar, compatible bone marrow), and transplants can sometimes cause severe side effects, including occasional life-threatening illness or death (Wiebking *ethic* ٢٠١٧).Couple that with world-renowned diagnostic experts and the most advanced diagnostic techniques to enable physicians to detect sickle cell anemia as early as possible and patients are experiencing improved outcommes, faster response to treatment and fewer side effects (Memish and Saeedi,٢٠١١). Some treatments that reduce HbS polymerization like GBT٤٤٠ (Voxelotor) is an oral small molecule designed to increase the oxygen affinity of HbS, shifting the oxygen dissociation curve of oxy-HbS to the left (Metcalf*ethic* ٢٠١٧) Nutritional Supplements Like Omega-٣ fatty acids have been purified from fish oil and tested for benefits as antioxidant, antithrombotic, and anti-inflammatory benefit. It decreased pain and decreased platelet activation in adults with sickle cell anemia (Daak*ethic* ٢٠١٢). Folic acid is widely prescribed for SCD with the rationale that increased erythropoiesis (Dixit*ethic* ٢٠١٨).

٢,٩Vitamin D

٢.٩.١ Introduction

Vitamin D (VD) is one of the lipophilic vitamins. The most important forms of VD are cholecalciferol (vitamin D٣, VD٣) and ergocalciferol (vitamin D٢, VD٢) (Basu *ethic* ٢٠١٥). It is the main form and is available in some natural dietary products (egg yolk, flesh of fatty fish, and fish liver oils), food fortified with VD, and many forms of dietary supplements. VD٢ is of plant origin and present in low amounts, e.g., in some mushrooms. It being less potent than VD٣, is rarely present in commercial preparations and fortified food. Despite that, it is a good alternative for vegans and vegetarians (Basu*ethic* ٢٠١٥). However, the main source of VD is endogenous synthesis from٧-dehydrocholesterol in the human skin after sun exposure. Part of VD is stored in adipose and muscle tissue, and part of it gets hydroxylated. Independent of the source, VD٣ and VD٢ act as hormone precursors since they require two stages of metabolism: First to ٢٥-hydroxy VD (٢٥(OH) D, calcidiol) in the liver; then to ١α, ٢٥-dihydroxy VD (١, ٢٥(OH) D, calcitriol) in the kidney (Wimalawansa,٢٠١٢). ٢٥(OH) D٣ is significantly less active than calcitriol and is transported in the circulation by binding to

VD binding proteins. The amount of circulating ٢٥(OH) D٣ is the most reliable measurement and is thought to reflect body VD status best (Wimalawansa,٢٠١٢).It is considered that most people are in sufficient or deficient in VD due to a lack of sun exposure, extensive use of sunscreens, which block VD synthesis, and poor dietary intake. Maintaining recommended serum levels, i.e., ٣٠–٦٠ ng/mL of ٢٥(OH) D٣, can be achieved through vitamin supplementation or food fortification without changing lifestyle to avoid impaired skeletal and overall health (Holick*ethic*٢٠١١).

٢,٩.٢ Vitamin D structure

Vitamin D has a secosteroid structure in which a bond (C٩–C١٠) in ring B of the steroid structure is broken. Vitamin D٣ and vitamin D٢ are produced by the photochemical reaction of ٧-dehydrocholestrol and ergosterol with ultraviolet light B (naturally with sunlight), and subsequent heat isomerization, respectively. These two chemical reactions (not enzymatic reactions) are essential for vitamin D synthesis. In human, these reactions of ٧-dehydrocholestrol occur in the skin (Yoshihiko *ethic*٢٠١٦).

٢,٩.٣ 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 ٥٠٪ of a dose of vitamin D is absorbed (McBeth *et al*, ٢٠١٠).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 (Levin *ethic* ٢٠١١).

٢,٩.٤ 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, ٧-dehydrocholesterol) to produce previtamin D, which is then slowly isomerized to vitamin D٣ It is the natural form of vitamin D produced in skin, and vitamin D٢ is derived from irradiation of ergosterol, which occurs to some degree in plankton under natural conditions and is used to produce vitamin D ٢ from the mold ergot (which contains as much as ٢٪ ergosterol) (Glover *ethic* ٢٠١٢)..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 rolein several other physiologic systems. Its use may well prevent several degenerative diseases, and it may also play a role as an anticancer agent (Gupta *ethic* ٢٠١١).

٢,٩.٥ Vitamin D physiology

Vitamin D was initially described as a substance that was able to cure rickets and was termed 'D' as it was the fourth in the sequence of vitamins discovered (Macdonald *ethic* ٢٠١١). The main two isoforms are vitamin D٣ (cholecalciferol) and vitamin D٢ (ergocalciferol) that share a similar metabolism so that we will not differentiate between these isoforms unless otherwise stated. It has been roughly estimated that ultraviolet-B (UV-B)-induced production of vitamin D in the skin accounts for about ٨٠٪ of vitamin D supply, whereas dietary intake (e.g. fish, eggs or vitamin D-fortified food) plays usually only a minor role (Ross *ethic* ٢٠١١). In the circulation, vitamin D metabolites are mainly bound to vitamin D-binding protein (DBP) and to a lesser extent to albumin and lipoproteins with only a small fraction (less than ١٪) circulating in its unbound (free) form .Although some tissues can take up DBP-bound vitamin D metabolites by the megalin–cubilin system, most cells seem to be dependent on free vitamin D metabolites that diffuse through the cell membrane to get access to the intracellularly located VDR (Pludowski *ethic* ٢٠١٧).Therefore, measurements of free ٢٥(OH)D might be useful in special conditions with significantly altered DBP levels (e.g. pregnancy, liver cirrhosis or hormonal contraceptive intake), but more data are needed to clarify the clinical significance of free ٢٥(OH)D. Vitamin D catabolism is initiated by ٢٤-hydroxylation of vitamin D metabolites that are finally excreted in the bile and urine (Reid , ٢٠١٨).

٢,٩.٦ Vitamin D in Bone health

Bone mass acquisition is influenced by both genetics and lifestyle-related factors, such as vitamin D status, physical activity and calcium intake (Golden and Abrams, ٢٠١٤).Vitamin D contributes significantly to bone mineralization by promoting intestinal calcium and phosphorus reabsorption (Savino *ethic* ٢٠١١).Moreover, vitamin D stimulates skeletal calcium and phosphorus and renal calcium reabsorption. Besides the direct regulation of calcium-phosphorus metabolism, vitamin D also indirectly promotes bone mass accrual stimulating the development of muscle tissue (Gallo *ethic* ٢٠١٣).Vitamin D status seems particularly important for bone health during adolescence. Indeed, duodenal expression of ٢٥-hydroxyvitamin D٣-١α-hydroxylase is higher in adolescents than in children and adults, representing a metabolic adaptation to promote dietary calcium absorption for the growing bone (Bagnoli*ethic* ٢٠١٣)**.**

٢,٩.٧ Consequences of vitamin D deficiency

Skeletal manifestations the commonly known consequences of Vitamin D deficiency are rickets in children and osteomalacia and osteoporosis in adults (Hazell *ethic* ٢٠١٥).In

children, it causes defective mineralization of bone due to imbalance between calcium and phosphorous in the bone, resulting in rickets and external skeletal deformity. It also causes muscle weakness and bone pain. In adults, inadequate dietary intake of Vitamin D leads to poor absorption of calcium from diet and increased calcium resorption from the bone and kidney and reduces bone mineral density resulting in osteoporosis and osteomalacia, muscle weakness and increased risk of falls. It is theorized that Vitamin D may increase muscle strength, thereby preventing falls. Many studies have shown an association between low Vitamin D concentrations and an increased risk of fractures and falls in older adults (Suryanarayana *ethic* ٢٠١٨).

٢,٩.٧.١ Depression

Vitamin D deficient patients took significantly longer duration for recovery than nondeficient persons. It signifies the importance of treating hypovitaminosis D for the effective management of depression (Kumar *ethic* ٢٠١٣).

٢,٩.٧.٢Parkinson's disease

Vitamin D insufficiency was seen in patients with Parkinson's disease (PD). Evidence suggests VDR as a genetic risk factor for PD, thereby underlining the potential importance of Vitamin D in PD. As Vitamin D status is a modifiable factor, Vitamin D acts as a potential preventive/therapeutic strategy for this disorder. However, there is a need for further studies on VDR as well as its interaction with Vitamin D levels in PD (Kapil *ethic* ٢٠١٧).

٢,٩.٧.٣ Infectious disease

Infectious disease such as tuberculosis, upper respiratory tract infections of viral origin, i.e., influenza is seen in individuals with Vitamin D deficiency (Chowdhury *ethic* ٢٠١٧).

٢,٩.٧.٤ Autoimmune diseases

Vitamin D is a potent modulator of immune system, and it is involved in regulating cell proliferation and differentiation. It was shown in a case–control study that Vitamin D deficiency was considerably higher in Type ١ diabetic (٩١٪) children when compared to nondiabetic (٨٥٪) children. Supplementation of Vitamin D resulted in ٣٠٪ reduction in the risk of developing Type ١ diabetes mellitus. Lower levels of Vitamin D were found to be associated with rheumatoid arthritis (Gunjaliya *ethic* ٢٠١٥).

٢,٩.٧.٥ Cancer

Vitamin D has a protective role in certain tissues by promoting apoptosis and inhibiting angiogenesis. Low level of Vitamin D in stores, such as lung, breast, colorectal, prostate, ovary, pancreas and esophagus, are associated with cancers. Vitamin D decreases cell

proliferation and increases cell differentiation. It stops the growth of new blood vessels and has significant anti-inflammatory effects (Misra *ethic* ٢٠١٧).

٢,٩.٧.٦ Heart disease

In the Framingham Heart Study, patients with low Vitamin D concentrations (<١٥ ng/Ml) had a ٦٠٪ higher risk of heart disease (through the renin-angiotensin hormone system) than those with higher concentrations. Severe Vitamin D deficiency is seen in patients with acute myocardial infarction and it is associated with many of its risk factors (Roy *ethic* ٢٠١٥).

٢,٩.٧.٧ Type ٢ diabetes mellitus

Vitamin D deficiency has been associated with increased risk of type ٢ diabetes mellitus, insulin resistance, and decreased insulin production, and hence, it has been associated with syndrome X (Borkar *ethic* ٢٠١٠). A trial of non diabetic patients aged ٦٥ years and older found that those who received ٧٠٠ IU of Vitamin D (plus calcium) had a smaller rise in fasting plasma glucose over ٣ years versus those who received placebo. Evidence reveals that Vitamin D reduces the risk of progression and development of type ٢ diabetes mellitus (Parameaswari *ethic*٢٠١٢).

٢.١٠ Treatment/Management

Adults who are vitamin D deficient require ٦٠٠٠ IU/day of vitamin D٣ for ٨ weeks or ٥٠,٠٠٠ IU of vitamin D٣ once weekly for ٨ weeks. When the serum ٢٥ (OH) D levels exceeds ٣٠ ng/mL, a ٢٠٠٠ IU/day maintenance dose is recommended. Adults who are vitamin D deficient who are at high risk for obesity, taking certain medications, have a malabsorption syndrome, or African American or Hispanic are recommended to take at least ١٠,٠٠٠ IU daily. Once serum ٢٥(OH) Dlevel exceeds ٣٠ng/mL, ٣٠٠٠ to ٦٠٠٠ IU/day maintenance dose is recommended. Children who are vitamin D deficient require ٢٠٠٠ IU/day of vitamin D٣ or ٥٠,٠٠٠ IU of vitamin D٣ once weekly for ٦ weeks (Holick *ethic* ٢٠١١). When the serum ٢٥(OH)D level exceeds ٣٠ ng/mL, ١٠٠٠ IU/day, maintenance treatment is recommended according to the American Academy of Pediatrics, infants who are breastfed and children who consume less than ١L of vitamin D-fortified milk will need ٤٠٠ IU of vitamin D supplementation (Tripkovic ethic ٢٠١٢).

٢,١١ Relation between Vitamin D in children with SCD:

Importantly, since over three decades, a growing body of studies, have reported links between deficiency in vitamin D(٢٥-hydroxy vitamin D; ٢٥-OHD, de facto a hormone), an endocrine organ dysfunction commonly detected in SCA patients, and its health consequences (Hyacinth *ethic*٢٠١٠).Nevertheless, genetics, social, geographical, seasonal,nutritionaland physiological influences, among others, are interconnected and contribute to the life adaptation, specie evolution, chronic diseases epidemiology and their clinical complexity (Hyacinth *ethic*٢٠١٠) .Hence, one should always avoid any generalization considering that

sometimes HbSS patients matched with HbAA control patients may elicit no significant differences in vitamin D levels, and a slight bone mineral density (BMD) decrease could be found in SCA pre-adolescent female children independently of disease severity, vitamin D deficiency, low calcium intake or bone hyper-resorption but maybe due to abnormal bone formation (Kavanagh *ethic* ٢٠١١).In general, the etiologies of vitamin D deficiency are poorly defined in SCA patients (e.g. often attributed to increased skin melanin concentrations, lower dietary intake, reduced levels of physical activity, highly prevalent bone resorption markers), but often result in bony changes and bone fragility (e.g. rickets, osteomalacia, incomplete ossification, low BMD, osteoporosis, osteonecrosis, fracture risk, chronic musculoskeletal pain and weakness, low BMD associated-vasoocclusive crises, hyperparathyroidism (Kavanagh *ethic* ٢٠١١).

٢,١٢ Previous studies

In ٢٠١٩ Adegokeand his college study in Nigeria mean serum ٢٥-OHD of the children with VOC was significantly lower than those in steady state (٢٨,٩±٨,٢ ng/ml vs.٣٧,١±١٢,٣ ng/ml *P.value* (٠,٠٠٤) (Adegoke *ethic* ٢٠١٩).

In ٢٠١٨ Ali Aljama and other study in Saudia Arabia Of those, ٨٢٪ (n=٥٢٣) had suboptimal ٢٥(OH) D (٠-<٣٠ ng/mL), and ٦٧٪ were deficient (٠-<٢٠ ng/mL). Patients with any SCD crisis (٢٠,٧٪, ١٤٤/٦٩٤) had lower ٢٥(OH) D (median, IQR: ١٠,١ ng/mL [٨,٦] ng/mL) compared to patients without crisis (٧١,٠٪, ٤٩٣/٦٩٤) (١٥,٧ ng/mL [١٨,٢] ng/mL) (*P*<.٠٠١) (Aljama *ethic* ٢٠١٨).

In ٢٠١٨ Adegoke and other study in Nigeria vitamin D deficiency (VDD) has been linked to anemia among sickle cell disease (SCD) correlations were significantly positive p.value (٠,٠١٧) VDD may play a role in SCD pathogenesis (Adegoke *ethic* ٢٠١٨).

In ٢٠١٨ Kaitlyn and other study in Canda shown low serum level ٢٥-hydroxyvitamin D (٢٥OHD) concentrations in Canadian Children with Sickle Cell Disease (Samson *ethic* ٢٠١٨).

Study in Salmaniya Medical Complex, Bahrain, Fifty-one patients with SCA had VDD, a prevalence of ٧٣,٩٪ and serum level of vitamin D <٥٠ nmol/L. In general, the prevalence of VDD in SCA varies between ٦٥٪ and ١٠٠٪ depending on the season and geographical area١٤,١٥. Seventeen (٢٤,٦٪) VDD patients had VD insufficiency and ٢٩ (٤٢٪) patients had VD deficiency. (Taysir G, et al, ٢٠١٧).

In ٢٠١٥ de Oliveira and other study in meta-analysis in Brazil eleven articles were selected among the ١٨ found. In ٦ of the ١١ studies, serum levels of vitamin D in children and/or adolescents with sickle-cell anemia were low (De Oliveira *ethic* ٢٠١٥).

Wykes and his colleagues in year ٢٠١٤ in U.K London vitamin٢٥-OHD levels in ٨١ children with SCA and looked for statistical associations with biochemical, haematological and clinical parameters. In a separate group of regularly transfused children with SCA, we compared changes in ٢٥-OHD blood concentrations following treatment with either high-dose intramuscular ergocalciferol (n = ١٥) or ٤ days of high-dose oral cholecalciferol (n = ٦٤). Ninety-one percent of children with SCA had ٢٥-OHD levels <٢٠ μg/L. The ٢٥-OHD levels were negatively correlated with increasing age (P < ٠,٠٠١) (Wykes *ethic* ٢٠١٤).

Jackson and his colleaguesin ٢٠١٢ in USA vitamin D deficiency is known to be common among patients with sickle cell anemia (SCA). Vitamin D levels were measured in ١٣٩ children (aged ٧,٩ to ١٥,١ years) to study its association with SCA morbidities; severe deficiency <١٠ ng/mL was present in ٦٤,٠٪ and only ٢,٢٪ were sufficient (>٣٠ ng/mL) (Jackson *ethic* ٢٠١٢).

Carmen and his colleagues in ٢٠١٢ in Madrid, Spain Vitamin D deficiency is highly prevalent in children with sickle cell disease Fifty-six percent of children had levels of ٢٥(OH) vitamin D of <٢٠ ng/ml, whereas ٧٩ and ١٨% of them had levels of <٣٠ and <١١ ng/ml, respectively (Gharrido *ethic* ٢٠١٢).In ٢٠١١ Osunkwol and other study in USA vitamin D deficiency is known to be common among patients with sickle cell anemia (SCA). Vitamin D levels were measured in ١٣٩ children (aged ٧,٩ to ١٥,١ years) to study its association with SCA morbidities; severe deficiency <١٠ ng/mL was present in ٦٤,٠٪ and only ٢,٢٪ were sufficient (>٣٠ ng/mL) (Osunkwo*ethic* ٢٠١١).

In ٢٠١١ Sadat and other study the Eastern Province of Saudi Arabia estimated ٩٢٪ of female, and ٧٠,٩٪ of male SCDP had VDD defined as ٢٥(OH)D level <٢٠ ng/mL (Sadat ethic٢٠١١).

In Jeddah study by Jalaluddin A. ٢٠٠٣, Fifty-one SCD patients of both sexes shows no significant difference between sexesand found serum concentrations of ٢٥(OH) Vitamin D in patients groups were significantly lower than the healthy matching controls (P<٠,٠١ and P<٠,٠٠١), respectively. (Jalaluddin A. ٢٠٠٣)

Chapter III

Materials and Methods

٣،١. Study design

This is case control study.

٣.٢ Study area and duration

The study was conducted in ALFaraby Center in Dammam-Kingdom Saudi Arabia, during the period from the November ٢٠١٨ to December ٢٠١٩.

٣.٣ Study population

Eighty-eight subjects were enrolled in the present study, and then classified as ٤٤ Patients with SCD as case group and ٤٤ apparently healthy as control group.

٣،٤ Inclusion Criteria

In case group children diagnosed SCD patient were included, while in control group was choose healthy individuals with the same age and gender of case group.

٣،٥ Exclusion Criteria

Any patient not disease by sickle cell and have normal vit D

٣،٦ Ethical Approval:

The study was approved b**y** Sudan University of Sciences and Technology. The consent after verbally and read by participant and parents of children.

٣.٧ Procedure of sample collection

Patients were either sat or lid down on an examination table, the arm was positioned on the armrest so that the vein identified become under some tension and its mobility was reducing. The skin was cleaned with ٧٠٪ ethanol and allowed to dry, personal details were checked up on the forms and on blood vials, tourniquet was applied to the arm, tight sufficiently to distend the vein, but not rightly to cause discomfort. ٣ ml of blood samples were taken from the superficial vein of the fore arm. Blood was collected in K٢EDTA, blood sample was analyzed by CELL-DYN Emerald and vein puncture Blood (٣ml) was collected by standard procedure, from the study groups, into sterile containers without anticoagulant and preserved at – ٢٠ degrees centigrade for vitamin D٣-OH measured. The blood was collected in tri sodium citrate (for erythrocyte sedimentation rate (ESR) in fast detector in half hour.

٣،٨ CELL-DYN Analyzer

The CELL-DYN Emerald is an automated hematology analyzer intended for in vitro diagnostic was use in the clinical laboratory. It was menu-driven and controlled by a microprocessor. The CELL-DYN Emerald was aspirates blood from an opened collection tube held up to the aspiration probe (Grimaldi ٢٠٠٠). The CELL-DYN Emerald can aspirate blood from several types of collection devices which contain K٢EDTA, the CELL-DYN Emerald provides (Grimaldi, ٢٠٠٠).

٣,٨.١ Principle of operation

The instrument aspirates whole blood specimen. The blood was mixed with ٢mL of diluent and ٠,٣٨mL of Lyse in the WBC counting chamber. The lyse reagent destroyed the RBC and resultant stroma and perforates the WBC cytoplasmic membrane allowing the cytoplasm to escape. The WBCs were counted directly by impedance and the Differential measurands are obtained from the graph (Grimaldi ٢٠٠٠). The instrument aspirates ٢٠µL of the dilution from the WBC Counting chamber and adds ١,٥mL of diluent to the RBC Counting Chamber for the RBC/PLT dilution. The Hematocrit (HCT) is the ratio of red blood cells to plasma and was expressed as a percentage of the whole blood volume. It is derived from the volume of the RBCs that are counted during the measurement cycle. The mean cell volume (MCV) is the average volume of individual RBCs (Grimaldi ,٢٠٠٠). Platelets were counted directly by impedance in the RBC Counting Chamber at the same time as RBCs. PLT – Platelet Count MPV – Mean Platelet Volume **(**Grimaldi ٢٠٠٠).

٣,٨.٢ Quality Control Methods and Materials

Internal QC Methods consist of running commercial control material or retained patient specimens. Commercial controls contain fixed cells and are assayed by the manufacturerto determine expected recovery ranges. Abbott recommends CELL-DYN Control Materials for use on the CELL-DYN Emerald System. A tri-levelcontrol is available that provides three levels of monitoring for each measure and; the number of controls used is determined by each laboratory.Accessoriesfor information on CELL-DYN Controls, used with the CELL-DYN Emerald System.Patient controls are retained patient specimens with results that fall within thelaboratory's defined ranges. They are tested by the laboratory to establishrecovery against defined target ranges. They provide an accurate and cost-effectivemeans of evaluating system performance.External QC methods use resources available outside the laboratory to assesssystem performance. These programs use a peer-review process to allow alaboratory to compare its performance with that of other laboratories. For example, in the USA, laboratories are required to participate in proficiency testing programs.Proficiency testing provides independent validation of a laboratory's internal QC program (Grimaldi, ٢٠٠٠).

٣,٩ Principle of Hemoglobin Electrophoresis

The first hemolysate from the EDTA blood was made and then it was run for electrophoresis where Hb was separated in different bands cellulose acetate or starch gel electrophoresis wasrun on the hemolysate at pH of ٨,٦, then Hb is quantified by elution and spectrophotography or by a densitometer,HbF was acid and alkali resistant so need to quantify by another method. Electrophoresis this is a migration of charged solutes or particle in liquid medium under the influence of the electric fiel,positive ions (cations) moves towards the cathode,negative ions (anions) moves towards the anode. (Hanaor *ethic*٢٠١٢)

٣,١٠ Erythrocyte Sedimentation Ratemethod (ESR)

One point twenty-eight ml of blood was mix with sodium citrate well and fill the tube to the mark (٠) on the rack exactly vertical for ٣٠ minutes, the reading nearest the clear plasma above the upper limit of the column of sedimentary cells as mm/١/٢ hr (Bray *ethic* ٢٠١٦).

٣,١١ Measurement of ٢٥-OH Vitamin D (Euroimmun Kits) by ELISA

٣,١١.١ Principle of method for vitamin D ELISA

This ELISA test kit is designed for in vitro determination of ٢٥-OHvitamin D in human serum. In the first analysis step, the calibrator andpatients samples are diluted with biotin-labelled ٢٥-OH vitamin D andadded to microplate wells coated with monoclonal anti -٢٥-OHvitamin D antibodies. During the incubation unknown amount of ٢٥-OH vitamin D in the patient samples and known amount of biotin-labelled ٢٥-OH vitamin D compete for the antibody binding sides inmicroplate wells plate. Unbound ٢٥-OH vitamin D is removed by washing. For the detection of bound biotin-labelled ٢٥-OH vitamin D a second incubation is performed using peroxidase labeledtreptavidin. In the third incubation using peroxidase substrate tetra methyl benzidine (TMB) The bound peroxidase promotes acolorreaction.The color intensity inversely proportional to the ٢٥-OH vitamin D concentration in sample.Result for the sample can be calculated directly using standard curve test procedure: ١٢٠ min / ٣٠ min / ١٥ min (room temperature), fully automatable (Fairclough, ٢٠١٥).

٣,١١.٢ Sample dilution

Reagents Ready for use, with the exception of the wash buffer (١٠ x) and biotin (١٠٠ x) Color-coded solutions. Test procedure :١٢٠ min / ٣٠ min / ١٥ min (room temperature), fully automatable. Alongside the reliable diagnosis of vitamin D deficiency, the ٢٥- O H Vitamin D ELISA is also useful for monitoringof therapy, sincethe effect ofdrugs may vary andvitamin D deficiency syndromes require treatment over a longer period oftime, often over yearsor even decades. As opposed to antibodies used in other commercial test systems, the novel monoclonal antibody used in this test is equally specific for both forms of ٢٥-OH vitamin D (١٠٠ %).

٣,١٢ Data analysis

The collected data was analyzed to obtain the mean, standard division, Frequency and excreted *P. value* of the sampling using statistical package for social science (SPSS) computer programmed version ٢٤ with one away anovaand T-test.

Chapter IV

Result

A total of eighty-eight volunteers were enrolled in this study, male ٣٠/٨٨, (٣٤,١٪) and female ٥٨/٨٨, (٦٥,٩ %.) They classified to ٤٤ case with male ١٤/٤٤, (٣١,٨٪), female ٣٠/٤٤, (٦٨,٢٪). While ٤٤ control male ١٦/٤٤, (٣٦,٤٪), female ٢٨/٤٤, (٦٣,٦٪) showed Table ٤,١.

The mean values of serum OH-vitamin D in case ١٢ ±٣ while compared to control ٢٧ ± ٩ showed in Table ٤,٢.

In case group Hemoglobin electrophoresis in SCD patient showed numberof HbAS was ١٦ (٣٦٪) , HbSS was ١٩ (٤٣٪) and HbSC was ٩ (٢١٪) of total ٤٤ patient (١٠٠٪) .Mean of ٢٥-OH vitamin D with Hb electrophoresis were HbAS (١٤,١ ± ١,٨) HbSS (٩,٤ ±٢,٥) and HbSC (١٣,٨±١,٠),showed in Table ٤,٣.

Mean values of complete blood count in cases and controls, in cases the main Hb concentration is (٧,١ ± ١,٢) g/dl then we divided Hb to the three ranges: mean Hb mild (٨,٨±٠,٦) g/dl, Hb moderate (٦,٨ ± ٠,٦) g/dl and Hb severe (٥,٧±٠,٢) g/dl, compared with controls (١٣,١±١,٤) g/dl. PCV in SCD the mean values (٢١,١±٣,٧) % compared to control (٤٠,٥±٣.٨) %. RBC in cases (٣,٠±٠,٧٦) mill/mm^{٣} compared to controls (٤,٦٧±٠,٥١ mill/mm$^{٣)}$ The mean of MCV (٧٤,٦ ±١٣,٧) fl, in case and control (٨٦,٥±٤,٣) fl). MCH among case vs. control with mean (٢٤,٤±٤,١) g/dl, (٢٧,٧±٢,٥) g/dl respectively. The average of MCHC for cases (٣٣,٧±٢,٤) g/dl and control (٣١,٩±١,١) g/dl. TWBC count among cases (١٣٧٠٥±٢٨١٥ while controls (٧٤٣٩±٢٥٩٢) cell/mm^{٣}. Platelets counts in cases (٥٢٦,٧±١٣١ × ١٠^{٣}/µl) compared to control (٢٧٢,٥±٤١ × ١٠^{٣}/µl). Mean values of ESR (٤٢,٣±١٦,١) in cases compared with controls (٢٢,٢±٧,٣), showed in Table ٤,٤.

Mean of ٢٥-OH vitamin D with age, gender, history of disease, type of treatment and Blood transfusion among study volunteers.Correlation of ٢٥-OH vitamin D with age group in cases compared to control, in cases group age < ٥ year (١٣,٣±١,٥), while in ٥-١٠ years (١١,٨±٢,٩), in age group more than ١٠ years (١٢,٧±٣,٣), in control group age <٥ years (١٦±٣,٢), while in ٥-١٠ years (١٧±٢,٠), in age group more than ١٠ years (١٨,٠±٣,٣), while in case male ١١,٩±٣,٠ while female ١٢,١±٣,١ compared to control in male ١٦ ± ٢,٢ while female ١٣ ± ٣,٢. History of disease were categorized into three groups as following: ١-٥ years, ٦-١٠ years and >١٠years. Correlation of ٢٥- OH-vitamin D with type treatment, the most common of treatment are hydroxyurea and L-glutamine. Time of blood transfusion in SCD patients were

categorized into three groups as follows: (١-٤) per ١ Months, (١-٤) per ٢ Months, (١-٤) per ٣ Months) showed in Table ٤,٥.

Mean of ٢٥-OH vitamin D crisis like chest pain,painful, leg ulcer vasocrisis hemolytic, acute chest syndrome and without crisis among study volunteers with mean (١٢,٥±٢,٩), (١٢,٦±٣,٥), (١٤,٢±٠,١), (١٠,٥±٢,٦). (٩,٧±٣,٧), (١١,٩±٢,٠), and (١٣,٧±٢,١) respectively.showed in Table ٤,٦.

Table ٤,١: Distribution of gender among study volunteer.

Gender	Case	Control	Total
Male	١٤ (٣١,٨٪)	١٦ (٣٦,٤٪)	٣٠ (٣٤,١٪)
Female	٣٠ (٦٨,٢٪)	٢٨ (٦٣,٦٪)	٥٨ (٦٥,٩٪)
Total	٤٤ (١٠٠٪)	٤٤ (١٠٠٪)	٨٨/٨٨(١٠٠٪)

Table ٤,٢: Mean ٢٥-OH vitamin D level amongcase and control.

Sample	٢٥-OH Vitamin D (ng/ml)	
	Mean ±Std. Deviation	*p.value*
Case	١٢ ±٣	٠,٠٠
Control	٢٧ ± ٩	

Table ٤,٣: Correlation between mean of vit D and Hb electrophoresis:

Hb electrophoresis	N	vitD	
		Mean	Std. Deviation
HbAS	١٦ (٣٦٪)	١٤,١	١,٨
HbSS	١٩ (٤٣٪)	٩,٤	٢,٥
HbSC	٩ (٢١٪)	١٣,٨	١,٠
Total	٤٤ (١٠٠٪)	١٢,٠	٣,٠
ANOVA			
٢٥-OH vitamin D			
Sig. ٠,٠٠٠			

Table ٤,٤: Mean of Complete Blood Count parameter, ESR among study volunteer (case and control).

Sample	Case	Control	*p.value*
	Mean ± STD	**Mean ± STD**	
TWBC	١٣٧٠٥ ± ٢٨١٥	٧٤٣٩ ± ٢٥٩٢	٠,٠٠
RBC	٣ ± ٠,٧٦	٤,٦٧ ± ٠,٥١	٠,٠٠
Hb	٧,١ ± ١,٢	١٣,١ ± ١,٤	٠,٠٠
Hb Mild	٨,٨ ±٠,٦		
HbModerate	٦,٨ ± ٠,٦		
HbSever	٥,٧ ± ٠,٢		
PCV	٢١,١ ± ٣,٧	٤٠,٥ ± ٣,٨	٠,٠٠
MCV	٧٤,٦ ± ١٣,٧	٨٦,٥ ± ٤,٣	٠,٠٠
MCH	٢٤,٤ ± ٤,١	٢٧,٧ ± ٢,٥	٠,٠٢
MCHC	٣٣,٧ ± ٢,٤	٣١,٩ ± ١,١	٠,٠٣
PLT	٥٢٦,٧ ± ١٣١	٢٧٢,٥ ± ٤١	٠,٠٠
ESR	٤٢,٣ ± ١٦,١	٢٢,٢ ± ٧,٣	٠.٠٣

Table ٤.٥: Mean of ٢٥-OH vitamin Dlevel with age, gender, history of disease, treatment and Blood transfusion among study volunteers.

Variable		٢٥-OH vitamin D		*p.value*
Age group		Mean	Std. Deviation	
< ٥ year		١٣,٣	١,٥	٠,٢
٥-١٠ year		١١,٨	٢,٩	
> ١٠ year		١٢,٧	٣,٣	
Gender	Male	١١,٩	٣,٠	٠,٨
	Female	١٢,١	٣,١	
History of disease				
١-٥ year		١٢,٤	٢,٩	٠,١
٦-١٠ year		٨,٩	٤,٤	
> ١٠ year		٩,٥	٣,٤	
Type of Treatment				
Hydroxyurea		١٢,٠	٢,٩	٠,٥
L-glutamine		١١,٢	٣,٧	
No		١٣,٣	٢,٨	
Blood transfusion				
(١-٤) ١ months		١١,٣٨٦	٣,٣٣٦٧	٠,١
(١-٤) ٢months		١٣,١١٣	١,٩٥٦٥	
(١-٤) ٣months		١٣,٢٢٣	٢,١٣٤٢	

Table ٤.٦: Mean of ٢٥-OH vitamin Dcrisis among study volunteers.

Blood crisis	**٢٥-OH vitamin D**		*p.value*
	Mean	Std. Deviation	
Chest pain	١٢,٥	٢,٩	٠,١
Painful	١٢,٦	٣,٥	
Leg ulcer	١٤,٢	٠,١	
Vaso crisis	١٠,٥	٢,٦	
Hemolytic	٩,٧	٣,٧	
Acute Chest syndrome	١١,٩	٢,٠	
No	١٣,٧	٢,١	

Chapter V

٥,٠ Discussion

Sickle red blood cells are prone to breakage (membrane rupture) which causes a much shorter life span of these cells. Hematological indices revealed that individuals with SCD have low significant associated with Hb, RBC count,PCV,MCH ,MCV,higher MCHCelevated platelets count was associated SCD; agreement with study in Sudan (Elberirethic٢٠١٨).

In present study the mean values of serum OH-vitamin D in case ١٢ ±٣ while compared to control ٢٧ ± ٩ statistical significant (*P.value* ٠,٠٠) was agreement with studyin Saudi Arabia Ali Aljama and his group was present sever deficiency of (OH) vitamin D in Saudi children with SCD (Aljama *ethic* ٢٠١٨) , Also in Canada shown low serum level ٢٥-hydroxyvitamin D (٢٥OHD) concentrations in Canadian Children with Sickle Cell Disease (Samson *ethic* ٢٠١٨) and also study in U.K London Ninety-one percent of children with SCA had ٢٥-OHD levels <٢٠ µg/L.

(OH) vitamin D levels were associated with the hemoglobin variants (HbAS, HSS, and HbSC),in present study was lower in HbSS was statistical significant agreement with recent study in Nigeria(Adegoke *ethic* ٢٠١٩) also in Saudi Arabia Ali Aljama and his group was present sever deficiency of (OH) vitamin D in Saudi children with SCD (Aljama *ethic* ٢٠١٨).While disagreement withother study was reported (OH) vitamin D levels were associated with the hemoglobin variants (HbAS, HSS, and HbSC) ,in present study was lower in HbSS was statistical insignificant (Yawn *ethic* ٢٠١٤).

The ٢٥-OH vitamin D levels were negatively correlated with increasing age (*P*< ٠,٠٠١) (Wykes *ethic* ٢٠١٤). The difference in our finding compared to other reports insignificant with (*P.value* ٠,٠٢) may be due do the sample size is relatively small, thus limiting the statistical power of our findings.

In our study, no difference found between levels of serum (OH) vitamin D in male and females was agreement with study in Spain (OH) vitamin D deficiency is highly prevalent in children with sickle cell disease Fifty-six percent of children had levels of ٢٥(OH) vitamin D of <٢٠ ng/ml, whereas ٧٩ and ١٨ % of them had levels of <٣٠ and <١١ ng/ml, respectively (Gharrido *ethic* ٢٠١٢).

The present study showed that SCD was significant associated raised WBC count as compared to controls with (*P.value* ٠.٠٠). White blood cells are now well known to be involved in pathophysiology of SCD agreement with study in USA (Osunkwol *ethic* ٢٠١١). Other author have also shown the importance of leukocytosis to clinical outcomes of early SCD related death, clinically overt stroke and acute chest syndrome, the results were expected the degree of chronic hemolysis, higher of infections and chronic pain in sickle cell patients (Elberir *ethic* ٢٠١٨).

٥,١ Conclusion

Vitamin D was statistical significant decrease in Saudi children patients with sickle cell disease.

Also lower values of hemoglobin concentration, Red blood cell counts, mean cell volume packed cell volume and mean cell hemoglobin; but higher values of mean cell hemoglobin concentration, white blood cells count; platelets count and erythrocyte sedimentation rate compared to control groups.

٥,٣ Recommendations

١. Vitamin D must be evaluated in children with sickle -cell anemia to avoid complication of diseases lead to osteomyelitis.

٢. The study recommended vitamin D protocol supplements for sickle cell anemia patients.

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

Chapter VI

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Appendices

Appendix (I) Questionnaire

Sudan University of Sciences and Technology

College of Graduate Studies

Estimation Level of Vitamin D ٢٥-OH among Saudi Children with Sickle Cell Disease in Dammam, Kingdom of Saudi Arabia.

قياس مستوى فيتامين د٢٥ - او أتش لدي الأطفال السعوديين المصابين بالأنيميا المنجلية

في الدمام-المملكة العربية السعودية

Questionnaire

١. Id:……..……..... …….

٢. Age: A. Less than ٥ yrs. ( ) **B.** ٥-١٠ yrs. ( ) **C.**> ١٠ year ( )

٣. Sex: Male ( ) Female ( )

٤. History of disease .A ١-٥ yrs. ( ) **B.**٦-١٠ yrs. ( ) **C.**≥١٠ yrs. ( )

٥. Family history Yes, ( ) No ( )

If Yes How many years? **A**. ١-٥ yrs. ( ) **B.**٦-١٠ yrs. ( ) **C.**> ١٠ year ( )

٦. Treatment and Type of it ……

٧. Blood transfusion time A. once. ( ) **B.**Twice. ( ) **C.** More. ( )

٨. History of crisis Yes, ( ) No. ( )

If yes specify…….....

Appendix II

A .Full blood count (CBC)results:

Parameter	**Measure**
TWBCs cell/mm^{٣}	
RBCs (mill/mm$^{٣)}$	
Hb g/dl	
PCV%	
MCV fl	
MCH pg	
MCHC g/dl	
Plats X١٠^{٣} /µl	

B. ESRResult:…… mm/hr.

C.HB electrophoresis from data records

Variants	**Results**
HbAS	
HbSS	
HbAC	

D.Vitamin D٣ levels (ng/ml) :

Appendix (III) Material and equipment

A. Requirements:

- Ethylene-diamine- tetra-acetic acid K٢EDTA.
- Sodium citrate
- Plain container
- Cotton
- ٧٠٪ Alcohol
- Vaccum Holder with their needles.
- Tourniquet.

B. Technical data:

Coating anti-٢٥-OH vitamin D (monoclonal). Calibration Quantitative, in nanograms per millilitre (ng/ml).Calibrator ١: ٠ ng/ml, Calibrator ٢ : ٤ ng/ml, Calibrator ٣ : ١٠ ng/ml, Calibrator ٤: ٢٥ ng/ml, Calibrator ٥: ٦٠ ng/ml and,Calibrator ٦: ١٢٠ ng/ml

C. Reagents:

Ready for use, with the exception of the wash buffer (١٠ x) and biotin (١٠٠ x) color-coded solutions.

Appendix (IV)

Cell-Dyn Emerald Hematology Analyzer

Appendix (V)

٢٥OH working sheet

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25-OH Vitamin D ELISA

- **Optimised and reliable detection of 25-OH vitamin D_3 and D_2**
- **Excellent correlation of the measurement results with reference methods (LC-MS/MS and HPLC)**
- **Fully automatable on all open ELISA platforms**

Technical data

Coating	Anti-25-OH vitamin D (monoclonal)
Calibration	Quantitative, in nanograms per millilitre (ng/ml) Calibrator 1: 0 ng/ml — Calibrator 4: 25 ng/ml Calibrator 2: 4 ng/ml — Calibrator 5: 60 ng/ml Calibrator 3: 10 ng/ml — Calibrator 6: 120 ng/ml
Sample dilution	Serum or plasma; 20 µl; 1:26 in sample buffer containing biotin
Reagents	Ready for use, with the exception of the wash buffer (10 x) and biotin (100 x). Colour-coded solutions
Test procedure	120 min / 30 min / 15 min (room temperature), fully automatable
Measurement	450 nm. Reference wavelength between 620 nm and 650 nm
Test kit format	96 break-off wells, kit includes all necessary reagents
Order number	**EQ 6411-9601**

Clinical significance

There are two forms of vitamin D: vitamin D_3, which is mainly produced in the skin and vitamin D_2 which is acquired by consumption of vegetable foods. Both forms are bound to a binding protein in the blood stream and metabolised in the liver into 25-OH vitamin D_2 or D_3, a storage form. It is only in the kidney that the biologically active metabolite 1,25 dihydroxy vitamin D, which has the function of a hormone (D hormone) is synthesised. The D hormone regulates calcium intake from the intestine, bone mineralisation, differentiation of osteoblasts and synthesis of bone matrix.

Vitamin D deficiency is a worldwide problem with serious health consequences. The serum level of 25-OH vitamin D, which shows the highest concentration of all vitamin D metabolites due to its storage function, is the best indicator of the vitamin D supply of the human organism. Already a small vitamin D deficiency with a 25-OH vitamin D level of 12–30 ng/ml (30–75 nmol/l) in the blood leads to a reduced calcium intake and thus to a secondary increase in parathyroid hormone and increased bone resorption. A vitamin D deficiency is therefore one of the most important risk factor for senile osteoporosis. A severe vitamin D deficiency with a level of <12 ng/ml (<30 nmol/l) leads e.g. to the clinical image of rickets in children or osteomalacia in adults, which are characterised by disrupted bone formation or insufficient matrix mineralisation. Moreover, hypovitaminosis D constitutes a risk factor for numerous further diseases.

Diagnostic application

Alongside the reliable diagnosis of vitamin D deficiency, the 25-OH Vitamin D ELISA is also useful for monitoring of therapy, since the effect of drugs may vary and vitamin D deficiency syndromes require treatment over a longer period of time, often over years or even decades. As opposed to antibodies used in other commercial test systems, the novel monoclonal antibody used in this test is equally specific for both forms of 25-OH vitamin D (100 %).

Autoimmune diagnostics · Infection diagnostics · Allergy diagnostics · Antigen detection · Molecular genetic diagnostics · Automation

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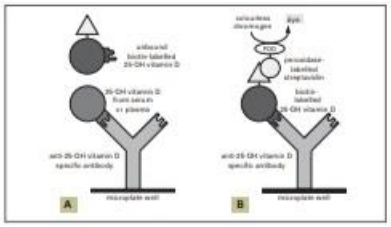
Test principle

25-OH vitamin D from the patient sample is bound by anti-25-OH antibodies on the microplate. Free antibody binding sites are occupied by labelled 25-OH vitamin D. The intensity of the colour formed after addition of the chromogen/substrate solution is measured using a photometer. The colour intensity is inversely proportional to the 25-OH vitamin D concentration in the serum or plasma.



A Binding of 25-OH vitamin D in serum or plasma → no colour reaction
B No 25-OH vitamin D present in serum or plasma → colour reaction

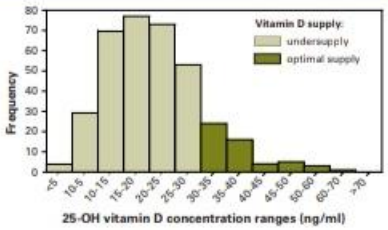
Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 samples. The intra-assay CVs are based on 40 measurements for each serum and the inter-assay CVs on four measurements repeated in ten different runs.

No.	Intra-assay variation, n = 40		No.	Inter-assay variation, n = 4 x 10	
	Mean value (ng / ml)	CV (%)		Mean value (ng / ml)	CV (%)
1	10.8	4.9	4	16.6	7.8
2	24.6	6.9	5	43.5	7.0
3	64.1	3.2	6	67.8	8.6

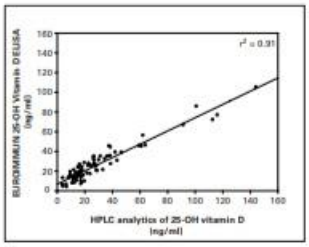
Reference range

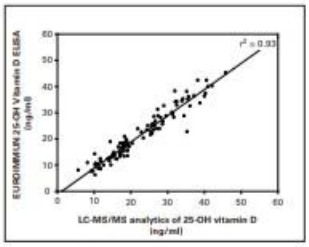
Sera from 359 healthy blood donors (aged 13–99 years) were investigated using the EUROIMMUN 25-OH Vitamin D ELISA. The mean 25-OH vitamin D concentration was 20.9 ng/ml (at 5th–95th percentile: 8.2–37.4 ng/ml). The vitamin D supply was optimal in 53 blood donors (30–70 ng/ml) and deficient in 306 blood donors (< 30 ng/ml).

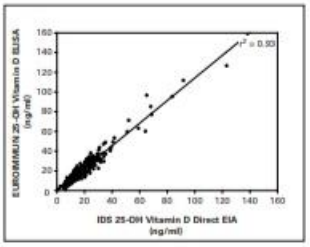


Correlation of the EUROIMMUN 25-OH Vitamin D ELISA with HPLC, LC-MS/MS and IDS ELISA

The EUROIMMUN 25-OH Vitamin D ELISA was compared with different reference tests and showed the following correlations: HPLC, $r^2 = 0.91$, n = 80; LC-MS/MS, $r^2 = 0.93$, n = 100; IDS 25-OH Vitamin D Direct EIA, $r^2 = 0.93$, n = 231. The correlation of the obtained results was very high.







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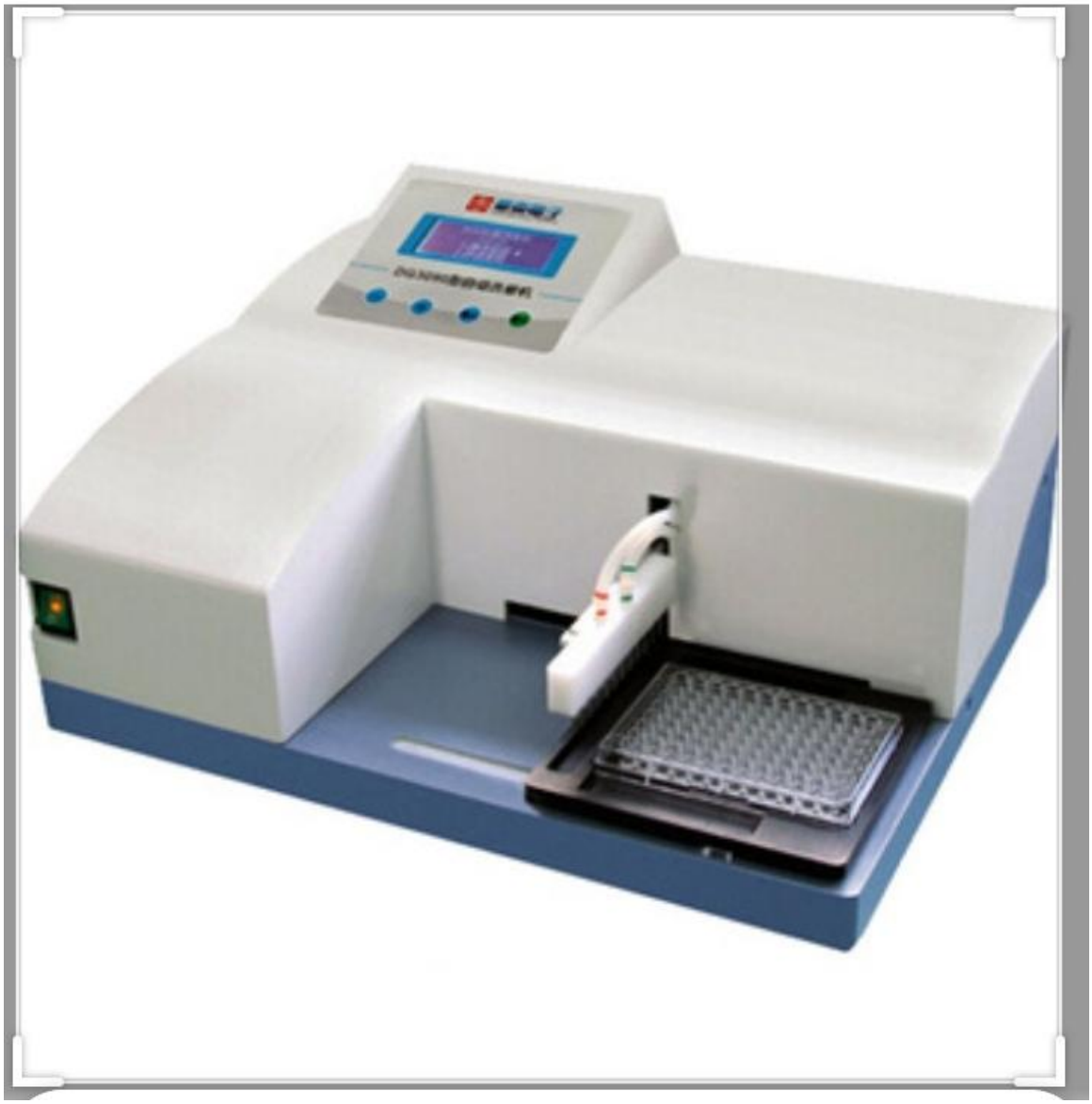
Appendix (VI)

A



ELISA washer

Appendix (VI)

B

Plate of vitamin D Kit