Sudan University for Sciences and Technology

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Determination of the effectr of vitamin D deficiency on hematological parameters in Khartoum state

تحديد العلاقه بين نقصان فيتامين د ومتغيرات الدم بولايه الخرطوم

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Submitted by

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الآيه

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

قال تعالى:

"رَبِّ قَدۡ ءَاتَيۡتَنِي مِنَ ٱلۡمُلۡكِ وَعَلَّمۡتَنِي مِن تَأۡوِيلِ ٱلۡأَحَادِيثِۚ فَاطِرَ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضِ أَنتَ وَلِيِّۦ فِي ٱلدُّنۡيَا وَٱلۡأٓخِرَةِۖ تَوَفَّنِي مُسۡلِمٗا وَأَلۡحِقۡنِي بِٱلصَّٰلِحِينَ**"**

صدق الله العظيم

سوره يوسف الآيه **(101)**

DEDICATION

To my… beloved mother and my dear father

Who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve your prayers have been answered.

To my …brothers and sisters

Who has a constant source of support and encouragement during all way and made sure that I give it all it takes to finish that which I have started.

To my… best friend

Gophran, Zohida, Walaa who have supported me throughout the process. I will always appreciate their support.

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Abstract

Vitamin D deficiency is a common condition that affects many metabolic activities that may cause a significant abnormalities. Vitamin D deficiency (VDD) is generally associated abnormal hematopoiesis and an outcome of varying degrees. Some studies reported an association between VDD and some hematological and non hematological parameters. Hematopoiesis occurs in the bone marrow and is strictly regulated with the help of various cytokines, hormones, growth factors, and even vitamins to supply for a steady state of the circulating red blood cells (RBC), white blood cells (WBC), and platelets (PLT). Lack or deficiency of any of these regulatory factors can potentially slow down the hematopoietic process and lead to a certain abnormality in the production of any one or more of these three cell lines .This study evaluation the haematological parameters among vitamin D deficiency .A total of 130 Sudanese subjects were enrolled in this study, 65 patients with diagnosed with vitamin D deficient disease and 65 healthy volunteers as a control (43%) of control were males and (56%) were females. (28%) of patients were males and (73%) were females all subjects diagnosed with vitamin D deficiency. Blood samples were collected from all participants in Ethylene Diamine tetra acetic acid (EDTA) anticoagulant container, and analyzed by automated hematological analyzer. Patients' data was collected from patients' medical files, and analyzed by statistical package for social sciences (SPSS), version 20.The result showed statically significant association between some hematological parameters among study group significant (decreased of MCH in case group *P.value* =(0.01). There was statistically significant difference in gender distribution when compared in case and control groups.*(P.value=0.04*) The study showed significant statistical difference between mean of RBCs and MCHC and VD level in patients (decreased in deficient level). (*P.value* =0.03, 0.01) Respectively. The result showed no statically significant association other RBCs indices and VD level in patients p.value (0.19, 0.11, 0.5, 0.7) respectively. The result showed no statically significant association between WBCs and differential leucocytes count and vitamin VD level in patients p.value(0.3, o.8, 0.2, 0.5, 0.4) respectivelly. The also showed no statically significant association between platelet count and vitamin D level in patients (P.*value* =0.2) . The result showed no statically significant association between VD level and gender of patients. *(P. value*= 0.1) .The result showed significant statistical difference between mean of MCHC and gender of patients (decreased in

female), *P.value*=0.03.The result showed no statically significant association between RBCs and other RBCs indices and gender of patients (p.v=(0.44, 0.85, 0.72, 0.66, 0.06, 0.03) respectively. The result showed significant statistical difference between mean of neutrophils and gender of patients (decreased in female), (*P.value*=0.04). The result showed no statically significant association between WBCs and other differential leucocytes count and gender of patients p.value=(0.7, 0.3, 0.4, 0.9) respectively. The result showed no statically significant association between platelet count and gender of patients. (P.*value* =0.1)

المستخلص

نقص فيتامين د هو حالة شائعة تؤثر على العديد من الأنشطة الأيضية التي قد تسبب تشوهات كبيرة. يرتبط نقص فيتامين د (VDD) عمومًا بتكوين الدم غير الطبيعي ونتيجة بدرجات متفاوتة. ذكرت بعض الدراسات وجود ارتباط بين VDD وبعض المعلمات الدموية وغير الدموية. يحدث تكوين الدم في نخاع العظام ويتم تنظيمه بشكل صارم بمساعدة السيتوكينات المختلفة والهرمونات وعوامل النمو وحتى الفيتامينات لتوفير حالة مستقرة من خلايا الدم الحمراء (RBC) وخلايا الدم البيضاء (WBC) والصفائح الدموية (PLT). يمكن أن يؤدي نقص أو نقص أي من هذه العوامل التنظيمية إلى إبطاء عملية تكوين الدم ويؤدي إلى بعض الشذوذ في إنتاج أي واحد أو أكثر من هذه الخطوط الخلوية الثلاثة. تم تسجيل ١٣٠ سودانيًا في هذه الدراسة ، وتم تشخيص ٦٥ مريضًا يعانون من نقص فيتامين (د) و ٦٥ متطوعًا سليمًا كعنصر تحكم (٤٣٪) من الذكور و (٥٦٪) من الإناث. كان (٢٨٪) من المرضى ذكور و (٧٣٪) إناث جميع الأشخاص الذين تم تشخيصهم بنقص فيتامين (د). تم جمع عينات الدم من جميع المشاركين في حاوية الإيثيلين ديامين تترا حمض الخليك (EDTA) ، وتم تحليلها بواسطة محلل الدم الآلي. تم جمع بيانات المرضى من الملفات الطبية للمرضى ، وتحليلها بالحزمة الإحصائية للعلوم الاجتماعية (SPSS) الإصدار ٢٠ ، وأظهرت النتيجة ارتباطًا ذا دلالة إحصائية بين بعض متغيرات الدم لدى مجموعة الدراسة معنويًا (انخفاض صحة الأم والطفل في مجموعة الحالة P.value. = (٠.٠١). توجد فروق ذات دلالة إحصائية في توزيع الجنس عند مقارنتها في المجموعة الضابطة والحالة (P.value = ٠.٠٤) وأظهرت الدراسة فروق ذات دلالة إحصائية بين متوسط عدد كرات الدم الحمراء ومستوى MCHC و VD في المرضى (انخفاض في مستوى النقص). (P.value = ٠.٠٣، ٠.٠١) على التوالي. لم تظهر النتائج ارتباط ذو دلالة إحصائية بين مؤشرات كرات الدم الحمراء الأخرى ومستوى VD في المرضى ص. تعداد كرات الدم البيضاء والكريات البيض التفاضلية ومستوى فيتامين VD في المرضى ص. القيمة (٠.٣ ، ٨.o ، ٠.٢ ، ٠.٥ ، ٠.٤) الاحترام ، كما لم تظهر أي علاقة ذات دلالة إحصائية. بين عدد الصفائح الدموية ومستوى فيتامين د في المرضى (P.value = ٠.٢). أظهرت النتائج عدم وجود علاقة ذات دلالة إحصائية بين مستوى VD وجنس المرضى. (P. value = ٠.١). أظهرت النتائج فروق ذات دلالة إحصائية بين متوسط MCHC وجنس المرضى (انخفض في الإناث) ، P.value = ٠.٠٣ ، وأظهرت النتيجة عدم وجود ارتباط ذي دلالة إحصائية بين كريات الدم الحمراء ومؤشرات كرات الدم الحمراء الأخرى وجنس المرضى (pv = (٠.٤٤، ٠.٨٥، ٠.٧٢، ٠.٦٦، ٠.٠٦، ٠.٠٣) على التوالي وأظهرت النتائج فروق ذات دلالة إحصائية بين متوسط العدلات وجنس المرضى (انخفض عند الإناث) ، (Pvalue = ٠.٠٤) وأظهرت النتائج. لا توجد علاقة ذات دلالة إحصائية بين كرات الدم البيضاء وعدد كريات الدم البيضاء التفاضلية الأخرى ونوع المرضى ص. القيمة = (٠.٧ ، ٠.٣ ، ٠.٤ ، ٠.٩) على التوالي ، ولم تظهر النتيجة ارتباطًا ذا دلالة إحصائية بين عدد الصفائح الدموية ونوع المرضى. ٠.١).

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List of Abbreviation

Symbols	Meaning
VDD	Vitamin D deficiency
RBCs	Red blood cells
WBCs	White blood cells
PLT	Platelet
HB	Hemoglobin
HCT	Hematocrit
MCV	Mean cell volume
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MPV	Mean platelet volume
HSCs	Hematopoietic stem cell
B.M	Bone marrow
CFU	Colony forming unit
BFU-E	Burst-forming unit- erythroid
EDTA	Ethylene Di amine tetra acetic acid
UVB	Ultra Violet Bulb
OR	Odd ratio
DC	Direct current
ELISA	Enzyme linked immune sorbent assay
SPSS	Statistical package for social sciences
USA	United state of America
G-CSF	Granulocyte Colony Stimulating Factor

CHAPTER I

1-Introduction

1. 1 Introduction

The blood system contains more than ten different blood cell types (lineages) with various functions: Leukocytes represent many specialized cell types involved in innate and acquired immunity. Erythrocytes provide O_2 and CO_2 transport, whereas megakaryocytes generate platelets for blood clotting and wound healing. All blood cell types arise from hematopoietic stem cells (HSCs) that reside mainly in the bone marrow (BM), a major site of adult hematopoiesis. Blood is one of the most regenerative and plastic tissues, and millions of “old” blood cells are replen-ished with new ones each second during life. In emergency situations such as anemia or infections, blood cell counts rapidly increase. The cell number then declines back to normal after recovery. The life times of various mature blood cell types range from hours to years (Rieger *et al*., 2012).

Vitamin D, a lipid-soluble vitamin, plays an essential role in maintaining skeletal integrity and function, electrolyte reabsorption, and immune system regulation among other health benefits. Vitamin D exists in two primary variants, vitamin D2 and D3. The hormonally active form of vitamin D, 1,25-dihydroxy vitamin D3 (cholecalciferol), plays a significant role in clinical medicine mainly due to its potent effects on calcium homeostasis and bone metabolism (Nair *et al.,* 2012).

The primary source of vitamin D3 is sunlight, which contains ultraviolet rays that synthesize cholecalciferol in the skin (Nair *et al.,* 2012). Alternative sources of vitamin D3 include animal products, such as fatty fish, while vitamin D2 can be obtained mainly from dietary plant and fungal sources such as mushrooms (Dedeoglu *et al.,* 2014).

Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements (Holick *et al.,* 2006). A diet high in oily fish prevents vitamin D deficiency (Bouillon, 2001). Solar ultraviolet B radiation (wavelength, 290 to 315 nm)

penetrates the skin and converts 7-dehydrocholesterol to previtamin D3, which is rapidly converted to vitamin D3 (Holick, 2006). Because any excess previtamin D3 or vitamin D3 is destroyed by sunlight,Vitamin D from the skin and diet is metabolized in the liver to 25-hydroxyvitamin D , which is used to determine a patient's vitamin D status (Holick *et al.,* 2006). 25-hydroxyvitamin D is metabolized in the kidneys by the enzyme 25-hydroxyvitamin D-1α-hydroxylase to its active form, 1,25-dihydroxyvitamin D ((Holick *et* al., 2006).

1.2 Rationale

Vitamin D deficiency is a common condition that affects many metabolic activities that may cause significant abnormalities. Hematopoiesis occurs in the bone marrow and is strictly regulated with the help of various cytokines, hormones, growth factors, and even vitamins to supply for a steady state of the circulating red blood cells (RBC), white blood cells (WBC), and platelets (PLT). Lack or deficiency of any of these regulatory factors can potentially slow down the hematopoietic process and lead to a certain abnormality in the production of any one or more of these three cell lines .Vitamin D deficiency (VDD) is generally associated abnormal hematopoiesis and an outcome of varying degrees.recently vitamin D deficiency became common disease in Sudan , Some studies reported an association between VDD and some hematological and non hematological parameters .To our knowledge, there is no enough data in Sudan addressing evaluation or assessment variations hematological parameters among VDD patients. This study aimed to find out the relationship between VDD and hematological parameters.

1.3 Objectives:

1.3.1 General objective

To determine the association between vitamin D deficiency and hematological parameters among Sudanese patients**.**

1.3.2 Specific objectives:

1. To measured hematological parameters and VD level in study group.
2. To compare the hematological parameters between study group .
3. Association between gender among study group.
4.To associate between vitamin D level an hematological parameters among VDD patients
5. To associate between vitamin D level and patients Gender**.**
6. To detection association between hematological parameters and patients Gender.

CHAPTER II

literature review

٢.١ Hematopoeisis

The process that leads to the production and regulation of blood cells is called hematopoiesis. It consists of mechanisms triggering differentiation and maturation of hematopoietic stem cells, Located in the bone marrow, hematopoietic stem cells are undifferentiated cells, unobservable directly (even though they can be tracked by markers), with unique capacities of differentiation (the ability to produce cells committed to one of blood cell types) and self-renewal (the ability to produce an identical cell with the same properties). Under the action of growth factors (molecules acting like hormones playing an activator/inhibitor role), hematopoietic stem cells produce differentiated cells throughout cell divisions until blood cells (white cells, red blood cells, and platelets) are formed and ready to enter the blood stream (Adimy *et al*., 2006).

2.1.1 Leucopoiesis:

We are interested here in leukopoiesis, the process by which white blood cells (also known as leukocytes) are produced, which is a sub-process of hematopoiesis. Like other blood cells, white cells are originated from a pool of hematopoietic stem cells. Under the action of mainly G-CSF (Granulocyte Colony Stimulating Factor), a growth factor only acting on the leukocyte line, hematopoietic stem cells differentiate in progenitors (the so-called CFU, Colony Forming Units), which in turn will produce precursor cells after a consequent number of divisions. After a few divisions late, leukocytes are formed and leave the bone marrow to enter the blood stream (Adimy *et al*., 2006).

vitamin D has been implicated in a broad range of physiological systems including bone and mineral metabolism, cellular proliferation and differentiation, and erythropoiesis (Haroon et al., 2012) These and other findings lead to the proposition that population supplementation with vitamin D will be beneficial (Chung *et al*., 2011). Sim and colleagues have suggested that

vitamin D has a role in erythropoiesis, and that deficiency brings a risk of anaemia, with lower haemoglobin and higher usage of erythrocyte-stimulating agents (Sim *et al*., 2010).

2.1.2 White blood cell:

White blood cells (WBC) are a heterogeneous group of nucleated cells that can be found in circulation for at least a period of their life. Their normal concentration in blood varies between 4000 and 10,000 per microliter. They play a most important role in phagocytosis and immunity and therefore in defense against infection (Cline *et al*., 1983) .

2.1.3 Erythropoiesis:

Human erythropoiesis is a complex, multi-step process, from the multipotent hematopoietic stem cell (HSC) to the mature erythrocyte (Orkin, 2000),in which HSCs differentiate into more committed erythroid progenitors, from a common myeloid progenitor the megakaryocytic-erythroid progenitor and finally the burst-forming unit- erythroid (BFU-E). BFU-Es are the first progenitor cells committed solely to the erythroid lineage (Gregory and Eaves, 1977). These BFU-Es further differentiate into the colony forming unit-erythroid (CFU-E), following which, terminal differentiation occurs.The second phase of erythroid maturation involves the differentiation of the nucleated precursors from proerythroblasts to basophilic, polychromatophilic and orthochromatic erythroblasts. This phase is characterized by the gradual accumulation of hemoglobin, progressive decrease in cell size and nuclear condensation ultimately resulting in enucleation (RD 1964).The final phase of erythroid development involves the maturation of the reticulocyte into erythrocytes. It is during this stage that the erythrocyte acquires its biconcave shape through extensive membrane remodeling and will circulate in the blood stream until it regulated and complex process originating in the bone marrow from a multipotent stem cell and terminating in a mature, enucleated erythrocyte.Every second, the human body generates 2 million red blood cells, through the process of erythropoiesis.The first steps of erythroid differentiation involve an engagement is removed by the macrophages within the reticuloendothelial system (Gifford *et al.,* 2006).

The link between serum 25(OH)D and several important hematological parameters may point to an inhibitory role of vitamin D in the regulation of erythropoiesis in adolescents. What is Known, The physiological effects of vitamin D on calcium homeostasis and bone metabolism

have been established. However, much less is known about the impact of circulating vitamin D on erythropoiesis. What is New, data from the KiGGS study in German adolescents demonstrated significant associations between serum vitamin D concentrations and red blood cell indices. further studies should be conducted to decipher the underlying mechanisms of vitamin D on erythropoiesis (Zhang., 2013).

2.1.4 Platelet

Platelets are small cell fragments that circulate in the blood and are involved in hemostasis, leading to the formation of blood clots The average platelet size (volume) in blood increases when the body is producing more platelets, so MPV is a good measurement of overall platelet function (Khode *et al*., 2012).

2.2 Vitamin D:

Vitamin D a seco-steroid, can either be made in the skin from a cholesterol-like precursor (7-dehydrocholesterol) or by exposure to sunlight or can be provided pre-formed in the diet .Vitamin D is required to maintain normal blood levels of calcium and phosphate, Vitamin D achieves this after its conversion to the active form 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], or calcitriol. This active form regulates the transcription of a number of vitamin D-dependent genes coding for calcium-transporting proteins and bone matrix proteins.Vitamin D also modulates the transcription of cell cycle proteins, that decrease cell proliferation and increase cell differentiation of a number of specialized cells of the body (e.g., osteoclastic precursors, enterocytes, keratinocytes, etc.). This property may explain the actions of vitamin D in bone resorption, intestinal calcium transport, and skin. Vitamin D also possesses immuno-modulatory properties that may alter responses to infections in vivo. The cell differentiating and immuno-modulatory properties underlie the reason why vitamin D derivatives are now used successfully in the treatment of psoriasis and other skin disorders (Feldman *et al*., 1997).

2.2.1 Sources of Vitamin D:

Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements (Holick et al 2006) version made in the skin is referred to as vitamin D_3 whereas the dietary form can be vitamin D_3 or a closely related molecule of plant origin known as vitamin D_2 . Because vitamin D can be made in the skin, it should not strictly be called a vitamin, and some nutritional texts refer to the substance as a prohormone and to the two forms as cholecalciferol (D_3) or ergocalciferol (D_2).From a nutritional perspective, the two forms are metabolised similarly in humans (Blunt *et al.,* 1968).

2.2.2 Functions of Vitamin D:

that are in turn needed for the normal mineralisation of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed. Approximately 33% of women 60 to 70 years of age and 66% of those 80 years of age or older have osteoporosis (Boonen *et* al., 2006). Vitamin D deficiency causes muscle weakness (Holick *et* al., 2006), Skeletal muscles have a vitamin D receptor and may require vitamin D for maximum function (Holick *et* al., 2006) .

2.2.3 Definition of Vitamin D defiency:

vitamin D deficiency is defined by most experts as a 25-hydroxyvitamin D level of less than 20 ng per milliliter (Holick *et al*., 2006) . intestinal calcium transport increased by 45 to 65% in women when 25-hydroxyvitamin D levels were increased from an average of 20 to 32 ng per milliliter (Heaney *et* al., 2003). Given such data, a level of 25-hydroxyvitamin D of 21 to 29 ng per milliliter (52 to 72 nmol per liter) can be considered to indicate a relative insufficiency of vitamin D, and a level of 30 ng per milliliter or greater can be considered to indicate sufficient vitamin D (Dawson *et al*., 2005). With the use of such definitions, it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency ((Holick *et al*., 2006). According to several studies, 40 to 100% of U.S. and European elderly men and women still

living in the community (not in nursing homes) are deficient in vitamin D (Holick *et al*., 2006). More than 50% of postmenopausal women taking medication for osteoporosis had suboptimal levels of 25-hydroxyvitamin D — below 30 ng per milliliter (Holick *et al*., 2005).

Rickets in children and osteomalacia in adults are the classic manifestations of profound vitamin D deficiency. In recent years, however, non-musculoskeletal conditions—including cancer, metabolic syndrome, infectious and autoimmune disorders—have also been found to be associated with low vitamin D levels (Pearce *et al*., 2010).

2 .2.4 Epidemiology :

In Europe, where very few foods are fortified with vitamin D, children and adults would appear to be at especially high risk (Holick *et* al., 2006). People living near the equator who are exposed to sunlight without sun protection have robust levels of 25-hydroxyvitamin D — above 30 ng per milliliter (Vieth *et* al., 2004). Also at risk were pregnant and lactating women who were thought to be immune to vitamin D deficiency since they took a daily prenatal multivitamin containing 400 IU of vitamin D (Hollis et *al*., 2004). 73% of the women and 80% of their infants were vitamin D–deficient (25-hydroxyvitamin D level, <20 ng per milliliter) at the time of birth (Lee et *al*., 2007).

2.2.5 Causes:

2.2.5.1 Reduced synthesis of cholecalciferol in the skin

Reduced sun exposure can result from ageing, veiling, illness or immobility (staying indoors). As people age, their ability to synthesis echolecalciferol from sun exposure decreases. Also, people with dark skin synthesise less cholecalciferol from sun exposure than do people with light skin (Vanlint *et al*., 2005) **,**Disorders of malabsorption**,** Small bowel disorders – especially coeliac disease and inflammatory bowel disease, infiltrative disorders (for example lymphoma, granuloma) and small bowel resection − can cause malabsorption of vitamin D. Other conditions

such as pancreatic disorders (chronic pancreatitis, cystic fibrosis) or biliary obstruction (primary biliary cirrhosis) can have the same effect (Vanlint *et al*., 2005). Enhanced degradation of 25-hydroxyvitamin D , Drugs such as rifampicin and anticonvulsants enhance the degradation of vitamin D which may contribute to or exacerbate vitamin D deficiency (Vanlint *et al.*, 2005).

Factors that affect cutaneous production of vitamin D include latitude, season, time of day, air pollution, cloud cover, melanin content of the skin, use of sunblock, age and the extent of clothing covering the body (Levis *et al*., 2005).

2.2.6 Groups at highest risk of vitamin D deficiency

Older people in residential care or those who are hospitalised, particularly people with hip fractures, are at risk of vitamin D deficiency, as are people living in institutional facilities. This is partly explained by age-related thinning of the skin and partly due to reduced sunlight exposure. Other at-risk groups include dark-skinned women (especially if veiled), ethnic minorities (Asian, Middle-Eastern origin) and refugees (Brock *et al*., 2004).

2.2.7 Risk factors for vitamin D deficiency:

major risk factors for vitamin D deficiency include inadequate sunlight exposure, inadequate dietary intake of vitamin D-containing foods, and malabsorption syndromes such as Crohn's disease (Dedeoglu, 2014). Although clinically apparent manifestations of vitamin D deficiency, such as rickets in children or periosteal bone pain in adults, are uncommon in most developed countries, recent literature suggests that subclinical, asymptomatic vitamin D deficiency still plays a notable role in contributing to several of the leading causes of death (Dedeoglu, 2014).

2.2.8 Factors affecting vitamin D levels:

Factors that affect cutaneous production of vitamin D include latitude, season, time of day, air pollution, cloud cover, melanin content of the skin, use of sunblock, age and the extent of clothing covering the body. When the sun is low on the horizon, atmospheric ozone, clouds and particulate air pollution deflect UVB radiation away from the surface of the Earth (Levis *et al*., 2005).

2.2.9 Clinical features of vitamin D deficiency

Muscle pain,proximal muscle weakness,Rib,hip,thigh and foot pain are typical fractures (Wood *et al*., 2016).

2.3 Diagnosis:

Vitamin D status is most reliably determined by assay of serum 25-hydroxyvitamin D (25-OHD) (Pearce *et al.*, 2010).

2.4 Treatment:

Three options exist for the treatment of vitamin D deficiency: sunlight, artificial UVB light or supplements; all have drawbacks. A total of 15 min of summer noonday sun or artificial UVB radiation (such as tanning beds) on both sides of the bare body will input ~ 10,000 IU of vitamin D into the systemic circulation of most light-skinned adults. One or two such exposures a week should maintain 25(OH)D levels in healthy ranges. Those who chose UVB light for vitamin D repletion, from either sunlight or artificial sources, should avoid sunburns, which are associated with malignant melanoma..The treatment of choice for human vitamin D deficiency is human vitamin D, colecalciferol, also known as vitamin D3 (Houghton L Aand Vieth R 2006).Rickets and osteomalacia should be treated with high strength calciferol (ergocalciferol or colecalciferol) for 8-12 weeks, followed by regular vitamin D supplements (Pearce *et al.*, 2010).

2.5 Pervious study:

Many previous studies investigated the association between VDD patients and complete blood count . Vitamin D has a number of pleiotropic effects in a variety of tissues, in addition to its well-known effects on mineral metabolism. To determine whether it has an effect on erythropoiesis, we studied the association of the components of the vitamin D axis with the prevalence and severity of anemia in chronic kidney disease. We measured the concentrations of 25-hydroxyvitamin D (25D), 1,25-dihydroxyvitamin D (1,25D), and hemoglobin in a cross-sectional study of 1661 subjects in SEEK, a multi-center cohort study of chronic kidney disease patients in the United States, of whom 41% met the criteria for anemia. The mean hemoglobin concentrations significantly decreased with decreasing tertiles of 25D and 1,25D. These linear

trends remained significant after adjustment for age, gender, ethnicity, diabetes, and parathyroid hormone. In similarly adjusted models, the lowest tertiles of 25D and 1,25D were independently associated with 2.8- and 2.0-fold increased prevalence of anemia compared with their respective highest tertiles. Patients with severe dual deficiency of 25D and 1,25D had a 5.4-fold prevalence of anemia compared with those replete in both. Our study shows that 25D and 1,25D deficiency are independently associated with decreased hemoglobin levels and anemia in chronic kidney disease. Whether this association is causal requires further study (Pate *et al.*, ٢٠١٠).

A cross-sectional study carried out as part of the screening and early evaluation of kidney disease project. Vitamin D was measured in subjects recruited at 2 screening camps in Riyadh, Saudi Arabia, between March to May 2008. Subjects from the 2 large commercial centers in Riyadh aged ≥18 years and Saudi nationals were invited. The study sample comprised of 488 subjects. The mean age of the subjects was 37.43) 11.32 (years, of which 50.2%) n=245(were males. Twenty-nine percent of subjects were in the vitamin D deficiency group, 22.7% were in the relative insufficiency group, and 47.5% had normal levels of 25-hydroxy vitamin D. We observed that female gender was an independent predictor of vitamin D deficiency or insufficiency) odds ratio OR(2.992; 95%), confidence intervals (2.069-4.327). Anemia was also a predictor for vitamin D deficiency or insufficiency OR: 3.16; 95% CI 2.02-4.92 (. Age was positively correlated with vitamin D levels) Pearson correlation=0.183, p<0.000.Vitamin D deficiency is common in healthy Saudi adults. This is more pronounced in females and in the younger age groups. Wearing of traditional clothes, deliberate avoidance of the sun, and inadequate dietary intake are likely to be the principal causes of low vitamin D levels (Alsuwadia *et al.*, ٢٠١٣).

The study carried out by Arnson in U.S.A reported mean of WBCs=13.9, and HB=12 g/dl in VDD patients respectively (Arnson *et al*., 2012). A study by Recepin Ankara (turkey)). Increased platelet counts were found in people with low vitamin D levels. Also observed, there was no significant correlation between vitamin D and age, gender,vitamin D groups (deficiency and insufficiency) (Recep *et al*., 2020).

a study in India carried out by randib, result showed no association between vitamin D deficient and anemia reported mean of |(Hb 7-9.9 g/dl) and moderate (Hb 10-12g/dl), but showed increased risk for moderate anemia among vitamin D deficiency (Randib *et al* ., 2019).

Other study conducted by Marah in British, reported vitamin D measurement in South Asians White Europeans mean of vitamin D level (21.7, 30.8) respectively. Result showed significant between VDD and Hematology indices , white cell count, haemoglobin, mean cell volume, mean cell haemoglobin and platelets(*P.V* =0.001),(Marah *et al*., 2012).

CHAPTER III

Materials and Methods

3.1 Materials

3.1.1 Study design

This was a hospital based case control study.

3.1.2 Study area and duration

Study was conducted at Asia hospital Khartoum, Sudan, from February 2020- march 2021.

3.1.3 Study population

one hundred thirty subjects were collected. Sixty five were vitamin D deficiency dignosed and sixty five were healthy volunteer .

3.1.4 Inclusion criteria

Vitamin D deficieny patients, diagnosed with Vitamin D deficiency using vitamin D assay .

3.1.5 Exclusion criteria

Pegnant women.

3.1.6 Ethical consideration

This study was approved by faculty of medical laboratory science, Sudan University for Sciences and Technology. Verbal informed consent was obtained from each participant before samples collection..

3.1.7 Data collection

Patients data (sex ,Complete blood count ,vitamin d level) were collected from patients medical files.

3.1.8 Sample collection

Blood samples (3ml) were collected from each patients upon their consent container.

3.2 Methods

3.2.1 Laboratory methods

3.2.1.1 Hematological parameters estimation (CBC)

This automated hematological analyzer (CBC Sysmex XP_300) can run 20 parameters per sample.It measured the cell count using direct current (DC) detection method and measures hemoglobin concentration by the Non-Cyanide method.

Complete blood count (CBC) is requested to give information about the cells in the patient's blood to diagnosis a medical condition and monitor medical conditions or treatment (VERSO,1964). Blood count of various types of blood cells has been used for clinical purpose since the 19^{th} century and using automated equipment for CBC was developed in 1959s and 1960s (Buttarello and plebani,2008).

Automated procedure of CBC

Blood cells are counted by using direct detection methods with concidence correction. Automatic discrimination separate the cell population based on complex algorithms. The intensity of the electronic pulse from each analyzed blood cell is proportional to the cell volume ,the Sysmex cell counter analyzes RBCs,WBCs and differential, platelets with uncompromised precision and accuracy.

Quality control

The reliability of instruments and reagents were monitored by quality control.

3.2.1.2 Vitamin D assay

Vitamin D deficiency and insufficiency has become a pandemic health problem with a consequent increase of requests for determining circulating levels of 25-hydroxyvitamin D [25(OH)D]. However, the analytical performance of these immunoassays, including radioimmunoassay and Enzymw linked immunosorbant assay (ELISA), is highly variable, and even mass spectrometric methods, which nowadays serves as the gold standard for the quantitatively determination of 25(OH)D, do not necessarily produce comparable results, creating limitations for the definition of normal vitamin D status ranges. To solve this problem, great efforts have been made to promote standardization of laboratory assays, which is important to achieve comparable results across different methods and manufacturers. In this

review, we performed a systematic analysis evaluating critically the advantages and limits of the current assays available for the measure of vitamin D status, i.e., circulating 25(OH)D and its metabolites, making suggestions that could be used in the clinical practice. Moreover, we also suggest the use of alternatives to blood test, including standardized surveys that may be of value in alerting health-care professionals about the vitamin D status of their patients.

3.3 Statistical analysis

Data was analyzed by statistical package for social sciences (SPSS), version20. Qualitative data was represented as frequency and Percentage. Quantitative data was presented as mean ± SD. Association between qualitative variables was tested using Pearson's Chi square ($\chi 2$) and Fisher's exact tests, relationship between qualitative variables and Quantitative variables was tested using independent T-test.

CHAPTER IV

RESULTS

A total of 130 Sudanese subjects were enrolled in this study, 65 of patients diagnosed with vitamin D deficiency and 65 healthy volunteers as a control group. Seventeen of patients were males(26%) and forty eight were females(73%),(Figure 4:1).Twenty eight of control were males(43%) and thirty seven were females(56%). (Figure 4:2)according to vitamin D level, insufficient levels of patients (82%) were males and (64%)were females, deficient level (17%) of patients were males and (33%) were females,(figure 4:3).

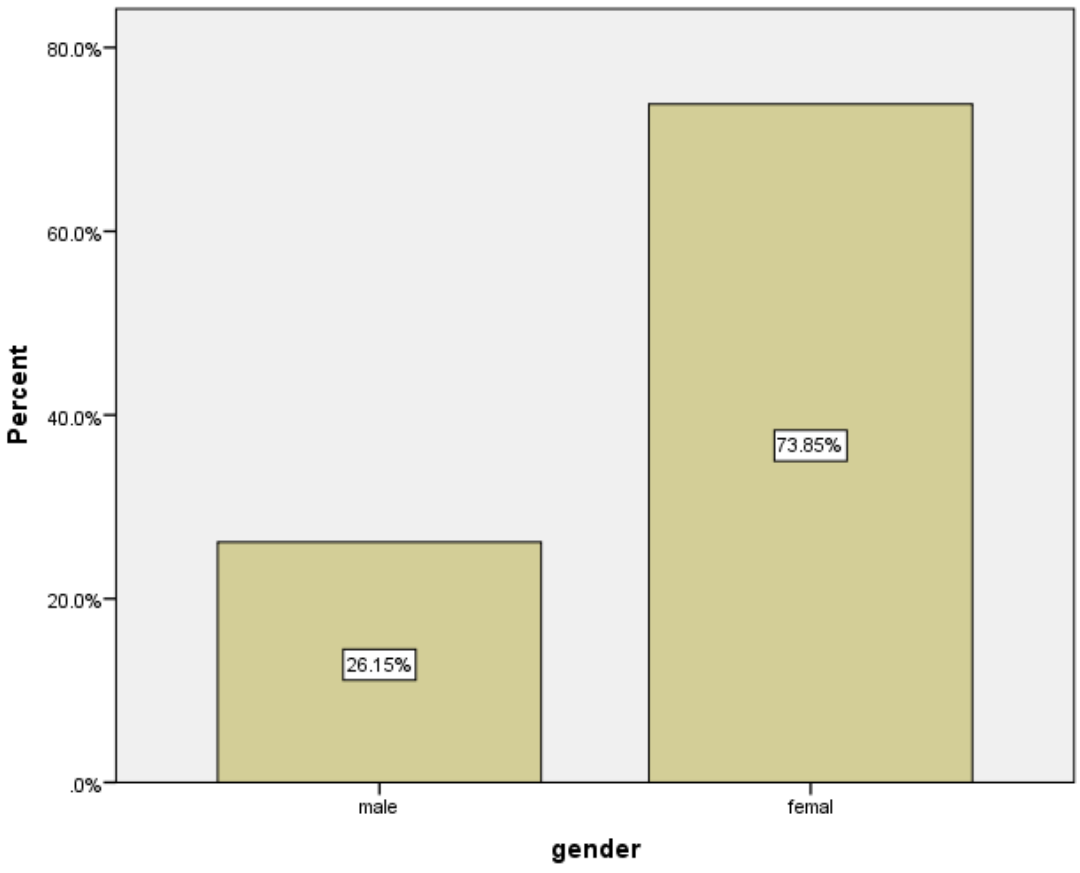

Figure 4:1 gender distribution among VDD patients.

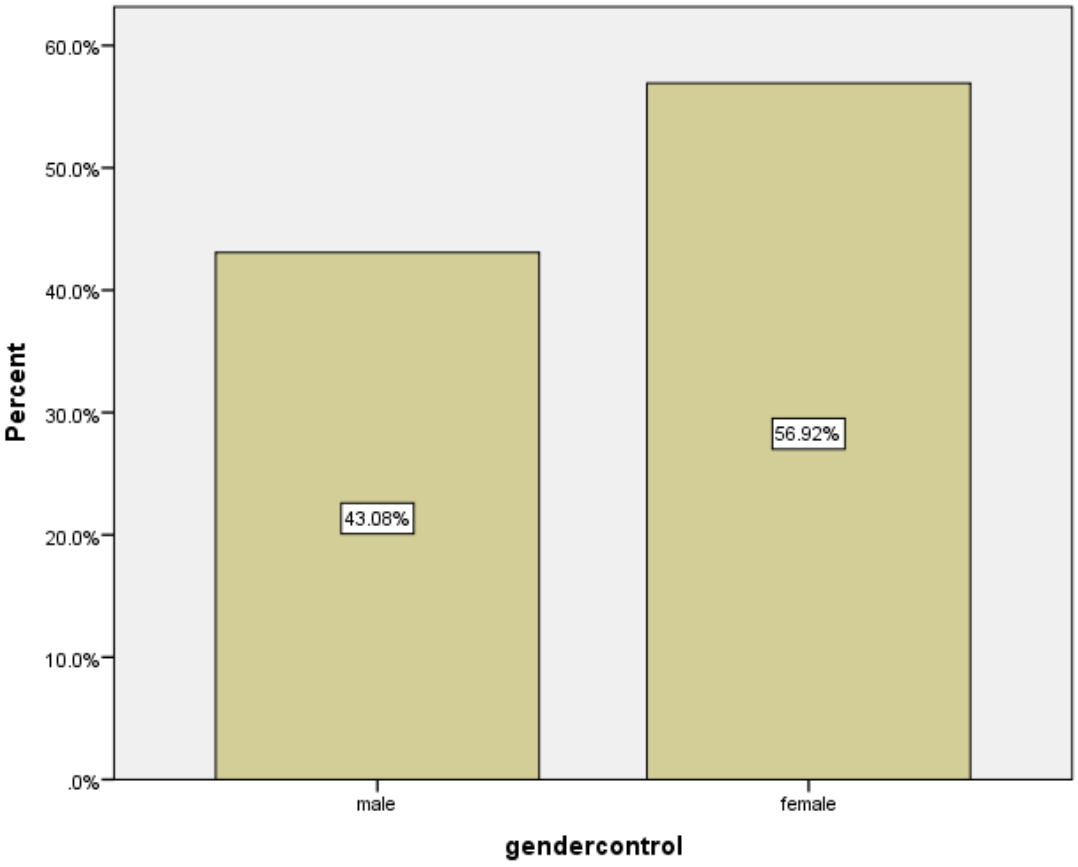

Figure 4:2 Gender distribution among control group

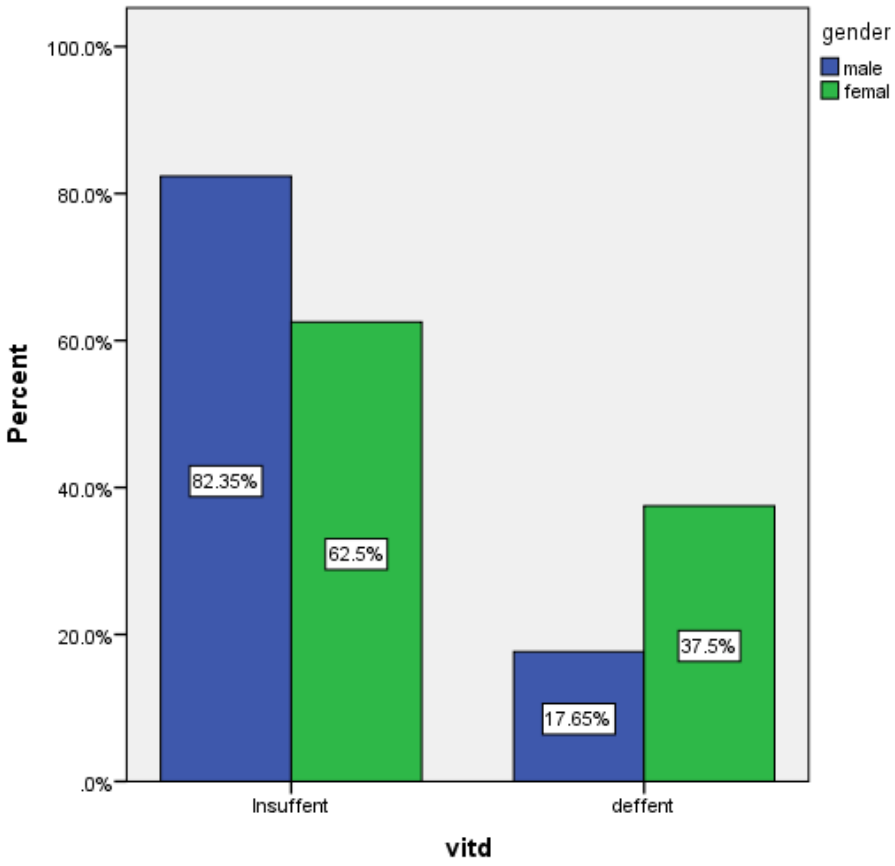

Figure 4:3 VD level and gender of patients

The result showed statically significant association between some hematological parameters among study group, significant (decreased of MCH in case group *P.value*,=(0.01) . Table 4:1 comparative between hematological parameters among study group.

There was statistically significant difference in gender distribution when compared case and control groups. P.value=(0.04). Table 4:2 Association between gender distribution among study groups.

4:1 comparative between hematological parameters among study group

Variables/ Parameters	Mean± SD		*P.value*
	Control	Case	
RBCs	4.8±1	4.6±0.6	0.1
Hb	13.5±2	14.7±16	0.5
HCT	41±6	36±8	0.8
MCV	83±9	91±8	0.4
MCH	28±3	26±2	0.01
MCHC	34±9	32±1	0.1
WBCs	7±3	7.7±4	0.5
Neutrophil	52±12	51±11	0.9
Lymphocyte	37±11	44±37	0.1
Monocyte	5±2	4.9±2	0.1
Eosinophil	3±5	2.6±1	0.3
Platelet	292±13	312± 86	0.2

Table4:2Association between gender distribution among study groups

Gender	VD		*P.value*
	Control	Case	
Male	20.9%	13.2%	0.04
Female	28.7%	37%	

The study showed significant statistical difference between mean of RBCs and MCHC and VD level in patients (significant decreased in deficient level). *(P.value*=0.03, 0.01)Respectively .
The result showed no statically significant association between RBCs and other RBCs indices and VDD patients.
Table 4:3 comparative between RBCs and RBCs indices and VDD patients.
The result showed no statically significant association between WBCs and deferential leucocytes count and VD level in patients. Table 4:4 association between WBCs and deferential leucocytes count and VD level in patients.
The result also showed no statically significant association between platelet count and VD level in patients. (P.*value* =0.2). Table 4:5 association between platelet count and VDD patients.
The result showed no statically significant association between VD level and gender of patients, *(P.value* =0.1) **Table 4:6** association between VD level and gender of patients.

Table 4:3 Association between RBCs and RBCs indices and vitamin D levels.

Variables/ Parameters	Insufficient	Deficient	*P.value*
RBCs	4.7±0.5	4.2±.0.9	0.03
Hb	16±19	11±2	0.19
HCT	37±7	33±10	0.11
MCV	96±108	80±11	0.5
MCH	26.5±3	31±1.5	0.7
MCHC	32±1.4	23±1.6	0.01

Table 4:4 Association between WBCs and deferential leucocytes count and VDD patients.

Variables/ Parameters	Insufficient	Deficient	*P.value*
WBCs	7.2±2.2	9±.8.1	0.3
Neutrophils	52%±12	51%±10	0.8
Lymphocytes	39%±11	55%±64	0.2
Monocytes	5%±2.6	4%±2.5	0.5
Eosinophil	2.6%±1.3	2.4%±1.1	0.4

Table 4:5 Association between platelet count and vitamin d level of patients

Variables	PLT count		*P.value*
	Mean	**SD**	
Insufficient	320	95	0.2
Deficient	296	63	

Table4:6Association between VDD and gender among case study.

Variables	VD		*P.value*
	Insufficient	**Deficient**	
Male	21.5%	4.6%	0.1
Female	46.2 %	27.7|%	

The result showed significant statistical difference between mean of MCHC and gender of patients (significant decreased in female). *(P.value*=0.03) .The result showed no statically significant association between RBCs and other RBCs indices and gender of patients. Table 4:7 association between RBCs and RBCs indices and gender of patients. The result showed significant statistical difference between mean of neutrophils and gender of patients (significant decreased in female), (*P.value*=0.04) . The result showed no statically significant association between WBCs and other differential leucocytes count and gender of patients. Table (4:8)

association between WBCs and differential leucocytes count and gender of patients. The result showed no statically significant association between platelet count and gender of patients (P.*value* =0.1) Table 4:9 association between platelet count and gender of patients.

Table 4:7 association between RBCs and RBCs indices and gender of patients

Variables/ Parameters	Male	Females	*P.value*
RBCs	4.5±0.7	4.6±.0.6	0.44
Hb	15.4±11.9	15±17	0.85
HCT	37±310	36±8	0.72
MCV	83.6±7.9	94±104	0.66
MCH	27±3	26±2.3	0.06
MCHC	33±1	23±1.6	0.03

Table 4:8 Association between WBCs and deferential leucocytes count and gender of patients

parameters Variables/	Females	Male	P.value
WBCs	7.9%±.2.6	7.4±2.4	**0.7**
Neutrophils	50%±11	65%±12	**0.04**
Lymphocyte	41%±10	52%±73	**0.3**
Monocyte	5%±2.5	4%±2.7	**0.4**
Esinophil	2.6%±1.2	2.6%±1.5	**0.9**

Table 4:9 association between platelet count and gender of patients

Variables	PLT count		*P.value*
	Mean	**SD**	
Male	287	86	0.1
Female	321	85	

Chapter V

Discussion, Conclusion, and Recommendations

5.1 Discussion

This study investigated the evaluation of hematological parameters among vitamin D deficient patients.

The study showed significant statistical difference between mean of RBCs and MCHC and VD level in patients (decreased in deficient level). (*P.value*=0.03, 0.01) Respectively. The result showed no statically significant association between RBCs indices and VDD patients. Agreement with in young north India children reported by randib chowdury , no association between VDD and anemia but showed increased risk for moderate anemia among VD deficient patients (Randib.,*et al* 2019).

The result showed no statically significant association between WBCs and differential leucocytes count and vitamin D level. The also showed no statically significant association between platelet count and vitamin D level. (P.*value* =0.2)., in study our result disagree, Increased platelet counts were found in people with low vitamin D levels (***P.V***=0.008). (Recep*et al* .,2020). Also disagree with study conducted in In South Asians by marwah ,study reported association between VDD Increased platelet counts *(PV*=0.008).(Marwah *e t al*., 2012) .

The results showed that,significant statistical difference between mean of MCHC and gender of patients (decreased in female). *(P.value*=0.03) .The result showed no statically significant association between RBCs and other RBCs indices and gender of patients. Our study disagreement study conducted in In South Asians by marwah ,study reported association between VDD and some haematological parameters ,in female, decreased Hb ,MCV,and MCH, *(PV*<0.001). also reported, *(*Marwah *et al*., 2012)

The result showed significant statistical difference between mean of neutrophils and gender of patients (decreased in female).(*P.value*=0.04). The result showed no statically significant association between WBCs and other deferential leucocytes count and gender of patients.Our study disagreement study conducted in In South Asians by marwah ,study reported association between VDD and female: increased WBCs,P.*value* =(0.001)).(marwah *et al*., 2012) The result showed no statically significant association between platelet count and gender of patients. (P.*value* =0.1) disagreement study conducted in In South Asians by marwah ,study reported association between VDD and female Increased platelet counts *(PV*=0.008).(marwah *et al*., 2012)

The result showed no statically significant association between VD level and gender of patients. *(P.value* =0.1) ,This in agreement with a study carried out by Recepin Ankara, there was no significant correlation between vitamin D level and gender of vitamin D groups (deficiency and insufficiency).(Recep *et al*., 2020) .

.Our study reported normal WBCS =9 ±8 and decreased Hb=11±2 retrospectively, this is result disagreement with study conducted by Arnson In U.S.A. showed, Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients.A reported in VDD patients there was increased WBCs=13.9, and normalHb=12 (Arnson *et al*., 2012).

5.2 Conclustion

The result showed significant association between Vitamin D deficiency and some hematological parameters.

5.3 Recommendation

1-Further studies should be conducted to associate between peripheral blood picture and VD level and conducted with large sample size.

2-Future studies should be conducted to identify association between hematological parameters and genotypes variation of VDD.

3-Future studies should be conducted to correlate between VD level and age of patients .

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Appendixes

Sysmex XP-300™ Automated Hematology Analyzer

Reagents :

Diluents

it is a kind of reliable isotonic diluents which can dilute white blood cells (WBC), red blood cells (RBC), platelet(PLT) and hemoglobin(HB), keep the shape of cells during test process, offer appropriate background value and clean WBC and RBC micro-aperture and tubes operationmanual

Lyse:

Lyse is a new-type reagent without NaN3 complex and cyanide which can dissolve RBC instantly with minimum ground substance complex, transform the membrane of WBC to diffuse cytoplasm, and then WBC shrinks to form membrane –bound nuclei, transform the hemoglobin to form hemo-compound which is suitable for the measurement in the condition of 540 n m wavelength and avoid the pollution caused by cyanide (Operation manual, 2015).

Cobas E 411:

The cobas e411 analyzer is a fully automated analyzer that uses a patented Electrochemil uminescence (ECL) technology for immunoassay analysis. It is designed for both quantitative and qualitative in vitro assay determinations for a broad range of applications including , Anemia bone cardiac and tumor markers , hormones and infectious diseases . The analyzer is available as a rack or disk sample handling system.

Sysmex XP-300™ Automated Hematology Analyzer

Cobas E411