Sudan University of Science and Technology

College of Graduate Studies

Assessment of Serum Uric Acid and Albumin among Sudanese Patients with Deep Vein Thrombosis

تقييم مستوي حمض اليوريك و الألبيومين في مصل الدم لدى السودانيين المصابين بمرض تخثر الوريد العميق

A dissertation submitted in partial fulfilment for the requirement of M.Sc degree in Medical Laboratory Sciences

(Clinical Chemistry)

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بسم الله الرحمن الرحيم

قَالَ تَعَالَى :

﴿وَ إِذَا مَرِضْتُ فَهُوَ يَشْفِينِ﴾

سورة الشعراء الآية : ﴿80﴾

DEDICATION

To symbol of love &giving my parents…

…….

To every patients suffers from DVT…

……

To my beautiful nation SUDAN …

……

To the origin of creation and excellence SUST

……..

ACKNOWLEDGMENTS

First, I thank Allah who help me to accomplish this work.

I would like to express my appreciation to my supervisor**. Nuha Elgaili Aubaker** who has cheerfully answered our queries, provided me with materials, checked my examples assisted me in a myriad ways with the writing and helpfully commented earlier draft of this project. I am very proud to join you in this project; it's really great honour for me.

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Abstract

Background: Deep venous thrombosis (DVT) is a common condition estimated to affect around 100000 patients each year in the UK.. it is associated with increased mortality in some thrombotic disorders .

Objectives: This study conducted to assess the serum uric acid, and albumin in DVT patients.

Methods: This was case control study based on 50 DVT patients as cases, and 50 apparently healthy individuals as controls (age and gender were matched between two groups) Blood sample was taken from each participant to prepare the serum . The serum uric acid and albumin concentrations were measured using Bio system BTS-400 spectrophotometer.The data obtained was subjected to analysis using statistical packaged for social science computer program (SPSS version 20).

Results: The result showed that, (70%) of patients at age range between (20-40) years, (24%) between (41-60)years and (6%) between (61-80) years. (80%) of patients were females while (20%) were males. The levels of albumin was significantly increase in DVT patients compared to control group and there was no significant difference in uric acid level in DVT patients compared to control group : albumin (mean ± SD: 4.1 ± 0.65 g/dl versus 3.5±0.49 g/dl, P .value= (0.000), uric acid (mean ±SD: 3.85 ±1.8 mg/dl versus 3.88 ± 1.2, P .value = (0.929). There were negative correlation between albumin , serum uric acid and age. (r = -0.334 , *p* .value=0.018 and r =-0.339 *p* .value=0.016)respectively , there were no correlation between the albumin ,Serum uric acid and duration of disease (r= - 0.026**,** P. value =0.858) , (r= - 0.018**,** P. value =0.903) respectively .

Conclusion: The results of this study concluded that , the levels of albumin is increased in DVT patients and the level of it increased in females than males. There were negative correlation between albumin, serum uric acid and age.

المستلخص

الخلفيه :يعتبر تخنثر الوريد العميق من الحالات الواسعة الانتشار حيث قدر تأثيره بحوالي 100000 مريض سنويا بالولايات المتحده , وقد وجد انه يصاحب ازدياد عدد الوفيات في بعض امراض التخنثر المختلفه .

الاهداف : قمت بهذه الدراسه لتقييم مستوي اليورك اسيد وسيرم الالبيومين في مرضي تخنثر الوريد العميق .

المواد والطرق: أجريت هذه الدراسة في 50 مريض بتخنثر الدم العميق و50 متطوع من الأصحاء كعينات ضابطه واخذت عينة الدم من كل الافراد لتحضير مصل الدم وتم قياس حمض اليورك و الألبيومين باستخدام جهاز البايوسيستم -400 وتم تحليل البيانات إحصايئا باستخدام برنامج (اس بي اس اس اصدار 20).

النتائج: أوضحت النتائج أن **70 %** من المرضي في مدي عمري بين **(20-40)** سنه و **24 %** بين **(41-60)** سنه و**6 %** بين **(61-80)** سنه . **80%** من المرضي اناث و **20%** ذكور . مستويات الالبيومين زادت , بينما لا يوجد فرق معنوي في مستوي سيرم اليورك اسيد في المرضي مقارنة مع المتطوعين الأصحاء مع قيمة ." المتوسط ± الإنحراف المعياري للمرضى مقارنة بمجموعة التحكم ". بالنسبة للالبيومين : (4،1 ± **0،65** جرام / ديسلتر مقابل3,**5** ± **49**، جرام / ديسلتر وكان الاحتمال الاحصائي للمقارنة 0.000). لليورك اسيد : (**58**،3 ± **1،8**مقابل **3.88** ± 1،2 ملجرام / ديسلتر وكان الاحتمال الاحصائي للمقارنة 0.929). اعتمادا العلاقه بين العمر وكل من الالبيومين و سيرم اليورك اسيد كانت ضعيفه وذات دلاله معنويه (معامل بيرسون للإرتباط = -0،334 ومستوى المعنوية = 0،018 و معامل بيرسون للإرتباط = -**0،339** ومستوى المعنوية = 0،0**16**) علي التوالي . أيضا لم توجد علاقه واضحه بين فترة الاصابه بالمرض وكل من الالبيومين وسيرم اليورك اسيد (معامل بيرسون للإرتباط = -0،026 ومستوى المعنوية = 0،**858** و معامل بيرسون للإرتباط = -**0،018** ومستوى المعنوية = 0،903) علي التوالي.

الخلاصه : خلصت الدراسه الي ان مستويات الالبيومين تزيد مع مرضي تخنثر الدم وكذلك مع الاناث اكثر من الذكور. واخيرا وجدت علاقه عكسيه بين الالبيومين ومصل اليورك اسيد باخذ العمر في الاعتبار.

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Abbreviation:

Conc : Concentration

DVT : deep venous thrombosis

KDa : Kilo Dalton

PE : Pulmonary Embolism

UA : Uric Acid

UK : United Kingdom

VTE : Venous Thromboembolism

CHAPTER ONE

Introduction, rationale and objectives

1.1 Introduction

Thrombosis is the formation of a blood clot in the vasculature ; two types of thrombosis are known: arterial and venous thrombosis , Arterial thrombosis is mainly composed of platelets with small amounts of red cells and white cells whereas venous thrombosis is composed of fibrin clot and red cells (**Betty,2007)** . Deep venous thrombosis (DVT) is a common condition estimated to affect around 100 000 patients each year in the UK. It can lead to death through pulmonary embolism and rarely limb loss through phlegmasia cerulean dolens **(Prakash *et al* ., 2016).**

Uric acid is the breakdown product of purines. Increased uric acid levels promote oxygenation of low-density lipoprotein cholesterol and facilitate lipid peroxidation, it may stimulate vascular smooth cell proliferation, and reduce vascular nitric oxide production. Moreover, higher uric acid levels may be associated with increased platelet adhesiveness predisposing to thrombus formation (**Tavish *et al* ., 2018**).

Albumin is a negatively charged, water soluble protein (molecular weight 66.3 kDa) that has a single polypeptide chain of 580 amino acids. It is synthesized primarily in liver except in early fetal life, when it is synthesized largely by the yolk sac **(Burtis *et al*., 2006)**. , recent studies have shown evidence that low serum albumin, being an acute phase reactant, may reflect inflammation, which is sometimes linked to VTE occurrence . Or, low serum albumin may reflect renal loss of albumin and anti-thrombotic proteins, and thus the same hypercoagulable state apparent in the nephrotic syndrome. In neither case would low serum albumin be considered a direct cause of venous thromboembolism (VTE) (**Aaron *et al*.,2010) .**

1.2 Rationale:

The venous thromboembolism (VTE), usually manifested by deep venous thrombosis (DVT) and pulmonary embolism (PE), is a common medical problem with an estimated incidence of 1-2 per 1000 person-years , Patients who develop VTE have high mortality rates of 11-30% . Increased serum uric acid levels may favor micro vascular diseases stimulating vascular smooth muscle cell proliferation and the serum albumin levels less than 2.0 to 2.5 g per dL (20 to 25 g per L) they seem to confer an increased risk of DVT **,** Therefore, increasing serum uric acid and albumin are expected to be positively associated with VTE (**Quintana *et al* , 2016)**, but, to the best of our knowledge, there are no published study assess this parameters in DVT in Sudan.

1.3 Objectives:

1.3.1 General objectives:

To assess serum uric acid and albumin levels in Sudanese patients with deep venous thrombosis.

1.3.2 Specific objectives:

1. To measure uric acid and albumin levels in study groups.
2. To compare the levels of uric acid and albumin in both study groups.
3. To correlate between biochemical parameters and study variables (age and duration of disease).

CHAPTER TWO

Literature review

2.Literature review :

2.1 Deep venous thrombosis:

Thrombosis is the formation of a blood clot in the vasculature. Two types of thrombosis are known: arterial and venous thrombosis. Arterial thrombosis is mainly composed of platelets with small amounts of red cells and white cells whereas venous thrombosis is composed of fibrin clot and red cells. Thrombosis may result from vascular injury, platelet activation, coagulation activation, defects in the fibrinolytic system, and defects in physiological inhibitors. Arterial and venous thrombosis along with complicating thromboembolism is the most important cause of death in the developed countries (**Betty , 2007)**. Deep venous thrombosis (DVT) is a common condition estimated to affect around 100 000 patients each year in the UK. It can lead to death through pulmonary embolism and rarely limb loss through phlegmasia cerulean dolens. The chronic sequelae of DVT, known as post-thrombotic syndrome (PTS), includes persistent pain, swelling or ulceration that occurs in around half of patients within 2 years of a DVT. PTS is associated with significant morbidity and, together with venous thromboembolism, carries a financial burden to the NHS costing an estimated about 1 billion per year to treat(**Prakash *et al*., 2016)**.

2.1.1 Signs and symptoms of deep venous thrombosis:

DVT can occur without the patient showing any signs or symptoms. Several factors determine presentation of a DVT, including the size of the thrombus, which can extend to occlude both proximal and distal veins, the ability of collateral blood vessels to cope with transporting blood to bypass the thrombus, and the severity of vascular occlusion (blockage) and

inflammation caused by the thrombus , so more likely to cause symptoms when it obstructs venous outflow, resulting in inflammation of the vein wall and surrounding tissue ,common symptoms of a DVT are warmth, redness, pain and swelling in the affected limb. When a patient reports these symptoms, a clinician should undertake a physical examination of the whole limb to observe for signs suggestive of DVT. These include tenderness on palpation, warmth, erythema, cyanosis, oedema and superficial venous dilation that can present as prominent collateral veins these signs and symptoms are not specific to DVT and can be present in numerous other conditions. Therefore, clinical judgement about the likelihood of DVT should also take into account the patient's individual risk factors for DVT, concurrent illnesses and medication, medical and surgical history, and demographic characteristics **(Bonner *et al* ., 2014) .**

2.1.2 Diagnosis of deep venous thrombosis:

Firstly : Compression/duplex ultrasonography of femoral and popliteal veins has both sensitivity and specificity of 97% in detecting DVT in a symptomatic patient. It is sensitive for proximal vein thrombosis but less sensitive for calf vein thrombosis.

Secondly : Impedance plethysmography records the electrical impedance of the calf region following a temporary occlusion of proximal veins. The sensitivity of this method is 96%, 50% and 38% for the diagnosis of acute DVT of the proximal, popliteal and distal veins, respectively (**Narani , 2010).**

Thirdly : Contrast venography remains the gold standard for the diagnosis of DVT. It is able to detect all clinical forms of DVT, including thrombosis in calf veins, pelvis and inferior vena cava.

Fourthly : Radionuclide ascending venography assesses the "thrombus burden" in the femoral, iliac, caval and pulmonary circulation. It has a sensitivity of 90% and specificity of 92% in detecting DVT in the proximal leg veins.

Finally: Plasma D-dimer is a marker of cross-linked fibrin degradation products. A negative D-dimer result can exclude DVT and PE in a patient with suspected VTE (**Narani , 2010).**

2.1.3 Causes of deep venous thrombosis:

Rudolph Virchow in 1856 described the factors that predispose to DVT, which are relevant even today. Virchow's triad comprises of 3 factors: venous stasis, damage to venous wall and hypercoagulability (**Narani ., 2010) .**

2.1.4 Pathophysiology and risk factors deep venous thrombosis:

The pathogenesis of VTE is often described by Virchow's triad, which proposes that venous thrombosis is the result of at least one of three etiologic factors: hypercoagulability, alterations in blood flow, and endothelial injury or dysfunction. Risk factors for VTE reflect these underlying pathophysiologic mechanisms, and between 75 and 96 percent of patients with VTE have at least one risk factor. If VTE is suspected, risk factors should be assessed to determine the pretest probability. Some factors suggest greater risk of VTE than others **(Wilbur and Shian, 2012).**

2.1.5 Treatment of deep venous thrombosis:

The mainstay of treatment for VTE is anticoagulation. Anticoagulants do not dismantle thrombi directly; rather, they prevent propagation of the thrombus while the endogenous fibrinolytic system works to decrease the clot burden. In most cases, treatment of VTE should be based on confirmatory diagnostic studies, but if the pretest probability is high enough, anticoagulation may be started empirically pending definitive testing. Before initiation of anticoagulation, baseline laboratory studies should be obtained including urinalysis, Hemoccult, hemoglobin, hematocrit, platelet count, prothrombin time, International Normalized Ratio (INR), activated partial thromboplastin time (aPTT), blood urea nitrogen, and creatinine . Parenteral agents for the acute treatment of VTE include weight-based unfractionated heparin (either intravenously or subcutaneously dosed); weight-based low-molecular-weight heparin (LMWH) , and the synthetic anti-Xa agent, fondaparinux . Intravenous unfractionated heparin typically is administered by an initial bolus (loading) dose followed by a continuous infusion; ensuing dose adjustments should be made using a standard nomogram . Weight based, subcutaneous unfractionated heparin also may be used with aPTT monitoring and dose adjustment to maintain the aPTT in therapeutic range **(Minichiello and Patrick ., 2008).**

2.2 Albumin:

2.2.1 Chemistry of albumin :

Albumin is a negatively charged, water soluble protein (molecular weight 66.3 kDa) that has a single polypeptide chain of 580 amino acids. It is synthesized primarily in liver except in early fetal life, when it is synthesized largely by the yolk sac. **(Burtis *et al*., 2006).**

2.2.2 Distribution of albumin :

Albumin is synthesized in the liver at a rate of 9 to 12 g/day and is the most abundant protein in the plasma. Albumin also exists in the extravascular (interstitial) space. The total amount of extravascular albumin exceeds the total intravascular amount by about 30%; however, the concentration of albumin in plasma (albumin mass/plasma volume) is much greater than its concentration in the interstitial space**(Bishop *et al* ., 2018).**

2.2.3 Physiology of albumin:

Albuminhas an important role in maintaining colloidal oncotic pressure and preventing edema. Since water moves freely through cell membranes and into the intravascular space by osmosis, the high concentration of proteins in intravascular fluid allows movement of water into vessels, and normal blood pressure and cardiac output allow circulation to evenly distribute the fluids. If the concentration of albumin is significantly decreased, fluids accumulate in interstitial spaces and cause edema. Normal protein concentration in blood vessels allows fluid to flow freely from intracellular to interstitial to intravascular spaces. Albumin also serves as a transport molecule for various substances. **(Arneson and Brickell , 2007).**

2.2.4 Functions of albumin:

Albumin is important in regulating the flow of water between the plasma and tissue fluid by its effect on plasma colloid osmotic pressure (oncotic pressure). When the concentration of albumin is significantly reduced, the plasma osmotic pressure is insufficient to draw water from the tissue spaces back into the plasma. This leads to a build-up of fluid in the tissues, referred to as oedema. Albumin also has important binding and transport functions. It binds and inactivates substances including calcium, bilirubin, fatty acids, urate, hormones, and magnesium, and also drugs such as penicillin, salicylates, sulphonamides, and barbiturates. When albumin levels are reduced, toxic effects can develop from an increase in unbound substances. Albumin diffuses easily through damaged membranes and is more readily filtered out by the kidneys than most globulins because its molecules are smaller. **(Cheesbrough , 2009).**

2.2.5 Clinical Significance of albumin:

2.2.5.1 Hypoalbuminemia:

Hypoalbuminemia may result from decreased synthesis, increased catabolism (use or loss), or combinations of these. Decreased synthesis may be primary (as in analbuminemia) or acquired (as in inflammation and liver disease). Also hypoalbuminemia may occur due to urinary loss, gastrointestinal loss, ascites or protein calorie malnutrition. **(Burtis *et al* ., 2006).** Non-nutritional causes of hypoalbuminemia, such as tissue injury, hepatic disease, gastrointestinal disorders, and volume overload, can affect the specificity of this marker **(Thomas *et al*., 2009) .**

2.2.5.2 Hyperalbuminemia:

Increased serum albumin levels are seen only with dehydration or after excessive albumin infusion. **(Bishop *et al* ., 2010)**

2.3 :Uric acid :

2.3.1 Chemistry of uric acid :

Uric acid is the product of catabolism of the purine nucleic acids. Although it is filtered by the glomerulus and secreted by the distal tubules into the urine, most uric acid is reabsorbed in the proximal tubules and reused. Uric acid is relatively insoluble in plasma and, at high concentrations, can be deposited in the joints and tissue, causing painful inflammation. Purines, such as adenine and guanine from the breakdown of ingested nucleicacids or from tissue destruction, are converted into uric acid, primarily in theliver**(Bishop *et al* ., 2018).**

2.3.2 Distribution of uric acid:

Uric acid is transported in the plasma from the liver to the kidney, where itis filtered by the glomerulus. Reabsorptionof 98% to 100% of the uric acidfrom the glomerular filtrate occurs in the proximal tubules. Small amounts of uric acid are secreted by the distal tubules into the urine. Renal excretionaccounts for about 70% of uric acid elimination; the remainder passes into the GI tract and is degraded by bacterial enzymes **(Bishop *et al* ., 2018).**

2.3.3 Physiology and Synthesis of uric acid:

Uric acid is the end product of an exogenous pool of purines and endogenous purine metabolism **(Chaudhary *et al.*, 2013)**. Most uric acid is derived from the metabolism of endogenous purine **(Schlesinger, 2005)**, endogenous production of uric acid mainly from the liver, intestines and other tissues like muscles, kidneys and the vascular endothelium **(Chaudhary *et al.*, 2013)**. The liver produces uric acid by degrading dietary and endogenously-synthesized purine compounds **(Jung *et al.*, 2010)**. Initially, adenosine monophosphate (AMP) is converted to inosine via two

different mechanisms; either first removing an amino group by deaminase to form inosine monophosphate (IMP) followed by dephosphorylation with nucleotidase to form inosine, or by 16 first removing a phosphate group by nucleotidase to form adenosine followed by deamination to form inosine. Guanine monophosphate (GMP) is converted to guanosine by nucleotidase. The nucleosides, inosine and guanosine, are further converted to purine base hypoxanthine and guanine, respectively, by purine nucleoside phosphorylase (PNP). Hypoxanthine is then oxidized to form xanthine by xanthine-oxidase (XO), and guanine is deaminated to form xanthine by guanine deaminase. Xanthine is again oxidized by xanthine oxidase to form the final product, uric acid **(Maiuolo *et al.*, 2016)**.

2.3.4 Functions of uric acid:

Uric acid is an alarm initiating the inflammatory process that is necessary for tissue repair, a scavenger of oxygen free radicals, a mobilizer of progenitor endothelial cells and supporter of adaptive immune system **(Nery *et al.*, 2015)**. It is thought that UA provides 60% of free-radical scavenging capacity in plasma. It is considered one of the most prominent antioxidants in the blood of humans **(Nery *et al.*, 2015)** , and its antioxidant properties are as powerful as those of ascorbic acid **(So and Thorens, 2010).** Uric acid can activate antigen presenting cells, leads to an increased T cell response **(Nery*et al.*, 2015)** .Finally, increase in UA can maintain blood pressure in conditions of low salt ingestion **(Álvarez and Macarrón ., 2010)**.

2.3.5 Clinical Significance of uric acid:

2.3.5.1 Hypouricemia:

Hypouricemia is generally defined as a serum uric acid (SUA) concentration of less than 2.0 mg/dL , It has no recognizable symptoms that require treatment. It is characterized by increased uric acid clearance or decreased

uric acid production **(Son *et al* .,2016).**and so is seen in congenital xanthine oxidase deficiency (xanthinuria), severe liver disease and renal tubular disorders such as the Fanconi syndrome. It can also result from excessive medication with allopurinol and the use of uricosuric drugs such as probenecid (**Marshall,2012)**. It has no recognizable symptoms, and therefore requires no treatment. However, it is a biochemical finding that deserves attention since it may be associated with primary or secondary tubulopathies and other underlying conditions **(Martín and Nieto ., 2011)**.

2.3.5.2 Hyperuricemia:

Hyperuricemia is defined as serum uric acid level of more than 7 mg/dL and blood levels of uric acid are causally associated with gout, as implicated by evidence from randomized clinical trials using urate lowering therapies**(Quintana ., *et al* 2016)**.Hyperuricaemia may occur because of increased formation of uric acid, decreased excretion, or a combination of both (**Marshall ,2012)**. In 25% to 30% of these patients, hyperuricemia is a result of overproduction of uric acid, although hyperuricemia may be exacerbated by a purine-rich diet, drugs, and alcohol. Plasma uric acid concentration in affected individuals is usually greater than 6.0 mg/dL. Patients with gout are susceptible to the formation of renal calculi, although not allpersons with abnormally high serum urate concentrations develop this complication. Patients with hemolytic or megaloblastic anemia may exhibit elevated uric acid concentration. Increased urate concentrations may be found following ingestion of a diet rich in purines (e.g., liver, kidney, sweetbreads, and shellfish)or as a result of increased tissue catabolism due to inadequate dietary intake(starvation) **(Bishop *et al* ., 2018).**Hyperuricemia is associated with deleterious effects on endothelial dysfunction, oxidative metabolism, platelet adhesiveness, hemorheology, and aggregation. The

atherosclerotic plaque contains a considerable amount of uric acid which may increase platelet adhesiveness and potentiate thrombus formation **(Quintana *et al* ., 2016).**

2.3.5.2.1 Gout:

Acute gout is characterized by severe joint pain of rapid onset associated with swelling and redness. The risk of gout increases with increasing plasma urate concentrations and thus with age. At any particular urate concentration, the risk is similar in males and females. However, although hyperuricaemia is a prerequisite for the development of gout, gout by no means always complicates hyperuricaemia. Indeed, some 85% of people with hyperuricaemia remain asymptomatic throughout life **(Marshall ,2012)**.

2.3.5.3 The Relationship between uric acid, albumin and deep vein thrombosis:

Deep venous thrombosis (DVT) is a common condition estimated to affect large numbers of patients each year in the UK. It can lead to death through pulmonary embolism and rarely limb loss through phlegmasia cerulean dolens**(Prakash *et al*., 2016)** . Hypoalbuminemia was a modest marker of increased DVT risk (**Aaron *et al*.,2010)**. Hypoalbuminemia, particularly ,2.8 g/dl, is the most significant independent predictor of venous thrombotic risk **(Lionaki *et al* .,2012).**Hyperuricemia is associated with deleterious effects on endothelial dysfunction, oxidative metabolism, platelet adhesiveness, hemorheology, and aggregation. The atherosclerotic plaque contains a considerable amount of uric acid which may increase platelet adhesiveness and potentiate thrombus formation **(Quintana *et al* ., 2016).**

CHAPTER THREE

Materials and methods

3.1 Materials:

3.1.1 Study approach:

Quantitative method was used to estimate uric acid and albumin levels in Sudanese patients with Deep Venous Thrombosis in Khartoum state during the period from January to March 2019.

3.1.2 Study design:

This is cross sectional hospital base case control study.

3.1.3 Study area:

This study was conducted in Omdurman teaching hospital in Khartoum state.

3.1.4 Study population:

The study included patients with deep vein thrombosis of case and health individuals

3.1.5 Sample size:

A total of 100 samples were collected (50 patients and 50 apparently healthy individual serve as control (age and sex matched with test group).

3.1.6 Inclusion Criteria:

Patients who diagnosed with deep vein thrombosis and healthy individuals were included in this study (age and genders were matched between two groups) .

3.1.7 Exclusion criteria:

Smoker, diabetic, hypertensive patients , renal diseases , gout disease and any disease that contribute to changes in thrombus formation

3.1.8 Ethical consideration:

Verbal consent was obtained from all participants and reassurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose

3.1.9 Data collection:

The clinical data were obtained and recorded on questionnaire sheet.

3.1.10 Sample collection and processing:

Venous blood samples were collected from participants , by using 70% alcohol as disinfectant to clean the area of collection and using tourniquet to

make vein more prominent. Three ml of venous blood was taken in plane container and serum was harvested after centrifugation 3000 RPM and was stored at -20 C^o until analysis.

3.2 Methods:

3.2.1 Estimation of Albumin:

3.2.1.1 Principle of the method: (Bromcresol green)

Albumin in the presence of Bromcresol green at a slightly acid pH ,produce a color change of the indicator from yellow-green to green-blue .

The intensity of the color formed is proportional to the albumin concentration in the sample.

3.2.1.2 Procedure of albumin:

-Three test tubes were prepared as follow:

	Blank	Standard	Sample
WR (ml)	1.0	1.0	1.0
Standard (µl)	-	5	-
Sample (µl)	-	-	5

- The tubes were mixed and incubated for 5 min at 37^{o}C or 10 min at 15-25^{o}C.

-The absorbance (A) of the samples was read , against the Blank in filter (620).

3.2.1.3 Calculation:

Serum $\frac{(A)\text{Sample} - (A)\text{ Blank}}{(A)\text{Standrd} - (A)\text{Blank}} \times 5$ (Standard conc) = g/dl Albumin in sample.

3.2.2 Uric acid :

3.2.2.1 Principle of the method:

Uric acid in the sample originates , by means of the coupled reactions described below , coloured complex that can be measured by spectrophotometry .

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{uricase} \text{Alantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{DCFS} \xrightarrow{peroxidase} \text{Quinoneimine} + 4H_2O$$

3.2.2.2 Procedure of uric acid:

-Three test tubes were prepared as follow:

	Blank	Standard	Sample
Distilled water	25 µl		
Standard (µl)	--	25 µl	--
Sample (µl)	--	--	25 µl
WR (ml)	1.0 ml	1.0 ml	1.0 ml

-- The tubes were mixed and incubated for 5 min at 37°C or 10 min at 16-25°C.

- The absorbance (A) of the samples was read against the Blank at filter (520) . The colour is stable 30 minutes.

3.2.2.3 Calculation:

Serum $\frac{(A)\text{Sample}}{(A)\text{Standrd}} \times 5$ (Standard conc) ×Sample dilution factor = Sample concentration.

3.2.3 Quality control:

Two levels of control material were analysed with each batch of samples. In addition, these controls were run with each new calibration, new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. Quality control scheme and procedures for corrective action was used when controls do not recover within the acceptable tolerances.

3.2.4 Data Analysis:

Data was analyzed to obtain mean, standard deviation and correlation of the parameters using statistical packaged for social science (SPSS) computer programme version 20, student *t* test used to compare means, and Person's correlation were applied for correlation between variables.

CHAPTER FOUR

Results

4. Results:

The present study involved 50 cases of DVT patients in addition to 50 control subjects collected from Omdurman Teaching hospital in order to assess serum uric acid and albumin .

The results of the biochemical parameters of serum albumin and uric acid are given in tables and figures as follows.

Table (4-1) and **Table (4-2):** Shows age and gender distribution in case group . (70%) of patients between (20-40) years, (24%) between (41-60) years and (6%) between (61-80) years. (80%) of patients were females while (20%) were males.

Table (4-3): Illustrates mean concentration of albumin and uric acid in patients and control groups. The levels of albumin was significantly increase in DVT patients compared to control group and there was no significant difference in uric acid level in DVT patients compared to control group : albumin (mean ± SD: 4.1 ± 0.65 mg/dl versus 3.5±0.49 mg/dl, *P* .value= (0.000), uric acid (mean ±SD: 3.85 ±1.8 mg/dl versus 3.88 ± 1.2) *P* .value = (0.929)

Table (4-4): Shows the mean of albumin and uric acid levels according to gender. The albumin was significantly decreased in male than female and there was no significant difference in uric acid level according to gender, albumin (mean ± SD : 3.6 ± 0.55 g/dl versus 4.2 ± 0.63 g/dl , *P* .value = 0.013). uric acid (mean ± SD : 2.9 ± 1.65 mg/dl versus 4.09 ± 1.85 mg/dl , *P* .value = 0.070).

Figure (4-1): Shows correlation between albumin level and age of DVT patients, There was weak negative correlation (r. value= -0.334 and *p* .value=0.018).

Figure (4-2): Shows correlation between uric acid level and age of DVT patients, There was negative correlation (r. value=-0.339 and *p* .value=0.016).

Figure (4-3): Shows correlation between the level of albumin and duration of disease (r = 0.026**,** *P*. value =0.858), there was no correlation.

Figure (4-4): Shows correlation between the level of uric acid and duration (r= 0.018**,** *P*. value =0.903) , there was no correlation.

Table (4-1) : Age distribution in case group .

Variable	Number	Percentage
Age 20 -40 Age 41-60 Age 61-80	35 12 3	70% 24% 6%

Table (4-2) gender distribution in case group

Variable	Number	Percentage
Sex male Sex female	10 40	20% 80 %

Table (4.3): Comparison between mean of albumin and uric acid level among patients with DVT

parameters	**Cases n=50**	**Control n=50**	*p*.value
Albumin (g/dl)	4.1±0.65	3.5±0.49	0.000
Uric acid (mg/dl)	3.85±1.8	3.88±1.2	0.929

Result given in mean± SD

P.value ≤ 0.05 consider significant.

Test used : Independent T.test

Table (4.4): comparison between mean of .albumin and uric acid according to gender

parameters	**Male n=10**	**Female n=40**	*p*.value
Albumin (g/dl)	3.6±0.55	4.2 ±0.63	0.013
Uric acid (mg/dl)	2.9 ±1.65	4.09±1.85	0.070

Result given in mean± SD

P.value ≤ 0.05 consider significant.

Test used : Independent T.test .

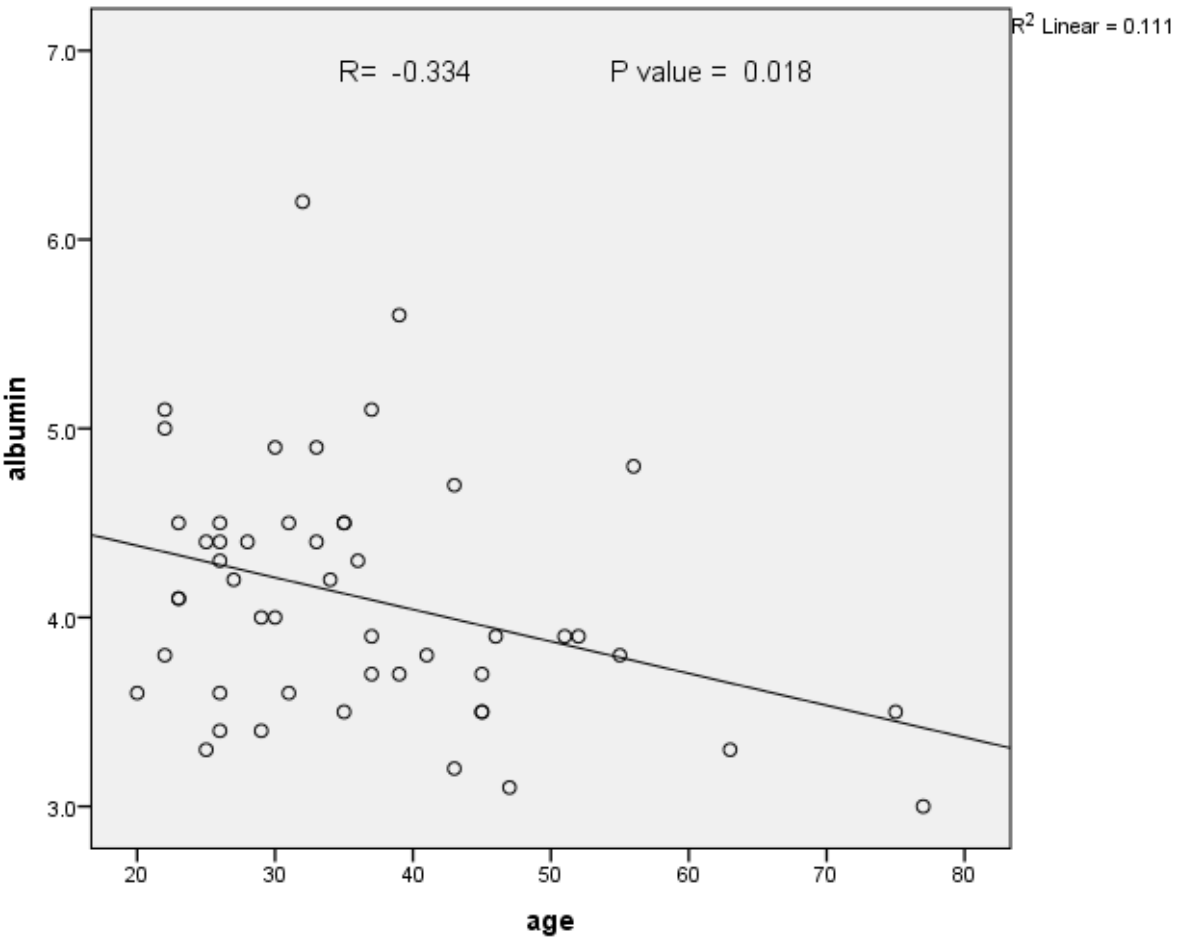

Figure (4-1): Correlation between albumin level and age in DVT patients, (r.value= -0.334 and *p*.value=0.018).

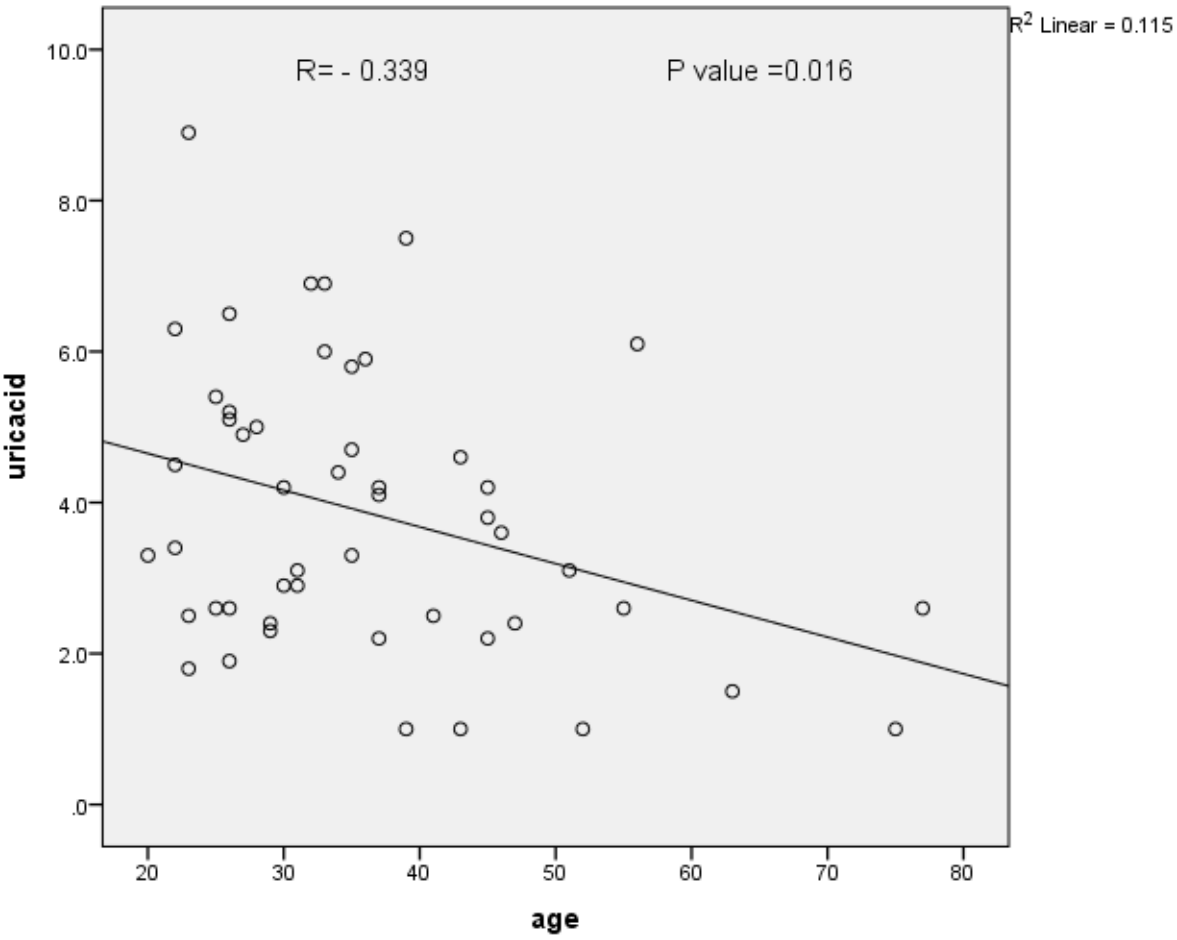

Figure (4-2): Correlation between serum uric acid level and age in DVT patients, (r. value=-0.339 and *p*. value=0.016).

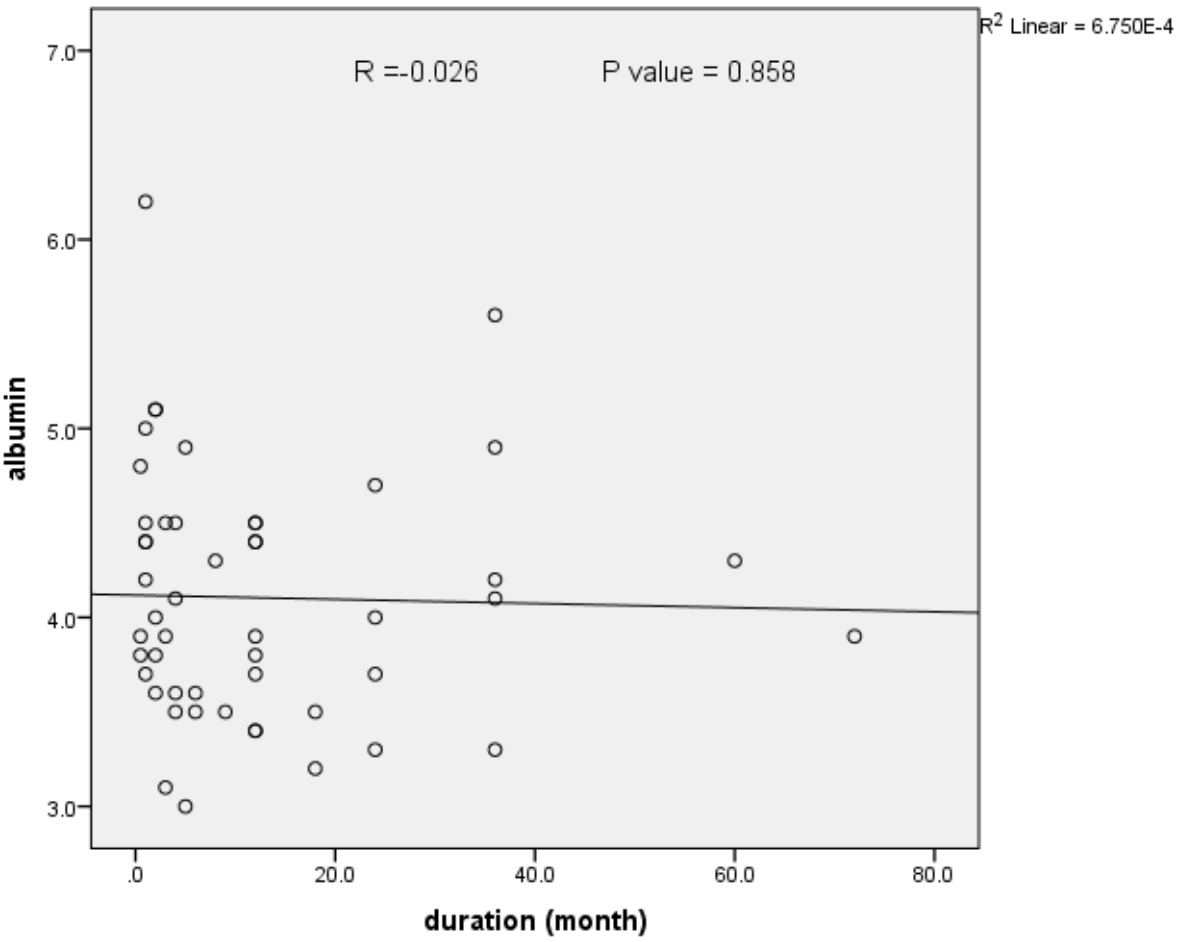

Figure (4-3): Correlation between albumin level and Duration of disease (month) in DVT patients , (r. value= -0.026 and *p*. value=0.858).

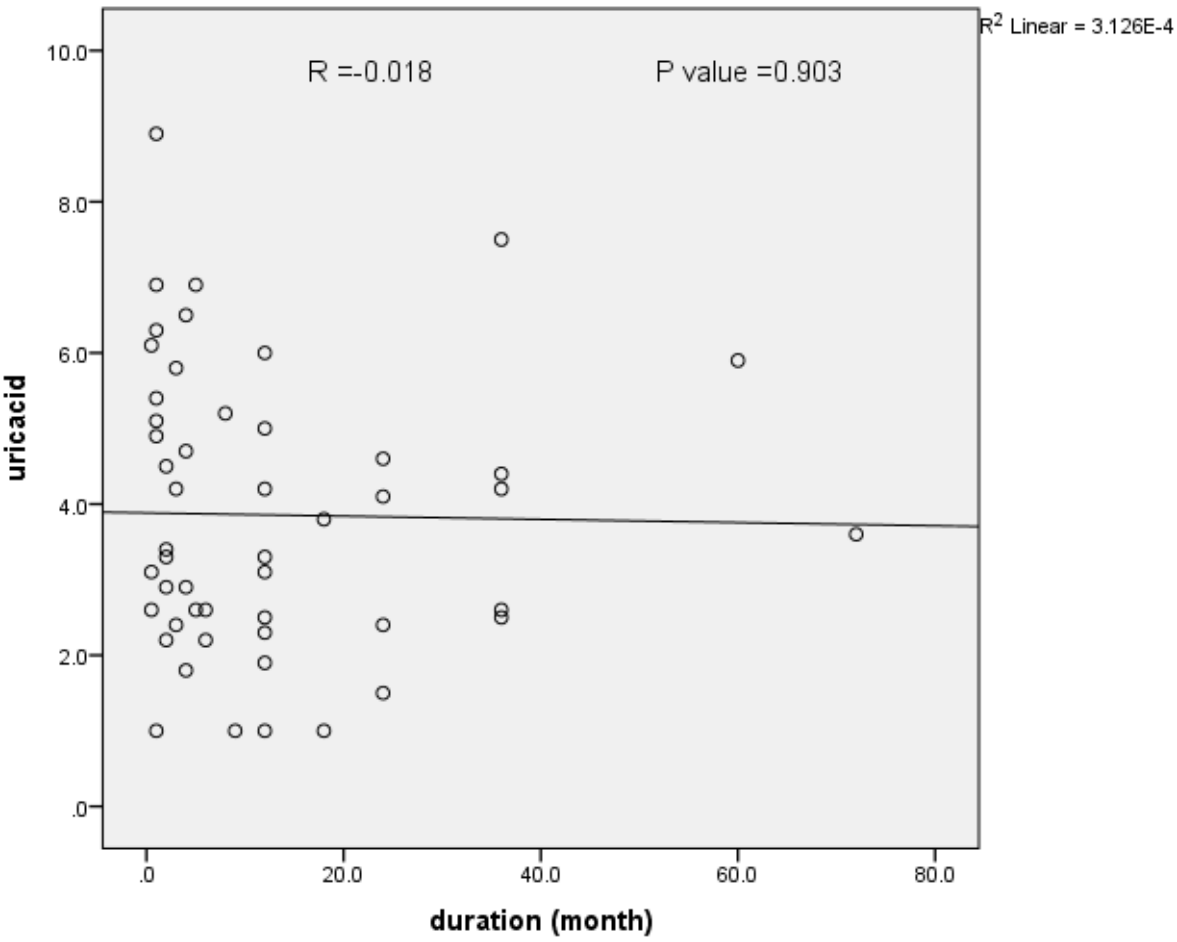

Figure (4-4): Correlation between serum uric acid level and duration of disease (month) in DVT patients, (r.value= -0.018 and *p*.value=0.903).

CHAPTER FIVE

Discussion, conclusion and recommendations

5-Discussion,Conlusion and Recommendations

5.1 Discussion :

Thrombosis is the formation of a blood clot in the vasculature ; two types of thrombosis are known: arterial and venous thrombosis , Arterial thrombosis is mainly composed of platelets with small amounts of red cells and white cells whereas venous thrombosis is composed of fibrin clot and red cells (**Betty,2007)** .

This study conducted to estimate the serum uric acid and albumin on DVT patients.

The result of present study showed that , (70%) of patients at age range between (20-40) years, (24%) between (41-60) years and (6%) between (61-80) years.(80%) of patients were females while (20%) were males. This result is agreed with study done by **(Silverstein *et al* ., 1998)** which reported that, the frequency of DVT was higher in females subjects than males with percent (130vs110 per 100000 respectively). The level of albumin was significantly increase in DVT patients compared to control group with *P*-value (0.000), This result agreed with study done by (**Idicula *et al*., 2009),** who observed that, serum albumin was significantly increased in DVT patients compared to control group. The elevation level of albumin is due to, the presence of inflammation in blood vessels during thrombus formation **(Bonner *et al* ., 2014)** .Serum uric acid level was insignificant difference in DVT patients compared to control group with *P*-value (0.929). This study disagreed with study carried out by (**Chiu *et al* ., 2016**), who reported that serum uric acid was significantly higher in patients with DVT compared to control group.

The result of this study showed that, there was insignificant difference in serum uric acid according to gender (*P*-value = 0.070). This result disagreed with previous study done by (**Arora *et al*., 2018**), which suggesting that serum uric acid was significantly higher in females when compared to the males patients with thrombotic complications . Albumin was significantly higher in female than male with *P*-value (0.013) , this result disagreed with recent study by (**Olson *et al* ., 2014**) , which suggesting that albumin was significantly lower in female than male groups.

In concerning to correlation, the correlation between albumin, serum uric acid and age were significant negative correlation (r = -0.334, *p* .value = 0.018 and r =-0.339,*p* .value =0.016) respectively, this result disagreed with study carried out by**(Weaving *et al* ., 2016 ; Umara *et al* .,2018)**, which found positive correlation between the albumin , serum uric acid and age.There were no correlation between albumin, serum uric acid and duration of disease, this result similar to another result , which reported that, there were no correlation between the albumin , serum uric acid and duration of disease **(Lionaki *et al* .,2012 ; Quintana *et al*., 2016)**.

5.2 Conclusion:

The results of this study concluded that , the levels of albumin is increased in DVT patients and the level of it increased in females than males. There were negative correlation between albumin, serum uric acid and age.

5.3 Recommendations:

- T he serum albumin and uric acid must be check for DVT patients to maintaining them in wright way as possible .
- Researchers should design studies to investigate markers that can predict the risk for DVT early and the risk for future adverse events.

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APPENDICES

University for Sciences and Technology

College of Graduate Studies

Questionnaire

No. ( )

Research title: Assessment of uric acid and albumin among Sudanese patients with deep venous thrombosis

General Information:

Name:……...……..……area:……..…

Age:……...

Gender: Male ☐ Female ☐

Duration of disease: ……

Use of treatment: Yes ☐ NO ☐

Type of of treatment:**……..**

Laboratory Investigations:

- Serum Uric acid: ……...mg/dl
- Serum Albumin:……..…… g/dl

-Date …….. -Tel No: …..……

-Signature ……