بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Determine the Relationship between ABO Blood Groups and Von Willebrand Factor Antigen Level Among Healthy Donors in Khartoum State.

تحديد العلاقه بين فصائل الدم ومستوى عامل فون ويلبراند المستضد عند المتبرعين الاصحاء في ولاية الخرطوم.

A thesis submitted in partial fulfillment for the requirement of the degree of M.Sc in Hematology and Immunohematology.

Presented by:

Ebtehal Omer Alhag Ali Mohamed

Supervisor:

Dr. Ibrahim Khider Ibrahim

2017

بسم الله الرحمن الرحيم

قال تعالى :

(لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ)

صدق الله العظيم

سورة البقره الايه (286)

Dedication

This Research is dedicated to:

To my father who always encouraged me .

To My mother has always made my life bright and beautiful .

To my brothers and sisters who have always been the source of my strength.

To my Lovely friends who always supported me.

Acknowledgement

Praise to Allah Who gave me Health , Strength and Patience to carry out this work

Iam very grateful thanks to my supervisor Dr.Ibrahim Khider for his supervision and continuedgave me the advices beside his great valuable efforts .

And I am a very grateful for his tolerance and dealing with me ,and helping to complete this work .

Finally I am very grateful who everhelped me.

Abstract

This is observation descriptive cross sectional study was carried out to study the Relationship between ABO blood groups and von Willebrand factor antigen level among healthy Donors was conducted in National Public Health Laboratory (Central Blood Bank) in Khartoum state during the period from January 2017 to May 2017. The study included sixty samples from healthy volunteer blood donors ,1.8ml of venous blood was with drown from each healthy donors, placed in tri sodium citrate containerthen separate the sample by centrifuge and extract plasma to measure von willebrand factor antigen level using Enzyme Linked Immunosorbent Assay. For each specimen ABO and RhD antigen were already determined and the personal data was recognizing by questionnaire.

The results was analyzed by statistical package for social science computer program (SPSS) version 16.the results obtained from healthy donors showed that the mean of von willebrand factor antigen level within different ABO blood groups is (0.741 IU/ml) in blood group A, (0.573 IU/ml) in blood group B, (0.913 IU/ml) in blood group AB and (0.425 IU/ml) in blood group O.

The results showed there was statistically significant differences of mean vWF:Ag within different ABO blood groups (P. value 0.000)that subjects with non-O blood group had a level significantly higher than those of group O, plasma vWF levels were lowest in group O (mean vWF:Ag 425 IU/ml) and highest in group AB subjects (mean vWF:Ag 913 IU/ml) while there was no statistically significant difference between the plasma vWF: Ag concentration and Age (P. value 0.077) and there was no statistically significant difference between the plasma vWF: Ag concentration and RhD Antigen (P. value 0.583) .

ملخص الدراسه

أجريت هذه الدراسة المقطعية الوصفية لدراسة العلاقة بين فصائل الدم ومستوي عامل فون ويلبراند المستضد عند المتبرعين الأصحاء تم إجراؤها في المعمل القومي للصحة العامة (بنك الدم المركزي) في ولاية الخرطوم خلال الفترة من يناير 2017 إلى مايو 2017

وشملت الدراسة ستين عينه من المتبرعين بالدم الأصحاء تم اخذ 1.8مليلتر من الدم الوريدي من كل متبرع صحي وتم وضعه في وعاء يحتوي على مانع تجلط ثلاثي سترات الصوديوم ثم فصل العينه بواسطه جهاز الطرد المركزي وإستخلاص المصل الدموي وذلك لقياس عامل فون ويلبراند المستضد عن طريق استخدام (ELISA) هذا بعد تحديد فصيلة الدم وعامل الريصص وتم جمع المعلومات الشخصيه للمتبرعين بواسطة استبيان. وتم تحليل النتائج من خلال الحزمة الإحصائية لبرنامج الحاسوب للعلوم الاجتماعية الاصدار 16 النتائج التي تم الحصول عليها من المتبرعين الأصحاء أن متوسط مستوى المستضد فون ويلبراند ضمن فصائل الدم المختلفه هو (0.741 وحدة دولية / مليلتر) في فصيلة الدم (A), (0.573 وحدة دولية / مليلتر) في فصيله الدم (B) , (0.913وحدة دولية / مليلتر) في فصيله الدم (AB) و (0.425 وحدة دولية / مليلتر) في فصيله الدم (O) .

أظهرت النتائج وجود فروقات ذات دلالة إحصائية في متوسط عامل فون ويلبراند المستضد بين فصائل الدم المختلفه (القيمه المعنويه 0.000) حيث كان اعلي في الأشخاص غير الفصيله (O) مقارنه بالأشخاص فصيلة (O) كان متوسط عامل فون ويلبراند المستضد أعلي في الفصيله (AB) وأقل في الفصيله (O) حين لم يكن هناك فرق ذودلالة إحصائية بين عامل فون ويلبراند المستضد والعمر(القيمه المعنويه 0.077) ولم يكن هناك فرق ذودلالة إحصائية بين عامل فون ويلبراند المستضد ومستضد RhD (القيمه المعنويه 0.583).

Table of Contents

List of Tables

List of abbreviations

ADAMTS13: A disintegrin and metalloproteinase with thrombospondin type-1 repeats-13

Arg: Arginine

Asp: Aspartate

BT: Bleeding Time

CSF: Cerebrospinal fluid

Cys: cysteine

DDAVP: 1-deamieno-8-D-arginine vasopressin

ELISA: Enzyme-linked immunosorbent assay

Glu: Glutamine

Gly: Glycine

GP Ib: glycoprotein Ib

His: Histidine

Ig: Immunoglobulin

LRG: leucine-rich

Lys: Lysine

Met: Methionine

PTT: Partial thromboplastin time

RBC: Red blood cell

Tyr: Tyrosine

Val: Valine

vWF : von Willebrand Factor

vWF:Ag: von Willebrand Factor Antigen

vWD: von Willebrand Disease

CHAPTER ONE

Introduction & Literature Review

1.1General Introduction:

Von Willebrand's factor levels vary with the blood group (William Kern, 2002). found that 66 percent of the total variation in plasma VWF levels was genetically determined and that 30 percent of this genetic component was explained by ABO blood group (Orstavik et al ,1985).

Von Willebrand factor is one of the few non-erythrocytic proteins that express ABO antigens. ABH oligosaccaride structures have been identified on the N-linked oligosaccharide chains of VWF located in the A1 domain, which contains the binding site for platelet glycoprotein Ib (Matsui , Titani and Mizuochi ,1992).

The importance of VWF glycosilation in mediating the effect of ABO blood group on VWF levels is well demonstrated by the observation that aberrant endothelial expression of a glycosyltranferase (N-acetylgalactosaminyltranferase) results in altered VWF glycosilation (Mohlke et al ,1999)

The mechanism by which ABH determinants on plasma VWF influence plasma VWF levels is still unknown and several hypotheses have been made during the last years (Jenkins and O'Donnell JS,2006). While the *in vitro* removal of A and B antigens from purified plasma VWF decreases VWF activity but not its antigenic level or binding to collagen (Sarode et al,2000) ,the detection of lower levels of all three in type O individuals suggests that ABO antigens *in vivo* influence the rate of synthesis or proteolysis/clearance of the whole VWF molecule rather than its function or multimeric structure (Moeller et al,2001).

Recent studies have suggested that ABO blood group determinants may be important in influencing the susceptibility of plasma VWF to proteolysis by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 repeats-13) metalloprotease (Jenkins and O'Donnell ,2006). purified VWF from

individuals of different ABO blood groups and then incubated with human plasma-derived ADAMTS13. Mulimeric analysis and collagen-binding assays both demonstrated that proteolysis was significantly faster for group O VWF compared to non-O VWF (Bowen ,2003) .Thus, while carbohydrate structure has previously been shown to protect VWF from proteolytic degradation in plasma (Federici et al,1984) , the recent literature data show that a reduction in the number of sugars on the oligosaccharide chains of VWF is associated with an increased susceptibility to cleavage by ADAMTS13 (Jenkins and O'Donnell ,2006). Indeed, Bombay phenotype was found to be associated with an increased susceptibility to ADAMTS13 proteolysis (O'Donnell et al,2005).

The pathogenic mechanism by which ABO blood group influences the susceptibility to cleavage by ADAMTS13 is still unknown but two N-linked potential glycosilation sites (asparagines 1515 and 1574) are located in close proximity to the ADAMTS13 cleavage site (Tyr1605-Met1606 bond within the A2 domain). Thus, the oligosaccharide chain composition may be involved in stabilizing the conformation of this VWF region, such that the removal of terminal sugar allows the A2 domain to adopt a conformation more permissive for ADAMTS13 cleavage (Bowen ,2003).

1.2 Literature Review:

1.2.1 Von Willebrand Factor

Von Willebrand's factor is synthesized by endothelial cells and megakaryocytes. It circulates in plasma complexed with the factor VIII clotting factor. It circulates as multimers of various sizes, with molecular weights up to 20 million daltons. The large multimers are required for normal vWF function; a decrease or absence of the high-molecular-weight multimers results in a bleeding disorder despite the presence of normal levels of total vWF (William Kern, 2002) .

vWF is involved in platelet adhesion to the vessel wall and to other platelets (aggregation). It also carries factor VIII and used to be referred to as factor VIII related antigen (VIII-Rag). It is a large cysteine-rich glycoprotein, with multimers made up on average of 2-50 subunits, with a molecular weight (MW) of 0.8-20 x 106. vWF is encoded by a gene on chromosome 12 and is synthesized both in endothelial cells and megakaryocytes, and stored in Weibel-Palade bodies and platelet a granules respectively. Plasma vWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously secreted and a minority is stored in Weibel-Palade bodies. The stored vWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of desmopressin (1-deamieno-8-D-arginine vasopressin, DDAVP). The vWF released from Weibel-Palade bodies is in the form of large and ultra large multimers, the most adhesive and reactive form of vWF. They are in turn cleaved in plasma to monomeric vWF and smaller multimers by the specific plasma metalloprotease, ADAMTS-13 (Hoffbrand. Moss and Pettit ,2006) .

vWF is a large multimeric glycoprotein that circulates in plasma and is also found in platelets and the Weibel-Palade bodies of endothelial cells. Mature vWF is a 2050–amino acid subunit that is disulfide linked into large multimers. It contains three adjacent A domains in the N-proximal half of the peptide that collectively regulate the adhesion of platelets to subendothelial matrix. In this respect, the A 1 and A 3 domains bind to different matrix collagens, whereas the A 1 domain contains the binding site for the gpIb complex . The A 1 domain is the primary role player in platelet adhesion because this part of the molecule is believed to change its conformation in response to immobilization and high shear forces, thus making it a high-affinity ligand for the gpIb complex receptor . It has also been suggested that shear stresses may induce conformational changes in the gpIb complex that may be important in increasing its affinity for vWF . To further add to the mystery behind the mechanism of shear dependence in gpIb–vWF interaction, in vitro activation of vWF and binding to the gpIb complex occur with generally very low affinity without shear, whereas this shear-free binding exhibits high affinity in the presence of the vancomycin-like antibiotic ristocetin and viper venom proteins, such as botrocetin. Interestingly, studies incorporating anti-gpIba and anti-VWF domain A 1 antibodies have suggested that ristocetin and botrocetin each appear to use different receptor and ligand-binding sites to facilitate vWF–gpIb complex interaction . The binding interaction between vWF and gpIb appears to involve at least three distinct regions within the N-terminal 282 residues of gpIb. Each of these regions appears to be responsible for either direct binding to vWF or modulating its affinity for the ligand . In this respect, one region (His 1 to Glu 282) that includes a cluster between residues Asp 252 and Arg 293 containing sulfated tyrosine residues and important anionic residues appears to be predominantly responsible for vWF–gpIb complex interaction in the presence of botrocetin over ristocetin . The second region contains the disulfide loop between Cys 209 and Cys

248 along with two naturally occurring mutations (Gly 233 to Val and Met 239 to Val) and two additional mutation sites identified in the laboratory (Asp 235 to Val and Lys 237 to Val) that can individually lead to expression of the pseudo- or platelet-type von Willebrand disease phenotype . This disorder is associated with a gain-of-function gpIba on platelets that adheres to vWF in the presence of lower concentrations of ristocetin (0.3 to 0.5 mg/ml) than is required for the wild-type phenotype (1.5 mg/ml). It is analogous to type 2B von Willebrand disease, in which high-molecular-weight vWF multimers are absent from the plasma, and similar gain-of-function mutations have been localized to the Cys 509 to Cys 695 disulfide loop of vWF exon 28. The third region includes the N-terminal flanking sequence to the leucine-rich repeat (LRG) motifs and the LRGs themselves. Mutations involving single amino acid residues within these LRGs account for some cases of the congenital bleeding disorder Bernard-Soulier syndrome in which the gpIb complex binds poorly or not at all to vWF.Evidence using mammalian Chinese hamster ovary cells expressing loss-of-function proteins and anti-gpIba monoclonal antibodies has suggested that the more N-terminal LRGs may play a more direct role in interaction with vWF (John . Greer , John Foerster , John Lukens,2003) .

1.2.2 Conditions associated with increased Levels of Von Willebrand Factor

Increased levels of vWF have been associated with stress, inflammation, postsurgical states, pregnancy, renal disease ,diabetes, rheumatoid disorders, scleroderma, and Raynaud phenomena. vWF may be an indicator of vascular endothelial status. Drugs such as 1-deamino-8-D-arginine vasopressin(DDAVP), steroids, and hormones may also result in elevated levels of vWF (Mary Turgeon,2010) .

Von Willebrand's factor levels vary over time in a given individual; they are increased with estrogen, during stress, and with liver disease. These levels also increase dramatically during pregnancy (as does the level of the factor VIII coagulant protein). If you strongly suspect vWD but the assay shows a normal level, consider repeating the test at another time. If the patient is a pregnant woman, repeat the work-up after she has delivered (several weeks after, to allow time for the vWF level to decrease) (William Kern, 2002).

Von Willebrand's factor levels vary with the blood groups; they are lowest in patients with blood group O, higher in patients with groups A, B, or AB. Always be a little suspicious of the diagnosis of vWD in people with blood group O (William Kern, 2002).

1.2.3 Types of Von Willebrand Disease:

1.2.3.1 Hereditary Von Willebrand Disease

The most important disease of platelet adhesion is von Willebrand disease (vWD). Discovered in 1926 by Dr.Eric von Willebrand, vWD is the most prevalent inherited bleeding disorder worldwide, affecting 1% to 3% of the world population by conservative estimates. In random studies of children investigated for epistaxis and women investigated for menorrhagia ,vWD was the most frequent cause of bleeding. Von Willebrand initially described a family of 12 children of which 10 had excessive nosebleeds, gum bleeds, and menorrhagia. One of the youngest girls died at age 13 during her fourth menstrual cycle of uncontrollable bleeding. vWD is an autosomal dominant disorder marked by easy bruising, nosebleeds, heavy menses, and excessive bleeding after tooth extraction or dental procedures. Type O individuals have a lower plasma concentration of vWF than other blood types. For many patients, the variabilities in clinical symptoms and laboratory presentations

have contributed to the under diagnosis of this disorder. Women may represent a significant yet underserved subset of individuals affected by vWD, since menorrhagia is a frequent presenting feature of this disease. According to Luscher, vWD may be the underlying cause in 9% to 11% of cases of menorrhagia,16 yet it is often not considered as a possible diagnosis by obstetricians and gynecologists. As a disease entity, vWD is fairly complex with few clear-cut and consistent diagnostic clues (Betty Ciesla,2007) .

1.2.3.1.1 Etiology

Von Willebrand disease may be an acquired or inherited disorder. The congenital disorder is autosomally dominant in most cases. Inherited abnormalities in von Willebrand disease are associated with a defect of the vWF gene on chromosome 12, but in some patients, the coexistence of an impaired response of plasminogen activator and telangiectasia suggests the presence of a regular defect or more extensive endothelial abnormalities. In several families, a large vWF gene deletion has been identified as the basis for von Willebrand disease. More than 20 distinct clinical and laboratory subtypes of von Willebrand disease have been described . Three broad types of von Willebrand disease are recognized. In addition, a platelet-type von Willebrand disease (pseudo–von Willebrand disease) is caused by an abnormal platelet receptor for VWF. In addition, acquired von Willebrand disease may complicate other diseases such as lymphoproliferative and autoimmune disorders, and proteolytic degradation of vWF complicates myeloproliferative disorders. Variant forms of von Willebrand disease can be identified by their patterns of genetic transmission and the vWF abnormalities in the plasma and the cellular compartment. Distinguishing between various subtypes of von Willebrand disease is important in determining appropriate therapy (Mary Turgeon,2010).

1.2.3.1.2 Epidemiology

Von Willebrand disease is recognized as one of the most common hereditary bleeding disorders in humans. The exact incidence is difficult to determine because milder forms are often not clinically recognized, but it has been estimated to have a Prevalence as high as 1% in the general population. No racial or ethnic predisposition has been determined. Both genders are affected, but there is a higher frequency of clinical manifestation in women (Mary Turgeon,2010) .

1.2.3.1.3 Pathophysiology

VonWillebrand disease is characterized by abnormal platelet function, expressed As a prolonged bleeding time. This is a consistent finding and may be Accompanied by decreased factor VIII procoagulant activity. vWF circulates in the blood in two distinct compartments, with two types of cells being responsible for vWF production. Vascular endothelium is the primary source of the synthesis and release of plasma vWF; the other type of cell that synthesizes vWF is the megakaryocyte. Approximately 15% of circulating vWF is produced in the megakaryocyte. vWF circulates in platelets, being stored primarily in the alpha granules, in association with factor VIII procoagulant protein (VIII:Ag). Platelet vWF is released from the alpha granules by various agonists and subsequently rebinds to the GP IIb/ IIIa complex. The site synthesis of VIII:Ag remains unknown, although the liver is thought to play an important role. VWF is a large, adhesive, multimeric GP present in plasma, platelets, and subendothelium. It is synthesized as a large precursor that consists of a signal peptide, a propeptide (von Willebrand antigen II), and the vWF subunit. It has the two main functions of regulating coagulant activity (VIII:C) and aiding in adhesion of platelets to subendothelial cell walls following vessel damage. In circulating blood, vWF is

part of a noncovalent bimolecular complex with the factor VIII procoagulant protein. This complex stabilizes factor VIII and protects it from rapid removal from the circulation. The vWF portion represents more than 95% of the mass of the complex and therefore controls the molecular stereochemistry. The vWF consists of repeating multimers, with the smallest circulating multimer thought to be a dimer or tetramer. Circulating vWF undergoes proteolytic cleavage under physiological conditions; thus, it can be distinguished from platelet vWF, which is not proteolyzed. The pathogenesis of von Willebrand disease is based on quantitative or qualitative abnormalities, or both, of vWF. When an abnormality is present, the decreased factor VIII procoagulant activity is attributable to the reduced concentration of vWF. vWF is essential in providing the basis for formation of a normal platelet thrombus. vWF binds to specifi c sites on the platelet, namely GP Ib and GP IIb/IIIa, while concurrently binding to the subendothelium of damaged vessel walls, forming a bridge. Patients with decreased levels of vWF, especially the larger multimeric forms, will lack adequate bridging action that produces prolonged bleeding times. Qualitative or quantitative abnormalities of vWF result in decreased adhesion and are responsible for the bleeding associated with von Willebrand disease. The significance of vWF in the regulation of VIII:C remains unclear. The increase in VIII:C following infusion of purified vWF suggests a possible role of vWF in the synthesis, release, or stabilization of VIII:Ag. Therefore, decreased levels of vWF may prolong the rate of blood clotting (Mary Turgeon,2010) .

1.2.3.1.4 Three main subtypes of VWD have been defined:

1.2.3.1.4.1 Type 1:

This is by far the most common (≥70% of cases of clinicalvWD). There is a decrease in the concentration of VWF in the plasma(ie, a quantitative defect), but all sizes of multimers, including the veryhigh-molecular-weight multimers, are present. The inheritance patternis autosomal dominant (William Kern, 2002) .

1.2.3.1.4.2 Type 2:

In Type 2 vWD, there is a qualitative defect in vWF, not a quantitative defect. Several different subtypes of VWD Type 2 are described. Inheritance is autosomal dominant in most cases, although a few types display autosomal recessive inheritance (William Kern, 2002) .

1.2.3.1.4.2.1 Type 2A:

In Type 2A, there is a deficiency of the high-molecular weight multimers of vWD, but the absolute level of total vWF is normal. This is the most common variant of VWD Type 2 (William Kern, 2002) .

1.2.3.1.4.2.2 Type 2B:

In Type 2B, the vWF appears to have an abnormally increased affinity for the vWF receptor (GP Ib-IX/V) on the platelet surface. Too much vWF is tied up on the platelet surface, rather than being in the plasma, and is therefore not able to bind to subendothelial collagen. The diagnosis of Type 2B vWD is made by demonstrating increased platelet agglutination with ristocetin rather than decreased ristocetin agglutination,.Mild thrombocytopenia is common (William Kern, 2002) .

1.2.3.1.4.2.3 Type 2M:

In Type 2M vWD, there is a mutation that affects some important functional domains of the protein (think "**M**" for **M**utation) (William Kern, 2002) .

1.2.3.1.4.2.4 Type 2N (vWD Normandy):

In Type 2N, there is a deficiency of the binding site for factor VIII on the vWF, so it cannot act as a chaperone for factor VIII. Consequently, the factor VIII half-life and plasma levels are decreased, and the patients clinically resemble mild hemophilia A. von Willebrand's disease Type 2N should be suspected when the family history does not fit the typical X-linked recessive inheritance pattern in a patient who otherwise appears to have hemophilia A. The diagnosis is established by demonstrating decreased affinity of vWF for factor VIII. (William Kern, 2002).

1.2.3.1.4.3 Type 3:

In Type 3 vWD, there is a total or near-total absence of vWF in the plasma. Type 3 vWD is rare. The patients have markedly decreased factor VIII levels (since they have no vWF to act as chaperone for factor VIII), and thus they may have bleeding manifestations resembling hemophilia. The inheritance pattern is autosomal recessive; some cases appear to represent homozygous Type 1 vWD (William Kern, 2002).

1.2.3.2 Acquired Von Willebrand Disease

Von Willebrand disease is occasionally seen as an acquired condition. Associations have been made with lupus erythematosus and other autoimmune disorders as well as myeloproliferative disorders. The presence of a circulating antibody to vWF may be implicated in some cases. Another mechanism responsible for decreased amounts of vWF in acquired states is the absorption of the coagulation component onto abnormal cell surfaces. Hemorrhagic complications are generally more severe in patients with acquired von Willebrand disease. Bleeding from mucous membranes is more common and reflects the much lower levels of vWF activity in these individuals. vWF activity is typically 20% or less of normal (Mary Turgeon,2010) .

1.2.3.3 Pseudo–von Willebrand Disease

This is a rare disorder in which patients resemble those with von Willebrand disease because of low levels or absence of large multimeric forms of vWF in the plasma. Patients with pseudo–von Willebrand disease have a platelet abnormality in which spontaneous platelet aggregation occurs. Low levels of larger multimers result from increased consumption during platelet aggregation (Mary Turgeon,2010) .

1.2.4 Clinical Manifestations of von Willebrand's Disease

Most cases of vWD present with the typical picture of a primary hemostatic defect: mucocutaneous bleeding (epistaxis, bleeding gums), easy bruising ,and immediate bleeding from cuts, incisions, and dental extractions .Most patients have a mild to moderate bleeding tendency. The severity of illnessin different patients is highly variable, and it can also vary over time inindividual patients. The clinical phenotype can vary between different members of the same family .Many patients

have relatively minor bleeding, and It is common for Type 1 vWD not to be diagnosed until the patient has a major hemostatic challenge as an adult, such as a dental extraction. The laboratory manifestations of vWD can also be highly variable; sometimes laboratory tests must be repeated several times to make a firm diagnosis. As noted, the inheritance pattern of most cases of vWD is autosomal dominant. Type 3 vWD presents with a mixed picture. Since the factor VIII levels are low, the patients may have hemarthroses, muscle hematomas, and other manifestations of defects of secondary hemostasis in addition to mucocutaneous bleeding (William Kern, 2002) .

1.2.5 Laboratory Diagnosis of von Willebrand's Disease

The most important diagnostic tests for vWD are the bleeding time, ristocetin cofactor assay, a quantitative assay of vWF concentration (ELISA or Laurell rocket immunoelectrophoresis), ristocetin-induced platelet aggregation, and agarose gel electrophoresis to determine whether the high-molecular- weight multimers are present or absent. Not all of these tests may be needed in every case. In vWD Type 1, the BT will usually be prolonged. The PTT may also be slightly prolonged due to decreased factor VIII concentration. The absolute level of vWF is decreased; the vWF multimer pattern is normal (the high molecular- weight multimers are present, but the concentration of all sizes of multimers is decreased). Platelet aggregation with ristocetin and the ristocetin cofactor activity are decreased; platelet aggregation with other agents is usually normal. Addition of normal plasma to platelets from patients with vWD corrects the aggregation deficiency, confirming that the deficiency is in the plasma, not the platelets. Type 2A is diagnosed by demonstrating an absence of the high-molecular-weight multimers by agarose gel electrophoresis. The absolute concentration of vWF is usually normal Type 2B is diagnosed by demonstrating increased platelet

agglutination with ristocetin. Plasma from patients with Type 2B will agglutinate platelets at very low ristocetin concentrations, which will not induce agglutination in normal people. The absolute level of vWF is decreased; agarose gel electrophoresis demonstrates a decrease in high-molecular-weight multimers (because they are all stuck on the platelets). Mild thrombocytopenia is common. Diagnosis of Type 2M requires sophisticated techniques, which are not widely available; therefore, specimens must usually be sent to reference laboratories that specialize in coagulation. Diagnosis of Type 2N may be suspected from the clinical history (resembling a mild or moderate case of hemophilia) and inheritance pattern (autosomal, not X-linked). Definitive diagnosis of Type 2N requires an assay of the affinity of vWF for factor VIII (available in some specialized reference laboratories). Type 3 is diagnosed by demonstrating a total or near-total absence of vWF in the plasma (William Kern, 2002) .

1.2.6 Treatment of von Willebrand's Disease

Most cases of vWD Type 1 can be very successfully treated with desmopressin acetate (DDAVP), which causes release of preformed vWD from endothelial cells. Desmopressin acetate is cheap, is safe, and has no infectious risk. It can be administered either intravenously or intranasally; the advantage of the intranasal method is that patients can easily treat themselves .Desmopressin acetate has been considered contraindicated in vWD Type 2B since it may induce thrombocytopenia (William Kern, 2002) .

There are two types of DDAVP nasal sprays: one for diabetes insipidus and one for vWD. The preparation for vWD is 10 times more concentrated than the one for diabetes insipidus.

Other types of vWD, or a patient with Type 1 requiring major surgery, may need replacement therapy. There are no commercially available vWF concentrate preparations, but some factor VIII concentrate preparations contain enough vWF to be effective. However, not all factor VIII concentrates contain significant amounts of vWF, and the high-molecular-weight multimers may be lacking. Humate-P has been recommended as having the most normal distribution of multimer sizes, but some other brands of factor VIII also contain significant amounts of vWF. Recombinant factor VIII has no vWF and would not be useful in patients with vWD (William Kern, 2002) .

The treatment of choice used to be cryoprecipitate since it is high in the vWF/factor VIII complex. However, there is no way to sterilize cryoprecipitate, unlike factor VIII concentrates, which are intensively treated. Factor VIII concentrates have therefore replaced cryoprecipitate as the treatment of choice for vWD requiring factor replacement (William Kern, 2002) .

1.2.7 The ABO Blood Group System

1.2.7.1 Historical Perspective

Karl Landsteiner truly opened the doors of blood banking with his discovery of the first human blood group system, ABO. This marked the beginning of the concept of individual uniqueness defined by the RBC antigens present on the RBC membrane. The ABO system is the most important of all blood groups in transfusion practice. It is the only blood group system in which individuals predictably have antibodies in their serum to antigens that are absent from their RBCs. This occurs without any exposure to RBCs by transfusion or pregnancy. Due to the presence of these antibodies, transfusion of an incompatible ABO type can result in the almost immediate lysis of donor RBCs. This produces a very

severe, if not fatal, transfusion reaction in the patient. Even today, transfusion of the wrong ABO group remains the leading cause of death reported to the Food and Drug Administration (FDA).Testing to detect ABO incompatibility between a donor and potential transfusion recipient is the foundation on which all other pretransfusion testing is based. ABO forward and reverse grouping tests are required to be performed on all donors and patients. ABO grouping is the most frequently performed test in the blood bank. There is always a reciprocal relationship between the forward and reverse type; thus, one serves as a check on the other. In 1901 Landsteiner drew blood from himself and five associates, separated the cells and serum, and then mixed each cell sample with each serum. He was inadvertently the first individual to perform the forward and reverse grouping. Forward grouping (front type) is defined as using known sources of commercial antisera (anti-A, anti-B) to detect antigens on an individual's RBCs. Reverse grouping (back type) is defined as detection of ABO antibodies in the patient's serum by using known reagent RBCs; namely, A1 and B cells (Denise Harmening ,2005) .

It has been postulated that bacteria are chemically similar to A and B antigens. Bacteria are widespread in the environment, providing a source of constant exposure of individuals to A-like and B-like antigens. This exposure serves as a source of stimulation of anti-A and anti-B. All other defined blood group systems do not regularly have in their serum "naturally occurring" antibodies to antigens they lack on their RBCs. Antibody production in most other blood group systems requires the introduction of foreign RBCs by transfusion or pregnancy, although some individuals can occasionally have antibodies present that are not related to the introduction of foreign RBCs. (These antibodies are usually of the IgM type and are not consistently present in everyone's serum). Performance of serum

grouping is, therefore, unique to the ABO blood group system. The regular occurrence of anti-A and/or anti-B in persons lacking the corresponding antigen(s) serves as a confirmation of results in RBC grouping. The frequency of these blood groups in the white population is as follows: group O, 45 percent; group A, 40 percent; group B, 11 percent; and group AB, 4 percent. Therefore, O and A are the most common blood group types, and blood group AB is the rarest. However, frequencies of ABO groups differ in a few selected populations and ethnic groups .For example, group B is found twice as frequently in Blacks and Asians as in Whites, and subgroup A2 is rarely found in Asians (Denise Harmening ,2005).

On the basis of the agglutination pattern, he named the first two blood group antigens A and B, using the first letters of the alphabet. RBCs not agglutinated by either sera were first called C but became known as "ohne A" and "ohne B" (ohne is German for "without") and finally O. In 1907, Jansky proposed using Roman numerals I, II, III, IV for O, A, B and AB respectively, and in 1910, Moss proposed using I, II, III and IV for AB, B, A and O, respectively. (Marion Reid and Christine Lomas Francis ,2004) .

These numerical terminologies were used respectively in Europe and America until 1927 when Landsteiner suggested, in order to avoid confusion, to use throughout the world the symbols A, B, O and AB (Marion Reid and Christine Lomas Francis ,2004) .

1.2.7.2 Expression Of ABO Antigens

On RBCs, Saliva and all body fluids except CSF (in secretors) , platelets (adsorbed from plasma) Tissues, on most epithelial cells (particularly glandular epithelia) and on endothelial cells. Broad tissue distribution (often termed "histo-blood group" antigens) (Marion Reid and Christine Lomas Francis ,2004) .

1.2.7.3 Inheritance of the ABO Blood Groups

The theory for the inheritance of the ABO blood groups wasfirst described by Bernstein in 1924. He demonstrated that an individual inherits one ABO gene from each parent and that these two genes determine which ABO antigens are present on the RBC membrane. The inheritance of ABO genes, therefore, follows simple mendelian genetics. ABO, like most other blood group systems, is codominant in expression. One position, or locus, on each chromosome 9 is occupied by an A, B, or O gene. The O gene is considered an amorph as no detectable antigen is produced in response to the inheritance of this gene. The designations A and B refer to phenotypes, whereas AA, BO, and OO denote genotypes. In the case of an O individual, both phenotype and genotype are the same, because that individual would have to be homozygous for the O gene. An individual who has the phenotype A (or B) can have the genotype AA or AO (or BB or BO). The phenotype and genotype are the same in an AB individual because of the inheritance of both the A and B gene (Denise Harmening ,2005) .

1.2.7.4 Formation of A, B, and H RBC Antigens

The formation of ABH antigens results from the interaction of genes at three separate loci (ABO, Hh, and Se). These genes do not actually code for the production of antigens but rather produce specific glycosyltransferases that add sugars to a basic precursor substance . A, B, and H antigens are formed from the same basic precursor material (called a paragloboside) to which sugars are attached in response to specific enzyme tranferases elicited by an inherited gene. The precursor substance on erythrocytes is referred to as type 2. This means that the terminal galactose on the precursor substance is attached to the N-acetylglucosamine in a beta 1 → 4 linkage. A type 1 precursor substance refers to a

beta 1 → 3 linkage between galactose and Nacetylglucosamine . ABH antigens on the RBC are constructed on oligosaccharide chains of type 2 precursor substance The ABH antigens develop as early as the 37th day of fetal life but do not increase much in strength during the gestational period. The RBCs of the newborn have been estimated to carry anywhere from 25 to 50 percent of the number of antigenic sites found on the adult RBC. As a result, reactions of newborn RBCs with ABO reagent antisera are frequently weaker than reactions with adult cells. The expression of A and B antigens on the RBCs is fully developed by 2 to 4 years of age and remains constant for life. As well as age, the phenotypic expression of ABH antigens may vary with race, genetic interaction, and disease states (Denise Harmening ,2005**)** .

1.2.7.5 Interaction of Hh and ABO Genes

Individuals who are blood group O inherit at least one H gene (genotype HH or Hh) and two O genes. The H gene elicits the production of an enzyme, alpha-2-L-fucosyltransferase, which transfers the sugar L-fucose to an oligosaccharide chain on the terminal galactose of type 2 chains. The sugars that occupy the terminal positions of this precursor chain and confer blood group specificity are called the immunodominant sugars. Therefore, L-fucose is the sugar responsible for H specificity (blood group O) . The O gene at the ABO locus, which is sometimes referred to as an amorph, does not elicit the production of a catalytically active polypeptide, and therefore the H substance remains unmodified. As a result, the O blood group has the highest concentration of H antigen. The H substance (L-fucose) must be formed for the other sugars to be attached in response to an inherited A and/or B gene. The H gene is present in more than 99.99 percent of the random population. The allele of H, “h,” is quite rare, and the genotype, hh, is extremely rare. The term “Bombay” has been used to refer to the phenotype that

lacks normal expression of the ABH antigens because of the inheritance of the hh genotype. The hh genotype does not elicit the production of alpha 2-Lfucosyltransferase; as a result, L-fucose is not added to the type 2 chain, and H substance is not expressed on the RBC. Even though Bombay (hh) individuals may inherit ABO genes, normal expression, as reflected in the formation of A, B, or H antigens, does not occur. In the formation of blood group A, the A gene (AA or AO)codesfor the production of alpha-3-N-acetylgalactosaminyltransferase,which transfers an N-acetyl-D-galactosamine(GalNAc) sugar to the H substance. This sugar is responsible for A specificity (blood group A). The A-specific immunodominant sugar is linked to a type 2 precursor substance that now contains H substance through the action of the H gene. The A gene tends to elicit higher concentrations of transferase than the B gene. This leads to the conversion of practically all of the H antigen on the RBC to A antigen sites. As many as 810,000 to 1,170,000 antigen sites exist on an A1 adult RBC in response to inherited genes. Individuals who are blood group B inherit a B gene (BB or BO) that codes for the production of alpha 3-D-galactosyltransferase, which then attaches D-galactose (Gal) sugar to the H substance previously placed on the type 2 precursor substance through the action of the H gene.This sugar is responsible for B specificity (blood group B) . Anywhere from 610,000 to 830,000 B antigen sites exist on a B adult RBC in response to the conversion of the H antigen by the alpha-3-Dgalactosyltransferase produced by the B gene. When both A and B genes are inherited, the B enzyme (alpha-3-D-galactosyltransferase) seems to compete more efficiently for the H substance than the A enzyme (alpha-3-N acetylgalactosaminyltransferase). Therefore, the average number of A antigens on an AB adult cell is approximately 600,000 sites, compared with an average of 720,000 B antigen sites (Denise Harmening ,2005) .

1.2.7.6 Formation of A, B, and H Soluble Antigens

ABH antigens are integral parts of the membranes of RBCs, endothelial cells, platelets, lymphocytes, and epithelial cells.ABH-soluble antigens can also be found in all body secretions.Their presence is dependent on the ABO genes inherited as well as the inheritance of another set of genes (secretorgenes) that regulate their formation. Eighty percent of therandom U.S. population are known as secretors because they have inherited a secretor gene (SeSe or Sese). The inheritance of an Se gene codes for the production of a transferase (alpha-2-L-fucosyltransferase) that results in the modification of the type 1 precursor substance in secretions to express H substance. This H substance can then be modified to express Aand B substance (if the corresponding gene is present) in secretions such as saliva. For example, a group A individual who is a secretor (SeSe or Sese) will secrete glycoproteins carrying A and H antigens. The Se gene does not, however, affect the formation of A, B, or H antigens on the RBC. It is the presence of the Se-gene–specified alpha-2-L-fucosyltransferase that determines whether ABH-soluble substances will be secreted (Denise Harmening ,2005) .

Table (1-1) Fluids in which A, B, and H Substances Can Be Detected in Secretors (Denise Harmening ,2005**)**

Saliva
Tears
Urine
Digestive juices
Bile
Milk
Amniotic fluid

Pathologic fluids: pleural, peritoneal ,pericardial ,ovarian cyct

Table (1-2) Comparison of ABH Antigens onRBCs and in Secretions (Denise Harmening ,2005)

ABH Antigens on Red Cells	**A, B, and H Soluble Substances**
RBC antigens can be glycolipids, glycoproteins, or glycosphingolipids	Secreted substances are glycoproteins
RBC antigens are only synthesized on type 2 precursor chains	Secreted substances are primarily synthesized on type 1 precursor chains
Type 2 chain refers to a beta 1→4 linkage in which the number one carbon of the galactose is attached to the number three carbon of the N-acetylglucosamine sugar of the precursor substance	Type 1 chain refers to a beta-1→3 linkage in which the number one carbon of the galactose is attached to the number three carbon of the N-acetylglucosamine sugar of the precursor substance
The enzyme produced by the H gene (alpha-2-L-fucosyltransferase) acts primarily on type 2 chains, which are prevalent on the RBC membrane	The enzyme produced by the Se gene (alpha-2-Lfucosyltransferase) preferentially acts on type 1 chains in secretory tissues

1.2.7.7 A and B subgroups

The A (and AB) phenotype can be subdivided into A1 and A2 (and A1B and A2B). In a European population, about 80% of group A individuals are A1 and 20% A2 . A1 and A2 differ quantitatively and qualitatively. A1 red cells react more strongly with anti-A than A2 cells. In addition, A2 red cells lack a component of the A antigen present on A1 cells and some individuals with the A2 or A2B phenotype produce an antibody, anti-A1, which agglutinates A1 and A1B cells but not A2 or A2B cells. Anti-A1 is seldom reactive at 37°C and generally considered clinically insignificant. There are numerous other ABO variants, involving weakened expression of A or B antigens (A3, Ax, Am, Ael, B3, Bx, Bm, Bel), but all are rare (Michael Murphy, Derwood Pamphilon and Weatherall ,2005) .

1.2.7.8 The Bombay Phenotypes (Oh)

The Bombay phenotype was first reported by Bhende in 1952 in Bombay, India. It represents the inheritance of a double dose of the h gene producing the very rare genotype hh. As a result, the ABO genes cannot be expressed, and ABH antigens cannot be formed as there is no H antigen made in the Bombay phenotype . More than 130 Bombay phenotypes have been reported in various parts of the world. These RBCs are devoid of normal ABH antigens and therefore fail to react with anti-A, anti-B, and anti-H. In RBC testing using anti-A and anti-B, the Bombay would phenotype as an O blood group. However, the RBCs of the Bombay phenotype (Oh) do not react with the anti-H lectin (Ulexeuropaeus), unlike those of the normal group O individual, which react strongly with anti-H lectin. Bombay serum contains anti-A, anti-B, anti-A,B, and anti-H. Unlike the anti-H found occasionally in the serum of A1 and A1B individuals, the Bombay anti-H can often

be potent and reacts strongly at 378C. It is an IgM antibody that can bind complement and cause RBC lysis. Transfusing normal group O blood (with the highest concentration of H antigen) to a Bombay recipient (anti-H in the serum) would cause immediate cell lysis. Therefore, only blood from another Bombay individual will be compatible and can be transfused to a Bombay recipient. ABH substance is absent in saliva.. When family studies demonstrate which ABO genes are inherited in the Bombay phenotype, the genes are written as superscripts (OhA, OhB, OhAB) (Denise Harmening ,2005) .

1.2.7.9 ABO Antibodies

Individuals normally produce antibodies directed against the A and/or B antigen(s) absent from their RBCs. These antibodies have been described as naturally occurring because they are produced without any exposure to RBCs. The ABO antibodies are predominantly IgM, activate complement, and react at room temperature or colder. ABO antibodies produce strong direct agglutination reactions during ABO testing. The production of ABO antibodies is initiated at birth, but titers are generally too low for detection until the individual is 3 to 6 months of age. Therefore, most antibodies found in cord blood serum are of maternal origin. Results of serum ABO testing before 3 to 6 months of age cannot be considered valid because some or all of the antibodies present may be IgG maternal antibodies that have crossed the placenta. As a result, it is logical to perform only forward grouping on cord blood from newborn infants. Antibody production peaks when an individual is between 5 and 10 years of age and declines

later in life. Elderly people usually have lower levels of anti- A and anti-B; antibodies may be undetectable in the reverse grouping. ABO antibodies can cause rapid intravascular hemolysis if the wrong ABO group is transfused and can result

in death of the patient. Although anti-A (from a group B individual) and anti-B (from a group A individual) contains predominantly IgM antibody, there may be small quantities of IgG present. Serum from group O individuals contains not only anti-A and anti-B but also anti-A,B, which reacts with A and B cells. Anti-A,B antibody activity, originally thought to be just a mixture of anti-A and anti-B, cannot be separated into a pure specificity when adsorbed with either A or B cells. For example, if group O serum is adsorbed with A or B cells, the antibody eluted will react with both A and B cells. Anti-A,B therefore possesses serologic activity not found in mixtures of anti-A plus anti-B. The predominant immunoglobulin class of antibodies in group O serum is IgG. Knowledge of the amount of IgG anti-A, anti-B, or anti-A,B in a woman's serum sometimes allows prediction or diagnosis of hemolytic disease of the newborn caused by ABO incompatibility. Testing RBCs with reagent anti-A,B is not required as a routine part of RBC testing. It is believed by some, however, that anti-A,B is more effective at detecting weakly expressed A and B antigens than reagent anti-A or anti-B. Reagent anti- A,B can be prepared using blended monoclonal anti-A and anti–B, polyclonal human anti-A,B or a blend of monoclonal anti-A, anti-B and anti-A,B. Both immunoglobulin classes of ABO antibodies react preferentially at room temperature (20–24C) or below and efficiently activate complement at 37C (Denise Harmening ,2005) .

1.3 Previous Studies :

In 2016 Akpan IS and Essien EM study ABO blood group status and Von Willebrand Factor antigen levels in a cohort of 100 blood donors in an African population stated that A comparison of the mean vWF: Ag levels of the various ABO blood groups showed that the differences between their means were statistically significant ($p < 0.05$). subjects with non-O blood group had a level significantly higher than those of group O. There was no statistically significant difference between the plasma vWF: Ag concentration and the RhD positive and RhD negative blood groups.(Akpan and Essien ,2016).

In July 2014 Asuquo James I, Okafor Ifeyinwa M, Usanga Esien A and Isong Idongesit , study Von Willebrand Factor Antigen Levels in Different ABO Blood Groups in a Nigerian Population stated that a multiple comparison of the mean VWF:Ag of all the blood groups The result confirmed that all the non-O blood groups had levels significantly higher than that of group O while the differences between the non-O blood groups were not statistically significant ($p>0.5$). the distribution of VWF:Ag levels among the various ABO blood groups showed that the differences between their means were statistically significant ($p<0.05$). The distribution of mean VWF:Ag levels among age groups were not statistically significant ($p>0.05$). (Asuquo James , Okafor Ifeyinwa , Usanga Esien and Isong Idongesit ,2014)

In September 2007 Massimo Franchini, Franco Capra, Giovanni Targher, Martina Montagnana, and Giuseppe Lippi study the Relationship between ABO blood group and von Willebrand factor levels stated that the influence of ABO blood groups on plasma VWF levels have consistently reported that group O subjects have lower plasma VWF levels than non-O individuals, plasma VWF levels were

lowest in group O (mean VWF:Ag 75 IU/dL) and highest in group AB subjects (mean VWF:Ag 123 IU/dL). (Massimo Franchini, Franco Capra, Giovanni Targher, Martina Montagnana, and Giuseppe Lippi , 2007)

In September 2006 Jenkins PV and O'Donnell JS, study ABO blood group determines plasma von Willebrand factor levels stated that, an association between ABO histo-blood group and risk of thrombosis has been recognized. Blood group non-O (A, B, and AB) individuals have consistently been found to demonstrate increased incidence of both arterial and venous thrombotic disease, compared to group O individuals. This increased risk is attributable to the fact that ABO blood group influences plasma levels of a coagulation glycoprotein named von Willebrand factor (VWF). VWF levels are 25 percent higher in non-O compared to group O individuals. (Jenkins and O'Donnell , 2006)

In August 2001 James O'Donnell and Laffan MA , study The relationship between ABO histo-blood group and von Willebrand factor stated that ABO histo-blood group is a major determinant of plasma levels of von Willebrand factor. Blood group O individuals have significantly (approximately 25%) lower plasma levels of both glycoproteins. This association is of clinical significance. Low plasma levels of vWF have long been established as causes of excess bleeding. Conversely, there is accumulating evidence that elevated VWF levels may represent an important risk factor for ischaemic heart disease and venous thromboembolic disease (James O'Donnell and Laffan ,2001)

In 1987 Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR study The effect of ABO blood group on the diagnosis of von Willebrand disease Stated that. The presence of the ABO blood group has a significant influence on VWF:Ag values; individuals with blood group O had the lowest mean VWF:Ag level (74.8

U/dL), followed by group A (105.9 U/dL), then group B (116.9 U/dL), and finally group AB (123.3 U/dL). Multiple regression analysis revealed that age significantly correlated with VWF:Ag levels in each blood group. (Gill , Endres-Brooks , Bauer , Marks ,and Montgomery ,1987)

1.4 Rationale:

There is von Willebrand factor antigen level variation in healthy population This difference makes it difficult to diagnose the von Willebrand disease .One of these Variables is the ABO blood groups this variation lead to Difficulty in diagnosis .

Some studies have found differences between different ABO blood groups regarding to concentration of vWF These differences make us have a problem in diagnosing people which they have less quantative of vWF, If we say that a person have Deficiency in vWF We need to keep in mind the ABO blood groups.

In this research, we want to see the mean of vWF:Ag within different blood groups, And to help the doctors to Access to accurate diagnosis .

Most of the published works on the relationship between ABO blood groups and the levels of von Willebrand factor have been carried out world wide. Very little work on this subject has been done on Sudan there is need for studies to be carried out on Sudanese population. This study is aimed at ascertaining if the reported the relationship between ABO blood groups and plasma levels of von Willebrand factor antigen reported in other centres is the same in sudan. .

1.5 Objectives:

1.5.1 General Objective:

To study the association of ABO blood groups and vWF:Ag level among healthy Doners in Khartoum state.

1.5.2 Spesific Objectives:

1- To measure the vWF:Ag level among study groups using ELISA technique.

2- To determine and compare the mean of vWF:Ag level among different ABO blood groups.

3- To study the association of vWF:Ag level with different ABO phenotype of studied subject

4- To correlate the vWF:Ag level with participant`s age.

5- To study the association of vWF:Ag level with RhD antigen.

CHAPTER TWO

Materials and Methods

Materials and methods

2.1 Study design:

Observation descriptive Cross sectional study.

2.2 Study area:

National Public Health Laboratory (Central Blood Bank) in Khartoum state.

2.3 Study population:

Healthy volunteer blood donors in Central Blood Bank in Khartoum state.

2.4 Study period:

From January2017 to May 2017

2.5 Sample size:

60 samples from healthy volunteer blood donors.

2.6 Sampling technique:

Simple random sample.

2.7 Data collection:

Data collection method; will be collected using questionnaire.

2.8 Data analysis:

The data was computed and analyzed to obtain the mean, standard deviation and frequencies using statistical package for social science computer program (SPSS).

2.9 Ethical consideration:

It was considered that all information obtained from participants was kept as highly confidential data and specimen's results were not permitted.

The participators were provided with information about the study and any risk which may be arised especially when the collection technique was applied.

2.10 Method of blood sample collection:

2.10.1 Requirements:

Trisodium citrate containers.

Cotton

Alcohol (70%).

Disposable syringes

Sterile plain containers

Sterile blue tips

Automated pippte

2.10.2 Procedure:

The person was either sit or lied up right on an examination table then the arm was positioned on the armrest so that the vein identified is under some tension and its mobility was reduced, after that the skin was cleaned with 70% ethanol and allowed to dry, to avoid stinging when the skin is penetrated. And avoid using the

tourniquet to the arm then collected 1.8ml was placed in a container containing 0.2ml of 3.2%Ttrisodium citrate. The samples for the estimation of vWF:Ag level were centrifuged immediately at 5000 for 15 minutes then plasma extracted into sterile plain container and stored at -20 until analysis.

2.11 Estimation of vWF:Ag :

Will be measured by using Enzyme Linked Immunosorbent Assay (ELISA).

2.11.1 Procedure:

According to manufacturer instructions (see Appendices).

CHAPTER THREE

The Results

The Results

Table 4.1 showed Donors with blood group O were the majority with a total of 20 subjects (34%) while group AB had the least with 8 subjects (13%). Subjects with blood group A were a total of 17 Subjects (28%), while subjects with blood group B accounted for 15 (25%).

Table (3.1) showed the Frequency of ABO blood groups

Blood group	Frequency (N)	Percentage (%)
O	**20**	**28**
A	**17**	**25**
B	**15**	**13**
AB	**8**	**34**
Total	**60**	**100**

The statistical analysis of the results showed that the mean of vWF:Ag antigen level among subjects of groups A,B,AB and O was (0.741±0.0507 , 0.573±0.0458 , 0.913±0.0354 and 0.425± 0.0444) respectively .

Table 4.2 showed that subjects with non-O blood group had a level significantly higher than those of group O. plasma vWF levels were lowest in group O (mean vWF:Ag 425 IU/ml) and highest in group AB subjects (mean vWF:Ag 913 IU/ml). And There was statistically significant differences of mean vWF:Ag within different ABO blood groups. (p.value <0.05).

Table (3.2) Distribution of von-Willebrand factor antigen concentration (vWF: Ag, IU/ml) among the ABO blood groups

	Normal range of vWF:Ag concentration (0.5 -1.5 IU/ml)		
Blood groups	**Mean of vWF:Ag**	**Standard Deviation**	**P.value**
A	**0.741**	**0.0507**	**0.000**
B	**0.573**	**0.0458**	
AB	**0.913**	**0.0354**	
O	**0.425**	**0.0444**	

Table 3.3 showed There was no statistically significant difference between the plasma vWF: Ag concentration and Age (p.value >0.05)

Table (3.3) Relationship between Plasma vWF:Ag concentration IU/ml and Age

		Age
Vwf:Ag	**Pearson correlation**	**0.230**
	p.value	**0.077**

Table 3.4 showed There was no statistically significant difference between the plasma vWF: Ag concentration and RhD Antigen (p.value >0.05)

Table (3.4) Relationship between Plasma vWF:Ag concentration IU/ml and Rh D Antigen

	RhD antigen	N	Mean	Standard Deviation	P.value
vWF:Ag	RhD Postive	54	0.543	0.3815	0.583
	RhD Negative	6	0.633	0.3882	

CHAPTER FOUR

Discussion, Conclusion and Recommendations

Discussion, Conclusion and Recommendations

4.1 Discussion:

This is observation descriptive cross sectional study was carried out to study the Relationship between ABO blood group and von willebrand factor Antigen level among healthy Sudanese population was conducted in National Public Health Laboratory (Central Blood Bank) in Khartoum state during the period from January 2017 to May 2017. The study included 60 samples from healthy volunteer blood donors.

The mean of vWF:Ag antigen statically significant difference within ABO blood groups A,B and AB all within normal range except group O slightly lower than the Normal range.

The study showed There was statistically significant differences of mean vWF:Ag within different ABO blood group (p.value significant <0.05) these result agreed with Akpan IS and Essien EM (2016) stated that A comparison of the mean vWF: Ag levels of the various ABO blood groups showed that the differences between their means were statistically significant (p <0.05).and with Asuquo James I, Okafor Ifeyinwa M, Usanga Esien A and Isong Idongesit (2014) stated that the distribution of VWF:Ag levels among the various ABO blood groups showed that the differences between their means were statistically significant (p<0.05).

The study showed that subjects with non-O blood group had a level significantly higher than those of group O, plasma vWF levels were lowest in group O (mean vWF:Ag 425 IU/ml) and highest in group AB subjects (mean vWF:Ag 913 IU/ml) these result agreed with Akpan IS and Essien EM (2016) stated that subjects with non-O blood group had a level significantly higher than those of group O and with

Asuquo James I, Okafor Ifeyinwa M, Usanga Esien A and Isong Idongesit (2014) stated that the non-O blood groups had levels significantly higher than that of group O and with Massimo Franchini, Franco Capra, Giovanni Targher, Martina Montagnana, and Giuseppe Lippi (2007) stated that reported that group O subjects have lower plasma VWF levels than non-O individuals, plasma VWF levels were lowest in group O (mean vWF:Ag 75 IU/dL) and highest in group AB subjects (mean vWF:Ag 123 IU/dL).and with Jenkins PV and O'Donnell JS (2006) stated that vWF levels are 25 percent higher in non-O compared to group O individuals and with James O'Donnell and Laffan MA (2001) stated that Blood group O individuals have significantly (approximately 25%) lower plasma levels of both glycoproteins. and with Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr ,and Montgomery RR (1987) stated that individuals with blood group O had the lowest mean vWF:Ag level.

The study showed There was no statistically significant difference between the plasma vWF: Ag concentration and Age (p.value >0.05) these result agreed with Asuquo James I, Okafor Ifeyinwa M, Usanga Esien A and Isong Idongesit (2014) stated that The distribution of mean VWF:Ag levels among age groups were not statistically significant (p>0.05).

The study showed There was no statistically significant difference between the plasma vWF: Ag concentration and RhD Antigen (p.value >0.05) these result similar to Akpan IS and Essien EM (2016) stated that There was no statistically significant difference between the plasma vWF: Ag concentration and the RhD positive and RhD negative blood groups

4.2 Conclusion:

This study has concluded that von willebrand factor antigen level is lower in individuals of blood group O than non-group O individuals.

The Age, RhD antigen do not affect the level of von willebrand factor antigen.

4.3 Recommendations:

We recommend that separate reference ranges of von willebrand factor antigen should be established for the various ABO blood groups phenotypes and also in interpreting vWf:Ag results .

ABO blood groups should be taken into consideration specially in diagnosis of von willebrand disease .

Another study may be conducted with more sample size.

References

References

Akpan IS and Essien EM.(2016) ABO blood group status and Von Willebrand Factor antigen levels in a cohort of 100 blood donors in an African population, *International Journal of Biomedical Research*, Journal DOI: 10.7439/ijbr.

Asuquo James I, Okafor Ifeyinwa M, Usanga Esien A and Isong Idongesit.(2014) Von Willebrand Factor Antigen Levels in Different ABO Blood Groups in a Nigerian Population , *International Journal of Biomedical Laboratory Science* (IJBLS) Vo1 3(No.24-28)

A.V.Hoffbrand. P.A.H.Moss and J.E.Pettit .(2006*) Essential Haematology* . 5th Ed. Garsington Road, Oxford OX4 2DQ, UK: Blackwell.

Betty Ciesla.(2007) *Hematology in Practice.* Arch Street Philadelphia, PA 19103 : F. A. Davis Company.

Bowen DJ,(2003) An influence of ABO blood group on the rate of proteolysis of von Willebrand factor by ADAMTS13. J Thromb Haemost. 1:33–40. doi: 10.1046/j.1538-7836.00007.x.

David C. Calverley and Lori J. Maness.(2003) Platelet Function in Hemostasis and Thrombosis . in John P. Greer , John Foerster , John N. Lukens (eds.). *Wintrobe's Clinical Hematology.*11th Ed : Lippincott Williams & Wilkins .

Denise M. Harmening.(2005) *Modern Blood Banking and Transfusion Practices*. 5th Ed. Arch Street Philadelphia, PA 19103 : F. A. Davis Company.

Federici AB, Elder JH, De Marco L, Ruggeri ZM, Zimmerman TS.(1984) Carbohydrate moiety of von Willebrand factor is not necessary for maintaining

multimeric structure and ristocetin cofactor activity but protects from proteolytic degradation. J Clin Invest. 74:2049–2055.

Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr ,and Montgomery RR.(1987) The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood. ;69:1691–1695.

Jenkins PV and O'Donnell JS.(2006) ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? Transfusion. ;46:1836–1844. doi: 10.1111/j.1537-2995.00975.x

Marion E. Reid and Christine Lomas Francis.(2004) *The Blood Group Antigen*. 2nd Ed. 84 Theobald's Road, London WC1X 8RR, UK, 525 B Street, Suite 1900 San Diego, California 92101-4495, USA : Academic Press.

Mary L. Turgeon.(2010) *Clinical Hematology Theory and Procedures.* 5th Ed. Walnut Street, Philadelphia, PA 19106 : Lippincott Williams & Wilkins.

Massimo Franchini, Franco Capra, Giovanni Targher, Martina Montagnana, and Giuseppe Lippi.(2007) the Relationship between ABO blood group and von Willebrand factor levels Thromb J. 5: 14 Published online. doi: 10.1186/1477-9560-5-14

Matsui T, Titani K, Mizuochi T.(1992) Structures of the asparagine-linked oligosaccharide chains of human von Willebrand factor. Occurrence of blood group A, B, and H(O) structures. J Biol Chem. 267:8723–8731.

Michael F. Murphy, Derwood H. Pamphilon and D.J. Weatherall.(2005). *Practical Transfusion Medicine*. 2nd Ed. 9600 Garsington Road, Oxford OX4 2DQ, UK : Blackwell.

Moeller A, Weippert-Kretschmer M, Prinz H, Kretschmer V.(2001) Influence of ABO blood groups on primary hemostasis.Transfusion.41:56–60. doi: 10.1046/j.1537-2995.41010056.x.

Mohlke KL, Purkayastha AA, Westrick RJ, Smith PL, Petryniak B, Lowe JB, Ginsburg D.(1999) Mvwf, a dominant modifier of murine von Willebrand factor, results from altered lineage-specific expression of a glycosyltransferase. Cell. 96:111–120. doi: 10.1016/S0092-8674(00)80964-2.

O'Donnell JS and Laffan MA. (2001) The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. Transfus Med.;11:343–351. doi: 10.1046/j.1365-3148..00315.x.

O'Donnell JS, McKinnon TA, Crawley JT, Lane DA, Laffan MA.(2005) Bombay phenotype is associated with reduced plasma-VWF levels and an increased susceptibility to ADAMTS13 proteolysis. Blood.106:1988–1991.doi: 10.1182/blood-02-0792.

Rstavik KH, Magnus P, Reisner H, Berg K, Graham JB, Nance W.(1985) Factor VIII and factor IX in a twin population: evidence for a major effect of ABO locus on factor VIII level. Am J Hum Genet. 37:89–101.

Sarode R, Goldstein J, Sussman II, Nagel RL, Tsai HM,(2000) Role of A and B blood group antigens in the expression of adhesive activity of von Willebrand factor. Br J Haematol. 109:857–64. doi: 10.1046/j.1365-2141..02113.x.

William Kern. (ed). (2002) *PDQ Hematology* . ISBN:1-55009-176-x .

Appendices

Sudan University of Science & Technology

Collage of Medical Laboratory Science

The Relationship between ABO blood groups and von Willebrand factor antigen level among healthy Donors in Khartoum state.

QUESTIONNAIRE

Name:

……

ID number

……

Age

……

Healthy volunteer blood donors :

Yes

☐

No

☐

Investigation :

ABO Blood group

……

vWF antigen level

……

.