بسم الله الرحمن الرحيم

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Measurement of Plasma Von Willebrand Factor Antigen Level among Sudanese Patients with Type 2 Diabetes Mellitus

قياس عامل مستوى الفون ويليبراند المستضد لدي المرضى السودانيين المصابين بداء السكري من النوع الثاني

Athesis Submitted in Partial Fulfillment for the Degree of M.Sc in Hematology and Immuno-hematolog

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Dedication

To my father who taught memean of life

To my mother who lactating me the mean of patience

To my kind husband and my beautiful children

To my brothers and sisters who always encouraged me

To my lovely friends who participated withmeinThis study.

Acknowledgement

Thanks in the first and end for Alla , Special grateful thanks for my kind supervisiorDr.Ibrahimkhider Ibrahim who gave me much of his time and effort to instruct and help me to complete this dissertation , Special thanks toDr.waleed (Department of immunolog .ALU) who helpedme in the practical of this study.Finaly I am very gratefule to every onewho helped me.

Abstract

This is observational analytical case control study was carried out to estimate plasmavwf (Ag) level in type 2 diabetic patient.It was conducted in Alribat national hospital in Khartoum state during the period from January 2017 to August 2017.The study included 80 samples;40 samples from type 2 diabetic patient (cases) and 40 samples from healthy individual (control) ;2.5 ml of venous blood was collected in avacotainer tube containing 0.25ml of 0.38% tri sodium citrate and then separated by centerfugation and plasma was collected to measure von willebrand factor using Enzyme linkageimmunosorbent Assay. The results was analyzed by statistical package for social science computer program (spss) version 16.The data obtained through questionnaire.

The results showed that there is statisticaly significant difference in the mean of vwf(Ag) level between cases and control *p.value*=0.002 and the mean of vwf (Ag) among cases =0.76 IU/ml and the mean of vwf (Ag) level among control =0.66 IU/ml.

In the present study I observed that there is strong positive correlation between vwf (Ag)level and the age of the diabetic patients *p.value*= 0.01and pearson correlation=0.89 and also show positive correlation between vwf (Ag) and the duration of the diabetes pvalue=0.03and pearson correlation=0.22; in addition there is appositive correlation vwf(Ag) level and fasting blood glucose *p.value*=0.04 and pearson correlation=0.15.

Finaly this study showed there is no significant difference in the mean of vwf (Ag) level between male and female*p.value*=0.064.

ملخص الاطروحه

اجريت هذه الدراسه الوصفيه التحليليه حاله وحاله ضابطه لقياس عامل الفون ويلبراند المستضد عند مرضى السكرى النوع الثانى والتى تم اجراؤها في مستشفى الرباط الوطنى في الخرطوم فى الفتره من يناير 2017 الي اغسطس 2017.

وشملت الدراسه ثمانين عينه ،اربعين عينه من اشخاص مرضي بالسكري النوع الثاني واربعين اخري من اشخاص اصحاء تم اخذ اثنين ونصف ملليلتر من الدم الوريدي من كل شخص وتم وضعها في وعاء يحتوي علي مانع تجلط ثلاثي سترات الصوديوم ثم تم فصل العينه بواسطة جهاز الطرد المركزي وتم تحليل النتائج(***ELISA***)المصل الدموي ودلك لقياس عامل الفون ويلبراند المستضد عن طريق استخدام خلال استخدام الحزمه اللأحصائيه لبرنامج الحاسوب للعلوم اللأجتماعيه الاصدار16 ؛جمعت البيانات بواسطة الاستبيان .

اظهرت نتائج التحليل الاحصائي وجود فرق دو دلاله احصائيه في متوسط عامل الفون ويلبراند المستضد بين مرضى السكري النوع الثاني والاشخاص الاصحاء (القيمه المعنويه ***0.002***)حيث كان متوسط مستوى الفون ويلبراند اعلي في مرضى السكري (0.76) مقارنه بمتوسط الفون ويلبراند في الاشخاص الاصحاء (0.66) .

اظهرت النتائج ايضا" وجود علاقه ايجابيه قويه بين مستوى الفون ويلبراند المستضد وعمر مريض السكرى النوع الثانى (القيمه المعنويه ***0.01***ومعامل الارتباط 0.89) ، كما اوجدت علاقه ايجابيه بين مستوي الفون ويلبراند المستضد ومدة مرض السكري عند المريض (القيمه المعنويه ***0.03***ومعامل الارتباط0.42). كدلك اوجدت علاقه ايحابيه بين مستوي الفون ويلبراند المستضد ومستوي السكر في الدم (القيمه المعنويه ***0.04***ومعامل الارتباط0.15).

واخيرا" اظهرت نتائج الدراسه عدم وجود فرق ذو دلاله احصائيه بين متوسط مستوي الفون ويلبراند المستضد بين النساء والرجال (القيمه المعنويه ***0.064***).

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List of abbreviations

ADMTS13: Adisintegrin and metalloproteinase with thrombospondin type-1repeat-13

AGES: Advanced glycation end product

BMI: Body mass index

DDAVP: 1-deamieno-8D-arginine vasopressin

ELISA: Enzyme linkage immunosorbent assay

HLA: Human leukocyte antigen

HUS: Hemolytic uremic syndrome

IVIG: Intra venous immunoglobulin

MGUS: Monoclonal gammopathy of undetermined significance

PDGF: Platelet derived growth factor

TTP: Throombotic thrombocytopenic purpura

T2DM: Type 2 diabetes mellitus

vWF: Von willebrand factor

vWF:Ag: Von willebrand factor antigen

Vwd: von willebrand disease

Chapter One

Introduction and Literature Review

Introduction and literature review

1.1 Introduction

Due to the increasing number of diabetics, this disease has acquired a character of "epidemic" in recent decades. In 2000, the number of diabetics worldwide was approximately 151 million; estimates are that in 2010 this will reach 221 million and by 2025, 324 million (Cheng 2005).

It is believed that changes in human behavior, environment and lifestyle are favoring an increase in the number of obese and diabetic individuals (Zimmet*et al* 2001).

Two types of diabetes mellitus are the most prevalent: type-1 diabetes is characterized by autoimmune destruction of pancreatic beta cells resulting in an absolute deficiency in insulin; and type 2 diabetes (T2DM), which corresponds to approximately 90% of cases of diabetes worldwide, is characterized by insulin resistance and/or reduced production of insulin (Zimmet*et al* 2001).

The costs involved in treating diabetes and associated complications are high, thus, heavy investments have been made in primary prevention (to reduce the incidence of DM) and secondary prevention (to reduce the immediate and long-term complications of diabetic patients) (Zimmet*et al* 2001).

The major complications resulting from T2DM are related to the microvascular and macrovascular systems (Yamada *et al* 2000).

The most common microvascular complications are nephropathy, retinopathy and neuropathy and the most important macrovascular complications include coronary artery disease, strokes and peripheral arterial disease (Brownlee 2001).

About 80% of diabetics die from thrombotic events with 75% to 80% of these deaths resulting from cardiovascular events (Carr 2001).

In general, diabetic patients present symptoms of hypercoagulability and hypofibrinolysis. However, correlations between the vascular complications related to diabetes and the degree of abnormality of the haemostatic system have not been clearly established (Yamada *et al* 2000). The endothelium consists of a single layer of cells lining the inside surface of blood vessels, hence establishing a barrier between the blood and the vessel (Sumpio*et al* 2002).

The endothelium contributes to maintaining the blood flow by preventing platelet aggregation, its anticoagulant properties and by stimulating the fibrinolytic system (Vonhinsberg 2001). Changes in the endothelium can activate inflammatory processes, which together with other factors such as hypertension and dyslipidemia, cause atherosclerotic plaques. These plaques may remain asymptomatic for years and not cause any clinical changes in diabetic patients (Grant 2007).

Hyperglycemia directly contributes to endothelial injury through irreversible glycation of collagen and other subendothelial structural proteins of the vessel, forming advanced glycation end products (AGEs) (Meigs*et al* 2000),

AGEs accumulate in the subendothelium over time influenced by increases in blood sugar levels and are directly related to atherosclerosis and renal failure (Takeuchim*et al* 2001) ,One marker of endothelial injury is the von Willebrand factor (vWF) (Duncan 1998).

Epidemiological studies show that high vWF levels predict the evolution or progression of cardiovascular disease; intervention studies have shown that treatment of hypercholesterolemia, hypertension, diabetes and

hyperhomocysteinemia reduce vWF levels as does smoking cessation (Hirano *et al* 2000).

An increase in vWF has been observed preceding T2DM (Meigs 2006) and there is a positive association between increased vWFand progression to microvasculaand (Hirano *et al* 2000).macrovascular (Blannand Lip 1998).dysfunction in diabetes.

Chronic hyperglycemia or the various associated metabolic abnormalities, such as hypertension, dyslipidemia and hyperinsulinemia may cause endothelial injury resulting in microvascular lesions characteristic of diabetes such as nephropathy. Additionally, there is a hypothesis that microalbuminuria in diabetic patients does not only indicate renal injury, but also widespread vascular damage (Hirano *et al* 2000). All these factors together explain the positive association between microalbuminuria, increases in TM and vWF, and cardiovascular events because, in diabetes, these factors are associated with microvascular and macrovascular lesions.(Yudkin 1998). Hemostatic system disorders may be simultaneously present (increases in endothelial injury markers such as vWF, which promotes platelet adhesion to the subendothelium; platelet hyperactivity; hypercoagulability with increased thrombin formation; increased fibrinogen levels, which participate in the formation of platelet and fibrin clots; and decreased fibrinolytic activity, with an increase in PAI-1 which results in a longer permanence of the fibrin clot). All factors may contribute to a greater or lesser degree in the high incidence of thrombotic events in diabetic individuals (Juhan-vague *et al*1996).

1.2 Literature Review

1.2.1 VonWillebrandFactor

1.2.1.1 VWF gene

The VWF gene provides instructions for making a blood clotting protein called von Willebrand factor. This protein contains regions that attach (bind) to specific cells and proteins during the formation of a blood clot. After an injury, clots protect the body by sealing off damaged blood vessels and preventing further blood loss.

Von Willebrand factor is made within endothelial cells, which line the inside surface of blood vessels, and bone marrow cells. The factor is made of several identical subunits. To facilitate binding to various cells and proteins, these subunits are cut into smaller pieces by an enzyme called ADAMTS13. Von Willebrand factor helps platelets stick together and adhere to the walls of blood vessels at the site of a wound. These groups of platelets form temporary clots, plugging holes in blood vessel walls to help stop bleeding. Von Willebrand factor also carries another blood clotting protein, coagulation factor VIII, to the area of clot formation (Franchini and Lippi 2007).

1.2.1.2 Health condition related to genetic change

More than 300 mutations in the VWF gene have been found to cause von Willebrand disease. Mutations in the VWF gene that reduce the amount of von Willebrand factor cause type 1 von Willebrand disease. People with type 1 von Willebrand disease have von Willebrand factor in their bloodstream, but at reduced amounts. Mutations that disrupt the function of the von Willebrand factor cause the four subtypes of type 2 von Willebrand disease. These mutations usually change

one of the protein building blocks (amino acids) used to make von Willebrand factor or problems with its function slows the formation of blood clots, which causes the factor, which can disrupt the factor's ability to bind to various cells and

proteins needed to form a blood clot. Mutations that result in an abnormally short, nonfunctional von Willebrand factor generally cause the more severe type 3 von Willebrand disease. A reduction in the amount of von Willebrandprolonged bleeding episodes seen in von Willebrand disease.(Franchini and Lippi 2007).

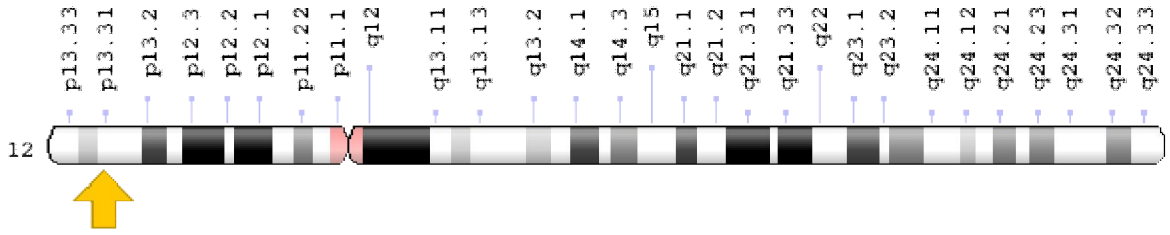
1.2.1.3 Other name for this gene

coagulation factor VIII VWF ,F8VWF andVWD

1.2.1.4 Chromosomal location

Cytogenetic Location: 12p13.31, which is the short (p) arm of chromosome 12 at position 13.31

Molecular Location: base pairs 5,948,874 to 6,124,675 on chromosome 12 (Homo sapiens Annotation Release 108, GRCh38.p7)(franchini and Lippi 2007) see figure(1.1).



Figure(1.1)Chromosomal location

1.2.1.5 Definition

Von Willebrand factor (vWF) is a bloodglycoprotein involved in hemostasis. It is deficient or defective in von Willebrand disease and is involved in a large number of other diseases, including thrombotic thrombocytopenic purpura, Heyde's syndrome, and possibly hemolytic-uremic syndrome. Increased plasma levels in a large number of cardiovascular, neoplastic, and connective tissue diseases are presumed to arise from adverse changes to the endothelium, and may contribute to an increased risk of thrombosis (Sadler 1998).

1.2.1.6 Synthesis

vWF is a large multimericglycoprotein present in blood plasma and produced constitutively as ultra-large vWF in endothelium (in the Weibel-Palade bodies), megakaryocytes (α-granules of platelets), and subendothelialconnective tissue (Sadler 1998).

1.2.1.7 Structure

The basic vWFmonomer is a 2050-amino acid protein. Every monomer contains a number of specific domains with a specific function; elements of note are (Sadler JE 1998) the D'/D3 domain, which binds to factor VIII, (Von Willebrand factor type D domain) the A1 domain, which binds to: plateletGPIb-receptor, heparin,possiblycollagen the A2 domain, which must partially unfold to expose the buried cleavage site for the specific ADAMTS13 protease that inactivates vWF by making much smaller multimers. The partial unfolding is affected by shear flow in the blood, by calcium binding, and by the lump of a sequence-adjacent "vicinal disulfide" at the A2-domain C-terminus (Jakobi*et al* 2011), the A3 domain, which

binds to collagen (Von Willebrand factor type A domain)the C1 domain, in which the RGD motif binds to platelet integrin $\alpha_{IIb}\beta_3$ when this is activated (Von Willebrand factor type C domain) the "cysteine knot" domain (at the C-terminal end of the protein), which vWF shares with platelet-derived growth factor (PDGF), transforming growth factor-β (TGFβ) and β-human chorionic gonadotropin (βHCG, of pregnancy test fame). (Von Willebrand factor type C domain) Monomers are subsequently N-glycosylated, arranged into dimers in the endoplasmic reticulum and into multimers in the Golgi apparatus by crosslinking of cysteine residues via disulfide bonds. With respect to the glycosylation, vWF is one of only a few proteins that carry ABO blood group system antigens.(Sadler1998) Multimers of vWF can be extremely large, >20,000 kDa, and consist of over 80 subunits of 250 kDa each. Only the large multimers are functional. Some cleavage products that result from vWF production are also secreted but probably serve no function (Sadler 1998) for structure see (figure 1.2).

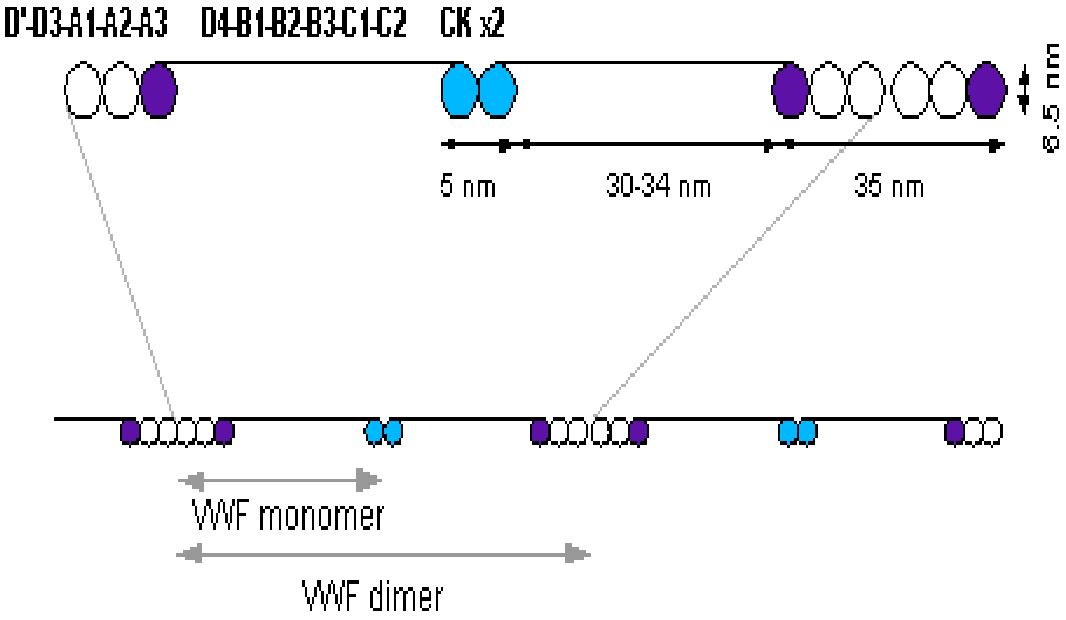


Figure (1.2) :Structure of VWF monomer and multimers

1.2.1.8 Function

Von Willebrand factor's primary function is binding to other proteins, in particular factor VIII, and it is important in platelet adhesion to wound sites.(Sadler 1998) It is not an enzyme and, thus, has no catalytic activity. vWF binds to a number of cells and molecules. The most important ones are (Sadler 1998) Factor VIII is bound to vWF while inactive in circulation; factor VIII degrades rapidly when not bound to vWF. Factor VIII is released from vWF by the action of thrombin. vWF binds to collagen, e.g., when it is exposed in endothelial cells due to damage occurring to the blood vessel. Endothelium also releases vWF which forms additional links between the platelets' glycoprotein Ib/IX/V and the collagen fibrils vWF binds to platelet gpIb when it forms a complex with gpIX and gpV; this binding occurs under all circumstances, but is most efficient under high shear stress (i.e., rapid blood flow in narrow blood vessels, vWF binds to other platelet receptors when they are activated, e.g., by thrombin (i.e., when coagulation has been stimulated). vWF plays a major role in blood coagulation. Therefore, vWF deficiency or dysfunction (von Willebrand disease) leads to a bleeding tendency, which is most apparent in tissues having high blood flow shear in narrow vessels. From studies it appears that vWF uncoils under these circumstances, decelerating passing platelets (Sadler1998) Recent research also suggests that von Willebrand factor is involved in the formation of blood vessels themselves, which would explain why some people with von Willebrand disease develop vascular malformations (predominantly in the digestive tract) that can bleed excessively.(Randi and Laffan 2017).

1.2.1.9 Catabolism

The biological breakdown (catabolism) of vWF is largely mediated by the enzyme ADAMTS13 (acronym of "*ad*isintegrin-like *a*nd *m*etalloprotease with *t*hrombo*s*pondin type 1 motif no. *13*"). It is a metalloproteinase that cleavesvWF between tyrosine at position 842 and methionine at position 843 (or 1605–1606 of the gene) in the A2 domain. This breaks down the multimers into smaller units, which are degraded by other peptidases(Levy *et al* 2005).

1.2.1.10 Role in disease

Hereditary or acquired defects of vWF lead to von Willebrand disease (vWD), a bleeding diathesis of the skin and mucous membranes, causing nosebleeds, menorrhagia, and gastrointestinal bleeding. The point at which the mutation occurs determines the severity of the bleeding diathesis. There are three types (I, II and III), and type II is further divided in several subtypes. Treatment depends on the nature of the abnormality and the severity of the symptoms(Sadler *et al* 2006) Most cases of vWD are hereditary, but abnormalities of vWF may be acquired; aortic valve stenosis, for instance, has been linked to vWD type IIA, causing gastrointestinal bleeding - an association known as Heydes syndrome. In thrombotic thrombocytopenic purpura (TTP) and hemolytic uremicsyndrome (HUS), ADAMTS13 either is deficient or has been inhibited by antibodies directed at the enzyme. This leads to decreased breakdown of the ultra-large multimers of vWF and microangiopathic hemolytic anemia with deposition of fibrin and platelets in small vessels, and capillary necrosis. In TTP, the organ most obviously affected is the brain; in HUS, the kidney (Moake 2004). Higher levels of vWF are more common among people that have had ischemic stroke (from blood-clotting) for the first time. Occurrence is not affected by ADAMTS13, and the only

significant genetic factor is the person's bloodgroup.High plasma vWF levels were found to be an independent predictor of major bleeding in anticoagulated atrial fibrillation patients.(Roldene*t al* 2011).

1.2.1.11 Von Willebrand Disease

Von Willebrand disease (VWD) is agenetic disorder caused by missing or defective von Willebrand factor clotting protein. VWF binds factor VIII, a key clotting protein, and platelets in blood vessel walls, which help form a platelet plug during the clotting process. The condition is named after Finnish physician Erik von Willebrand, a who first described it in the 1920s. VWD is the most common bleeding disorder, affecting up to 1% of the US population. It is carried on chromosome 12 and occurs equally in men and women (Rochester 2015) **.**

1.2.1.11.1 Inheretance of VWD gene

Von Willebrand disease can have different inheritance patterns.Most cases of type 1 and type 2 von Willebrand disease are inherited in an autosomal dominant patternsee (figure 1.3), which means one copy of the altered gene in each cell is sufficient to cause the disorder.Type 3, some cases of type 2, and a small number of type 1 cases of von Willebrand disease are inherited in an autosomal recessive pattern (figure 1.4), which means both copies of the gene in each cell have mutations. Most often, the parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they do not show signs and symptoms of the condition(Rochester 2015).

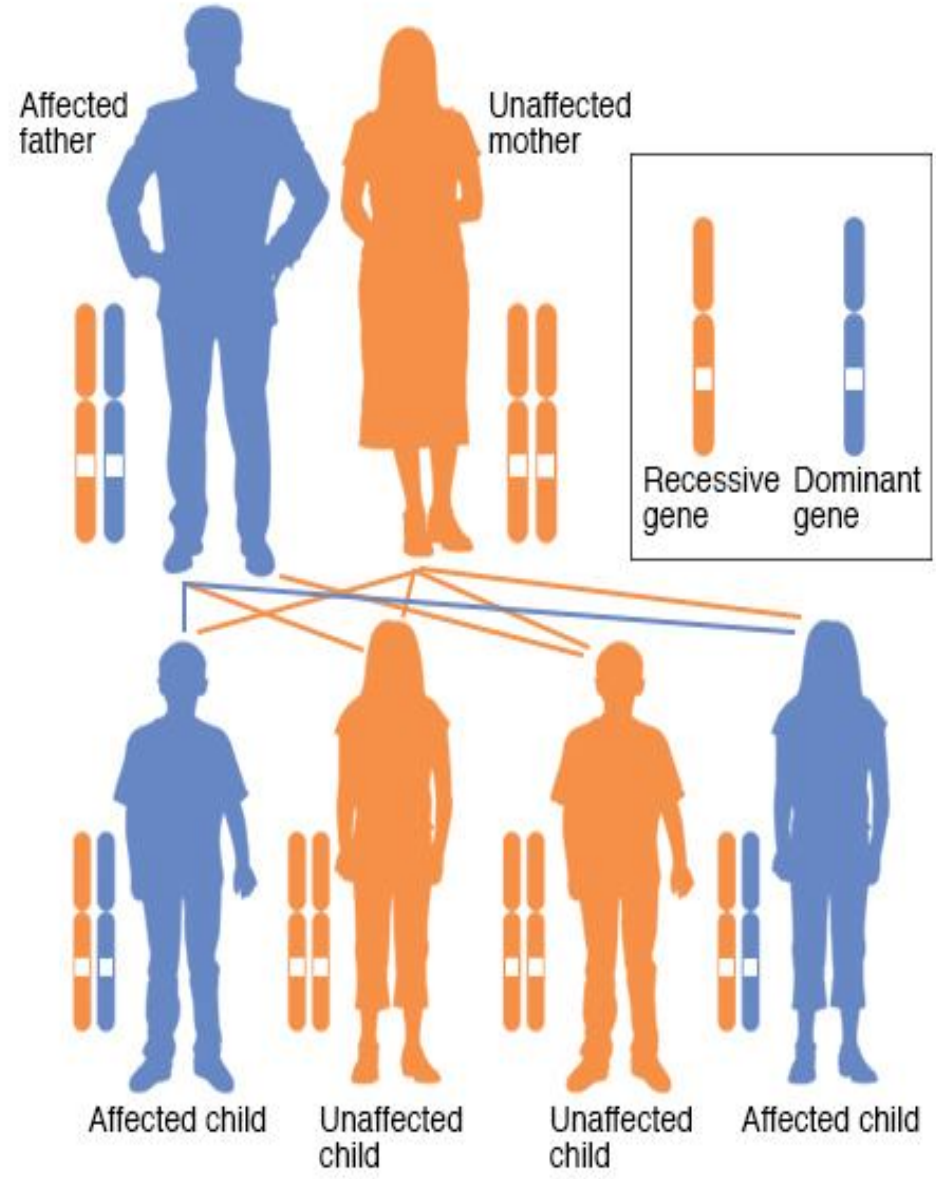


Figure (1.3):Autosomal dominant inheritance pattern

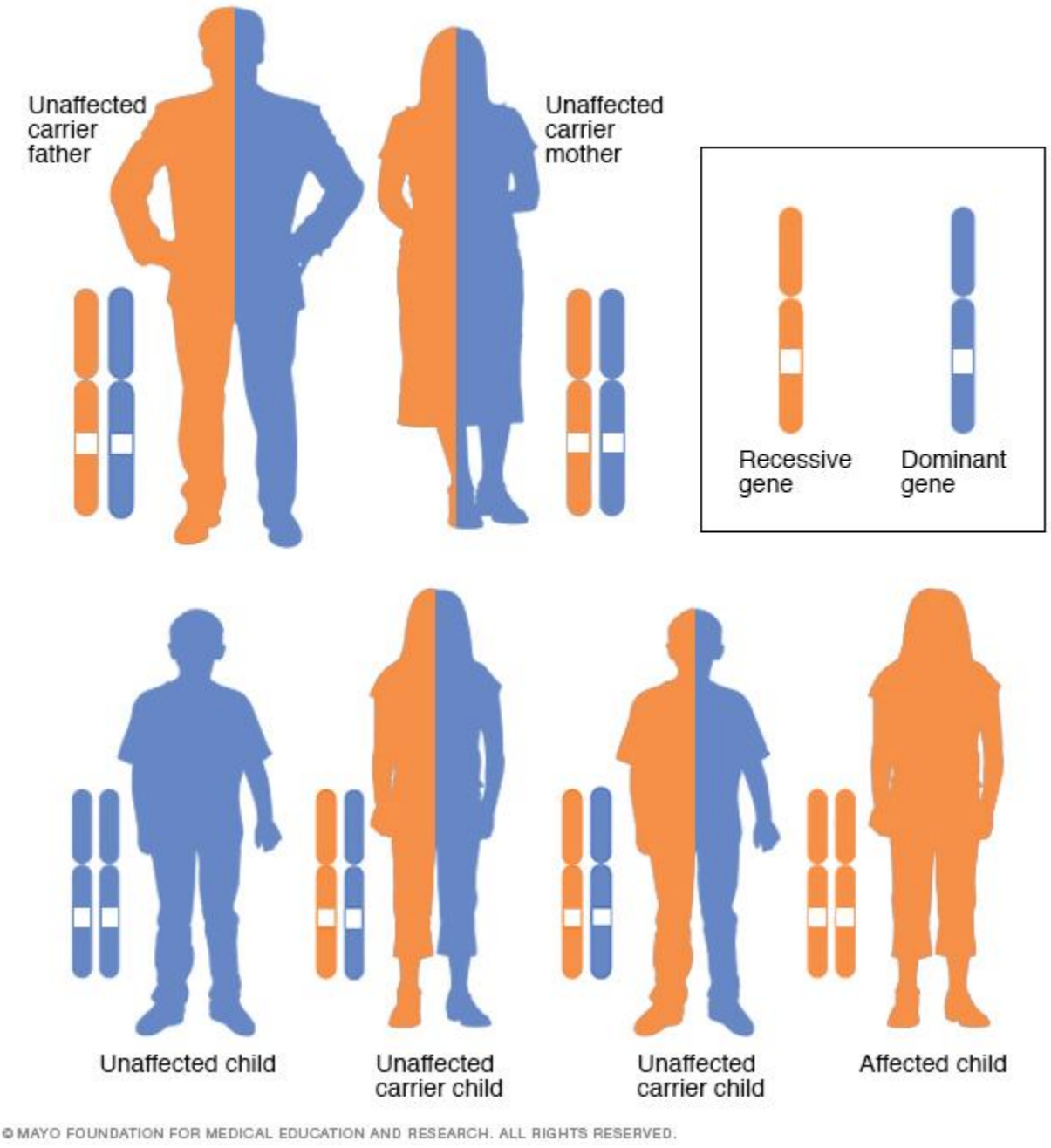


Figure (1.4):Autosomal recessive inheritance pattern

.2.1.11.2 Symptoms

People with VWD experience frequent nosebleeds, easy bruising and excessive bleeding during and after invasive procedures, such as tooth extractions and surgery. Women often experience menorrhagia, heavy menstrual periods that last longer than average, and hemorrhaging after childbirth (Francois 2017)..

1.2.1.11.3 Types of von Willebrand disease

1.2.1.11.3.1 Type 1 VWD

Type 1 VWD is the most common form, accounting for 75% of all cases of VWD. In Type 1 VWD, the von Willebrand factor (VWF) works normally, but there is not enough of it. Many people with Type 1 VWD have no symptoms at all until they experience a bad injury or an operation.

1.2.1.11.3.2 Type 2 von Willebrand disease

Type 2 VWD is less common than Type 1. It represents 20-25% of all cases.
In Type 2 VWD, the amount of VWF in people's blood is often normal. The problem is that the VWF does not work properly.
There are several sub-types of Type 2 VWD. It is important to get an exact diagnosis because the sub-types are treated differently.
Type 2A VWD is the most common sub-type. It represents 15-20% of all cases of VWD. In Type 2A VWD, the amount of VWF is often normal. However, because of a defect in the VWF protein, the platelets do not bind together well. The VWF does not act as a glue to hold the platelets in place to plug a hole in a blood vessel.
Type 2B VWD is the next most common. It represents about 5% of all cases of VWD. In Type 2B VWD, the VWF binds to platelets in the bloodstream, instead of binding at the site of the injury to the blood vessel. Then, the body removes these

large bundles of platelets from circulation. This causes a shortage of platelets. Type 2N VWD is much rarer. (The “N” stands for Normandy, France where the sub-type was first identified.) This is what doctors know about it.

• In Type 2N the VWF works normally with platelets. As a result, the grouping of platelets around the injury happens as it should.

• VWF also helps to carry around factor VIII in the blood and stabilize it so it can take part in the formation of a solid clot. In Type 2N the VWF does not transport factor VIII. As a result, factor VIII levels are low.

• Sometimes, because of the low factor VIII levels, Type 2N is mistaken for factor VIII deficiency hemophilia.

• In order for a child to get Type 2N, both parents must pass on the defective gene. There are several other extremely rare sub-types of Type 2 VWD, including Type 2M. The 'M' stands for 'Multimer', a part of the structure of the VWF molecule. In Type 2M, binding of the VWF to platelets is impaired.(Francois Laroche 2017).

1.2.1.11.3.3 Type 3 von Willebrand disease

Type 3 VWD is very rare. It affects about 1 in 500,000 people. However, it is the most severe type of VWD. People with Type 3 VWD have very little VWF in their blood. Because VWF transports factor VIII, they also have very low levels of factor VIII. As a result, bleeding can happen often and, if untreated, can be serious. Usually, in order for a baby to get Type 3 VWD, both parents must pass on the defective VWD gene. However, in some cases, the disease can result from a combination of one parent passing on the defective gene and a mutation in the child's gene inherited from the other parent.(Francois 2017).

1.2.1.11.3.4 Acquired VWd

This type of VWD in adults results after a diagnosis of an autoimmune disease, such as lupus, or from heart disease or some types of cancer. It can also occur after taking certain medications.Acquired von Willebrand's disease is a rare bleeding disorder that might be caused by other medical problems or medicines. It prevents blood from clotting properly. It is rarer than the inherited form of von Willebrand's disease.(Hillman *et al* 2011).

Table (1.1) ;Differential diagnosis between AVWS and VWD

Aspect	**In favor of AVWS**	**In favor of VWD**
Personal history	Late onest of bleeding Un eventful surgery before onest of bleeding	Early onest of bleeding No un eventful surgery or no previous high risk situation
Family history	Negative	Positive
AVWS associated disorder	Present	Absent
Laboratory evaluation	Presence of inhibitor or vwf antibody	Vwf mutation
Tretment response	Remission after treatment of underlying disorder Response to IVIG(in igG MGUS associate AVWS)	Normal recovery and half life of vwf containing sustained response to desmopression

VWD: von willebrand disease ***VWF***: von willebrand disease ***MGUS:*** monoclonal gammopathy of undetermined significance ***IVIG:*** intra venous immunoglobulin (Hillman *et al* 2011).

1.2.1.11.4 Diagnosis of VWD

Because many people with von Willebrand disease have mild signs and symptoms, the condition can be difficult to diagnose. If you have any indication of a bleeding disorder, your doctor may refer you to a blood disorders specialist (hematologist) .To evaluate you for von Willebrand disease, your doctor will likely ask you detailed questions about your medical history and check for bruises or other signs of recent bleeding.Your doctor will also likely recommend the following blood tests: (Rochester 2015).

Von Willebrand factor antigen. This test determines the level of von Willebrand factor in your blood by measuring a particular protein.

Ristocetin cofactor activity. This test measures how well the von Willebrand factor works in your clotting process. Ristocetin, which is an antibiotic, is used in this laboratory testing.

Factor VIII clotting activity. This test shows whether you have abnormally low levels and activity of factor VIII.

Von Willebrand factor multimers. This test evaluates the specific structure of von Willebrand factor in your blood, its protein complexes (multimers) and how its molecules break down. This information helps identify the type of von Willebrand disease you have (Rochester 2015).

1.2.1.11.5 Treatment-

Even though von Willebrand disease is a lifelong condition with no cure, treatment can help prevent or stop bleeding episodes. Your doctor may suggest one or more

of the following treatments to increase your von Willebrand factor, strengthen blood clots or, in women, control heavy menstrual bleeding:

Desmopressin This medication is available as an injection (DDAVP) or nasal spray (Stimate). It's a synthetic hormone, similar to the natural hormone vasopressin. It controls bleeding by stimulating your body to release more von Willebrand factor already stored in the lining of your blood vessels. DDAVP is usually effective in people with type 1 and some subtypes of type 2 disease. Many doctors consider DDAVP the first treatment to use in the management of von Willebrand disease. Some women use the nasal spray (Stimate) at the beginning of their menstrual periods to control excessive bleeding. It can also be effective when used before a minor surgical procedure.

Replacement therapies. These include infusions of prepared doses of concentrated blood-clotting factors containing von Willebrand factor and factor VIII (Humate-P, others). These therapies can be useful in all disease types. Your doctor may recommend them if DDAVP isn't an option for you or was ineffective.Another replacement therapy approved by the FDA for treating adults 18 and older is a genetically engineered (recombinant) von Willebrand factor product Because recombinant factor is made without plasma, it may reduce the risk of a viral infection or allergic reaction.

Contraceptives. For women, these can be useful for controlling heavy bleeding during menstrual periods. The estrogen hormones present in birth control pills can boost levels of von Willebrand factor and factor VIII activity. This effect is likely available with birth control patches, though further study is needed to confirm it.

Clot-stabilizing medications. These anti-fibrinolytic medications — such as aminocaproic acid (Amicar) and tranexamicacid can help stop bleeding by

slowing the breakdown of blood clots. Doctors often prescribe these drugs before or after a surgical procedure or tooth extraction.If your condition is mild, your doctor might recommend treatment only when you're undergoing surgery or dental work or when you've experienced trauma (Rochester 2015).

1.2.1.11.6 Risk factors

The main risk factor for von Willebrand disease is having a family history of it. A parent can pass the abnormal gene for the disease to his or her child.Most cases are "autosomal dominant inherited" disorders, which means you only need an abnormal gene from one parent to be affected. If you have the gene for von Willebrand disease, you have a 50 percent chance of transmitting this gene to your offspring.The most severe form of the condition (type 3) is "autosomal recessive," whichmeansboth of your parents have to pass an abnormal gene to you (Rochester 2015).

1.2.1.11.7 Complications

Complications of von Willebrand disease may include:

Anemia. Women who experience heavy menstrual bleeding can develop iron deficiency anemia.

Swelling and pain. If abnormal bleeding occurs in the joints or soft tissue, swelling and severe pain can result.

Death from bleeding. Rarely, someone with von Willebrand disease may experience uncontrolled bleeding that can be life-threatening and needs emergency medical attention(Rochester 2015).

1.2.1.11.8 Prognosis

For most affected individuals, vWD is a mild, manageable bleeding disorder in which clinically severe hemorrhage manifests only in the face of trauma or invasive procedures. However, significant variability of symptomatology exists among family members. In individuals with vWD types II and III, bleeding episodes may be severe and potentially life threatening. Individuals with type III disease who have correspondingly low FVIII levels may develop arthropathies, as more commonly seen in hemophilia A patients with comparably decreased FVIII levels. Levels of vWF normally increase with age. However, Sanders and colleagues found that although vWF levels increased with aging in patients with type I vWD, elderly patients with type I reported no change in their pattern of bleeding did not change. In patients with type II vWD, vWF levels did not increase with aging, and eldelry patients reported significantly more bleeding symptoms.(Sander *et al* 2014).

1.2.2 Diabetes Mellitus

1.2.2.1 Back ground

Diabetes mellitus is a complex metabolic disorder characterized by persistent hyperglycaemia (higher than normal blood glucose levels) resulting from defects in insulin secretion, insulin action or both. The two main types of diabetes are type 1 (formerly known as insulin-dependent diabetes) and type 2 (formerly known as non–insulin-dependent diabetes). Type 1 diabetes is caused predominantly by the autoimmune destruction of the insulin producing β-cells of the pancreatic islets, while type2 diabetes results from both impaired insulin secretion and resistance to the action of insulin. Diabetes is a major global health problem and in 2010 was estimated to affect 285 million individuals worldwide; this figure is projected to

rise to more than 400 million over the next two decades as a result of changing population demographics, such as ageing and urbanization, and changes in lifestyle, such as diet an exercise, and the associated increase in obesity (Richard and Neil 2012).

1.2.2.2 Prevalence of diabetes

The increaselargely represents an increase in the prevalence of type 2 diabetes, which accounts for ~90% of all cases of diabetes, but the prevalence of type 1 diabetes is also increasing.The prevalence of diabetes in the USA in 2010 was almost 27 million (12.3%), while 3.6 million people are affected by diabetes in the UK (7.4%) (Richard and Neil 2012).

1.2.2.3 Prediabetes (Borderline Diabetes)

Prediabetes, also commonly referred to as borderline diabetes, is a metabolic condition and growing global problem that is closely tied to obesity.

If undiagnosed or untreated, prediabetes can develop into type 2 diabetes; which whilst treatable is currently not fully reversible. Prediabetes is characterised by the presence blood glucose levels that are higher than normal(V.Rochester 2015).

1.2.2.4 Types of diabetes

1.2.2.4.1 Type 1 diabetes

Type 1 diabetes is caused by an absolute deficiency of insulin. In populations of white NorthernEuropean ancestry, it usually occurs as the result of a T-cell–mediated autoimmune destruction of the β-cells of the pancreas . By contrast, autoimmune type 1 diabetes is uncommon in non-Caucasian populations. With a better understanding of the pathogenesis of diabetes, it is recognized that other

genetic or acquired factors affecting pancreatic β-cell function can result in diabetes that presents in the same way as autoimmune type 1 diabetes . Furthermore, there may be a broad range of clinical manifestations that overlap between type 1 and type 2 diabetes (Richard and Neil 2012).

1.2.2.4.1.1 Aetiology

It is apparent that both genetic and environmental factors are important in the development of Type 1 diabetes

1. Genetic factor

Evidence for the importance of genetic factors comes from twin and family studies. The risk of developing diabetes increases with the number of family members with the condition (see table 1. 2). (Richard and Neil 2012).

Table(1.2);- Risk of developing type 1 diabetes for relatives of people with type 1 diabetes

Family member	Risk
Monozygotic twin	30-50% in other twin 65-70% if twin diagnosed before Age of 5 years
Diazygotic twin	15%
Sibbling with HLA genotype that is identical to the affected sibling	16-20%
Sibbling who shares one HLA gene	9%
HLA non identical sibling	3%
Mother	2%
Father	8%
Both parent	30%
General population	0.4%

2. Environmental factors

Although the genetic susceptibility to type 1 diabetes is inherited, only 12–15% of type 1 diabetes occurs in families with a history of diabetes and only 10% of HLA-susceptible individuals develop type 1 diabetes. This indicates that genetic factors do not account entirely for the development of type 1 diabetes.

1.2.2.4.2 Type 2 Diabetes

Type 2 diabetes is a heterogeneous disorder that results from the interaction of genetic predisposition and environmental factors, creating a combination insulin deficiency and insulin resistance (Richard and Neil 2012).

1.2.2.4.2.1 Aetiology

The risk factors for type 2 diabetes can be divided into those that are unmodifiable, and those that are environmental and therefore potentially changeable (Table 1.3).

Un modifiable	**Enviromental**
Family history-genetic	Obesity
Birth weight	Diet
Ethnicity	Physical activity
Age	urbanization
Past history of diabetes in pregnancy (gestational Diabetes)	

Table (1.3);- Risk Factors for Type 2 Diabetes

1.2.2.4.2.2Genetic predisposition

The heritability of type 2 diabetes is greater than for type 1 diabetes, and is estimated to account for 40–80% of total disease susceptibility . Many patients have a family history of diabetes, and monozygotic twin studies show a high concordance rate (60–90%). A maternal history of diabetes confers a higher risk of type 2 diabetes in the offspring than a paternal history, possibly through an effect of maternal hyperglycaemia during pregnancy(Richard and Neil 2012).

1.2.2.4.2.3 Environmental factors

The most important environmental risk factors for diabetes are obesity and physical inactivity.

** Obesity*

The massive explosion in obesity rates worldwide has largely been responsible for the increase in diabetes; it is estimated that up to 80% of all new casesof diabetes can be attributed to obesity. In the UK, the average body mass index (BMI) of a person with type 2 diabetes is 30.0 kg/m2, while in the USA, 67% of those with type 2 diabetes have a BMI of greater than 27 kg/m2, and 46% have a BMI of greater than 30 kg/m2. The risk of developing type 2 diabetes increases across the whole range of BMI, such that the risk in a middle-aged woman whose BMI is greater than 35 kg/m2 is 93.2 times greater than in a woman whose BMI is less than 22.5 kg/m2 . Similar changes are also seen in men.(Richard and Neil 2012).

** Physical inactivity*

Physical inactivity is also associated with an increased risk of diabetes. People who exercise for around 30 min/day have half the risk of developing diabetes

compared to those with a sedentary lifestyle. Although some of the difference can be explained by differences in adiposity, exercise itself accounts for approximately half of the effect.(Richard and Neil 2012).

** Intrauterine environment*

The intrauterine environment is important for the development of type 2 diabetes. Low birth weight.and thinness at birth are associated with increasing insulin resistance and diabetes in the offspring. In contradiction to this general observation, it appears that babies born to mothers with diabetes are also at increased risk of diabetes, despite the fact that thesebabies often have a high birth weight. Consequentlythe relationship between birth weight and subsequent risk of diabetes appears to be J-shaped.(Richard and Neil 2012).

1.2.2.4.2.4 Ageing

- The changing world demographics with its ageing population add a further explanation for the increase in diabetes as the prevalence increases with age. (Richard and Neil 2012).

1.2.2.4.2.5 Pathogenesis

Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and insulin secretion. In type 2 diabetes, both of these mechanisms break down with impaired insulin secretion through pancreatic β-cell dysfunction and impaired insulin action through insulin resistance (Richard and Neil 2012).

1.2.2.4.2.6 Prognosis

Type 2 diabetes is associated with premature mortality, predominantly through cardiovascular disease. Even after adjustment for other cardiovascular risk factors, diabetes is associated with a twoto three-fold increase in the risk of myocardial infarction or stroke.(Richard and Neil 2012).

1.2.2.4.3 Gestational diabetes

Gestational diabetes occurs when there is a high blood glucose level during pregnancy. As pregnancy progresses, the developing baby has a greater need for glucose. Hormone changes during pregnancy also affect the action of insulin, which brings about high blood glucose levels(Rochester 2015).

1.2.2.5 Causes of diabetes

The causes of diabetes are not known. The following factors may increase your chance of getting diabetes:

Family history of diabetes or inherited tendency

African-American, Hispanic, Native American, or Asian-American race, Pacific Islander or ethnic background

Being overweight (20 percent or more over your desired body weight)

Physical stress (such as surgery or illness)

Use of certain medications, including steroids and blood pressure medications

Injury to the pancreas (such as infection, tumor, surgery, or accident)

Autoimmune disease

High blood pressure

Abnormal blood cholesterol or triglyceride levels

Age (risk increases with age)

Alcohol (risk increases with years of heavy alcohol use)

Smoking

History of gestational diabetes or delivery of a baby weighing more than 9 pounds (4.1 Kg).

Pregnancy

It is important to note that sugar itself does not cause diabetes. Eating a lot of sugar can lead to tooth decay, but it does not cause diabete (Rochester 2015)..

1.2.2.6 Symptoms of diabetes

The symptoms of diabetes include

Increased thirst ,Increased hunger (especially after eating) ,Dry mouth ,Frequent urination ,Unexplained weight loss (even though you are eating and feel hungry) ,Weak, tired feeling ,Blurred vision ,Numbness or tingling in the hands or feet ,Slow-healing sores or cuts ,Dry and itchy skin (usually in the vaginal or groin area) ,Frequent yeast infections (Rochester 2015).

1.2.2.7 Symptoms of low blood sugar

Most people have symptoms of low blood sugar (hypoglycemia) when their blood sugar is less than 60 mg/dl. (Your health care provider will tell you how to test your blood sugar level.) When your blood sugar is low, your body gives out signs

that you need food. Different people have different symptoms. You will learn to know your

symptoms.Common early symptoms of low blood sugar include the following:

Feeling weak ,Feeling dizzy ,Feeling hungry ,Trembling and feeling shaky Sweating ,Pounding heart ,Pale skin and Feeling frightened or anxious (Rochester 2015).

1.2.2.8 Diagnosing of diabetes mellitus

Diabetes is diagnosed with fasting sugar blood tests or with A1c blood tests, also known as glycated hemoglobin tests. A fasting blood sugar test is performed after you have had nothing to eat or drink, Normal fasting blood sugar is less than 100 mg/dl (5.6 mmol/l). You do not have to be fasting for an A1c blood test (see table 1.4) (Inzucchi et al 2010).

	Normal	**Pre-diabetes**	**Diabetes**
Fasting Glucose Test	Less than 100	100-125	126 or higher
Random (anytime) Glucose Test	Less than 140	140-199	200 or higher
A1c Test	Less than 5.7%	5.7 - 6.4%	6.5% or higher

Table(1.4) Diagnosis of diabetes mellitus

1.2.2.9 Management of diabetes

There is no cure for diabetes, but it can be treated and controlled. The goals of managing diabetes are to:

Keep your blood glucose levels as near to normal as possible by balancing food intake with medication and activity.

Maintain your blood cholesterol and triglyceride (lipid) levels as near the normal ranges as possible by decreasing the total amount of fat to 30% or less of your total daily calories, and by reducing saturated fat and cholesterol.

Control your blood pressure. (Your blood pressure should not go over 130/80.)

Decrease or possibly prevent the development of diabetes-related health problems (Inzucchi et al 2010).

1.2.2.10 long-term complications of diabetes

Retinopathy (eye disease): All patients with diabetes should see an ophthalmologist (eye specialist) every year for a dilated eye examination. Patients with known eye disease or symptoms of blurred vision in one eye or who have blind spots may need to see their ophthalmologist more often.

Nephropathy (kidney disease): Urine testing should be performed every year. Regular blood pressure checks also are important because control of high blood pressure is essential in slowing kidney disease. Generally, blood pressure should be less than 130/80 in adults. Persistent swelling in the leg or feet also may be a symptom of kidney disease and should be reported to your doctor.

Neuropathy (nerve disease): Numbness or tingling in your feet should be reported to your doctor at your regular visits. Check your feet every day for redness, calluses, cracks, or breakdown in the skin tissue. If you notice these symptoms before your scheduled visits, notify your doctor immediately (Inzucchi et al 2010).

1.2.2.11Other long-term complications may include

Eye problems, including glaucoma and cataracts ,Dental problems High blood pressureand Heart disease (Inzucchi et al 2010).

1.3 Previous studies

Frankle*et al* (2008) .They concluded that higher levels of vWF were associated with risk of cardiovascular disease among frmingham offspring population with type 2 diabetes compared with control .

Seligman,*et al* (2000).Reported that type 2 diabetic patients with dyslipidemia have increased levels of ET-1 and vWF among American population compared to control groupe.

Ying Shao *et al* (2016).Reported that vwf was significantly high in type 2 diabetic patients among chinese patient compared to control.

Umadevi*et al* (2016).Reported that mean level of vwf were significantly increased in type 2 diabetic Bangalorian patients compared to normal.

Foss *et al* (2002). Reported that there was no significant difference in level of vwf between cases and control among people of Denmark.

Chen *et al* (2013),reported that vwf (Ag) correlated positively with age and duration of type 2 diabetic patient.also found that there is no difference in mean of vwf between male and female among liaocheng people.

N,samy.*et al* (2012).reported that there is highly significant elevation in plasma vwf in type 2diabetic patient with micro and macro albuminuria as compared to controls and diabetic patient with normal albuminuria also reported that there was positive significant correlation between vwf and duration of diabetic among eygptianpeople.and found that there is no difference in mean of vwf between male and female.

1.4 Rationale

There are many published reports addressing the role of vWF level in the development of macro vascular complication among diabetic patients. When we search in the literature we didn`t find any published reports from sudan addressing this problem so this research was conducted to examine the role of this factor in the development as such complication which may aid in the management of this complications.

1.5 Objectives

1.5.1General objective

To study the association between the vWF level and diabetes mellitus.

1.5.2 Specific objectives

1. To determine the level of vwf (Ag) in Type 2 Diabetic patient as well as normal individual (control) using ELISA technique.
2. To correlate the level of vwf (Ag) with patient`s gender, age,FBG and duration of disease.

Chapter Tow

Material and Method

Chapter Tow

Material and methods

2.1 Study design

It is observational analytical case control study

2.2 Study area and period

This study was carried out during the period from January to June 2017 at Al Ribat national hospital.

2.3 Study population

Sudanese patient suffering from type 2 diabetes at different age and sexwere recruited to participate in this study as well as apparently healthy volunteers were enrolled as control group.

2.3.1 Inclusion criteria

All patients with type 2 diabetes

2.3.2 Exclusion criteria

Patient with any other disease associated with a change in vWF level

2.4 Sample size

The sample was collected from 40 type 2 diabetic patients as (cases) and 40 normal individual as (control).

2.5 Sample technique

It is convenience non probability sample.

2.6 Data collection

A questionnaire was filled for each patient by direct interviewing (see appendix 1).

2.7 Ethical consideration

All patients were informed about the aim of the study and they gave their consent.

2.8 Method of blood sample collection

2.7ml of venous blood sample were collected using vacationer tube which contain 0.38% tri –sodium citrate and gently mixed and then separated immediately at 4500 rpm for 15 min after which platelet poor plasma (ppp) was collected in plain container and stored at -20 ˚C until analysis.

2.9 Estimation of Vwf: Ag

vWF level was determined for each participant by ELISA(see appendix 2) using Technozyme kit (Technoclone-Austria) (see appendix 3).

2.10 Data analysis

Data were collected manually in a master sheet and analysis was performed using computerized spss program.

Chapter three

Results

Results

3.1 Characteristic of the studied population

The percent study included 40 patients with type 2 diabetes (cases) and 40 normal individual was collected.

3.1.1The demographic data of diadetic patients

The studied patient characteristic are presented in table (3.1) and represent 14 male as 35% and 26 female as 65% of the total cases and the mean of age (years) was 53minimum 22 and maximum 75and of mean of duration of the disease about 7.6 years and minimum is one year and maximum is 24 years and the Fasting blood glucose mean is about 260g/dl of minimum of 93g/dl and maximum of 431g/dl.

	VWF(Ag) U/ml	**Age**	**duration**	**Fbg**
Mean	0.7660	52.9750	7.6000	260.1500
Std. Deviation	0.16441	12.69057	5.83447	76.35731
Minimum	0.40	22.00	1.00	93.00
Maximum	1.20	75.00	24.00	431.00

Table 3.1 The demographic data of diabetic patients

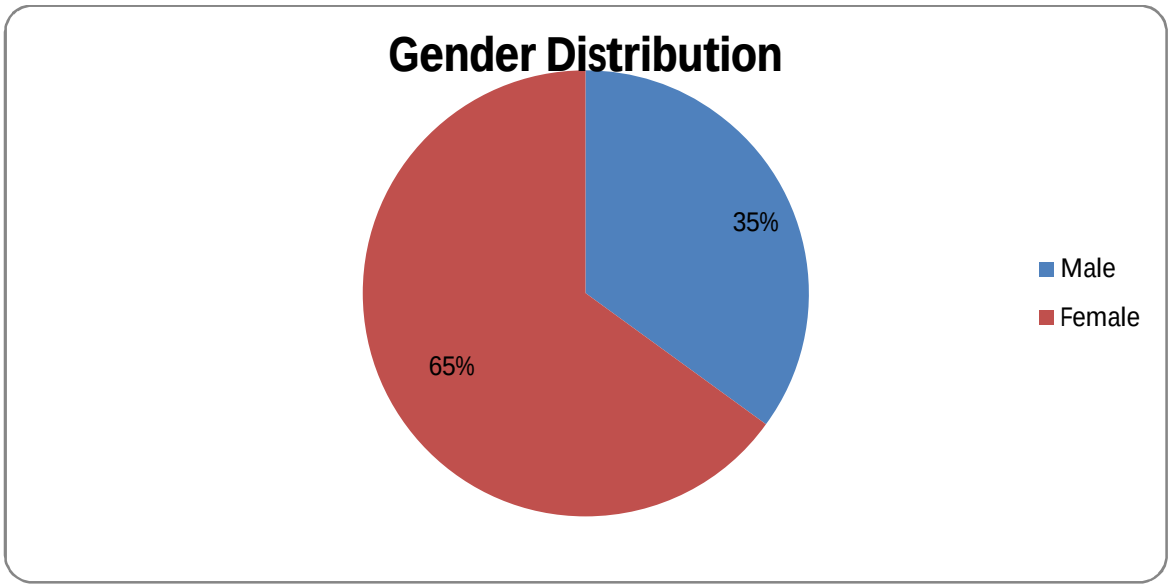


Figure 3.1 Gender distribution

3.2 VWF (Ag) among cases and control

The number of cases is 40 type 2 diabetic patient and 40 healthy individual as control of total of 80 represent in table (3.2) .The mean of vwf (Ag) concentration among patients was 0.766 ± 0.164 U/ml while among control group it was 0.665±0.136 U/ml ; the difference was statistically significant *P.value* : 0.002.

Study Group	VWF Ag Level U/ml (NR:0.5-1.5)		
	Mean	Std. Deviation	P.value
Type 2 Patients	0.766	0.164	0.002
Control	0.665	0.136	

Table (3.2) VWF (Ag) among cases and control

3.3 The Correlation between VWF and gender

The statistical analysis represent in table (3.3) found that the number of male about 16 and the mean of vwf (Ag)concentration about (0.74U/ml) and the number of female is about 26 of mean about (0.78U/ml) but there is no difference in vwf (Ag) concentration between male and female *p value*=0.064.

Gender	**VWF Ag Level U/ml (NR:0.5-1.5)**		
	Mean	**Std. Deviation**	**P.value**
Male	**0.7413**	**0.144**	**0.064**
Female	**0.7816**	**0.184**	

Table (3.3) The correlation between VWF(Ag) and gender

3.4 The correlation between VWF(Ag) and age

The statistical analysis showed that there is positive correlation between vwf (Ag) concentration and age of the patient *p.value*=0.01and pearson correlation = 0.890 see table (3.4).

3.5 The correlation between VWF(Ag)and the duration of the disease

The statistical analysis represent in table (3.4) showed that there is positive correlation between vwf (Ag) concentration and the duration of the disease *p value*= 0.03 and pearson correlation =0.42.

3.6 Correlation between VWF(Ag) and Fasting blood glucose

The result of the percent study found that there is positive correlation between vwf(Ag) concentration and fasting blood glucose *p value*=0.04 and pearson correlation =0.15 see table(3.4)

VWF(Ag) level U/ml (NR:0.5-1.5)				
	Mean	**STD deviation**	**p.value**	**Pearson correlation**
Age(years)	52.9	12.69	0.01	0.89
Duration of diabetes(years)	7.6	5.85	0.03	0.42
Fbg(mg/dl)	260.15	76.35	0.04	0.15

Table(3.4) The correlation between VWF(Ag) and Age,Duration of diabetes and FBG

Chapter four

Discussion,Conclusions and Recommendations

Chapter Four

Discussion, Conclusion and recommendation

4.1 Discussion

About 80% of diabetic die from thrombotic events with 75% to 80% of these deaths resulting from cardiovascular events which caused due to endothelial injury caused by glycation of collagen and other sub endothelial structural proteins of the vessels forming advanced glycation end product **(Meigs*et al*. 2000**).AGES accumulate in the subendothelial over time influenced by increases in blood sugar levels and are directly related to atherosclerosis and renal failure **(Takeuchi *et al.*2001).**

In present study we found that there is significant elevation in plasma vWF in type 2 diabetic when compared with control (*p.value*=0.002) which agree with**shao Y.*et al* (2016)** Who reported that vwf was significantly high in type 2 diabetic patient among Chinese patient and also agree with **Umadevi*et al* (2016)** who reported that mean level of vwf (Ag) were significantly increased in type 2 diabetic Bangalorian patients compared to control ;also agree with **Meigs*et al*(2006)** who reported that there is increase in the level of vwf in type 2 diabetic patient in comparison to control among Framingham offspring and disagree with finding from Denmark **Foss *et al* (2002)** who reported that there is no significant difference in the level of vWF between cases and control .The study also disagree with **Samy.*et al***(2012) she reported that there is no difference in plasma vwf between cases and control among Egyptian people.

This study also found there is no significant difference in plasma vwf between male and female(*p.value*=0.064) which is agree with **Chen *et al* (2013)** who

reported that there is no difference in the mean of vwf between male and female among `liaocheng people also agree with **Samy.*et al* (2012)** she also reported that there is no difference in the mean of vWF between male and female among Egyptian people.

Finaly;this study present strong positive correlation between vwf and age of the patient (*p.value*=0.01and pearson correlation= 0.89) and also there is positive correlation between vWF and duration of diabetic(*p.value*=0.03and pearson correlation=0.22) which agree with reports published by **Chen *et al*(2013)** who reported that vWF correlate positively with age and duration of diabetic patients among liaocheng people. And also agree with **Samey.*et al* (2012)** she reported that there is positive significant correlation between vWF and duration of type 2 diabetic disease among Egyptian people.

4.2 Conclusions

This study concluded that

- VWF is significantly greater among type 2diabetic patients compared to healthy individuals.
- Gender has no effect on the vWF level among study group.
- There is a positive correlation between vWF level and patient`s age.

4.3 Recommendations

- Further studies with large sample size should be carried out in vWF among diabetic patients to provide more definitive information about its effect
- Further studies must be done in the other coagulation parameter and endothelial marker to evaluate its changes in diabetes.
- More intensive study must be done in all types of diabetes.

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Appendices

Appendix (1):

Estimation of Plasma Von Willebrand Factor Antigen Level Among Sudanese Patients with Type 2 Diabetious Mellitus

Questionnaire

Date:…./……/2017

Serial number:……

Name:…….

Age:……..yrs

Gender: Male…….Female……..

Do you eat or drink any thing this morrning?.....

Duration of disease:……..yrs

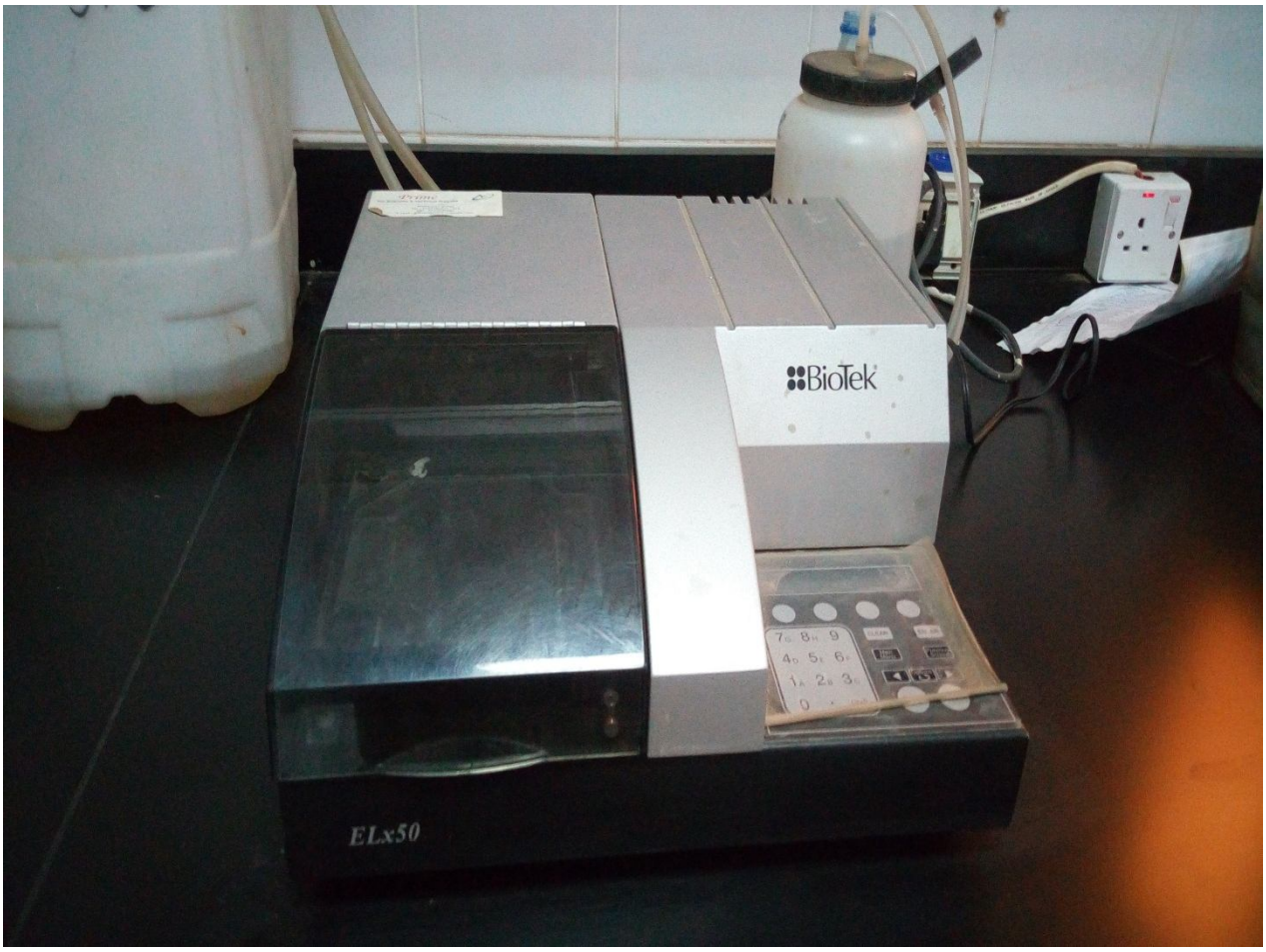
Do you make any physical exercise before coming to the hospital?.....

Do you have any other disease/s?.....

Result of fasting blood glucose:……g/dl

Appendix(2):

Equipement for performing ELISA test




Culture sample
تنبيه هام للجميع
الرجاء إغلاق الجهاز بعد الانتهاء و فصله من الكهرباء
ANAN