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**Association of Diabetes Mellitus Type2 with ABO and A Subgroup and
Rhesus D Blood Group in Khartoum State**

ارتباط النوع الثاني من مرض السكري مع فصائل الدم وفروع الدم أ والعامل الريصي في ولاية
الخرطوم

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الآية

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قال تعالى :

(وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَىٰ) (5)

صدق الله العظيم

(سورة الضحى الآية (5))

Dedication

To my lovely mother

To my husband who encouraged and support me

To my family

Acknowledgment

First of all I thanks ALLAH for helping me to complete this work. Special thanks to my supervisor Dr. Munsoor Mohammed Munsoor for his support.

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Abstract

This was an analytical case control study, conducted in Al Waledain health center in Burri Al Lamab in Khartoum state, during the period from August to November 2019 . Aimed to investigate the association of Diabetes mellitus type2 with ABO and A sub group and Rhesus D blood groups in Khartoum state . Total of 100 subjects are involved in this study 50 diabetic type2 subjects as case and another 50 non diabetic subjects as control matching in age and sex .

Three ml of EDTA anticoagulated blood was taken , Slide agglutination test for the determination of ABO and A subgroup and Rhesus D blood groups were used , D^u technique was performed to Rhesus negative results . Data were analyzed by using SPSS program version 20 , chi square test and correlation was calculated to show any association of diabetes mellitus type2 with ABO and A subgroup and Rhesus blood groups.

The study include both sexes with 43/100(43%) male while 57/100(57%) was female.

The mean age of case was (51.44 ± 13.824) years and in the control was (43.60 ± 17.287) years . The percent of sex in case male was (21%) , and female was (29%) , and in control male was (22%) and female was (28%) .

The distribution of ABO blood group of both case and controls group was O(23% vs 24%) , A1(20% vs 14) , B(7% vs 9%) , A1B(0% vs 2%) , A2B(0% vs1%) ,(p.value = 0.436) the results showed there was no significant variation in the distribution of ABO blood group between type2 diabetic subjects and control . Also no significant variation (p.value = 0.647) was found in the frequency of Rhesus with type2 diabetic subjects .

The O blood group give risk factor for diabetes mellitus type2.

There was strong positive correlation between age of case and control groups (sig = 0.00 , r = 0.844) . and negative correlation between age and sex among case and control (sig = 0.370 , r = - 0.091) .

Finally conclude there was no association between ABO and A sub group / Rhesus factor with diabetes mellitus type2. The O blood group was risk factor for diabetes mellitus type2. And there was strong positive correlation between age of case and control and negative correlation between age and sex among case and control .

المستخلص

هذه دراسة حالة مرضية وحالة ضابطة تحليلية ، اجريت في مركز صحي الوالدين بيري اللاماب في مدينة الخرطوم في الفترة من شهر اغسطس الى شهر نوفمبر سنة 2019م , الهدف منها التحقيق من وجود ارتباط للنوع الثاني من مرض السكري مع فصائل الدم وفروع الدم أ والعامل الريصي في ولاية الخرطوم . مجموع 100 شخص شاركوا في هذه الدراسة , خمسون شخص مرضى بالنوع الثاني من السكري , وخمسون من الاصحاء كحالة ضابطة متطابقين في العمر والجنس .

تم اخذ 3 مل من الدم في مادة مانعة للتجلط (ثنائي امين الايتلين رباعي حمض الخليك) اجريت اختبارات زمر الدم والعامل الريصي باستخدام طريقة الشرائح واستخدام محاليل تحتوي علي امصال مضادة , واجري فحص تأكيدي لكل عينة سالبة العامل الريصي , وحللت البيانات باستخدام نظام الحزم الاحصائية للمجتمع النسخة 20 , حسب مربع كاي والارتباط لمعرفة العلاقة بين ارتباط النوع الثاني من مرض السكري مع فصائل الدم وفروع الدم أ والعامل الريصي . شملت الدراسة الجنسين الذكور بنسبة 43 % والنساء بنسبة 57 % , متوسط اعمار المرضى (51.44 ± 13.824) سنة والاصحاء (43.60 ± 17.287) سنة . نسبة الجنس في المرضى (الرجال 21%) و (النساء 29%) والنسبة في الاصحاء (الرجال 22%) و (النساء 28%) .

توزيع فصائل الدم بين مرضى السكري النوع الثاني والاصحاء كالاتي : O (23% مقابل 24%) , A1 (20% مقابل 14%) , B (7% مقابل 9%) , A1B (0% مقابل 2%) , A2B (0% مقابل 1%) , (P.value = 0.436) , اظهرت النتائج انه لا يوجد اختلاف ذو دلالة معنوية في تردد فصائل الدم بين مرض السكري النوع الثاني والاصحاء . أيضا اظهرت الدراسة انه لا يوجد اختلاف ذو دلالة معنوية (p.value 0.647) في تردد العامل الريصي بين مرضى السكري النوع الثاني .

فصيلة الدم O لديها عامل خطورة للإصابة بالنوع الثاني من مرض السكري هنالك ارتباط موجب قوي بين العمر في الاصحاء والمرضى (sig = 0.00 , r = 0.844) . وارتباط سالب بين العمر والجنس في المرضى والاصحاء (sig = 0.370 , r = - 0.091) خلصت الدراسة على انه لا توجد علاقة ذات دلالة معنوية بين فصائل الدم والعامل الريصي مع مرض السكر النوع الثاني . فصيلة الدم O لديها عامل خطورة للإصابة بالنوع الثاني من مرض السكر . وهنالك ارتباط موجب قوي بين العمر في الاصحاء والمرضى , وارتباط سالب بين العمر والجنس في المرضى والاصحاء .

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List of abbreviations :-

ADA	American Diabetes Association
BGS	Blood group system
DMT2	Diabetes Mellitus type2
FPG	Fasting plasma glucose
GAD	Glutamic Acid decarboxylase
HDFN	Hemolytic disease of fetus and newborn
HTRs	Hemolytic transfusion reactions
IAT	Indirect antihuman globulin
ICA	Islet cell antibodies
IDDM	Insulin dependent Diabetes Mellitus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISBT	International Society of Blood Transfusion
K2EDTA	Di Potassium Ethylene Diamine Tetra Acetic Acid
MDT	Multi-disciplinary team
MODY	Maturity onset diabetes of the young
NIDDM	Non-insulin dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
Rh	Rhesus
RHAG	Rhesus antigen
SPSS	Statistical package of social science
UK	United Kingdom
USA	United State of America

Chapter I

Introduction

1.1 Introduction:

Blood groups were discovered at the beginning of the twentieth century when Landsteiner noticed that plasma from some individuals agglutinated the red cells from others. For the next 45 years, only those antibodies that directly agglutinate red cells could be studied.

With the development of the antiglobulin test by Coombs, non – agglutinating antibodies could be detected and the science of blood group serology blossomed. There are now 339 authenticated blood group antigens, 297 of which fall into one of 33 blood group systems, (Daniels, 2013).

The ABO BGS is the most important in transfusion medicine as almost all normal, healthy people older than 3 months of age have naturally occurring antibodies to the ABO antigens that they lack. These antibodies were first called naturally occurring because they were thought to arise without antigenic stimulation,(Quinley, 2011).

Diabetes mellitus is a chronic metabolic disorder with vascular components that is characterized by disturbances in carbohydrate, lipid and protein metabolism. So, hyperglycemia and glycosuria reflect the major metabolic lesion in carbohydrate metabolism, with secondary metabolic disturbances in proteins (gluconeogenesis)and lipids (ketosis and hypercholesterolemia).(Sood, 2015).

1.2 Rationale :

Diabetes is the most worldwide problem in public health and is one of the most important contributing factor to early mortality and morbidity around the world .The causes of Diabetes are complicated but there are factor such as genetic , environmental factor , but still , limited studies are added about the relationship of ABO and Rhesus blood type with type2 Diabetes Mellitus (Abdulridha , 2018). There was previous study in Sudan done by Ali , A (2015) about association of diabetes mellitus type2 with ABO and Rhesus blood group in Khartoum state and not found association .

In my study I added sub group of A blood groups with ABO and Rhesus factor to more confirmed about any association to diabetes mellitus type2 and if T2DM has related with certain blood groups to help in early diagnosis of T2DM .

1.3 Objectives:

1.3.1 General objective :

To study the association of diabetes mellitus type2 with ABO blood groups and sub group of A and Rhesus D factor in Khartoum state .

1.3.2 Specific objectives :

1. To evaluate the relation between ABO and sub group of A and Rhesus D blood groups with diabetes mellitus type 2 patients .
2. To determine the distribution of ABO groups and sub groups of A and Rhesus D factor in diabetic and non-diabetic subject .
3. To compare ABO groups and sub group of A and Rhesus D factor with diabetes mellitus type2 according to sex .

Chapter II

Literature Review

2.1 Function and composition of blood:

Blood is classified as connective tissue with an excessive and complex liquid intercellular material . Blood is main transportation vehicle of the body . it carries oxygen and nutrients to tissues and waste products of metabolism, carbon dioxide and urea , to the lungs and kidneys . blood also has important homoeostatic function , pH of blood 7.4 blood plays vital protective function against infection by virtue of its leucocytes and antibodies (immunoglobulin's) in the plasma . furthermore , injury to blood vessels is followed by blood clotting , which stops further loss of this vital fluid .Blood consist of formed elements (blood cell) , of which there are three types :

Red cells (erythrocytes) , white cells (leucocytes) , platelets(thrombocytes), (Abdul Gader, 2000) .

2.2 What is a blood group:

In 1900, Landsteiner showed that people could be divided into three groups (now called A, B, and O) on the basis of whether their red cells clumped when mixed with separated sera from other people. A fourth group (AB) was soon found. This is the origin of the term 'blood group'. A blood group could be defined as, 'An inherited character of the red cell surface, detected by a specific alloantibody'. Blood groups do not have to be red-cell specific, or even blood-cell specific, and most are also detected on other cell types. Blood groups do have to be detected by a specific antibody: polymorphisms suspected of being present on the red cell surface, but only detected by other means, such as DNA sequencing, are not blood groups. Furthermore, the antibodies must be alloantibodies, implying that some individuals lack the blood group . Blood group antigens may be: proteins, glycoproteins, glycolipids. (Daniels and Bromilow, 2014).

2.3 Historical perspective of the ABO blood group system:

Karl Landsteiner discovered the ABO blood group system in 1900, which incited the beginning of modern blood banking and transfusion medicine. Landsteiner performed a series of experiments demonstrating serological incompatibilities between individuals. In 1901, using his blood and the blood of his colleagues, he mixed the serum of some individuals with other people's cells. Inadvertently, he was the first person to perform forward and reverse grouping. This series of experiments led him to discover three of the four ABO groups: A, B, and O,(Sheryl,2010).

Shortly after Landsteiner's initial discovery, his associates, Alfred von Decastello and Adriano Sturli, discovered the fourth blood group, AB. In later studies, Landsteiner correlated the presence of the ABO antigens on red cells and the reciprocal agglutinating antibodies in the serum of the same individual (A antigens on red blood cells and anti-B in the serum). This discovery was labeled Landsteiner's Law or Landsteiner's Rule. This rule is the basis for all transfusion therapy as well as a guideline for determining the compatibility of donor and recipients,(Sheryl, 2010) .

2.3.1 Blood group systems:

The International Society of Blood Transfusion recognizes 347 red cell surface antigens, 308 of which belong to one of 36 blood group systems . Each system represents either a single gene or two or three very closely linked homologous genes. Each system is genetically discrete from all others. In addition, there are 39 antigens that have not been included in systems, owing to inadequate genetic evidence. Most blood groups are inherited as Mendelian characters, although environmental factors may occasionally affect blood group expression. The 36 systems represent a total of 41 genes: MNS contains 3 loci, Rh, Xg and Ch/Rg 2 loci each, and the other 32 systems each contain a single gene. All of the genes are autosomal, except for *XG* and *XK*, which are on the X chromosome, and *CD99*, which is located on both the X and Y chromosomes. All 41 genes have been identified and sequenced,(Daniels *et al*, 2016).

2.3.2 ABO Antigens :

According to the ABO blood group system, there are four different kinds of blood groups: A,B, AB or O. The antigen on red cells may be A/B/AB or no antigen at all. There are two subgroups in group A, namely A1 and A2. A and B antigens are inherited as per the Mendelian laws. Each individual inherits two ABO genes, one from each parent and these genes determine the ABO antigens on their red blood cells. Absence of both antigens, A and B on the red cells is seen in blood group O.(Nayak,2012)

2.3.3 ABO Antibodies :

Anti-A and anti-B are found in the plasma of individuals who lack the corresponding antigen (group O have anti-A and anti-B; group A have anti-B; group B have anti-A; group AB have neither antibody). These antibodies are produced in response to environmental stimuli, such as plant and bacterial moieties (e.g. *E. coli* sugars), and are therefore termed naturally occurring antibodies. Antibody production begins after birth and is usually detectable by 4–6 months of age, reaches a peak at age 5–10 years, and then declines with increasing age.(Westhoff and Shaz 2013). The serum contains naturally occurring

antibodies against red cell A/B/AB antigens these are of IgM type and do not cross the placental barrier.(Nayak,2012).

Table (2.1) ABO phenotypes (blood groups) and genotype(Nayak, 2012).

Phenotype(blood group)	Antigens on RBCs	Antibodies on serum	genotype
A	A	Anti –B	AA or AO
B	B	Anti –A	BB or BO
AB	A and B	Neither	AB
O	Neither	Anti –A and anti-B	OO

2.3.4 Genetics of ABO system:

ABO system follows Mendelian law of inheritance. The locus for ABO grouping is a chromosome 9 which is occupied by one of three major allelic genes namely A, B and O. Each individual has a pair of chromosomes (one from each parent). The A and B genes are dominant, while O gene is recessive, thus, not detected directly and accordingly absence of A and B antigens on red cells indicates ‘O’ blood group. The expression of A and B genes appears to be dependent on another gene called H gene. H gene is inherited, independent of A, B and O genes. H gene is expressed both as homozygous (HH) and heterozygous (Hh). When no H gene is inherited, a (hh) phenotype results which is extremely rare. This is commonly called Bombay group. Bombay group individuals are homozygous for hh gene.(Sood 2010).

The ABO blood group genes code not for the antigens directly, which are carbohydrate in nature, but for the production of glycosyltransferases. Glycosyltransferases are enzymes that facilitate the transfer of carbohydrate (sugar) molecules onto carbohydrate precursor molecules. The transferase associated with each blood group is specific for a particular immunodominant sugar. The immunodominant sugar molecule completes the antigenic determinant when combined with the precursor substance,(Quinley, 2011).

Basic precursor substance is first converted into H substance (by transferase) under the influence of H gene. H substance is partially converted under the influence of A and B genes (and specific transferase) into A and B antigens. Some of the H substance remains unconverted. Since O group individuals do not have A and B genes, neither A nor B antigen is formed and these have only H substance (H substance remains unchanged),.(Nayak , 2012).

Table (2.2) the possible phenotypes and genotypes in ABO group system(sood, 2010):

Phenotypes	Genotypes
A1	A1A1,A1,A2-A1,O
A2	A2A2.A2O
B	BB,BO
A1B	A1B
A2B	A2B
O	OO

Table (2.3)transferases and immunodominant sugars of the ABO blood group system (Quinley,2011):

Gene	Glycosyletransferase	Immunodominant sugar
H	L-Fucosyletranseferase	L-Fucose
A	N-acetylegalactoseaminyle tranferase	N-acetylgalactoseamine
B	D-galactosyletransferase	D-galactose

2.3.5 Clinical importance of blood groups:

Blood groups are of great clinical importance in blood transfusion and in transplantation. In fact, the discovery of the ABO system was one of the most important factors in making the practice of blood transfusion possible. Many blood group antibodies have the potential to cause rapid destruction of transfused red cells bearing the corresponding antigen, giving rise to a haemolytic transfusion reaction (HTR), either immediately or several days after the transfusion. At their worst, HTRs give rise to disseminated intravascular coagulation, renal failure, and death. At their mildest, they reduce the efficacy of the transfusion . IgG blood group antibodies can cross the placenta during pregnancy and haemolyse fetal red cells expressing the corresponding antigen. This may cause alloimmune fetal haemolytic anemia, more commonly known as haemolytic disease of the fetus and newborn (HDFN). Many blood group antibodies have the potential to cause HDFN, but the most common culprits are D and c of the Rh system and K of the Kell system. (Daniels and Bromilow, 2014).

Table (2.4)clinical important blood group systems (Hoffbrands,2016) :

system	Frequency of antibodies	Cause of HTR	Cause of HDN
ABO	Almost universal	Yes (common)	Yes (usually mild)
Rh	Common	Yes (common)	Yes
Kell	Occasional	Yes (occasional)	Anemia not hemolysis
Duffy	Occasional	Yes (occasional)	Yes (occasional)
Kidd	Occasional	Yes (occasional)	Yes (occasional)
Lutheran	Rare	Yes (rare)	No
Lewis	Occasional	Yes (rare)	No
P	Occasional	Yes (rare)	Yes (yes)
MN	Rare	Yes (rare)	Yes (yes)
Li	Rare	Unlike	No

2.3.6 Sub group of A:

The A phenotype can be subdivided into A1 and A2. A1 is the more common phenotype in all populations. A1 and A1B red cells have a stronger expression of A antigen than A2 and A2B, respectively. With most anti-A reagents, A1 red cells agglutinate faster, give stronger agglutinates, and are agglutinated by higher dilutions of anti-A, than A2 cells. There is also a qualitative difference between A1 and A2. About 2% of A2 and 25% of A2B individuals produce an antibody called anti-A1 that reacts with A1 and A1B cells, but not with A2 or A2B cells. The usual serological interpretation of this is that both A1 and A2 cells have A antigen, but A1 cells have an additional antigen, called A1, absent from A2 cells (Dniels and Bromilow, 2014).

2.4 The Rhesus blood group system:

The term Rh refers not only to a specific red blood cell (RBC) antigen but also to a complex blood group system that is currently composed of nearly 50 different antigenic specificities. Although the Rh antibodies were among the first to be described, scientists have spent years unraveling the complexities of the Rh system and its mode of inheritance, the genetic control of the Rh system, and the biochemical structure of the Rh antigens.(Wiler, 2005).

2.4.1 History of the Rh System :

Before 1939, the only significant blood group antigens recognized were those of the ABO system. Transfusion medicine was thus based on matching ABO groups. Despite ABO matching, blood transfusions continued to result in morbidity and mortality. As the 1930s ended, two significant discoveries were made that would further the safety of blood transfusion and eventually result in defining the most extensive blood group system known. It began when Levine and Stetson¹ described a hemolytic transfusion reaction in an obstetrical patient. After delivery of a stillborn infant, a woman required transfusions. Her husband, who had the same ABO type, was selected as her donor. After transfusion, the recipient demonstrated the classic symptoms of an acute hemolytic transfusion reaction. A year later, Landsteiner and Wiener² reported on an antibody made by guinea pigs and rabbits when they were transfused with rhesus monkey RBCs. This antibody, which agglutinated 85 percent of human RBCs, was named Rh. By the mid-1940s, five antigens made up the Rh system. Today the Rh blood group system is made up of nearly 50 different specificities. (Wiler, 2005).

2.4.2 Nomenclature :

The Rh system was discovered in the 1940s, and several terminologies developed over the years. These reflected differences in thinking regarding the inheritance of the antigens. Fisher and Race believed that the Rh system consisted of three closely linked genes or alleles: D at one locus, C or c at the second, and E or e at the third, as reflected in the DCE terminology. This terminology is used most often in written discussions of the Rh system antigens. The Wiener terminology was based on the belief that the Rh antigens were the products of a single gene coding for an “agglutinin” composed of multiple “blood factors.” The names given to each of the five major Rh antigens were Rh₀, rh'_, rh''_, hr'_, and hr''_, but the original Wiener terminology is obsolete. A modified version is useful in spoken language to convey the Rh haplotype,(Quinley, 2011).

Table (2.5) Fisher Race Rh gene combinations (Mehdi, 2013)

Fisher Race		Short notions
Gene complex	Antigens	
CDe	C,D,e	R ₁
Cde	c,D,E	R ₂
cDe	c,D,e	R ₀
CDE	C,D,E	R _z
Cde	C,e	R
Cde	c,e	r'
cdE	c,E	r''
CdE	C,E	R _y

2.4.3 Type of Rh antigens:

The five commonly detected antigens are D, C, E, c and e, of which D is the most potent and highly immunogenic, followed by c and E. The commonly used terms of Rh+ (positive) and Rh – (negative) depends on the presence or absence of D antigen. Approximately 95% of Indians are Rh positive while only 85% of Caucasians (whites) show Rh positivity.(Mehdi, 2013).

2.4.4 The D weak or D^u phenotype:

It has been observed that certain D positive red cells are not agglutinated by all anti-D sera, but require antihuman globulin (Coomb's) sera in indirect Coomb's test (ICT) to show agglutination. The phenomenon is nothing but a weak expression of the D antigen. This particular D phenotype is called D^u. So, D^u is not a different antigen but a differing expression of D antigen.(Mehdi, 2013).

2.4.5 Rh Phenotypes with Diminished or Undetectable Rh Antigens:

The complexity of the Rh blood group system allows for existent phenotypes to be either decreased in the levels of detectable Rh antigens or completely absent in these antigens. These phenotypes have arisen as mutations. The most common of these phenotypes is Rh null. Rh null very rarely, an individual will present with a total lack of Rh antigens on the surface of the red cells. These individuals are known as Rh null. The most common cause of the Rh null phenotype results from mutations in the RHAG gene.(Whitlock,2010). The individuals lack not only D but all the Rh antigens. They have a type of haemolytic anemia caused by an abnormal cell membrane. These individuals are more prone to develop anti-D

antibody.(Mehdi,2013). D deletion Phenotypes. Rare Rh phenotypes exist with complete lack of C, c, E, and e antigens. The notation for this phenotype is written either as -D- or D--. These cells may exhibit increased amounts of the D antigen. These individuals can develop complex antibodies to the antigens that are not present on the red cell surface.(Whitlock, 2010).

2.4.6 Antibodies of the Rh system:

Naturally occurring antibodies ,Generally, Rh antibodies are only produced following immunization with red cells. However, anti-E is often naturally occurring; about half may occur without a history of pregnancy or transfusion. Rarely, naturally occurring anti-D and anti-Cw are found. All such naturally occurring Rh antibodies react optimally with enzyme-treated cells; if they react at 37°C by IAT, they are clinically significant.(Daniels *et al* , 2016).

The Rh antibodies are clinically significant and are capable of causing haemolytic transfusion reaction (HTR). Most of the Rh antibodies and specially anti-D require antigenic stimulus to develop. The Rh antibodies result from immunization by either transfusion or pregnancy. Only anti-C and anti-E can occur without known antigenic stimulus. The Rh antibodies are mostly IgG, and react best in enzyme or antiglobulin medium, but anti-C has been detected in saline test indicating its IgM nature. Generally, Rh antibodies do not bind complement.(Mehdi, 2013).

2.5 Diabetes Mellitus:

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. In 1979, the National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus.

1 This scheme included dividing diabetes into two broad categories: type 1, insulin-dependent diabetes mellitus (IDDM); and type 2, non-insulin-dependent diabetes mellitus (NIDDM).(Freeman,2010). Diabetes is a multisystem disease that carries significant morbidity and mortality from its chronic macro vascular and micro vascular complications . Throughout the course of ‘living with diabetes’ a number of acute complications can occur which require careful patient education and management. Furthermore, different life stages, from young people, pregnancy to elderly care, require the expertise of the whole diabetes multi-disciplinary team (MDT) . In 2012, 382 million adults worldwide were estimated to have diabetes and it is thought that 550 million will have diabetes before 2030. Currently, there are approximately 3 million people with diagnosed diabetes in the

UK and around 850 000 people with undiagnosed diabetes. In the UK, around 10% of the health service budget is spent on diabetes care and diabetes-related problems. Diabetes therefore carries enormous health burdens and economic implications that will continue to grow as its prevalence increases.(Rees *et al*, 2017).

2.5.1 Types of diabetes:

The types of diabetes have been classified by the WHO. Type 1 diabetes (previously referred to as insulin-dependent diabetes mellitus or IDDM) is due to absolute insulin deficiency and is usually an autoimmune disease leading to the destruction of the insulin-secreting beta cells in the pancreas. In some cases the cause of destruction of the beta cells is not known. Type 2 (previously known as non-insulin dependent diabetes mellitus or NIDDM) results from relative insulin deficiency that may be associated with varying degrees of insulin action defects known collectively as insulin resistance. For a practicing clinician the implication of this diagnosis is that patients with type 1 diabetes require insulin straight away and insulin should not be stopped as it is life-preserving. Type 2 patients can progress through several stages and may require insulin later on in their disease.(Holt and Kumar, 2010).

Table (2.6) classification of diabetes mellitus(Freeman, 2010):

Diabetes mellitus classification	Pathogenesis
Type 1	β Cell destruction Absolute insulin deficiency Autoantibodies <ul style="list-style-type: none"> • Islet cell autoantibodies • Insulin autoantibodies • Glutamic acid decarboxylase autoantibodies • Tyrosine phosphatase IA-2 and IA-2B autoantibodies
Type 2	Insulin resistance with an insulin secretory defect Relative insulin deficiency
Other	Associated with secondary conditions <ul style="list-style-type: none"> • Genetic defects of β-cell function • Pancreatic disease • Endocrine disease

	<ul style="list-style-type: none"> • Drug or chemical induced • Insulin receptor abnormalities • Other genetic syndromes
Gestational	<p>Glucose intolerance during pregnancy</p> <p>Due to metabolic and hormonal changes</p>

2.5.1.1 Type 1 Diabetes:

There is absolute insulin deficiency due to autoimmune destruction of β cells. There is also a genetic susceptibility to the disease. The main genetic abnormality is in the major histocompatibility complex on chromosome 6. This usually presents at a younger age.(Kumar,2016). The pancreatic islets of newly diagnosed patients with type 1 DM show characteristic histological features of autoimmune disease. Islet cell antibodies (ICA) are frequently present in the plasma (and may be detectable long before the condition presents clinically), together with antibodies to insulin, glutamic acid decarboxylase (GAD) and other proteins, which, like ICA, are sensitive markers of risk of progression to clinical diabetes in the apparently healthy members of patients' families.(Marshall *et al* 2012).

2.5.1.2 Type 2 Diabetes:

This occurs due to insulin resistance or insensitivity of tissues to insulin and relative insulin deficiency (and may later lead to absolute insulin deficiency). Obesity is a major risk factor for the disease. The insulin resistance seems to be caused by the toxic effects of increased lipid accumulation, which interferes with insulin signaling processes between receptor activation and cellular effects. Some studies have shown that obese individuals have less number of insulin receptors in muscle, adipose tissue and liver.They usually show an improvement in glucose tolerance with exercise.(Kumar,2016). Environmental factors are also important. Many patients with type 2 DM are obese, particularly tending to have visceral (intra-abdominal) obesity, which is known to cause insulin resistance, and have other features of the 'metabolic syndrome, Reduced physical activity also causes insulin resistance, and various drugs, including corticosteroids and other immunosuppressant's, protease inhibitors, thiazides in high doses and some 'atypical' antipsychotics and β -adrenergic antagonists, are

diabetogenic. The interaction between genetic and environmental factors in the pathogenesis of type 2 DM is exemplified by the high prevalence of the condition in certain ethnic groups.(Marshall *et al*, 2012).

2.5.2 Other types of diabetes:

Type 1, type 2, and gestational diabetes are the main types of diabetes. However, they're not the only ones. Other forms of diabetes occur because of mutations in single genes. You can inherit these gene mutations, or they can occur out of the blue. Maturity-onset diabetes of the young (MODY) and neonatal diabetes mellitus are two of the most common forms. Cystic fibrosis–related diabetes is another type of diabetes common in people with cystic fibrosis, which occurs because of scarring of the pancreas. This also destroys beta cells and stops insulin production.(ADA, 2018).

2.5.3 Gestational diabetes:

The term gestational diabetes was first coined in a 1957 study of 621 pregnant women who were tested for glucose intolerance with the 100 g, 3 h glucose tolerance test (GTT). Although this label was reserved for women who had the highest level of glucose intolerance, the term was subsequently generalized to identify pregnant women who had any degree of glucose intolerance with onset or first recognition during pregnancy.(Sacks,2018). Gestational diabetes is a type of diabetes that occurs during pregnancy. It usually goes away after giving birth but gives moms and their babies a lifetime risk of developing type 2 diabetes. Women with gestational diabetes are more likely to some day have type 2 diabetes. Their children are also more likely to develop type 2 diabetes and be obese. Women with gestational diabetes can take medications, eat healthy foods, and exercise to manage their blood glucose during pregnancy. Uncontrolled blood glucose during pregnancy can increase the risk of preeclampsia and injury during birth because babies are large. When you've had gestational diabetes, you have a two in three risk for it in subsequent pregnancies.(ADA ,2018).

2.5.4 Insulin :

Insulin is the primary hormone responsible for the entry of glucose into the cell. It is synthesized by the cells of islets of Langerhans in the pancreas. When these cells detect an increase in body glucose, they release insulin. The release of insulin causes an increased movement of glucose into the cells and increased glucose metabolism. Insulin is normally released when glucose levels are high and is *not* released when glucose levels are decreased.

It decreases plasma glucose levels by increasing the transport entry of glucose in muscle and adipose tissue by way of nonspecific receptors. It also regulates glucose by increasing glycogenesis, lipogenesis and glycolysis and inhibiting glycogenolysis. Insulin is the only hormone that decreases glucose levels and can be referred to as a hypoglycemic agent.(Freeman, 2010).

2.5.5 Diagnosis of diabetes mellitus:

Diagnosis of diabetes is based upon plasma glucose levels. Three ways to diagnose diabetes

are possible and each, in the absence of unequivocal hyperglycemia, must be confirmed, on a subsequent day. The 75 g oral glucose tolerance test (OGTT) is more sensitive and modestly

more specific than fasting plasma glucose (FPG) in the diagnosis of diabetes, but is poorly reproducible. Because of its ease, patient acceptability and lower cost, measurement of FPG is the preferred diagnostic test. The use of the hemoglobin A1c (glycosylated hemoglobin or HbA1c) for the diagnosis of diabetes was previously not recommended due to lack of global standardization and uncertainty about diagnostic thresholds. Presently, because of a worldwide move towards a standardized assay and with increasing evidence about the prognostic significance of HbA1c, it is included as a diagnostic test in the 2011 American Diabetes Association (ADA) guidelines.(Thomas and Vasan,2016). In symptomatic individuals (e.g. polyuria, polydipsia and unexplained weight loss), the diagnosis can be made based upon the WHO 2006 criteria: A random venous plasma glucose concentration ≥ 11.1 mmol/L, or A fasting plasma glucose concentration ≥ 7.0 mmol/L (whole blood ≥ 6.1 mmol/L), or Two-hour plasma glucose concentration ≥ 11.1 mmol/L after 75 g anhydrous glucose in an oral glucose tolerance test (OGTT). In 2011, the WHO recommended an HbA1c of 48 mmol/mol (6.5%) as the cut-off point for diagnosing diabetes. When HbA1c is ≥ 48 mmol/mol (6.5%), diagnosis should be confirmed with a second sample, unless the individual is symptomatic with plasma glucose levels ≥ 11.1 mmol/L (Rees *et al*, 2017).

2.6 Association of the ABO blood group with disease :

2.6.1 Bacterial infection :

Group O individuals are more susceptible than non - O to the severe effects of infection with enterotoxigenic *Escherichia coli* , responsible for millions of cases of gastrointestinal infections annually, and for some strains of the cholera bacterium, *Vibrio cholera* .This

effect could result from a binding preference of bacteria - derived heat labile toxins for A - or B - active structures over H antigen .(Daniels, 2013).

2.6.2 Malaria:

There is convincing evidence that a major selection pressure driving the evolution of the ABO polymorphism has been provided by Plasmodium falciparum malaria. The global distribution of ABO phenotypes shows that O is more common, relative to non - O, in those parts of the world where P. falciparum infection is endemic . Published evidence for group O individuals being more susceptible to P. falciparum infection .(Daniels, 2013).

2.6.3 Clotting :

For well over half a century statistical associations between clotting anomalies and ABO phenotype have been recognized, with thrombotic disease more common in A than in O individuals and bleeding more common in O than in A.(Daniels, 2013).

2.6.4 Pancreatic cancer :

Since the 1950s numerous statistical analyses have indicated that group A individuals are at a higher risk than group O for a variety of forms of cancer , although a recent meta - analysis suggested that the association between ABO and cancer is limited to exocrine pancreas malignancy.(Daniels, 2013).

2.7 Previous study:

The study of Aggarwal *et al* ,2018 . in Muzaffarnagar city . study the association of ABO and Rh blood groups with type 2 diabetes mellitus . finding increased frequency of blood group AB and O (18.26% vs. 10.31% and 34.61% vs. 29.31%) in diabetic. The association between blood group ABO and DM was statistically significant (Chi-square value - 8.24, $P < 0.04$). , also finding increased frequency of Rh+ blood group (96.15% vs. 95.54%) in diabetic but no statistically significant association was found among Rh blood group and DM (Chi-square value - 0.021, $P = 0.88$).

Study of Jaggi and Yadav, 2014. in India , A chi-square value for ABO blood groups was found to be highly significant in both T2DM patients and control subjects.($P < 0.005$).

blood group B (43.40%) and O (30.19%) were more numerous than controls and least frequent blood group was AB (6.60%). Blood group A (25.86%) and AB (12.07%) were more common in controls than in T2DM patients. Allele frequency for diabetics were in order $O > B > A$. A allele frequency (0.477) was found to be highest and B allele frequency (0.317) was lowest in controls. The frequency of D allele was higher in T2DM patients (0.755) than in controls (0.679). d allele frequency was found to be higher in controls (0.321) than T2DM patients (0.245).

study of Ali, 2015. in Khartoum state was found, there is no significant variation in the distribution of ABO blood group between diabetic type 2 subjects and the control group. The highest occurrence of diabetes mellitus type 2 was found in blood group O, followed by A, B and the least occurrence was found in blood group AB. The control group has the order of blood group as the diabetic subject O(42), A(33), B(21) and AB(4). No significant variation in the distribution of RH blood group was found between diabetic type 2 subjects and the control. The occurrence of DM type 2 subject was (94%) among the RH positive subjects, the healthy subjects showed higher frequency of RH positive (96%) than type 2 diabetic participants.

study of Meo *et al* , 2016. In Saudi Arabia . study the association of ABO and Rh blood groups with type2 diabetes Mellitus . They find blood group B was associated with high incidence of type 2 Diabetes and blood group O has a minimum association with type2 Diabetes . blood group A and AB were almost equal distributed in both diabetic and non-diabetic population . however , we were unable to find an association between Rh +ve and Rh -ve blood groups with type2 Diabetes Mellitus .

Study of Sharma *et al* , 2014 . in Jodhpur in India . study the association between ABO blood groups and Diabetes Mellitus . They find the most frequent blood group in Jodhpur city was found to be group B followed by O , A and AB in both males and females . also find no comparison occur between diabetic and control in males and females and total subjects irrespective of gender belonging to different blood groups . also find no significant association between type of blood groups and DM .

Study of Alanazi *et al* ,2018 . in Saudi Arabia . study the association of Diabetes Mellitus with ABO blood group and Rh . They find the frequency of blood groups in case O , B , A , AB . also found highly significant relation between DM and Rh blood group ,while insignificant relations between gender and DM with Rh blood group .

Study of Nagpal and Hegde ,2015 . in India. study the distribution of the ABO and Rh D blood type in patients with type2 Diabetes Mellitus . They find blood groups AB and B showed less common association , blood group A were more associated with DM , blood group O showed similar distribution among both groups . Higher percentage of diabetics than controls had Rh positive blood groups .

Chapter III

Materials and methods

3.1 Study design :

This study was been designed as an analytical case control study .

3.2 Study Area :

This study was done in Alwaldeain health center in Buri AL Lamab at Khartoum state.

3.3 Study Duration :

The study was carried out in period from August to November 2019.

3.4 Study population :

Sudanese patients with Diabetes Mellitus type2 in Al Waldeain health center . Healthy individuals who had no Diabetes Mellitus type2

3.5 Inclusion Criteria :

Individuals with type2 Diabetes Mellitus under treatment in Al Waledain health center .

3.6 Exclusion Criteria :

Non cooperative diabetic individuals .

3.7 ethical consideration :

An approval of this study was taken from medical ethical committee of medical laboratory college in Sudan university of science and technology each person participate in this study were informed about the aim of study and its importance , samples and information will be use just for purpose of this study and keep it confidential.

3.8 Sampling :

3ml of EDTA anticoagulated blood was taken .

3.8.1 Sample size :

One hundred subjects were collected . Fifty were suspected having Diabetes Mellitus type2, and Fifty healthy individual .

3.9 Data collection :

Data collected by non-self-administered .The questionnaire was design to obtain information about sex and age .

3.10 Sample processing :

Use whole blood as specimen.

- Collect 3 mL of blood in 3.6 mg K2EDTA vacutainer.
- Process the sample within 1 hour of collection.
- If a delay in testing is expected , red cells should be separated from the plasma, washed and stored in a red preservative

solution at 2–8°C for no longer than 35 days.

- Do not use insufficient sample.(Ramakrishnan and Sulochana , 2012).

3.10.1 Sample technique :

ABO slide agglutination test were done

3.10.1.1 Principle :

This test is based on the principle of direct hemagglutination. The erythrocytes of a person contain antigens on the surface of the membrane. When these antigens are allowed to react with the corresponding antibodies, antigen-antibody reactions are produced. Normal erythrocytes will clump or agglutinate when mixed with Anti A/Anti B/ Anti A1 , if they possess A/B/A1 antigens respectively .(Ramakrishnan and Sulochana , 2012).

3.10.1.2 Procedure :

- 1.On the section of slide labeled anti- A place one drop of antibody A.
- 2.On the section of slide labeled anti- B place one drop of antibody B.
- 3.On the section of slide labeled anti- A1 place one drop of antibody A1.
4. Place one drop of cells in each antibody containing circle.
5. Carefully mix each solution with a separate applicator stick.
6. Tilt slowly for one minute, then observe for the agglutination.
- 7.record result.

Interpretation: Agglutination of red blood cells is positive result.

A smooth suspension of RBCs at the end of 2 minutes is a negative result.

3.10.1.3 Quality Controls :

Positive and negative control must be included with every test .the anti-A reagent should be tested against group A(positive control) and B (negative control) cells, and the anti-B reagent against group B (positive control) and group A (negative control) cells .(Knowles, 2001).

3.10.1.4 Rh D antigen:

Antisera containing antibodies specific for the D antigen is used to test for the D antigen , anti-D antisera are monoclonal. The antibody contained in the antisera will attach to the D antigen on red cells and agglutinate if the cells possess the antigen.(Sheryl, 2010). If Rh(D) is negative D^u technique its perform

3.10.1.4.1 D^u method principle :

Cells with Du antigen are sensitized with anti D by incubating at 37°C for 30 minutes. This results in the adsorption of anti D on the surface of the cell without producing

hemagglutination. The presence of reacted antibody on the surface of D^U cells is recognized by using anti human globulin which reacts with coated antibody and brings about

hemagglutination .(Ramakrishnan and Sulochana , 2012).

3.10.1.4.2 Procedure

- Prepare 5% suspension of washed red cells with normal saline.
- Take 1 tube and label it as test (T)
- Place 2 drops of 5% cell suspension in the tube
- Add 1 drop of anti D in the tube labeled T
- Place the tube in the water bath at 37oC for 30 minutes.
- Remove the tubes from the water bath and wash the cells in normal saline 2–3 times.
- Add 2 drops of anti-human globulin to both the tubes. Mix gently
- Centrifuge the tubes at 1500 rpm for 1 minute and look for agglutination.

Interpretation of results: Du positive: If agglutination is present in the tube labeled T

Du negative: If no agglutination seen in the tube T.

3.10.2 Data analysis :

The data were analyzed by statistical package for social science (SPSS) computer software version 20 .

3.10.2.1 Statistical analysis :

Chi square test and correlation done to check the statistical significance, The *p-value* considered significant was ≤ 0.05 .

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Chapter VI

Results

4.1 Results :

The mean of age in the study group is 47.5 years .

The study include 43% males and 57% females .

Fifty diabetic type2 subjects as case and fifty non diabetic subjects as control were included in this study .

The significant level for all analysis at ($P \leq 0.05$).

Table (4.2) demographic data of study population :

Demographic data	Case No (50)		Control No (50)	
	Age (mean \pm SD)	51.44 \pm 13.824		43.60 \pm 17.287
sex	Male	Female	Male	Female
	N %	No 5	No %	No %
	21%	29%	22%	28%

Table (4.2) Showed the mean age of case was (51.44 \pm 13.824), and in the control was (43.60 \pm 17.287) . in case there was (21% male and 29% female) , and in control (22% male and 28% female).

Table (4.3) distribution of ABO and A sub group and Rhesus in case according to sex .

Blood group	Male	Female	Total %
O+ve	20.0 %	22.0 %	42.0 %
O-ve	0.0 %	4.0 %	4.0 %
A1+ve	12.0%	24.0 %	36.0 %
A1-ve	2.0 %	2.0 %	4.0 %
B+ve	6.0 %	4.0 %	10.0 %
B-ve	2.0 %	2.0 %	4.0 %
A1B+ve	0.0 %	0.0 %	0.0 %
A1B-ve	0.0 %	0.0 %	0.0 %
A2B+ve	0.0 %	0.0 %	0.0 %
A2B-ve	0.0 %	0.0 %	0.0 %
Total %	42.0 %	58.0 %	100.0 %

Table(4.3) Showed the distribution of ABO and A sub group and Rhesus in case according to sex, sex have not significant effect on distribution of ABO blood group in diabetic type2 , the distribution of sex in males and females was O , A1 , B . The frequency of diabetic type2 in ABO/Rh positive blood group in males and females as followed O (10 vs 11) , A1 (6 vs 12) , B (3 vs 2) while Rh negative blood group in males and females was O (0 vs 2) , A1 (1 vs 1) , B (1 vs 1) . There were 25 Rh positive females compare with 19 Rh positive males and 4 Rh negative females compared with 2 Rh negative males .

Table (4.4) Chi square table for association of ABO and A sub group in case and control .

Blood group	Case		Control		P . value
	No	%	No	%	
O	23	23%	24	24%	0.436
A1	20	20%	14	14%	
B	7	7%	9	9%	
A1B	0	0%	2	2%	
A2B	0	0%	1	1%	
Total	50	50%	50	50%	

Table (4.4) Showed the distribution of ABO and A sub group , There is no significant variation in distribution of ABO blood group between case and control.

The most frequent ABO blood group in case was O 23/100 (23%) followed by A1 20/100 (20%) , B 7/100 (7%) , A1B 0/100 (0%) and A2B 0/100 (0%) . In control showed that O 24/100 (24%) , A1 14/100 (14%) , B 9/100 (9%) , A1B 2/100 (2%) and A2B 1/100 (1%) .

Table (4.5) Chi square table for association of Rhesus in case and control .

Rh	case		control		P . value
	No	%	No	%	
Positive	44	44%	44	44%	0.620
Negative	6	6%	6	6%	
Total	50	50%	50	50%	

Table (4.5) Showed the distribution of Rhesus blood group in case and control , There is no significant variation in distribution of Rhesus blood group between case (50%) and control groups (50%) .

Odd ratio of case and control among Rhesus positive and negative

$$O R = 44*6 / 44*6 = 1.0$$

That mean the Rhesus blood group is not risk factor for diabetes mellitus type2.

Table (4.6) chi square table for association of Rhesus blood group in case

	Rh positive	Rh negative	Total	P . value
case male count	19.0	2.0	21.0	0.647
% of total	38.0 %	4.0 %	42.0 %	
Case female count	25.0	4.0	29.0	
% of total	50.0 %	8.0 %	58.0 %	
Total count	44.0	6.0	50.0	
% of total	88.0 %	12.0 %	100.0 %	

Table (4.6) Showed the association of Rh blood group in case , the result show no significant variation (p. value 0.647) . % of Rh positive male 38% , % of Rh positive female 50 % . % of Rh negative male 4.0 % , % of Rh negative female 8.0 %

Table (4.7) distribution of ABO and A sub group and Rh factor in case and control .

Blood group	case %	control %	Total %
O+ve	21 %	20 %	41.0 %
O-ve	2.0 %	4.0 %	6.0%
A1+ve	18 %	13 %	31.0 %
A1-ve	2.0 %	1.0 %	3.0 %
B+ve	5.0 %	9.0 %	14.0 %
B-ve	2.0 %	0.0 %	2.0 %
A1B+ve	0.0 %	1.0 %	1.0 %
A1B-ve	0.0 %	1.0 %	1.0 %
A2B+ve	0.0 %	1.0 %	1.0 %
A2B-ve	0.0 %	0.0 %	0.0 %
Total	50.0 %	50.0 %	50.0 %

Table (4.7) Showed the distribution of ABO and A sub group and Rhesus positive in case and control subjects as followed O (21 vs 20) , A1 (18 vs 13) , B (5vs 9) , A1B (0 vs 1) , A2B (0 vs 1) . Also showed distribution of ABO and A sub group and Rhesus negative in diabetic and control subjects O (2 vs 4) , A1 (2 vs 1) , B (2 vs 0) , A1B (0 vs 1) , A2B (0 vs 0).

Odd ratio of case and control against blood group

The odd ratio of O blood group of case and control give risk factor to diabetes mellitus type2

$$O R = 21*4 / 20*2 = 2.1$$

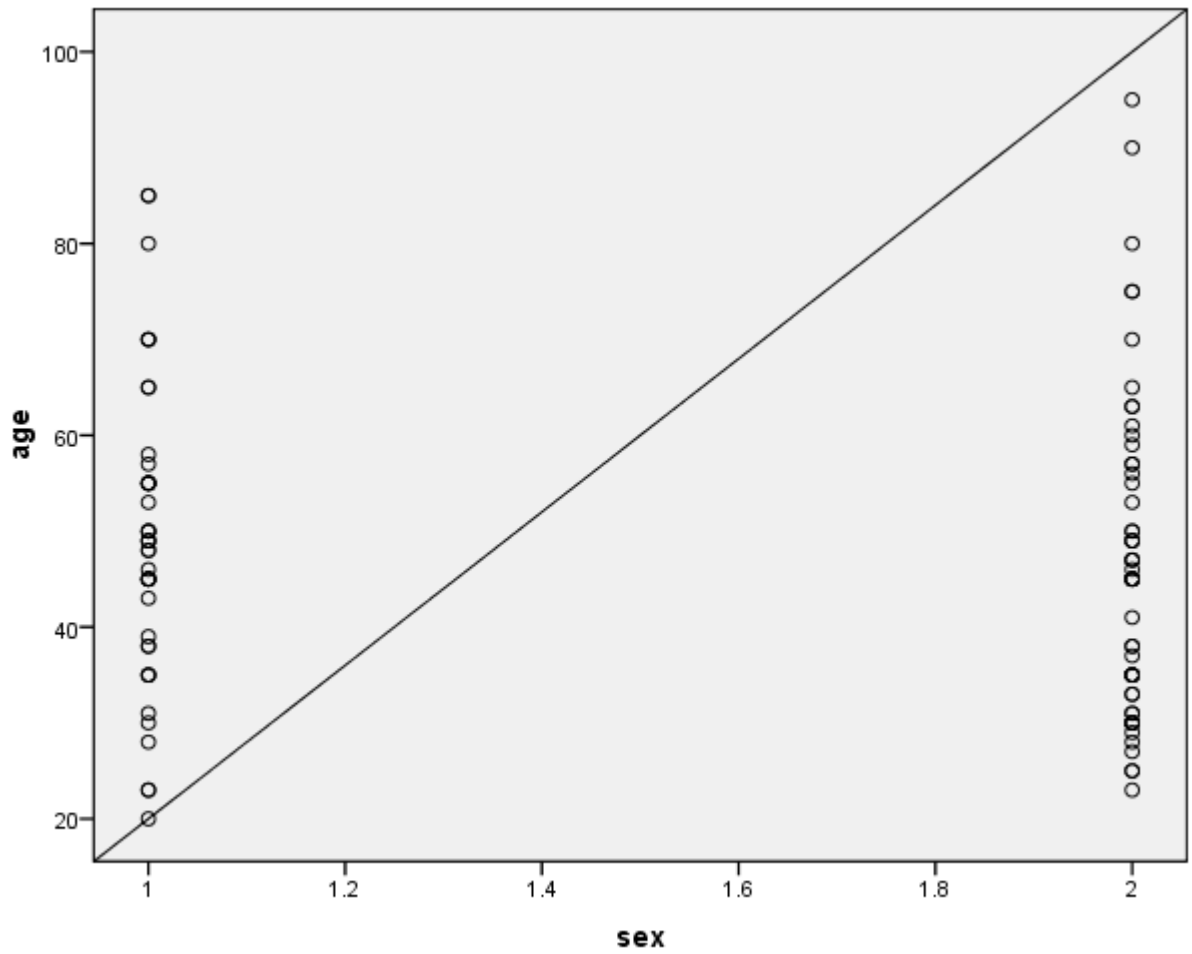


Figure 4.1 : The correlation between age and sex group among case and control (sig = 0.370 , r = - 0.091)

There was negative correlation between age and sex among case and control

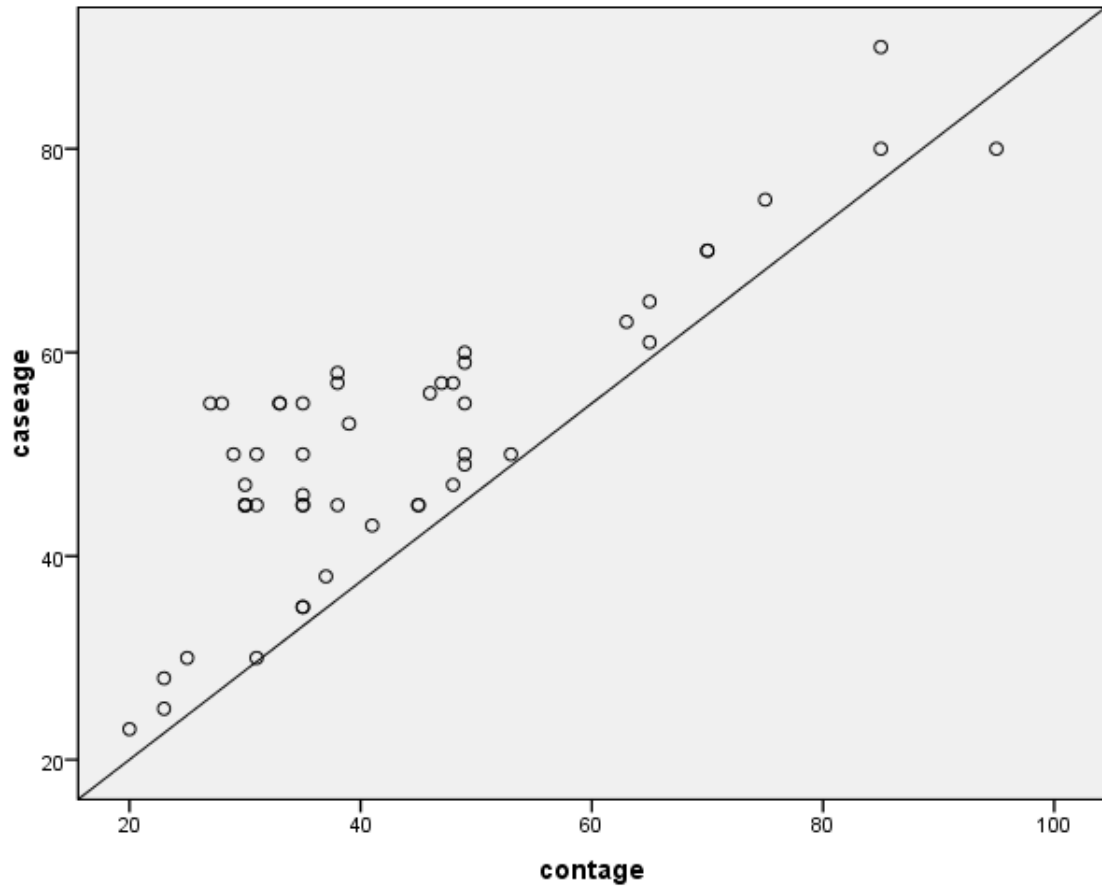


Figure 4.2 : The correlation between age group of case and age group of control (sig = 0.00 , r = 0.844).

There was strong positive correlation between age of case and control group .

Chapter V

Discussion, Conclusion and Recommendations

5.1 Discussion :

This study demonstrate that, blood group O has highest genotype followed by A1, B in diabetic patients , also there is no association between DMT2 and ABO/Rh blood groups , and the sex does not affect the distribution of ABO blood groups among DMT2 , This result agreement with Ali AA, (2015) in Khartoum state not found association between Diabetes mellitus type 2 and ABO/Rh blood group, and agree with the distribution of blood groups as O , A , B , And the sex did not affect the distribution of blood group , agreement of two study may be about same population enrolled in study .

the Rh positive is more prevalent than Rh negative , Nagpal and Hegde, (2015) in India , the result obtain revealed an association between Rh positive blood groups and DMT2 and negative association between blood group B and AB .this is not similar to our study because our study revealed there is no association between Rh positive blood groups and DMT2, but the study agree with the negative association between blood group B and AB . The disagree about association of Rh positive with DM2 may be about differences in gene expression of Indian population and Sudanese population

Another study by Meo SA *et al*,(2016) show blood group B has high incidence of type2 diabetes and blood group O has a minimum association with type2 diabetes ,and equal distribution of blood group A and AB , our study disagreement of this result because it showed blood group O has high prevalence of DMT2 then A1 ,and B . also Meo study show there is no association between Rh positive and Rh negative blood group with type2DM , our study agree with this result . most study do in different Sudanese ethnic group showed blood group O was high frequent rate than other blood group .

The possible explanation of variation between studies may be related to geographical factors have a role in genetic expression of ABO blood groups .

The prevalence of DM is higher in patients with Rh positive blood groups as compared to the Rh negative blood groups and diabetes is most prevalent in patients with O positive blood group , this result find out by Javid *et al* (2017) it's similar to our study .

Alanazi *et al* , (2018) in northern Saudi Arabia we could not find any evidence that particular ABO blood group was more susceptible to develop diabetes mellitus .this is on line with our study . while the study showed a highly significant relation between DM and Rh blood groups , our study find no association between DM and Rh blood groups .

5.2 Conclusion :

- The study shows no association of diabetes mellitus type 2 with ABO blood groups and subgroup of A and Rhesus D factor .
- The prevalence of ABO blood groups and subgroup of A and Rhesus D blood groups in diabetic type2 was $O > A1 > B$
- The distribution of ABO blood groups and subgroup of A and Rhesus D blood groups does not affect by gender
- The odd ratio of O blood group of case and control give risk factor to diabetes mellitus type2
- There was negative correlation between age and sex among case and control
- There was strong positive correlation between age of case and control group .

5.3 Recommendations :

This study recommended that :

1. Further studies by using large sample size and full investigation of ABO/Rh blood groups and diabetes mellitus
2. Molecular studies about gene expression of ABO/Rh blood groups of diabetes mellitus patients in different Sudanese ethnic groups .
3. Blood group O person need early checkup for DMT2 because at risk factor to DMT2 .

References

- Abdul Gader , M ; A .**(2000). The blood. in: Sukkar , M ; Y, El-Munshid , H ; A , and Ardawi , M ; S , ed , Concise human physiology , 2nd ed . U K : Black well, pp.15 – 16 .
- Abdulridha , K ; A .** (2018) . Evaluation of the relationship between ABO blood groups ,Rh factor and diabetes Mellitus type2 , *international journal of medical research and health sciences* ,**7** (11) , pp. 110-114.
- Aggarwal ,T ; Singh , D ; Sharma , B ; Siddiqui , S . and Agarwal , S.**(2018) . Association of ABO and Rh blood groups with type 2 diabetes mellitus in Muzaffarnagar city . *national journal of physiology , pharmacy and pharmacology* , **8** (2), pp . 167 – 170 .
- Alanazi , A ; A , Alkhidhr , S ; A , Alhadhari , Mohammed ; A , Al- Hathloul ,W ; A , Alsharif , J ; E , et al .** (2018) . Association of diabetes mellitus with ABO blood groups and Rh . *the Egyptian journal of hospital medicine* , **73** (4) , pp. 6535_ 6540 .
- Ali , A ; A .** (2015) . Association of diabetes mellitus type2 with ABO and Rhesus blood groups in Sudanese patients in Khartoum state , M.Sc. degree , Sudan university .
- American diabetes association .**(2018).managing type 2 diabetes, 1st ed ,Canada , John Wiley and sons , 12 – 13 .
- Daniels ,G .** (2013).Human blood groups , 3rd ed, Oxford , : John Wiley and sons , pp .1, 66 –68.
- Daniels , G , Bromilow , I.**(2014) . Essential guide to blood groups , 3rd ed, Oxford : John Wiley and sons , pp 1 , 3 , 23 .
- Daniels , G , Contreras , M , Allard , Sh .**(2016). Red cell immunohaematology . in : Hoffbrand , A , Higgs , D ; R , Keeling , D ; M , Mehta , A ; B , ed, post graduate haematology , 7th ed. Oxford : John Wiley and sons , p. 195 .
- Free man . V . S .** (2010) . Carbohydrates , in : Bishop , M ; L , Fody , E ; P , Schoeff , L ; E , ed, , clinical chemistry technique , principle , correlations , 6th ed. China : Lippincott Williams and Wilkins , pp. 313 – 315 .
- Hoffbrand , A ; V ,Moss, P; A .**(2016). Essential haematology, 7th ed, Oxford : John Wiley and sons, pp 336.
- Holt , T , Kumar , S .** (2010) . ABC of diabetes , 6th ed, Oxford : John Wiley and sons , pp . 1.
- Jaggi , S , Yadav , A ; S .** (2014) . Distribution of ABO and Rh (D) allele frequency among the type2 diabetes mellitus patients , *American international journal of research in formal , applied and natural sciences* , **5** (1) ,pp. 24 – 26 .

- Javed , M , Akhtar , N ; M , Muzaffer , S .** (2017) , frequency of ABO and Rh blood groups in patients with diabetes mellitus , *Pakistan journal of medical and health sciences* , **11** (1) , pp. 114_ 116 .
- Knowles , S ; M .** (2001) . Laboratory aspects of blood transfusion . in : Lewis , S ; M , Bain , B ; J , Bates , I , Dacie and Lewis practical hematology , 9th ed . London : Churchill Livingstone ,pp. 473 .
- Kumar , S ; S .** (2016) . physiology of diabetes mellitus . in : Thomas , N , Kapoor , N , Velavan , J , Vasani , S ,ed , A practical guide to diabetes mellitus , 7th ed . New Delhi : Jaypee brothers ,pp . 11 - 12 .
- Marshall , W ; J , Bangert , S ; K , Lapsley , M .** (2012) . Clinical chemistry , 7th, China : Elsevier , pp. 275 – 276 .
- Mehdi . S . R .** (2013) . essential of blood banking , 2nd ed, New Delhi , Jaypee brothers , 19– 21.
- Meo , S ; A , Rouq , F ; A , Suraya , F , Zaidi , S ; Z .** (2016), Association of ABO and Rh blood groups with type 2 diabetes mellitus , *European review for medical and pharmacological sciences* , **20** (2) , pp . 237_ 242 .
- Nagpal , B , and Hegde , U .** (2015) . The distribution of the ABO and Rh (D) blood type in patient with typeII DM , *international journal of advance research* , **3** (11) , pp . 1561_ 1565.
- Nayak , R , Rai , S , Gupta , A .** (2012) . Essential in hematology and clinical pathology , 1st ed , New Delhi : Jaypee brothers , pp . 500 – 502 .
- Quinley , E ; D .** (2011) . Immunohematology principles and practice , 3rd ed, China : Lippincott Williams and Wilkins , pp . 119 – 120 , 140 – 141 .
- Ramakrishnan , S , Sulochana , K ; N .** (2012) . Manual of medical laboratory techniques , 1st ed, New Delhi : Jaypee brothers , pp . 202 – 203 .
- Rees , A , Levy , M , Lansdown , A .** (2017) . Clinical endocrinology and diabetes at a glance , 1st ed, Oxford : John Wiley and sons , pp . 125 – 129 .
- Sacks , D ; A .** (2018) . Screening for gestational diabetes . in : McCance , D ; R , Maresh , M , Sacks , D ; A , ed , A practical manual of diabetes in pregnancy , 2nd ed . Oxford : John Wiley and sons , pp . 49 – 50 .
- Sharma ; S , Kumar ; J , Choudhary ; R , Soni; N,D .**(2014) , association between ABO blood groups and DM , *Scholar Journal of applied medical science* , **2** (1A) ,pp 34 – 37.
- Sheryl , A ; W .** (2010) . Immunohematology for medical laboratory technicians , 1st ed , USA : Cengage , pp . 89 , 115 .

- Sood , R .** (2010) . Hematology for students and practitioners , 6th ed, New Delhi : Jaypee brothers , pp . 196 , 209 , 628 .
- Sood , R .** (2015) . Concise book of medical laboratory technology methods and interpretations , 2nd ed , New Delhi : Jaypee , pp . 434 .
- Thomas , N , Vasani , S .** (2016) . Diagnosis of diabetes mellitus . in : Thomas , N , Kapoor , N , Velavan , J , Vasani , S , ed , A practical guide to diabetes mellitus , 7th ed .New Delhi : Jaypee brothers , pp . 29 .
- Westhoff ,C ; M , Shaz , B ; H .** (2013) . ABO and H blood group system . in : Shaz , B ; H , Hillyer , C ; D , Roshal , M , Abrams , C ; S ed . Transfusion medicine and hemostasis , 2nd ed . London : Elsevier , pp . 152 .
- Whitlock , S ; A .** (2010) . Immunohematology for medical laboratory technicians , 1st ed , USA : Delmar Cengage , pp . 117 – 118 .
- Wiler , M .** (2005) . The Rh blood group system . in : Harmening , D ; M , ed . Modern blood banking and transfusion practices , 5th ed . Philadelphia : F , A , Davis company , pp . 135 .

Appendices

Appendix 1:

Inform consent

(الموافقة المستنيرة)

الاسم : فاطمة ادم محمد علي

طالبة ماجستير في امراض الدم ومبحث المناعة الدموية بجامعة السودان للعلوم والتكنولوجيا
عنوان البحث : ارتباط النوع الثاني من مرض السكري مع فصائل الدم وفروع الدم أ والعامل الريصي في ولاية
الخرطوم

عزيزي المشارك الهدف من الدراسة هو معرفة مدى تأثير فصائل الدم في حدوث مرض السكري النوع الثاني .
وللوصول لذلك سأقوم بأخذ عينات دم وريدي منك ومن عدد 49 من المرضى المصابين بالسكري النوع الثاني .
وسيكون اخذ العينات بأمان تام ولا توجد أي مخاطر عليك . بعد ذلك ساجري عليها التحاليل المخبرية لمعرفة فصيلة
دمك .

سأقوم بملأ استبيان منك لمعرفة العمر والجنس وكل ذلك سيكون في سرية تامه , وسيتم استخدام المعلومات فقط من
اجل الدراسة .

وستكون مشاركتك في البحث طوعية ومن حقك الانسحاب من البحث في أي وقت .

انا المشارك بعد اطلاعي علي فكرة البحث واهدافه اوافق علي المشاركة وان يتم اخذ عينة
دم مني .

التوقيع

شكرا لتعاونكم

توقيع الباحث

Appendix 2:

general equipment and reagents :

% 70 alcohol

Cotton

Gloves

Syringe

Tourniquet

EDTA vacutainer

Glass slides

Wooden applicator

Pasteur Pipette

Centrifuge

microscope

glass tubes

Normal saline.

water bath 37°C

5% RBCs suspension.

Anti- A antibodies

Anti B- antibodies

Anti-A1 lectin

Anti D (Monoclonal IgM + IgG)

Antihuman globulin .

Appendix 3 :

**Sudan university of science and technology
College of graduate studies
Questionnaire**

Number :

Name :

Age :

Sex :

Blood group :

Rhesus :

Date :sig :