

بسم الله الرحمن الرحيم

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**Association of Diabetes Mellitus type2with ABO and
Rhesus Blood Groups in Sudanese Patients in
Khartoum State**

**ارتباط النوع الثاني من مرض السكري مع فصائل الدم والعامل الرصى في
ولاية الخرطوم**

**Adissertation Submitted for partial fulfilment of
M.Sc.degree in Medical Laboratory
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الآيه

قوله تعالى: {يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا
الْعِلْمَ دَرَجَاتٍ}

[المجادلة: 11]

Dedication

To

The greatest love.....my father

The love of lifemy mother

Those who always on my sidebrothers and sister

Candles that lighten my way.....teachers

My supporters through my ways.....friends

**The strivers in this life.....Haematology and
Immunohaematology pioneers**

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thanks for all

Abstract

This is a case control study .The objective of the study was to determine the association between ABO/Rhesus (Rh) blood group and diabetes mellitus type 2 in Sudanese patients. The study was conducted in Khartoum Teaching Hospital during the period January to July 2015Hundred diabetic subjects of both sexes and with different ages were enrolled randomly in this study and another hundred healthy non diabetic subjects of both sexes and different ages were taken randomly as a control group.

Venous blood samples were taken from all the participants and ABO/ RH blood groups was determined by direct and indirect tube methods. The datawere analyzed by one sample Chi square test using SPSS version 16 . The results showed that there was no significant difference in the frequency of ABO blood group between type 2 diabetic subjects and the control group (P.value = 0.85).The frequency and order of the ABO blood group distribution in both the diabetics and the control group was O (42%vs 44%) , A (33% vs 33%) , B (21% vs 18%) and AB (4% vs 5%).No significant variation (P.value = 0.37) was found in the frequency of Rh positivetype between the diabetic subjects (94%) and the control group (96%).The gender did not affect the order of distribution ABO blood group or the Rh type in the diabetic subjects.

It is concluded that there is no association between ABO/Rh blood group and DM type two or the gender.

المستخلص

هذه دراسة حالة انضباطية، الهدف من الدراسة تحديد وجود علاقة بين فصيلة الدم والعامل الريصي في المرضى السوانيين المصابين بمرض السكري النوع الثاني، أجريت هذه الدراسة في مستشفى الخرطوم التعليمي في الفترة من شهر يناير الي شهر يوليو سنة 2015 م. شملت هذه الدراسة مائة عينة من المرضى المصابين بالسكري النوع الثاني حسب الجنس واختلاف العمر جمعت بطريقة عشوائية ، واستخدمت مائة عينة كمحكم من الاصحاء مع اعتبار نفس الجنس واختلاف العمر. اخذت عينات دم وريدية وفحصت فصيلة الدم والعامل الريصي بالطريقة المباشرة وغير المباشرة. واطهرت النتائج التي حلت باستخدام البرنامج الاحصائي انه لا يوجد اختلاف ذو دلالة معنوية قي فصائل الدم والعامل الريصي عند المرضى المصابين بالسكري النوع الثاني والاصحاء، وكان التردد وترتيب التوزيع لفصائل الدم بين مرضي السكري النوع الثاني والاصحاء كالآتي:

O (42% مقابل 44%) , A (33% مقابل 33%) , B (21% مقابل 18%) and AB (4%)

اظهرت الدراسة انه لا يوجد اختلاف ذو دلالة معنوية في توزيع العامل الريصي بين (5% مقابل مرضي السكري النوع الثاني (94%) والاصحاء (96%). أظهرت الدراسة انه ليس هناك تأثير للجنس علي علاقة فصائل الدم والعامل الريصي مع مرضي السكري النوع الثاني.

خلصت الدراسة الي انه لا توجد علاقة ذات دلالة معنوية بين فصائل الدم والعامل الريصي مع مرضي السكري النوع الثاني وايضا لا يتاثر باختلاف الجنس .

List of contents

الاية	I
Dedication	II
Acknowledgment	III
Abstract	IV
المستخلص	V
List of contents	VI
List of tables	IX
List of abbreviations	X

Chapter one

Introduction and literature review

1.	Introduction	1
1.1.	Blood	2
1.1.1.	Blood group system	2
1.1.2	History of ABO discoveries	6
1.1.3	The importance of ABO blood group system	7
1.1.4	Classification of ABO blood group system	9
1.1.5	Antigens of ABO blood group system	11

1.1.6	Antibodies of ABO blood group system	12
1.1.7	Sub group of A	12
1.1.8	ABH secretor status	13
1.2.	The Rhesus system	13
1.2.1	Discovery of RH system	16
1.2.2	The nomenclature of RH system	16
1.2.3	The fisher –Race nomenclature	16
1.2.4	The Winner nomenclature	17
1.2.5	Rh blood group system antigens and antibodies	17
1.2.6	Other Rh blood group system antigens	18
1.2..7	Rh phenotype	18
1.3	Diabetes mellitus	19
1.3.1	Classification of Diabetes mellitus	19
1.3.1.1	Type 1 Diabetes	22
1.3.1.2	Type 2 Diabetes	23
1.3.2	Other specific types of Diabetes mellitus	23
1.3.3	Gestational Diabetes mellitus	23
1.3.4	Insulin	24

1.3.6	Diagnosis of Diabetes mellitus	25
1.4	Association of the ABO group with disease	25
1.5	Objective	28
1.5.1	General objective	28
1,5.2	Specific objective	28

Chapter Two

Materials and Methods

2.1	Study design	29
2.2	Study population	29
2.3	Inclusion criteria	29
2.4	Exclusion criteria	29
2.5	Data collection	29
2.6	Materials	29
2.6	Method s	30
2.6.1	Samples collection	30
2.6.2	ABO slide agglutination	30
2.6.2.1	Principle	30
2.6.2.2	Procedure	31

2.6.2.3	Interpretation	31
2.6.2,3	Control	31
2.6.3	RH (D) red blood cell type	32
2.6.3.1.	Principle	32
2.6.4	Du method(The indirect antiglobulin)	32
2.6.4.1	Principle	32
2.6.4.2	The technique of Du method	32
2.6.4.3	Requirements	33
2.6.4.4	Interpretation	33
2.9	Statistical analysis	34
2.10	Ethical consideration	34

Chapter Three

Results

3	Results	35
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Chapter Four

Discussion, Conclusion and Recommendation.

4.1	Discussion	41
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4.2	Conclusion	43
4.3	Recomondation	43
4.4	Refrences	44
	Appendics	

List of Table

Table No	Table	Page No
1.1	Blood group system recognized by ISBT	4
1.2	The ABO blood group system	8
1.3	The ABO group system	10
1.4	The most common Genotype	15
1.5	Diabetes mellitus classification	21
3.1	One sample Chi square table for association of blood group frequency with DM	37
3.2	One sample Chi square table for association of RH factor frequency with DM type 2	38
3.3.	Distribution of RH blood groups in diabetic type 2 as compared to controls	39
3.4	The Distribution ABO and RH in diabetic type 2 according Gender	40

List of Abbreviation:

AB	Antibody
Ag	Antigen
CAD	Coranary Artery Disease
DM	Diabetes mellitus
ID	Immunodifusion
IGG	Immunoglobulin G
IGM	Immunoglobulin M
ISBT	International Society of Blood transfusion
GDM	Gestational diabetes mellitus
K₂EDTA	Potassium Ethylene Diamine tetra Acetic Acid
LISS	Low Ionic Strength Saline
RBC	Red cell corpuscular
RH	Rhesus
SPSS	Statistical Package of Social Science
UK	United Kingdom
USA	United State of America

Chapter one

Introduction and Literature Review

1.1.INTRODUCTION:

Since the discovery of the ABO system in 1900, a multitude of blood group antigens have been identified and many different styles of terminology have been used. The International Society of blood transfusion (ISBT) recognizes 285 blood group antigens; 245 of these are classified into one of 29 blood group system. Forty years later, both Landsteiner and Wiener discovered Rh(D)antigen.(Garratty*et al*, 2000)

The genes of ABO and Rh(D) are located on chromosome 9 and 1 respectively. The bombardment of the red blood cells with A and /or B antigens occur as a consequence of the action of the glycosyltransferases enzymes that add specific sugars to the precursor substance. (John ,1996).

Diabetes Mellitus (DM) describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion or insulin action or both (WHO,1999) .

Many investigators have tried to identify a possible association between ABO & Rh blood groups and diabetes mellitus. The results have been variable, inconsistent and differed from one region to other. Some people have identified an association between blood groups and diabetes but there are studies where no association could be established.

1.2.Literature review :

1.2.1.Blood

Blood is a vital fluid of our body and as such the life line of human body. It is a red colored viscid fluid slightly salty in taste. Blood is alkaline in reaction PH 7.4 and specific gravity ranges from 1.052 to 1.060. In adult human, blood volume ranges between 4.5 to 6.0 liters and is approximately about one thirteenth of adult human body weight. Temperature of circulating blood is 37.7 C. Blood has two main components cells and plasma. Cells consist of 40 to 45% of the total amount of blood and plasma consists of 55 to 60% of total amount of blood. Cells are the formed elements and are of three types red cells (erythrocyte), white cells (leucocytes) and platelet (thrombocyte) and each has its own characteristic.(Talib, 1995)

1.2.2. Blood group system

Blood type(or blood group)is determined, in part, by the ABO blood group antigens present on red blood cell.

A blood type (also called ablood group) is a classification based on the presence or absence of inherited antigenic substances on the surface of red blood cell(RBCs).These antigens may be protein, carbohydrates, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell Surface antigens can stem from one allele (or very closely linked genes)and collectively from a blood group system(Manton ,1993)

The clinical importance of a blood group system in blood transfusion lies in the frequency of its antibodies and in the possibility that such antibodies will destroy incompatible cells *in vivo*. A B O system was the first to be recognized and remains the most important in transfusion and transplantation (histo - blood group system). The reason for this is that almost everybody over the age of about 6 months has clinically significant anti A and anti B in his or her serum if they lack the corresponding antigens on their red cells hence transfusions given without regard to ABO groups would result in incompatibility patient will have *in vivo* adverse hemolytic reactions. (Hoffbrand *et al.*, 2000)

ISBT recognized blood group system as shown in table(1.1)

Table (1.1) Blood Group System Recognized by ISBT (Hoff Brand *et al.*, 2000)

System number	System name conventional	System symbol ISBT	Chromosomal location	Gene(s)
001	ABO	ABO	gq34.1 – q34.2	ABO
002	MNS	MNS	4q28-q31	GYPA GYPB
003	P	P1	22q11.2-qter	P
004	Rh	RH	1p36.2-p34	PHD RHCE
005	Lutheran	Lu	19q12-q13	LU
006	Kell	KEL	7q33	KEL
007	Lewis	LE	19p13.3	FUT3
008	Duffy	Fy	1q22-q23	FY
009	Kidd	JK	18q11-q12	HUT11
010	Diego	DI	17q12-q21	SLC4A1
011	Yt	YT	7q22	ACHE
012	Xg	XG	Xp22.32	XG
013	Scianna	SC	1p36.2-p22.1	SC
014	Dombrock	DO	12p13.2-p12.1	GO
015	Colton	CO	7p14	AQP1
016	LW	LW	19p13.2-cen	LW
017	Chid/Rogers	CH/RG	6p21.3	C4A,C4P
018	H	H	19p13	FUT1

019	Kx	XK	Xp21.1	XK
020	Gerbich	GE	2q14-q21	GYPC
021	Cromer	CROM	1q32	GAF
022	Knops	KN	1q32	CR1
023	Indian	IN	11p13	CD44
024	Ok	OK	19pter –p13-2	OK
025	MER2	RAPH	11p15	MER2

International Society of Blood Transfusion (ISBT)

1.2.2.1. History of ABO discoveries :

Blood group and inherent differences in human blood from one individual to another were first discovered by a German scientist, Karl Landsteiner, in 1900, who took samples from his colleagues, separated the serum, and prepared saline suspension of the red cells.

When each serum sample was mixed with each red cell suspension, he noticed that agglutination of red cells had occurred in some mixtures and not in others (Neville, 1994).

Classification of blood groups was based on the realization that agglutination had occurred because the red cells possessed an antigen and the corresponding specific antibody was present in the serum, when no agglutination, either the antigen or the antibody was missing from the mixture. Landsteiner isolated and recognized separate antigens, now known as A and B. The antibody that reacted with the B antigen was known as anti B (Neville, 1994).

From these observations, Landsteiner recognized three separate groups, named according to the antigen present on the red cells.

Individuals who possessed the A antigen and B antigen were classified as belonging to group O. The symbol denoting zero or the lack of A and B antigen on the red cells. A fourth was discovered by Landsteiner's pupils von Decastlo and Sturli in 1900. The red cells of individuals in this group showed agglutination with both anti A and anti B, and the group was called AB. (Neville, 1994).

It was further observed that individuals who have the A antigen on their red cells also possess anti B in their serum, individuals who possessed the B antigen

had anti A in their serum, individuals who possessed neither A nor B antigens had anti A and anti B in their serum, individuals with both A and B antigens had neither anti A nor anti B in their serum .(Nevile *etal*,1994).

1.2.2.2.The importance of ABO blood group system:

Blood groups from a comparatively small field of study but have an important place in genetics, immunology , and anthropology and in clinical medicine .(Kathleen *etal*,1988)

the clinical importance of blood group antigens depends on the frequency of occurrence of the corresponding antibody and its ability to hemolyse red cells, on these criteria the ABO and RH systems are capable of causing severe intravascular hemolysis after an incompatible transfusion, the RH D antigen is the most immunogenic red cell antigen after A and B being capable of stimulating anti D production after transfusion or pregnancy in the majority of RH D negative individuals.(Dacie *etal*,1945).

The clinical significance of different red cell antibodies depends partly on their destructive capacity importance in blood transfusion practice owing to its great variety. Conversely, ABO and D antibodies are by far the most significant, due to their high frequency and destructive capacity.(Hoff Brand *et al.*, 2000).

Table (1-2) The ABO Blood Group System. (Hoff brand *et al.*, 2006)

Phenotype	Genotype	Antigens	Naturally occurring anti bodies	Frequency (UR) (%)
O	OO	O	Anti-A, Anti-B	46
A	AA or AO	A	Anti-B	42
B	BB or BO	B	Anti-A	9
AB	AB	AB	None	3

1.2.2.3. Classification of ABO blood group system:

Classification of blood groups was based on the realization that agglutination had occurred because the red cells possessed an antigen and corresponding specific antibody was present in the serum, When no agglutination had occurred, either the antigen or the antibody was missing from the mixture ,from these observation Landsteiner recognized four separate groups, named according to the antigen present on the red cells. Individuals who possessed the A antigen were classified as belonging to group A, individuals who possessed the B antigen were classified as belonging to group B, Red cells from certain individuals which showed no agglutination with either anti A or anti B and were classified as belonging to group O (the symbol O denoting zero or lack of A and B antigens on red cells),and the red cells of individuals which show agglutination with both anti- A and anti-B the blood group was called AB. (Race and Sunger,1975)

Table (1-3) The ABO Group System (Dacie and Lewis, 2001):

Blood group	Subgroup	Antigens on red cells	Antibodies in plasma
A	A ₁	A+A ₁	Anti-B (Anti-A ₁)
	A ₂	A	
B	-	B	Anti-A, Anti-A ₁
AB	A ₁ B	A+A ₁ +B	None Anti-A ₁
	A ₂ B	A+B	
O	-	(H) ⁺	Anti-A Anti-A ₁ Anti-B Anti-AB

1.2.2.4. Antigens of ABO blood group system :

They are mainly A, B and H antigens which are proteins in nature and various proteins are embedded in a mosaic pattern without any fixed position on fluid lipid layer of cell membrane. A, B and H antigen sites are greatest on band 3 of sialoglycoprotein and they are also found on polyglycosilceramides and the number of A, B simple glycolipid. The number of A, B and H antigens sites varies in newborn and adult. These antigenic sites are important because the antibody molecule gets attached to red cells at this site. The ABH antigens are widely distributed they are even found in animals plant and bacteria , in human body apart from the red cells it is also found in saliva , fluid of pseudomucinous ovarian cyst of secretors , meconium of secretor body, it was even discovered in Egyptian mummies , cornea and in the tissue epidermal and epithelial cells, in spermatozoa amongst blood component A,B and H were observed on normoblast, the A, B antigens occur on platelets , white cells and serum (Talib, 1995).

The O gene is a morph and does not transform the H-substance. Although there are six possible genotypes, the absence of a specific anti-O prevents the serological recognition of more than four phenotypes. The two major sub groups of A (A_1 and A_2). A_2 cells react more weakly than A_1 cells with anti-A and patients who are A_2B can be wrongly grouped as B. Naturally occurring anti bodies to A and/ or B antigens are found in the plasma of subjects whose red cells lack the corresponding antigens. (Hoff Brand *et al*, 2000)

1.2.2.5. Antibodies of ABO blood group system:

Naturally occurring antibodies occur in the plasma of subjects who lack the corresponding antigen and who have not been transfused or been pregnant. The most important are anti-A and anti B- they are usually immunoglobulin M (IgM), and react optimally at cold temperatures (4°C) so, although reactive at 37°C, are called cold antibodies. Immune antibodies develop in response to the introduction – by transfusion or by transplacental passage during pregnancy- of red cells that possess antigens that the subject lacks. These antibodies are commonly IgG, although some IgM , antibodies may also develop- usually in the early phase of an immune response. Immune antibodies react optimally at 37°C (warm antibodies). Only IgG antibodies are capable of transplacental passage from mother to fetus. The most important immune antibody is Rh antibody, anti-D. (Hoff brand *et al.*, 2000).

1.2.2.6. Sub groups of A:

In addition to the common phenotypes A₁ and A₂ numerous phenotypes with weak expression of A on the red cells have been found and multitude of names has been adopted. Most of these phenotypes can be fitted into the following categories: A₃, A_x, A_m, A_y and A_e. The serological characteristics of these phenotypes results from inheritance of a rare allele at the ABO locus, which can be detected when parried with B, but not with A₁ or A₂ .

1.2.2.7. ABH secretor status:

About 80% of the UK populations are ABH secretors as they have H antigen plus A or B according to their ABO genotype, in a water – soluble form in their body secretions. The remaining 20% are non – secretors and have no secreted ABH antigens, regardless of ABO phenotype (Hoff Brand *et al*, 2000).

1.3.The Rhesus System:

The Rhesus system is the second most clinically important and complex blood group system. It consists of 50 different antigens, but only 5 antigens D, C, c, E and e are inherited in various combinations and account for most of the Rh-related problems encountered in practice. The Rh antigen with the strongest antigenicity is the Rh (D) antigen. As simple rule, it can be noted that persons whose red cells express the D antigen are Rh (D) positive and individuals whose red cells lack the D antigen are Rh (D) negative. The different genotypes, their Rh status, and the frequency of these genotypes in Caucasians. About 85% of North American Caucasians are Rh (D) positive. After the discovery of the Rh system in 1940, various theories were postulated to explain the mode of inheritance and different nomenclatures were proposed. The Winner system proposed that the gene product was a single entity with multiple serological specificities (Denisand Hamening, 1998).

Hassan ,(2010), found in the majorSudanese ethnic groups ,that majority were (98%) were RH(D) positive and only 2% were RH negative .,

Bello,(2012), found gene frequency and RH(D) blood group alleles, in Lagos ,South –West Niegeria ,to be 97% of the sampled population were RH(D) positive, while 3% were RH(D) negative.

Table (1-4): The most common Rh genotypes (Hoff brand *et al.*, 2000)

CDE Nomenclature	Short symbol	Frequency %	RhD status
Cde/cde	Rr	15	Negative
CDe/cde	R ₁ r	31	Positive
CDe/CDe	R ₁ R ₁	16	Positive
cDE/cde	R ₂ r	13	Positive
CDe/Cde	R ₁ R ₂	13	Positive
cDE/Cde	R ₂ R ₂	3	Positive
Other genotypes		9	Positive(all most)

1.2.3.1. Discovery of Rh system

The Rhesus system is named after the Rhesus Macaque, following an experiment by Karl Landsteiner, Alexander S. Wiener in 1940, which showed that rabbits, when immunized with rhesus monkey red cells, produce an antibody that also agglutinates this factor. The significance of the Rh factor was soon realized by Dr. Philip Levine who made a connection between the Rh factor and the incidence of erythroblastosis, and Wiener realized adverse reactions from the Rh factor. Wiener then pioneered the exchange transfusion technique which saved the lives of many thousands of infants before intrauterine transfusion was invented which enabled much more severely affected fetuses to be successfully treated. (Denise Hamening, 1998).

1.2.3.2. The nomenclature of Rh blood group system

Several nomenclatures can be used to describe Rh genes and antigens. Fisher-Race nomenclature, which uses CDE terminology, is more commonly used for Antigens; Wiener nomenclature, which uses Rh designations, is favored for haplotypes and gene complexes. An individual who inherits (c and e gene) from one parent and (D and c and e genes) from the other parent expresses DC, c and e antigens on his or her erythrocyte. (Hoffbrand *et al.*, 2000).

1.2.3.2.1. The Fisher-Race nomenclature :

Fisher-Race theory states that there are three closely linked loci, each with a primary set of allelic genes D and d, C, c, E and e. These three loci are so closely linked that crossing over occurs only very rarely, and the three Rh genes are inherited as a complex. The Rh genes complex was assumed to possess closely linked genes, which could be assembled in eight different

ways: CDE, CDe, cDE, Cde, Cde, cDe, cdE, cde. International nomenclature, the Rh antigens are therefore named: C, D, E, c, d, e. the antigen (d) and its corresponding antibody has never been discovered and is thought not to exist. The symbol (d) is used to denote the absence of D antigen. All individuals who lack the D- antigen are Rh negative. Regardless of whether the C or E or both are present, the most frequent genotype among D- negative individuals is cde. The theory of Fisher- Race was confirmed when the two unknown reactions CDE were shown to be as predicted by Murray and Cowders in 1945 and when anti e was discovered by Mourant in the same year. The only weakness in the theory therefore was failure to find the expected antigen- d. Other antigens since found to be a part of Rh system have been classified using the same basic principle. (Nevillen., 1994).

1.2.3.2.2. The Weiner nomenclature:

Weiner visualized multiple allele determining his own particular antigen. The antigen comprises multiple factors depending on which genes are present and are recognized by which ever factors are detectable. The two genes (i.e. one paternal and one maternal), have been alike (homozygous) or different (heterozygous). Therefore, multiple allele are called R₁, R₂, R_O, r, r̂, r̂̂, RZ, RY. Rh- antigens are called Rho, h₁, RĤ, rĥ. In simple terms, for example the Rh₁ gene produces a complex antigen on the red cell that made up of at least three factors: Rĥ, Rho, rĥ. (Nevillen., 1994).

:1.2.3.3. Rh blood group system antigens and antibodies:

Rh antigen is a protein surrounded by lipid, Rh activity is not lost when lipid is extracted from red cells membranes (the lipid doesn't carry the antigenic

determinant but may be essential for confirmation of the determinants). (Nevillen., 1994).

In the Rhesus blood group system, naturally occurring Rhesus antibodies are not found in the serum of individuals lacking the corresponding Rhesus antigens. Rhesus antibodies are formed by immunization. The most important Rhesus antibodies is Anti- D, which can be formed when a Rh negative individual is transfused with Rh positive blood or when a Rh negative woman becomes pregnant with a Rh positive infant and the red cells of the baby pass into her circulation particularly at the time of delivery, stimulating the production of anti- D antibody. Such circulating anti- D will not become immediately harmful unless the individual receives a transfusion of Rh positive blood, in such a situation the donor's D antigen red cells will be hemolyzed by the anti- D. (Nevillen., 1994).

1.2.3.4. Other Rh blood group system antigens

Currently, 50 antigens have been described in the Rh group system, D, C, c, E and e antigens are the most important ones. The other antigens are much less frequently encountered or are rarely clinically significant. Each is given a number. (Mark, 2005).

1.2.3.5 . Rh Phenotypes:

The completeness with which the Rh phenotype can be determined depends on the anti sera available; if anti- c is available but not anti- C, samples can be classified as c positive (that is. cc or Cc) and c negative (that is. CC). If anti- C is also available, Cc can be distinguished from cc. If a sample is tested with anti- D, anti- C, anti-c and anti- E and gives positive reactions

with all for anti sera: the phenotype is written DCcE. Red cells that fail to react with anti-D are described as dd. Mountran's notation is occasionally misleading for example, although a negative reaction with anti-E usually implies that the cells are ee.(Mollison, 1997) .

1.3.Diabetes mellitus:

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, produce hyperglycemia and relative insulin deficiency, resistance or both. It affects more than 120 million by the year 2020, Diabetes is usually irreversible and although patients have a reasonably normal life style, its late complication result in reduced life expectancy and major health costs.(David *et al*,2005).

Diabetes is widely recognized as one of the leading causes of death. The rapid increase in diabetes parallels the increase in obesity and overweight. Recent information indicates that 5.5% in Northern of Sudan and 8.6% in Khartoum state have diabetes and the number is expected to rise.(Elbagiret *al*,2006).

1.3.1.Classification of Diabetes Mellitus:

In 1979, the national diabetes data group developed a classification and diagnosis scheme for diabetes mellitus. This scheme divides diabetes into two broad categories: type 1, insulin –dependent diabetes mellitus (IDDM); and type 2, non insulin dependent diabetes mellitus (NIDDM).

Therefore, the WHO guidelines recommend the following categories of

Diabetes:

.Type 1diabetes

.Type 2diabetes

.Other specific types of diabetes

.Gestational diabetes mellitus(GDM).(Bishop,2010)

Table(1.5):Diabetes mellitus classification(Bishop,2010)

Diabetes mellitus classification	Pathogenesis
Type 1	<p>Beta –cell destruction</p> <p>Absolute insulin deficiency</p> <p>Auto antibodies</p> <p>.islet cell auto antibodies</p> <p>.insulin acid decarboxylase auto antibodies</p> <p>.tyrosine phosphate auto antibodies</p>
Type 2	<p>Insulin resistance with an insulin secretary defect</p> <p>Relative insulin deficiency</p>
other	<p>Associated with secondary conditios</p> <p>.genetic defect of beta-cell function</p> <p>.pancreatic disease</p> <p>.endocrine disease</p> <p>.drug or chemical induces</p> <p>.insulin receptor abnormalities</p> <p>.other genetic syndromes</p>
Gestational	<p>Glucose intolerance during pregnancy due metabolic and hormonal changes</p>

1.3.1.1.Type 1 diabetes:

Type 1 diabetes is characterized by inappropriate hyperglycemia primarily a result of pancreatic islet beta cell destruction and a tendency to ketoacidosis. Type 1 diabetes mellitus is a result of cellular mediated autoimmune destruction of the beta cells of the pancreas, causing an absolute deficiency of insulin secretion. An upper limit of 110mg/dl on the fasting plasma glucose is designated as the upper limit of normal blood glucose. Type 1 constitutes only 10% to 20% of cases of diabetes and commonly occurs in childhood and adolescence. This disease is in individuals with a genetic predisposition and causes the immune destruction of the beta cells of the pancreas and, therefore, a decreased production of insulin. Characteristics of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency. This diabetic type is genetically related. One or more of the following markers are found in 85% to 90% of individuals with fasting hyperglycemia: islet cell auto antibodies, insulin auto antibodies, glutamic acid decarboxylase auto antibodies, and tyrosine phosphatase IA-2 and IA-2B auto antibodies. (Bishop, 2010).

Signs and symptoms include: polydipsia (excessive thirst), polyphagia (increased food intake), polyuria (excessive urine production), rapid weight loss, hyperventilation, mental confusion, and possible loss of consciousness (due to increased glucose to brain).

Complications include microvascular problems, nephropathy, and retinopathy. An increased heart disease is also found in patients with diabetes. (Bishop, 2010).

1.3.1.2.Type 2 diabetes:

Type 2 diabetes mellitus is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretory defect. This resistance results in relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region. This type of diabetes often goes undiagnosed for many years and is associated with a strong obesity, and lack of physical exercise. Characteristics usually include adult onset of the disease and milder symptoms than in type 1, with ketoacidosis seldom occurring. However, these patients are more likely to go into hyperosmolar coma and are at an increased risk of developing macrovascular and microvascular complications. (Bishop, 2010).

1.3.2.Other specific types of diabetes:

Other specific types of diabetes are associated with certain conditions (secondary), including genetic defect of beta-cell function or insulin action, pancreatic disease, diseases of endocrine origin, drug or chemical induced insulin receptor abnormalities, and certain genetic syndromes. The characteristic and prognosis of this form of diabetes depend on primary disorder. (Bishop, 2010).

1.3.3. Gestational diabetes mellitus (GDM):

GDM is any degree of glucose intolerance with onset or recognition during pregnancy. Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. However, this

disease is associated with increased perinatal complications and an increased risk for development of diabetes in later years. Infants born to mothers with diabetes are at increased risk for respiratory distress syndrome, hypocalcemia, and hyperbilirubinemia. Fetal insulin secretion is stimulated in the neonate of mother with diabetes. However, when the infant is born and the umbilical cord is severed, the infant's over supply of glucose is abruptly terminated, causing severe hypoglycemia. (Bishop, 2010).

1.3.4. Insulin:

Insulin is the primary hormone responsible for entry of glucose into the cell. It is synthesized by the beta cells of Islets of Langerhans in the pancreas. When these cells defect an increase in body glucose, the release of insulin causes an increased movement of glucose into the cells and increased glucose metabolism. Insulin when glucose levels are decreased. It decreases plasma glucose levels by increasing the transport entry of glucose in muscle and adipose 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 hypoglycemic agent. (Bishop, 2010)

1.3.5. Diagnosis of diabetes mellitus:

Diabetes mellitus is characterized by current or persistent hyperglycemia, and diagnosed by demonstrating any one of the following: (WHO, 2009).

- Fasting plasma level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance

- Symptoms of hyperglycemia and casual plasma glucose > 11.1 mmol/l (200mg/dl)
- Glycated hemoglobin (HbA1C) $> 6.5\%$

Two hours after a 75g oral load as in a glucose tolerance test.(WHO,1999)

1.4.Association of the ABO group with disease:

1.4.1.Breast cancer:

Association of ABO blood group and breast cancer ,the evidence for association of blood groups with breast cancer is controversial ,some blood groups showed positive association and others were negative.It appears that different blood groups are associated with breast cancer;blood group A apparently increases the risk for cancer . High frequency of breast cancer was found in blood group A followed by O and B.(Shikha *et al*,2015)

1.4.2.Coronary heart disease:

Many reports have appeared in recent years suggesting association between blood group with increased coronary heart disease (CHD),clinical studies in developed countries have shown that individuals of the A blood group phenotype are more susceptible to coronary heart disease(CHD),blood phenotype A is associated with substantially increased risk(CHD),(Hafeezullah *et al*,2005)

1.4.3.Diabetes mellitus :

A study investigating the association between ABO blood groups and diabetes mellitus done by Soni 2014, in Jodhpur, India. They did not find any significant difference between the frequency of the blood groups of control and diabetic patients type 1 or 2, frequency of ABO blood group. They also found that blood group B is the most common, AB and A were less seen in diabetics, They found a negative association with blood group O as well, The frequency of ABO blood groups was in the order of $B > O > A > AB$, in diabetic patients of both type 1 and 2.

Koley, 2008. in Punjab, India, did not find any association between the DM and blood groups ($P\text{-value} > 0.05$), The blood group B is more common (38.55%) in patients than the control (37.47%), blood group A was more often in the patients (27.98%) than the control (26.74%), but in the control group blood group O more numerous (26.32%) than patients (24.46%).

The relationship between ABO, RH with diabetes mellitus and obesity was investigated in Namakkal town by Ganesan and Gani (2014).

They found a significant difference between the patients and the control group in blood group B the frequency.

In Maghnia, Sahi, (2008) did not find any association between blood group and type 2 diabetes mellitus, It was also noted the blood group O was distributed with highest frequency among diabetic subject (52.85%), d allele frequency presence was higher in diabetics than in non diabetics (0.3778 vs 0.364 respectively) The allele frequency was exhibited in the order of $O > A > B$ in all the diabetic subject.

1.5.Objectives:

1.5.1.General objective:

To determine The association of ABO and Rh blood group systems inSudanese patients with diabetes mellitus type 2 .

1.5.2. Specific objectives:

- To determinethe frequency of ABO and Rh blood groups of patients with diabetes mellitus type 2.
- To determine frequency of ABO and Rh blood groups according to gender with diabetes mellitus type 2.
- To determine frequency of ABO and Rh blood groups according to age with diabetes mellitus type 2.

Chapter two

Materials and Methods

2. Materials and Methods

2.1. Study design:

This is case control analytical study conducted in Khartoum State during the period of January to July 2015 in Sudanese patients with diabetes mellitus diseases type 2.

2.2. Study population:

Hundered patients with confirmed diabetes mellitus diseases type 2 and (100) healthy individual (control)of both sex were enrolled in the study .

2.3. Inclusion criteria:

Patients who were confirm to have diabetes mellitus andunder treatment in Khartoum State Hospitals.

2.4. Exclusion criteria:

Non cooperative diabetic patients.

2.5. Data collection:

Data were collected using self administered pre- coded questionnaires basic . The questionnaires was specifically designed to obtain information about sex, age, type of diabetes mellitus disease and duration of the disease.

2.6. Materials:

General Equipment and reagents:

- Syringe
- Cotton and gloves
- 70% alcohol
- EDTA containers
- Slides
- Antibody A
- Antibody B
- Applicator sticks
- Pipettes

2.7. Methods:

2.7.1. Sample collection:

Two point five ml of venous blood were drawn after sterilization by 70% alcohol using 21 G needle with limited occlusion of the arm by the tourniquet. The blood was collected in K2 EDTA (Potassium Ethylene Diamine Tetra Acetate) and mixed gently. (Kathleen *et al.*, 1998).

2.7.2. ABO slide agglutination test:

2.7.2.1 Principle:

When red cells were mixed with various reagents of antisera (soluble antibody), agglutination occurs on the slides containing cells positive (possessing the antigen) for the corresponding antigen. No agglutination occurs in the red cells that did not contain the corresponding antigen (Walker, 1997).

2.7.2.2 Procedure:

1. On the section of the slide labeled anti- A one drop of antibody A was placed.
2. On the section of the slide labeled anti- B one drop of antibody B was placed.
3. One drop of cells was placed in each antibody containing circle.
4. the solution was mixed carefully with a separate applicator stick.
5. The slide was slowly tilted for one minute, then agglutination was observed.
6. Result were recorded.

2.7.2.3. Interpretation:

Agglutination (clumping) of the red blood cells is positive. No agglutination is negative- It is critical to read the results immediately as false positive can occur when the mixture begins to dry on the slide.

2.7.2.4. Controls:

Known positive (+ve) and negative (-ve) (RBCs positive and negative for A, B antigen) were included in accordance with the relevant guide lines of the quality assurance.

2.8. Rh (D) red blood cell typing:

2.8.1. Principle:

Rh (D) typing is based on the principle of agglutination. Normal human red blood cells possessing antigen will clump in the presence of antibody directed toward the antigens.

Agglutination of patient or control red blood cells with anti- D serum and no agglutination with the control reagent is a positive test result, which indicates the presence of the D antigen on the red blood cells. Absence of agglutination is a negative test result, which indicates the D antigen is not demonstrable.

If Rh typing is negative, Du typing is automatically performed.

2.8.2. Du Method (The indirect anti globulin):

2.8.2.1. Principle:

The indirect antiglobulin test is used for the detection of antibodies that may cause red cell sensitization in *vitro*. If both IgG antibodies and the corresponding antigens are present in serum, red cell mixture incubation will cause the antibody to attach antigenic receptor on red cell.

2.8.2.2. The Technique of Dumethod:

- Two drop of mixture (IgG and IgM) anti- D were placed in 10×75mm test tube.
- One drop of washed 5% suspension of the test cell was added.
- Mixed well, and the tube was incubated at 37c for 15 minutes in LISS.
- After incubation, the mixture was centrifuged and then the result was read and recorded.
- The mixture was washed 3-4 times in large volume of saline, and then each wash was decanted completely.
- Two drops of anti globulin reagent were added, mixed well and incubated for 4-5 minutes at room temperature.
- The mixture was centrifuged at 3400rpm for 15 seconds.
- The final results were read and recorded (Walker , 1997).

2.8.2.3. Requirements:

- Test tubes
- Water bath at 37C
- Anti- D sera
- Coomb's sera
- Pasteur pipette
- Microscope

- Bench centrifuge

2.8.2.4. Interpretation:

Agglutination in test sample and negative reaction in control sample shows a positive test and the sample are labeled Rh (d) positive.

2.9.Statistical Analysis:

The data was analyzed using Statistical Package for Social Sciences (SPSS version 16). The data were described as percent and frequency. Chi square test was used to determine the association between ABO/Rh blood groups and DM and gender.

2.10.Ethical consideration :

The study was approved by the Medical Ethical Committee of Medical Laboratories College- SUST . A written consent was obtained from the participants after they had been informed with the objectives, benefit and expected outcome of the study.

The participants were assured that the collected information will be kept confidential and will not be used for any other purpose than this study.

Chapter three

Results

3.Results

3.1.Basic data of the study group :

All the diabetic participant were found to be diabetes mellitus type 2.

The majority of the cases were females (61%)while the males were (39%).

The mean of age the study group is 59,9 years(SD +_10.0),and the mean of duration of diabetes mellitus was10,9 years(SD+_1.0).

3.2.Distribution of ABO blood group:

Table (3.1) shows that 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).

3.3.Distribution of RH blood group in type 2 diabetic subjects compared with the control :

It is presented in Table (3.2) which show that 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 subjectsshowed higher frequency of RH positive (96%) than type 2 diabetic participants.

3.4 Distribution of ABO and RH among healthy individuals and diabetic type 2 patients :

It is shown in Table(3.3.).The distribution of both ABO and RH positive type was as follows for the diabetic type2patient and the control group was O(38vs43),A(32vs 32),B (20vs 18) and AB (4vs 3) ,and the distribution of both ABO and RH negative for the diabetic control was :group O(4vs 1),A (1 vs 1),B (1vs 0) and AB (0 vs 2).

3.5. Distribution ABO and RH in diabetic type 2 Patients according to gender:

The Gender did not have asignificant effect on the distribution of ABO blood group among diabetic type 2 subjects and the order of distributionfor both sexes was O>A>B>AB.(table 3.5)

Table (3.4) shows the frequency of DM and ABO/ RH positive blood group in male and female as follows : O(15 vs 23) ,A(14 vs 18), B (7 vs 13) and AB (1 vs 3).While that of the blood group and Rh negative was :

O(0 vs 4) ,A (1 vs 0), B (1 vs 0) and AB (0 vs 0)in males and femalesrespectively .

There were 57 RH positive females compared with 37 males being RH positive, and 4 RH negative females compared with 2 RH negative males.

Table (3.1)One sample Chi square table for association of ABO blood group frequency with DM type 2:

Blood group	Diabetic type2	Non diabetics	p.value
A	33(33%)	33(33%)	0.85
B	21(21%)	18(18%)	
AB	4(4%)	5(5%)	
O	42(42%)	44(44%)	
Total	100(100%)	100(100%)	

significance level at ($P \leq 0.05$)

Table (3.2)One sample Chi square table for association of RH factor frequency with Diabetes Mellitus type 2:

Rh Factor	Diabetics	Non Diabetics	P.value
Positive	94 (4%)	96(96%)	0.37
Negative	6 (6%)	4 (4%)	
Total	100 (100%)	100 (100%)	

significance level at ($P \leq 0.05$)

Table (3.3) :Distribution of Rh blood groups in type 2 diabetics as compared to controls:

Blood Group	Dibeties %	Control %
A +ve	32	32
A –ve	01	01
B +ve	20	18
B –ve	01	00
AB +ve	04	3
AB –ve	00	02
O +ve	38	43
O –ve	04	1
Total	100	100

Table(3.4)Distribution ABO and Rh in Diabetic type 2 according
Gender:

Sex	A		B		AB		O		Total
	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	
Male	14	1	7	1	1	0	15	0	39
Female	18	0	13	0	3	0	23	4	61
Total	32	1	20	1	4	0	38	4	100

4.1.DISCUSION:

Dali *et al*, (2009) in Alergia did not find any association between ABO/RH blood group and diabetes mellitus. This accords with the findings of the current work, also they found the highest frequency among the diabetic subjects was of blood group O which is on line with this study.

Also in Iraq Jassim, (2012) found the highest occurrence was of blood group O, but they disagree with the current study in that they found a significant difference from the other groups.

Egawa, *et al*. (2013), in Japan, did not observe any association between ABO blood group and Diabetes mellitus and the order of the blood group distribution among the diabetic subject was A>O>B>AB which is different from that observed in this study.

Ganesan and Gani (2014) in India, agree with the current work as they found the highest frequency (40.16%) was of blood group O among the diabetic subject which is a bit lower than the observed frequency (42%) in this study. They found a significantly higher frequency of blood group B in the diabetic subjects than in the healthy ones which is controversial with the result of the current work.

Sherma *et al*, (2014), in India agree with the current work in that they did not find any association between Diabetes mellitus and ABO blood group and the order of the ABO blood group frequency was (B>O>A>AB) which is different from the present work.

The current study did not find any difference between the diabetic individuals and the control group with regard to RH factor frequency.

This is on line with many previous studies (Dali *etal*,2009, and Sherma*etal*,2014).In the current work the frequency of RH positive was (94%) which is higher than that registered by Dali(2009)(84.65%) and Sherma*etal*(2014)(90%).

The results of the current study and that of Dali 2011, and Sharma *etal* 2014 , agreed that blood group AB being of the least frequency among the diabetic patients.

The distribution order of blood group ABO in this study for both the diabetic and the healthy subjects is on line with the finding of Abo algasim*etal* (2007) in Dinka Sudanese ethnic group, and Hassan (2010) in the Sudanese major ethnic groups that is Danagla , Shaygia and Gaaleene.

The variation between the current work and the previous studies done abroad with regard to the distribution order of the ABO blood groups among the diabetic individuals maybe attributed to genetic or environmental factors.

4.2.Conclusion:

- No association was found between the ABO/Rh blood group and Diabetes mellitus type 2.
- The distribution order of ABO/Rh blood group in diabetic type 2 and healthy subject was $O > A > B > AB$.
- The gender did not affect the distribution of the ABO and RH blood group.

Recomondations :-

Further studies should be done to investigate the

1-relationship between ABO/RH blood group and diabetes mellitus type 2 with a large sample size.

2.role of the ethnic group and/or traditional in distribution of ABO/RH blood group and diabetes mellitus type 2.

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Appendix

Sudan University of science & Technology College of Medical

Laboratory Science

Department of Haematology

Questionnaire

Name:.....

Number :.....

Sex:.....

Phone No:.....

Duration of Diabetes:.....

Type of Treatment:

Insulin() Tablet() Diet()

Blood group:.....

Date:.....sig:.....