Introduction and literature review

1.1 General Introduction:

Blood group serology includes the study of antigenic molecule present on the various cellular and soluble component of whole blood together with that of the antibodies and lactins that recognize them and their interaction. However in practice, blood group serology is restricted to red cell antigens and their interaction with specific antibody. There are several blood group systems which are antigens present on the surface of red cell. Approximately 700 red cell antigens have been described.The most major blood group system is ABO blood group system (BGS) because of it is clinical significance, followed by Rh (BGS). Diabetes mellitus (DM) is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by a relative or absolute deficiency of insulin. DM is a leading cause of adult blindness and amputation, and a major cause of renal failure, heart attacks and strokes. [1]

Despite an ample supply of glucose, the body behaves as though starved, and glucose is over produced by the liver and underused by other tissue. As a result of the concentration of glucose in the blood often exceed the capacity of the kidney to reabsorb glucose, so some of it is spills in to the urine. The high concentration of glucose in urine is drowse water somatically from the body.[2]

1.2 Literature review:

1.2.1 Historical perspective of the ABO blood group system:

Karl Landsteiner discovered the ABO blood group system (BGS) 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. 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(Ag) on red cells and the reciprocal agglutinating antibodies in the serum of the same individual (e.g. 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. ABO grouping is one of the primary tests performed in the blood bank. Felix Bernstein discovered the group inheritance pattern of multiple alleles at one locus in 1924. This discovery explained the inheritance of ABO blood groups. Additionally, it was established that an individual inherits one ABO gene from each parent. These genes produce the antigens present on the surface of an individual's red cells. Like Landsteiner's discoveries, Bernstein's determination of inheritance patterns of the ABO group has played a major role in the knowledge

base for all blood group systems. In 1930, O. Thompson postulated a four-allele system of inheritance. This proposed system was based on the discovery of Emil Frieherr von Dungern and Ludwig Hirtzfeld in 1911

that the group A Ag can be divided into two subgroups, A1 and A2. Thompson expanded this premise and proposed the four allelic genes: A1, A2, B, and O. His expansion of Landsteiner's original findings enhanced the ability to provide safe blood for transfusion. [3]

1.2.2 ABO and H system antigens:

1.2.2.1 ABO antigens:

Antigens detected in blood bank testing, including ABO antigens, are located on the surface of the red blood cell. ABO antigens are also present on lymphocytes, thrombocytes, organs, endothelial cells, and epithelial cells. When Landsteiner performed his mixing tests, he detected the ABO antigens. The biochemistry and structure of ABO antigens are well-established. Antigens of the ABO system are well-developed in adults. They are detectable at 5 to 6 weeks of gestation. Newborns demonstrate weaker antigens, but ABO antigens are fully developed by two to four years of age. One factor contributing to the difference in ABO antigen strength between newborns and adults is the number of branched oligosaccharides. Adults demonstrate greater numbers of branched chains compared to newborns, who have more linear chains. The branched chains permit attachment of more molecules to determine H antigen specificity. Following H antigen development, the A and/or B specific molecule may be attached. Adults have more branched chains hence, the ability to add on more terminal sugars and produce more antigens. Newborns and infants have fewer antigen sites on their red cells.[3]

1.2.2.2 Inheritance of A, B, and H Antigens:

As Bernstein discovered, ABO antigens are inherited in a simple Mendelian fashion from an individual's parents. Each individual possesses a pair of genes. Each gene occupies an identical locus on chromosome 9. There are three possible genes that can be inherited. The three genes are: A, B, and O. A and B genes produce a detectable product

while the O gene is an amorph that does not produce a detectable product. The expression of the A and B genes is codominant.

The H antigen is required to produce A and/or B antigens. [3]

Table (1.1) Summery of ABO gene combination and phenotype

Combination	Phenotype
AO	A
AA	A
BO	B
BB	B
AB	AB
OO	O

The H gene is also inherited in Mendelian fashion and occupies a locus on chromosome 19. Each parent contributes one gene, either H or h. The possible genetic combinations are HH, Hh, or hh. Individuals who are genetically either HH or Hh will produce the H antigen, and it can be detected on their red cells. The frequency of occurrence of the H antigen in the Caucasian population is greater than 99.99%. Individuals inheriting an hh genotype do not produce the H antigen and have the bombay phenotype Oh. The plasma of an individual with a bombay phenotype frequently demonstrates an anti-H. [3]

1.2.2.3 Biochemical and structural development of A, B, and H antigens:

Expression of A, B, and H genes does not result in the direct production of antigens. Rather, each gene codes for the production of an enzyme known as a transferase. Each transferase catalyzes the transfer of a carbohydrate molecule to an oligosaccharide chain. The attached

carbohydrate provides antigenic specificity. The O gene codes for an enzymatically inactive protein and, hence, no antigen is produced.[3]

Table (1.2) Summaries of transferase enzymes for ABH antigen production

Gene	Transferase
H	α-L-fucosyltransferase
A	α-3-N-acetyl-D- galactosaminyl Transferase
B	α-3-D-acetyl-D-galactosyl Transferase
O	No Transferase Produced

1.2.2.4 Development of H antigen:

The H allele codes for the transferase, L-fucosyltransferase. This enzyme catalyzes the formation of the H antigen by transfer of L-fucose to either type. [3]

1.2.2.5 Development of A and B antigens:

The H antigen oligosaccharide chain serves as a precursor for both the A and B antigens. The A and B alleles each code for a transferase that attaches a sugar molecule to the terminal end of the H antigen oligosaccharide chain, which forms either the A or B antigen.The A allele codes for N-acetylgalactosamine transferase. This transferase attaches N-acetyl-D-galactosamine to the H antigen forming the A antigen. The B allele codes for D-galactosyltransferase. This transferase attaches D-galactose to the H antigen forming the B antigen. The product of the O allele is an enzymatically inactive protein. Hence, this allele produces no detectable antigen. Conversely, group O cells contain the most H antigen. This results from no conversion of H antigen to A and/or B antigens. In comparison, group A1B cells have the least amount of H antigen since quantitatively the most H is converted to A1 and B antigens. [3]

1.2.2.6 Secretor status:

The gene that controls the presence of ABH antigen in the secretions is called SeSe, in single Sese or double SeSe dose result in the presence of the antigens in secretion. Approximately 78% of adult are secretors, the remainder 22% are non secretor. [4]

1.2.2.7 A and B Subgroups:

The A (and AB) phenotype can be subdivided into A1 and A2 (and A1B and A2B). In a european population, about 80% of group A individuals are A1 and 20% A2. A1 and A2 differ quantitatively and qualitatively. A1 red cells react more strongly with anti-A than A2 cells. In addition, A2 red cells lack a component of the A antigen present on A1 cells and some individuals with the A2 or A2B phenotype produce anti-A1, an antibody that agglutinates A1 and A1B cells, but not A2 or A2B cells. Anti-A1 is seldom reactive at 37◦C and generally considered clinically insignificant. There are numerous other ABO variants, involving weakened expression of A or B antigens (A3, Ax, Am, Ael, B3, Bx, Bm, Bel), but all are rare. At least 60 subgroups of A and 30 subgroups of B have been recognized by molecular genetical methods, but the symbols Ax, Ael, etc., represent phenotypic characteristics and not single genetic entities. [5]

1.2.2.8 ABO antibodies:

ABO is the only blood group system that in a natural and consistent manner has antibodies (Abs) present in sera of people who lack the corresponding antigen from their red blood cells. With the exception of newborn infants under 5 months, deviations from this rule are extremely rare and related to disease. These antibodies are detected at about 3 months and increase their titer until the 5th to 10th year of life. Neonates may present the IgG type of ABO (Ab) that have maternal origin, because IgG (Ab) can cross the placenta. [6]

Antibodies directed against ABO antigens are the most important antibodies in transfusion medicine. This is a profound, but true statement. For this reason, ABO antibodies require detailed description. The ABO blood group presents a unique situation in immunohematology. It is the only example of a blood group where each individual produces antibodies to antigens not present on the red cells. These ABO antibodies were originally thought to be natural antibodies formed with no apparent antigenic stimulus. Since the antibodies are not stimulated by exposure to red cells, they may also be considered non-red cell stimulated antibodies. However, some form of an antigenic stimulus must exist. The proposed mechanism is environmental. These "naturally occurring" substances resemble A and B antigens and stimulate the production of complementary antibodies to the antigens that are not present on the red cell surface. Newborns have no ABO antibodies. When newborns are tested, only a forward group is performed. Newborns may exhibit passive ABO antibodies that have crossed the placental barrier. Reverse grouping of a newborn or umbilical cord serum indicates the blood group of the mother. The child will begin antibody production, and have a detectable titer, at three to six months of age. ABO antibody production peaks at age five to ten years of age and continues in immune competent individuals throughout life. Titers begin to wane in the elderly.[3]

Table (1-3) Antigens and antibodies of ABO blood group:

Blood Group	Antigens	Antibodies
A	A	Anti-B
B	B	Anti-A
O	Neither A or B	Anti-A, anti-B
AB	A and B	Neither anti-A or anti-B

1.2.2.9 Immunoglobulin class:

ABO antibodies are typically isoagglutinins. They are saline agglutinins with optimal reactivity at 4°C. These naturally occurring antibodies are mostly IgM isotype, but IgG and IgA classes of ABO antibodies have been detected. The development of IgG antibodies occurs without apparent antigen exposure via transfusion of incompatible red cells or fetal maternal incompatibility. [3]

1.2.2.10 Clinical significance of ABO antibodies:

ABO antibodies are capable of causing both hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTR). These issues explain the clinical significance of "naturally occurring" antibodies. HDFN usually presents itself with a maternal antibody of an IgG isotype that corresponds to an antigen on the surface of the baby's red cells. ABO hemolytic disease may affect a woman's first pregnancy. This is in contrast to Rh HDFN where the antigenic stimulation usually occurs in the first pregnancy and subsequent antigen-positive newborns are affected. Hemolytic transfusion reaction occurs when a recipient is transfused with red cells that are an ABO group incompatible with the antibodies in his or her serum. Because of the complement-binding ability of the ABO antibodies, this is always a life-threatening situation. As the recipient antibodies react with the incompatible red cells, complement is activated and in vivo hemolysis, agglutination, and red blood cell destruction occurs. ABO compatibility is also significant in solid organ transplantation. For most organs, an ideal scenario for transplant is an ABO compatible solid organ. Post transfusion antibody titer, and pheresis to reduce the titer of the incompatible antibody, will assist in achieving a positive outcome when an ABO incompatible organ is transplanted. [3]

1.2.3 Rh blood group system:

1.2.3.1 Introduction and historical back ground:

The Rh blood group locus is composed of two related structural genes, *RhD* and *RhCE.* [7]

Karl Landsteiner and Alexander Weiner discovered the D antigen in 1940. Philip Levine and R. E. Stetson discovered the first anti-D in 1939 from a stillborn fetus of a mother who had been transfused with her husband's blood during her pregnancy. [3]

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. Winner terminology was based on the belief that the Rh antigens were the products of a single gene coding for an "agglutinogene" composed of multiple "blood factors", the names given to each of the five major Rh antigens were Rh0, rh', rh", hr', hr". [4]

1.2.3.2 Rh antigens:-

The Rh blood group locus is composed of two related structural genes, *RhD* and *RhCE,* which encode the membrane proteins that carry the D, Cc and Ee antigens. The *RhD* gene may be either present or absent, giving the Rh D+ve or Rh D-ve phenotype, respectively. Alternative RNA splicing from the *RhCE* gene generates two proteins, which encode the C, c, E or e antigens. [7]

1.2.3.2.1 D antigen:

The D antigen is the primary antigen in the Rh system, when present on red cells, the individual is designated as "Rh positive." An individual may inherit one D gene from each parent, the inheritance of either one or two D genes will designate that person as "Rh positive."Conversely when no D gene is inherited from either parent, the individual is designated as "Rh negative". The D antigen is very antigenic. More than 80% of Rh negative (D negative) individuals transfused with Rh positive blood will

develop an anti-D on initial exposure. Rh positive individuals may be transfused with either Rh positive or Rh negative blood. Rh negative individuals, however, should always be transfused with Rh negative blood unless the situation is life threatening and only Rh positive blood is available. Exclusive administration of Rh negative blood is crucial for women of child-bearing age. Rh negative women who develop anti-D are likely to develop hemolytic disease of the fetus and newborn (HDFN) if an Rh positive infant is born to an Rh negative mother. [3]

1.2.3.2.2 D variant:

Some individual have changes in the *RHD* gene, these encode change in the protein which often cause variation in the expression of D antigen and include weak D, partial D phenotype. [4]

1.2.3.2.2.1 Weak D:

Weak D antigen expression is primarily found in person with a single *RHD* that has a mutation encodings an amino acid change, these are designated types 1 through 57, importantly types 1, 2 and 3 are more common and makeup 90% of the weak D cases.[4]

1.2.3.2.2.2 Partial D:

RBCs with a partial D are primarily due to inheritance of hybrid gene in which portions of *RHD* are replaced by the corresponding portions of *RHCE* this results in loss of some D epitops. The RBCs type as D positive but individuals make anti D following transfusion or pregnancy.[4]

1.2.3.3 Rh antibody:

Compared to the ABO system, individuals who lack any of the Rh antigens rarely develop antibodies to those antigens without red cell stimulation via pregnancy or transfusion. Antibodies to all of the Rh antigens may cause both HDFN and hemolytic transfusion reaction (HTR). The characteristics of all Rh antibodies are the same regardless of

the corresponding antigen. They are IgG antibodies that bind to their respective antigens at 37°C. They may display agglutination at 37°C and in the AHG testing phase. They do not bind complement and are enhanced by enzymes. [3]

1.2.4 Diabetes mellitus (DM):

Diabetes mellitus is metabolic disorder characterized by chronic hyperglycemia with disturbance in carbohydrate, fat and protein metabolism arising from defect in insulin secretion, action or both.[8]

1.2.4.1 Classification of diabetes mellitus:

1.2.4.1.1 Type I diabetes (IDDM):

Is a result of cellular-mediated autoimmune destruction of the β-cells of the pancreas, causing an absolute deficiency of insulin secretion .Type one constitutes only 10%to20% of all cases of diabetes and commonly occurs in childhood and adolescence .This disease is usually initiated by an environmental factor or infection in individuals with a genetic predisposition .Characterized of type one diabetes include abrupt onset ,insulin dependence and ketosis tendency.[9]

Treatment of the patient is dependent on exogenous insulin administration sustain life and prevent diabetic ketoacidosis. [10]

1.2.4.1.2 Type II diabetes (NIDDM):

Characterized by hyperglycemia as a result of an individual resistance to insulin with an insulin secretory defect .This resistance result in a relative not an absolute insulin deficiency, type two constitutes the majority of the diabetes cases .Most patients in this type are obese or have an increase body fat ,risk increase with an increase in age and lack of physical exercise .Adult onset with ketoacidosis seldom occurring.[9]

Treatment: patient may be controlled by diet, exercise, oral hypoglycemic agent or may require insulin administration. [10]

1.2.4.1.3 Other specific types of diabetes:

These are associated with certain conditions including genetic defects of β-cell function or insulin action, pancreatic disease, disease of endocrine origin, drug or chemical induced insulin receptor or abnormalities and certain genetic syndromes. [9]

1.2.4.1.4 Gestational diabetes mellitus (GDM):

Any degree of glucose intolerance recognition during pregnancy .Causes include metabolic and hormonal changes ,this disease is associated with increase prenatal complication and an increase risk for the development of diabetes in later years.[9]

1.2.4.2 Pathophysiology of DM:

On both type 1 and 2 diabetes ,the individual will be hyperglycemic which can be sever can also occur after the renal tubular transporter system for glucose become saturated, this happens when the glucose concentration in plasma exceeds roughly 180 mg/dl in an individual with normal renal function and urine output. As hepatic glucose over production continuous, the plasma glucose concentration reaches a plateau around 300 mg/dl to 500 mg/dl provide renal output is maintained glucose execration will match the over production causing the plateau.[11]

1.2.4.3 Clinical feature:

It includes sweating, drowsy, volume depletion, hyper ventilating, and neurological abnormality. [12]

1.2.4.4 Complication of diabetes mellitus:

1.2.4.4.1 Acute metabolic complication of diabetes mellitus:

❖ Hypoglycemia

This is the most commonly caused by accidental over administration of insulin or sulphonyleuria or meglitinides. [12]

❖ Diabetic ketoacidosis (DKA)

It may be precipitated by infection, acute myocardial infarction or vomiting. [12]

❖ Hyperosmolal non ketotic coma

In diabetic ketoacidosis there is always plasma hyperosmolality due to hyperglycemia and many of the symptoms, including those of confusion and coma are related to it.[12]

❖ Lactic acidosis

It may be due to the use of metformin in certain situation such as high dose in the very elderly, those with renal, liver or cardiac failure or dehydrated patient.[12]

1.2.4.4.2 long term effect of diabetes milletus:

❖ Microvascular disease

It is vascular disease occurring in the smallest arteries, arteriols and capillaries. Microvascular disease includes: Diabetic nephropathy. [8]

❖ Diabetic retinopathy

It may lead to blindness because of vitreous haemorrhage from proliferating retinal vessels. And maculopathy as a result of exudates from vessels or oedema affecting the macula. [13]

❖ Kidney disease [nephropathy]

Is a major cause of premature death in patients with diabetes, with deaths related to cardiovascular disease as well as renal failure.[8]

❖ Infections also more common for example urinary tract or chest infection and candida.[12]

❖ Diabetic neuropathy

Can be prepheral systemic sensory, peripheral painful or acute mononeuropathy. [12]

❖ Diabetic ulcer of the feet

Can lead to gangrene and amputation. [12]

❖ Skin disorders

Such as necrobiosis lipidica, and abscesses. [12]

1.2.5 Previous studies:

Some study was undertaken to examine the frequency of ABO and Rh BGS in healthy and diabetic patients.

In 2010, Dr. Fathelrahman Mahdi Hassan, study Frequency of ABO, and Rh(D) Blood Groups in Major Sudanese Ethnic Group, in Khartoum state and found that O blood group was more frequent and followed by A, B, and AB.[14]

In December 2012 Waleid M. Shahata , Hiba B. Khalil , Awad-Elkareem Abass, Ishag Adam Shahad M. Hussien, Blood group and Rhesus antigens among Blood donors attending the Central Blood Bank in Sudan and also found that O blood group is most frequent.[15]

In 2011 SM Dali, MA Aour, F Belmokhtar, R Belmokhtar, F Boazza, study The relationship between ABO/rhesus blood groups and type 2 diabetes mellitus in Maghnia, western Algeria,and also found that O blood group was distributed with the highest frequency among diabetic subjects.[16]

In 2008 Shyamal Koley study The Distribution of the ABO Blood Types in Patients with Diabetes Mellitus, and found that the most frequent blood group is B then A, O and AB.[17]

Rationale:-

Diabetes breakout in sudan in last years that give us inducement to study frequency of ABO and RH group in diabetic Sudanese patients, we do this study to be consider as basic study for other researchers who will detect if there is genetic association between the blood group and diabetes.

Objectives:-

General objective:
- To study frequency of ABO and Rh blood group antigenes in diabetic patients.

Specific objectives:
- To determine ABO antigens in diabetic patients.
- To determine Rh antigen in diabetic patients.
- To determine the most frequent antigens.

Materials and methods

2.1 Study design:

This is analytical descriptive cross sectional study.

2.2 Study area:

The study is conducted in Khartoum state in Alhajyousf area.

2.3 Study population and sample size:

500 Ethylene Di amine Tetra Acetic Acid (EDTA) venous blood were collected from diabetic patients, was conducted during November2013to january2014.

2.4 Ethical consideration:

- Study population was provided with enough knowledge about study and its values.

- The agreement was taken from population without compulsion.

2.5 Inclusion criteria:

Sample must be collected from diabetic patients.

2.6 Exclusion criteria:

non diabetic patients were excluded.

2.7 Statistical analysis:

Statistical analysis done using statistical package for the social sciences (SPSS) computerized program.

2.8 Sampling:

2.5ml of blood was collected from each patient in EDTA container

2.9 Principle:

The cells were mixed well with the antiserum and rocked for several minutes while observing for agglutination. Agglutination appears as a clumping of RBCs and may be visualized macroscopically.[18]

2.10 Requirement:

- Disposable 5ml syringe with needle.

- Tourniquet.
- 70% alcohol.
- Cotton.
- Slides.
- Anti sera (A, B, D, and A1).
- Capillary tube.
- 2.5 EDTA container.

2.11 Procedure:

- The venipuncture site was disinfected with 70% alcohol.
- The tourniquet was applied.
- The needle was inserted in to vein and the tourniquet was loosened.
- Blood was collected.
- Blood was carefully delivered from the syringe in to the EDTA blood container then mixed.
- The container was labeled with patient information.
- A, B, A1and D was labeled on clean dry slide.
- Small drop of homogenous, non hemolysed blood was placed on each mark.
- one drop of anti sera A, B, A1and D was added respectively.
- Mixed, rotated and after 3-5min and agglutination observed.
- Result was reported

Results

Table (3.1) Frequency of ABO BGs

ABO group	Frequency	Percent	Cumulative Percent
O	202	40.4	40.4
A	160	32.0	72.4
B	111	22.2	94.6
AB	27	5.4	100.0
Total	500	100.0	

Table 3.2 Frequency of ABO and ABO subgroup with Rh BGS

Blood group	Frequency	Percent	Valid Percent	Cumulative Percent
A1+ve	153	30.6	30.6	30.6
A2+ve	4	.8	.8	31.4
B+ve	110	22.0	22.0	53.4
O+ve	188	37.6	37.6	91.0
A1B+ve	26	5.2	5.2	96.2
A2B+ve	1	.2	.2	96.4
A1-ve	3	.6	.6	97.0
B-ve	1	.2	.2	97.2
O-ve	14	2.8	2.8	100.0
Total	500	100.0	100.0	

Table (3.3) Frequency of Rh (D)

Rh group	Frequency	Percent	Cumulative Percent
+ve	482	96.4	96.4
-ve	18	3.6	100.0
Total	500	100.0	

Discussion, Conclusion and Recommendations:

4.1 Discussion:

Descriptive study was conducted in Khartoum state during the period from November 2013 to june 2014 to study the frequency of ABO and Rh (D) blood groups on diabetic patients, 500 sample were collected from diabetic patients the result revealed the following:-

The most predominant ABO blood group is O (40.4%) followed by A (32.0%), B (22.0%), AB (5.4%), Majority (96.4%) of the subjects were Rh (D) positive and only (3.6%) were Rh negative, which was agree with SM. Dali, MA Aour, F Belmokhtar, R Belmokhtar, F Boazza study of the relationship between ABO/rhesus blood groups and type 2 diabetes mellitus in Maghnia in western Algeria ,which found that O blood group was distributed with the highest frequency among diabetic subjects(52.85%).

Our result was vary from study of The Distribution of the ABO Blood Types in Patients with Diabetes Mellitus which done by Shyamal Koley, That found the most common blood group is B (38.5%) followed by A (27.9%), O (24.4%), and lastly AB (9.0%).

This result was harmonized with the result of Dr. Fathelrahman Mahdi Hassan that study Frequency of ABO, and Rh(D) Blood Groups in Major Sudanese Ethnic Group in Khartoum state and found that O blood group was more frequent (52.7%) followed by A, B, and AB. Rh (D) result (98%) are positive, (2.0%) are negative.

Also concurrence with Waleid M. Shahata, Hiba B. Khalil, Awad-Elkareem Abass, Ishag Adam Shahad M. Hussien study of Blood group and Rhesus antigens among Blood donors attending the Central Blood Bank in Sudan that show O blood group is most frequent (51.5%), A (29.5%), B (16%), and AB (6%); whereas the frequency percentage of Rh antigens were D (93%).

4.2 Conclusion

The result of this study show that the O blood group is the most frequent in both Rh positive and negative, followed by A, B and then AB, the majority of patients are positive for Rh(D).

4.3 Recommendations

❖ It advisable that to increase sample size to get accurate reliable result.

❖ We recommend that to conduct study to detect if there is genetic association between DM and BGS.

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