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Frequency of Subgroup A Phenotypes (A1 and A2) Among Student of Medical Laboratory Science in Shendi University

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الآية

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قال تعالي:

﴿ إِنَّا فَتَحْنَا لَكَ فَتْحاً مُّبِيناً * لِيَغْفِرَ لَكَ اللَّهُ مَا تَقَدَّمَ مِن ذَنبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطاً مُّسْتَقِيماً ﴾

صدق الله العظيم

سورة الفتح الآية (١ – ٢)

Dedication

To my parents, brothers and sisters

To my wife and children

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List of abbreviation

Abbreviation	Mean
C3	Complement-3
DAT	Direct Antiglobulin Test
EDTA	Ethylene Diamine Tetra Acetic Acid
HDN	Haemolytic Disease of the Newborn
IgA	Immunologlobulin A
IgG	Immunologlobulin G
IgM	Immunologlobulin M
ISBT	International Society of Blood Transfusion
Lu	Lutheran Blood Group System
LW	Landsteiner and Wiener
Rh	Rhesus Blood Group System
RBCs	Red Blood Cells
LISS	Low Lonic Strength Solution

Abstract

This is descriptive analytical prospective cross sectional study conducted in Shendi University faculty of medical laboratory science and aimed to determine the prevalence of A subgroup (A1 and A2) among the students between March and August 2018.

A total of 100 peripheral blood samples were collected from different batches of the students with different age including both gender was used Slide method in stead of jell technique.

The result of the current study reveled and conclude that the sub group A1 was the most common within the students (frequency were 92%), wile the A2 sub group represent only 8% of the study population.

ملخص البحث

أجريت هذه الدراسة التحليلية في جامعة شندي كلية علوم المحتبرات الطبية والتي تهدف إلى تحديد مدى انتشار الزمر الفرعية لفصيلة الدم A (A1 & A2) وذلك في الفترة من مارس إلى أغسطس ٢٠١٨م، تم أخذ ١٠٠ عينة من أفراد أصحاء من الجنسين من أعمار مختلفة من طلاب جامعة شندي علوم المختبرات الطبية الذكور والإناث استخدمت فيها طريقة الشريحة بدلاً عن طريقة الجل تكنيك بالنسبة للنوعين.

كانت أهم نتائج الدراسة أن نسبة تحت قروب A1 هو النسبة السائدة في الطلاب وكانت نسبته (٩٢%)، بينما تحت قروب A2 بنسبة ضئيلة (٨%).

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1-1 Introduction

The date of 1901 represents the most important achievement in the history of blood transfusion through the discovery of the ABO blood groups by Karl Landsteiner. (Garratt G. Transfusion 2000).

ABO system of three allelic genes A, B and O. The ABO RBC antigens are cell surface heterosaccharides.

ABO Ags are not primary gene products but instead they are enzymatic reaction Products catalyzed by enzymes called glycosyltransferases. All normal individuals synthesize a common core glycan called the H Ag (type O) that is attached to a polypeptide backbone. Individuals who possess an a Allele gene product form an enzyme that adds a terminal Nacetylgalactosamine to some of their O Ags for the A Ag. The B allele enzyme adds a terminal galactose. Type O blood does not lack Aginstead Have H substance.

A1 red blood cells have about one million A antigens per cell. A2 red cells have only 250,000 A antigens per cell, or one-fourth the amount that A1 cells have.

The 'A' antigen on A1 and A2 subgroup blood cells is named 'Type 2 A' antigen; however, A1 subgroup blood cells also have two additional forms of antigen as well, 'Type 3 A' and 'Type 4 A', neither of which appear on A2 subgroup blood cells. (ABO blood group. Available at http://Len.wikipedia.com)

Blood from individuals expressing the A1 allele should not be transfused into non-A recipients (B or O) because they will mount an immune reaction to the "foreign" glycosphingolipid that is the product of the A1 enzyme. The decreased activity of the enzyme coded by the A2 allele results in much fewer H-antigen complexes bearing the key N-acetyl galactosamine on red blood cells. However, blood from an A2 donor may still cause an incompatible transfusion reaction if transferred into B or O recipients, so this should be avoided. When organs are transplanted between individuals, similar ABO compatibility/immune rejection considerations also apply, but there have been some documented cases of organs from A2 individuals being able to be transplanted into group O or B patients,

without the need for debilitating immune suppression. (ABO blood group. Available at http://Len.wikipedia.com)

1. 2 Justification

The (A) blood group is one of the ABO system groups. It's the second common type of ABO group in Sudanese after the (O) blood group.

Blood group A has two sub type A1 and A2, which has a very important role in the blood transfusion, but infortunately they don't screened in the blood banks as one of the routein investigations when blood prepared for donation. In addition to that the prevalence of A sub groups is unknown, so the current study intended to determine the frequency of the A sub groups within the students in the faculty of Medical Lab- sciences. The importance of this study is just to take an idea about the A sub groups at the limited level in the faculty of MLS. And in the future it could be applied at the level of whole city in order to know every body A blood group profile data to save money and time in blood transfusion practice.

1.3 Objectives

1.3.1 General Objective:

To determine the frequency of subgroup A phenotypes (A1 and A2) within the study population.

1.3.2 Specific Objectives:

- To determine the frequency of A1 subgroup.
- To determine the frequency of A2 subgroup.
- To determine the in frequency of A subgroup according to the gender.

Chapter Two

Literature Review

Literature review

2.1 Historical background:

Blood groups from comparatively small field of study but they have an important place in genetics, immunology, anthropology and in clinical medicine. They are also invaluable tools in forensic science. In 1901, Landsteiner, the father of blood groups discovered the ABO blood group system by testing the sera of a number of people against their own red cells and those of all the others. three different patterns of reaction were obtained (Kathleen E, 1988).

In 1902 Decatello and Sturli showed that failure or success depended upon properties to be found in the donors corpuscles and the recipients plasma. The four Landsteiner groups make – up what is known as the ABO system (Sir Lionel,1953).

In 1927 a series of experiments carried out by Landsteiner and Levine using rabbit was eminently successful revealed two systems M N and p. in 1940 landsteiner and Wiener immunized rabbits and guinea pigs with the blood of the Macacus rhesus monkey.

Thereafter through the stimulus of the second world war iso – immunization through blood transfusion on a large scale served a similar purpose and led t the discovery o Lutheran . Duffy and lewis systems (Kathleen E- 1988)

2-2 ABO system:

This consist of three allelic genes A,B and O. the A and B genes control the synthesis of specific enzymes responsible for the addition of single carbohydrate residues to a basic antigenic glycoprotein or glycolipid with a terminal sugar L-Fucose on the red cell, known as the H substance. The O genes is morph and does not transform the H substance. Although there are six possible genotypes, the absence of specific anti – O prevents the serological recognition of more than four phenotypes. The A, B and H antigens are present on most body cells including white cells and platelets are also. In 80% of the population who possess secretor genes, these antigens are also found in soluble from in

secretions and body fluids, e.g. plasma, saliva, semen and sweat (Hoffbrand & pettit, 2001)

In 1940 Landsteiner and Wiener reported the discovery of a human blood factor which they celled Rh, they immunized guinea pigs and rabbits with blood from the Macacus rhesus monkey and there by obtained an antiserum which , after suitable absorption , agglutinated not only the red cells of the rhesus monkey but also approximately 85% of a panel of blood samples from the white population of New York (Kathleen E,1988) .

In 1941 Levine and his co-workers, showed that not only an Rh negative mother become immunized to an Rh positive fetus in utero but also the antibody could then traverse the placenta and give rise to erythroblastosis fetalis or hemolyatic disease of the newborn. Moreover, they showed that these antibodies were warm antibodies reacting more strongly at 37°c than at lower temperature. Later work demonstrated that the animal anti- serum used by human anti – Rh but another antigen possessed by Rh positives and Rh negative persons but in much greater amount in Rh positives (Kathleen E,1988).

Se Levine suggested that the antigen defined by the original Rhesus antibody be called LW in honour of Landsteiner and Wiener, and the antibody anti LW (Hoffbrand & Pettit, 2001)

The Rh blood group loucs is composed of two related structural genes RhD and Rh EC, which encode the membrane proteins that carry the D, Cc and Ee antigen. The RhD gene may be either present or absent, giving the Rh D-or RhD- phenotype respectively . Rh antibodies rarely occur naturally, most are immune, i.e- they result from previous transfusion or pregnancy. Anti D is responsible for most of the clinical problems associated with the system and a simple subdivision of subjects into Rh D positive and Rh D negative using anti D is sufficient for routine clinical purposes (Hoffbrand & Pettit, 2001).

2-3 General introduction to Rh blood group system:

The Rh blood group system was the fourth system to be discovered , yet it is the second most important in blood transfusion , (Kathleen , E 1988).

In 1939 Levine and Stetson reported that an antibody was present in the serum of a patient after a severe reaction to transfusion of her husband blood (Hoffbrand &Pettit, 2001).

Levine and Stetson postulated that the antibody had arisen as the result from thee father (Kathleen E, 1988)

2.3.1 Rh antigens nomenclatures for the Rh blood group system.

2.3.1.1 Fisher-Rae nomenclature:

According to the theory put forward by fisher in 1943, the Rh system is composed of three closely linked allelic genes, each with two alleles, C and c, D and d and E and e. A person inherits a set of alleles of the three RH genes from each parent, these sets of alleles of closely linked genes are known as haplotype. (Mollison ,1983).

In term of nomenclature, the Rh antigens are therefore named C,D,E and e, The antigen d has never been discovered and the sympol d is used to denoe the absence of D. All individuals who lack the D antigen are said to be Rh negative, regardless of whether C or E antigen or both are present (Neville, 1994).

2.3.1.2 The Wiener nomenclature:

Wiener visualized multiple alleles at the single complex locus, each allele determining it own particular agglutinogen. The agglutinogens comprise multiple factors (depending on which genes are present) and recognized by whichever factors are detectable. The two genes (i.e one paternal and one maternal) may be alike (homozygous) or different (heterozygous). Therefore, multiple alleles may exist at this locus. The eight major alleles are called: R1,R2 ,r ,R0 ,r ,r and Rz and ry Wiener uses blod-face type to indicate factors, Roman type to indicate agglutinogens and italics, to indicate the genes . (Neville, 1994).

Table (2-1): Comparison of Fisher – Race and wiener nomenclatures.

Fisher – Race	Wiener
CDe	Rh1
Cde	Rh2
cDe	Rh0
CDE	Rz
Cde	R
cdE	R
Cde	R
CdE	Ry

2.3.1.3 The Rosenfield nomenclature:

Rosenfield and associates, in 1962 developed a mumetical terminology for the Rh groups. The nomenclature proposes a system that describes with particular antisera, is free of any genetic implication and give equal importance to positive and negative reactions . The antigens are numbered in chronological order of discovery and the system has certain obvious advantages .

Table (2-2):Comparison of nomenclatures of main antigens of the Rh system.

Wiener	Fisher-Race	Rosenfield
Rho	D	RH1
Rh	C	Rh2
Rh	E	Rh3
Hr	C	Rh4
Hr	E	Rh5

2.3.1.4 Tippet's theory:

Tippette theory suggests that there may be only two genes , one determining D and one determining C ,c,E and e. Tippette the recent genetic model of Rh inheritance.

Table (2-3): Tippetts genetic model applied to the eight common Rh gene complexes.

First	Second locus	Gene complex
D	Ce	Dce
D	Ce	DCe
D	cE	DcE
D	CE	DCE
D	Ce	DcE
D	Ce	dCe
D	cE	dcE
D	CE	dCE

2.3.2.0 The antigens of the Rh blood group system:

The Rh antigens is protein, the structure of which is maintained by surrounding lipid. Because of this, Rh Ag can not be extract from red cells in a soluble from. Rh activity not lost when lipid do not carry the antigenic determinants but may be essential for the conformation of the determinats (Neville,1994).

2.3.2.1 The antigens C,D,E,c and e:

Rh (D) can be considered the most clinically significant of all the red cell antigens after the A and B antigens of the ABO blood group system.

The antigens is highly antigenic, it has been shown that 50 percent of Rh-negative individuals form anti D following a single transfusion of Rh- positive blood, although the immunizing dose can be considerably less (Neville ,1994).

The antigens C,c and E, e for most practical purposes behave as pairs of antithetical antigens, although alternative alleles occur at the Cc, D, and Ee loci. Individuals who lack any of these Ags may be stimulated to produce the corresponding antibodies by transfusion or pregnancy . Of antibodies , anti-c, anti – E and anti – e occur fairly frequently either as single antibodies or in combination with one another or with Abs out side the Rh-system . Anti C is

rare as a single antibody , although it occurs more commonly in Rh negative subjects in combination with anti-D (Neville,1994).

2.3.3 Chemical approach to the D antigen:

Rh substances are not detectable in any appreciable amount in body fluids, so this source which has been so rewarding in the study of A ,B,H and lewis substances is not available for the chemical study of Rh and the amount of Rh substances which can be extracted from the red cell memberane is very small. It has been found that Rh antigens are more heat labile than A and B antigens, their PH stability also differs in that the Rh activity of dried red cell membrane is unstable below PH 5.8 and above PH 9.2 (Green,1965).

These characteristics are suggestive of a protein rather than carbohydrate structures might be connected with D specificity was obtained from the fact that the antigenicity is readily destroyed by treatment of red cells with dilute periodate at a concentration so low that even A and B antigens which are known to be carbohydrate ,are not destroyed (Kathleen E ,1988).

2.3.4. Alleles at the D locus (D^u):

A new allele at the D locus, called D^u was first described by Stratton (1946). Race and Colleagues (1948) defined Du as red cells that give some but not all of the reaction expected of D. Specifically that D^u red cells were agglutinated by some anti D sera and not by other, D^u cells when compared with normal D cells, take up only 7 to 25 percent as much anti D. The D^u antigen to be genetically inherited from laboratory stand point D^u may simply regarded as a weak from of the D antigen.

There are many grades, D^u, red cells of the higher grade of D are agglutinated by certain anti D sera, while red cells of the lower grad are detectable only by the in direct antiglobulin test, certain lower grade D^u may only be detectable by absorption and anti D from the red cells .(Neville, 1994).

2.3.4.1 The clinical single significances of D^u:

D^u is a much less effective antigen than D with respect to the stimulation of anti D, however, D^u red cells may be destroyed at an increased rate by anti D, and D^u infant can suffer from HDN if the mother possesses anti D .

In addition a severe hemolytic transfusion may result from the transfusion of D^u positive red cells to a recipient whose serum contains anti D (Neville,1994).

Table (2-4): The frequency of Rh (D) factor in various populations:

Population	Rh(D) Negative	Rh (D)Positive
European	Approx 35%	65%
Caucasian	16%	84%
American blacks	Approx 7%	93%
Native American Indians	1%	99%
African descent	Less 1%	Over 99%
Japanese& Chinese	Less 1%	Over 99%

Table (2-5): The frequency of Rh (D)in Sudanese ethnic group (%).

Ethnic Group	Rh(D) positive
Dangala	94
Halaween	89
Hadandawa	83
Miseria	83
Nuba	29
Shaigia	94
Alshokria	96

2-4 Inheritance of the ABO groups

According to the generally accepted theory of Bernstein the characters A,B and O are inherited by means of three allelic genes, also called A, B and O called A,

B and O. The O gene is considered to be silent since it does not appear to control the development of an antigen on the red cell Kathleen, E,1988).

In 1911 Van Dungeon and Herzfeld showed that A antigen could be divided into subgroups A_1 and A_2 and in 1930 Thompson proposed a four allele theory of inheritance to encompass these subgroups .Under the terms o the theory of Thompson , four allelic genes A1 , A_2 , B and O . Since each individual inherits one chromosome from each parent for each chromosome pair, two genes are inherited for each characteristic and these four allelic genes give rise to ten possible genotypes (Hoffbrand& Pettit , 2001).

Table (2-6) The ABO phenotypes and their corresponding possible genotypes

Phenotypes	Possible Genotypes
A_1	A1 A_1 A_1 O,A_1 ,A_2
B	BB,BO
O	OO
A_1 B	A_1 B
A2	A_2 A_2 ,A_2 O
A_2 B	A_2B

Blood group antigens are stable characteristics controlled by genes inherited in a simple Mundelein manner (Hoff brand & Pettit, 2001).

2.4.1 Blood group nomenclature

Logically, the first letter of the alphabet were discovered systems then there was a leap to M, N and P, still maintaining capital letters However the alleles associated with M N were named S and s. In some other systems the individual in whom the first antibody was detected . Thus Mrs. Kell, Messrs Lutheran and Duffy and Mr. Kidd have all leat their names to systems . Sometimes the first. Common allele are usually designated a and b, resulting in Fy b . Lu a Lu b etc, (Kathleen E,1988).

2.4.2 Classification of the blood groups

Classification of the blood groups was based on the realization had occurred because the red cells possessed an antigen and the 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 observations, Landsteiner recognized three separate groups, named according to the antigen present on the red cells (Neville, 1994).

Table (1-7): Classification of the ABO blood groups.

Antigen on red cells	Antibodies in serum	Blood group
A	Anti B	A
B	Anti A	B
Neither A nor B	Anti A and anti B	O
A and B	Neither anti A nor anti B	AB

2.4.3 The gene H , its role in expression of A and B genes

The expression of the A and B genes appears to depend on the action of another gene, Known as H . Most individuals are homozygous for this gene (i.e. HH) , although because its allele (h) is A morphic gene the heterozygote Hh cannot be recognized, the phenotype h (genotype hh), is extremely rare (the Bombay phenotype) .(Hoff brand & Pettit, 2001).

All humans contain RBCs with A –type blood also contain an additional enzyme (called A- type enzyme) which adds N- Acetylgactosamine to the O antigen. Humans with B-type blood contain another enzyme (called B- type blood contain another enzyme (called B- type enzymes) which adds Galactose to the O antigen . Humans with AB – type blood contain both A-type and B-type enzymes while humans with O-type blood lack both types of enzyme . According to the ABO blood typing system they are four differenced kind of blood typing A ,B and O(null) .(Nobel Web A B,2007).

2.4.4 Importance of ABO system

The 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 red cells in vivo . The ABO system was the first to be recognized , and remain the most important (Hoff brand & Pettit, 2001).

2.4.5 The Antigens of the ABO system

The majority of human bloods can be grouped into six main ABO phenotypes, although several other rare variants can distinguish serologically.

2.4.5.1 The subgroups of A

The distinction between the A1, and A2 subgroups anti A1 which will agglutinate A1 but not A2 red cells . A1 can be obtained in several ways:

1- Can be made by absorbing anti A (from group B people) with A2 cell .
2- It can be made from extract of the seeds of the Dolichos biflorus .
3- It is found in the serum of some A_2 and A_2 B persons .
4- Monoclonal anti – A1 is also available .

Anti A1 is not used routinely in most laboratories since it is not necessary to distinguish group A1 from group A_2 blood for most recipients. There is no specific antibody for A_2 red cells. Group B serum containing two antibodies : Anti A, which agglutinates both A_1 and A_2 red cells and anti A1 which agglutinates only A_1 red cells, the anti A component of group O serum also has both antibodies. The difference between the A_1 and A_2 subgroup is partly quantitative, the red cells of A_1 and A_1 B subjects have more A antigen sites than A_2 and A_2B subject respectively.

There is also evidence that is a qualitative difference between A_1 and A_2 cell:

(1) Hakomori other workers have obtained more polymorphic types of blood group A determinants from A1 red cells than from A_2 red cell.

(II) A_2 and A_2B people sometimes have anti A_1 in their serum.

For practical purposes, A_2 can be regarded as a weaker form of A_1 (Hoff brand & pettitm 2001).

Approximately 80 percent of blood group A individuals belong to the sub- group A_1 and 60 percent of group A B blood to the sub- group A_1 B, the remainder belong to the sub- group A_2 and A_2B Various grades of increasingly weak A agglutinogen known as A_3 (Friendenriech, 1936) A_4 , A_5 , A_6 (Jonsson and Fast, 1948) have been described as well as agglutinogen, named AO not agglutinated by potent anti – A from group B subject, but only by anti A from group O (Rasmussen, et al, 1952).

The recognition of the sub- groups necessarily modifies the concept of the complete safety of the universal donor and universal recipient (Sir Lionel , 1953).

2.4.5.2. The H antigen

The h gene segregates independently from ABO the H antigen is present to some extent on all red cells regardless of the ABO group (except Bomb red cells) but the amount of antigen varies with the ABO group as following O>A2 >A2,B> B>A1>A1B(Hoffbrand & Pettit , 2001) .

2.4.5.3 Development of the A, and H antigens

The A and B antigens can be detected on the red cells of very young fetuses , but their reactions are weaker than those of adult red cells. (Table 3) the number of A and B antigen site is less on cord red cells than on adult red cells, the H antigen is less well developed at birth than in adult life, After birth, the agglutinability of red cells anti A , anti B and anti H increase until about 3 years of age, and thereafter, in health remains stable throughout life (Hoffbrand &Pettit , 2001).

Table (2-8): Number of A and b antigen sit on red cells O Various A and B groups:

Blood group of red cell	Approximate number of A antigen
A_1 adult	1000.000
A_1 cord	300.00
A_1 B adult	500.000
A_1 B cord	220000
A_2 adult	250.000
A_2 cord	140.000
A_2B adult	120.000
Blood group of red cell	Approximate number of B antigen sites
B adult	700.000
A2B adult	400.000

2.4.5.4 The distribution of the A and B antigens

A and B antigens are not confined to red cells . There is evidence that they are present on white cells , platelets , epidermal and other tissue cells, they are also present in an alcohol- soluble form in plasma of people of suitable ABO groups whether they are secretors or non- secretors, and in the saliva and secretion of ABH secreretors (Hoffbrand & Pettit, 2001)

2.4.6. Rare ABO variants

Rare ABO varints are usually disclosed because an expected ABO Antibody is missing . Thus a sample typed as group O that has anti- B but no anti – A will usually prove to be a weak A variant. The presence of weak A or B antigens can be demonstrated either by using potent antisera or by absorption and elution (Hoff brand & Pettit, 2001).

2.4.7 ABH secretor status

The majorities of the British population is secretors of the appropriate A, B and H substances and have these antigens in all their body fluids. The saliva is normally tested in order to determine secretor status.

Approximately 20% of people are non secretors and lack these antigens in their body fluids regard less of the ABO group on their red cells . The term secretor applies to the H antigen together with the appropriate A and B antigen which are present in a water- soluble form in secretions . The gene responsible for secretion , se , can be regarded as an operator gene which causes the H antigen , and as consequence the appropriate A and B antigen , to be expressed in all body fluids . Se is active in homozygote and heterozygote and thus non- secretors must be homozygous for the allele se (Hoff-brand & Pettit, 2001).

2.4.8 Antibodies of the ABO system

1- Anti – A and Anti B

Development of anti A& B, sera taken from people over the age of about 6 months which do not contain the expected A and B antibodies are very rare . The person may have blood of rare sub group of A or may be blood – group chimera or may have a congenital absence of IgM. The ABO antibodies arise in response to A and B like antigens present in bacterial , viral or animal molecules which enter the body in various ways (Hoffbrand & Pettit , 2001)

Immune anti A and anti B can produced by persons of the appropriate ABO groups after immunization with suitable red cell, or blood group substances. Naturally occurring anti A and B are cold antibodies in that they react better at lower temperatures than at 37°C. The antibodies from group O person even before immunization usually have some IgG anti A, B After appropriate immunization the thermal characteristics of the antibodies change, but group A and B persons continue to produce anti bodies that are mainly of the IgM class, although some IgG is usually made. Some IgA anti A or anti B is produced following immunization with A or B substances. (Hoffbrand & Pettit, 2001)

Table (2-9): Some properties of immune and naturally occurring anti A and anti B.

	Naturally occurring	Immune	
	IgM	IgM	IgG
1-Complement binding at 37°C	++/++	+++	++
2- Agglutination of appropriate cells	+++	+++	+++
3- Cross the placenta	0	0	+++
4-Enhanced by anti IgG	0	0	+++
5-Inhibited by A or B substance (saliva)	+++	+++	0
6. 2-ME* or DTT** sensitive	+++	+++	0

* 2-Mereaptoethanol

** DTT, Dithiothreitol

2-Anti –A1

Anti A1, reactive at room temperature (18-22°C) can be found in the serum of 1-8% of group A2B persons. Most of these antibodies are more of a nuisance incompatibility test than of clinical importance, because they often do not agglutinate A1 red cells at 30°C and above and so are unlikely to result in increased in vivo red cell destruction . (Hoffbrand &Pettit , 2001).

3- Anti –H

Several forms of anti H exist

A- Clinically significant true anti H occurs in the serum of persons with Bombay blood and is very rare.

B- Normal incomplete cold antibody is present in all normal sera .

C- Anti H and anti H1 , commonly found in the serum of group A1,B and A1 B person , gives similar patterns of agglutination with adult and cord red cells.

Anti H is inhibited by secretor saliva and anti – H1 is not . (Hoffbrand & Pettit 2001)

Table (2-10): ABO blood groups and incidence (%)

Phenotypes	Whites	African Americans	Asians
A_1	43	19	27
A_2	10	8	Rare
B	9	19	25
A_1 B	3	3	5
A_2 B	1	1	Rare
O	44	49	43

Table (2-11): Allelic frequency of ABO groups studies in different population (%)

B.Group	Kuwait	Kenya	Nigeria	India	Turkey
O	66.78	69	48	32.5	22
A	16.08	15.8	24.43	24.7	22.2
B	14	12.6	23.88	37.5	38.2
AB	3.265	2.39	2.75	5.3	14.8

Table (2-12): The frequencies of ABO groups in a few selected populations . The percentage of various phenotypes

Population	O	A1	A2	B	A1B	A2B	Special characteristic
South American Indians	100	0	0	0	0	0	AIIO
Vietnamese	45	21.4	0	29.1	4.5	0	No 2 Bcommoner than A
Australian	44.4	55.6	0	0	0	0	No A2 orB
Germans	42.8	32.5	9.4	11.0	3.1	1.1	-
Bengalese	22	22.2	1.8	38.2	14.8	0.9	B Commonest
Lapps	18.2	36.1	18.5	4.8	6.2	6.2	Very high A2

Table (2-13): Blood groups frequency distribution (%)

B.Group	Whites	Blacks	Americans	Orientals	British population
O	45	49	79	40	46.7
A	40	27	16	28	41.7
B	11	20	4	27	8.6
AB	4	4	1	5	3

Table (2-14): The frequencies of ABO groups in some Sudanese tribes (%)

Blood Group	Alshokria	Arrakein
O	64	39
A	22	30
B	11	27
AB	3	4

2-5 Previous study

The frequency of A subgroups in the population from south India is. The aim of the study was to study the frequency of A subtypes and the prevalence of anti-A_1 antibody among this population.

Results of previous study:

A total of 40,113 patients' samples were typed for ABO, Rh group and A subgroups in our blood bank attached to a tertiary care hospital. Among 10,325 group A samples, 98.14% classified as A_1, 1.07% as A_2, and 0.01% as weak A; the remaining group A samples were from neonates and reacted poorly with anti A_1-lectin. The majority of AB samples (n=2,667) were of A_1B type (89.28%). However, the proportion of A_2B (8.99%) among AB samples was significantly higher than that of A_2 in group A samples ($p < 0.0001$). The prevalence of anti-A_1 antibodies among A_2 and A_2B samples was 1.8% and 3.75%, respectively, and none of them showed reactivity at 37°C.

Chapter Three

Materials and Method

Materials and methods

3.1: Study design

This is descriptive analytical prospective cross sectional study conducted in Shendi University faculty of medical laboratory science and aimed to determine the prevalence of A subgroup (A1 and A2) among the students.

3.2: Study population

A total of 100 peripheral blood samples were collected from different batch of students with different ages and gender.

3.3: Study area

The research was done in Shendi city, in faculty of Medical Laboratory Sciences which is located 172km north to the capital Khartoum.

Most of the population working in agriculture, simple industrial works, employer and trading.

The town consider as a centre of Galion tribe and other tribes. There were several general centers for different services and purposes, also there were university with various faculties such as faculty of medicine and health sciences Shendi has two big hospital. Educational hospital and Elmek Nimer university hospital and all of them have different department which provide a good health services for town's population.

3.4: Methods

They are two methods uses in blood group identification.

Slide method (direct).

Tube method (in direct).

3.4.1 Slide method

3.4.1.1 Principle

Aim to detect the present of antigen in the red cell.

- Aimed clearance.
- Data analysis.

3.4.1.2 Equipment and Reagent

Lancets.

Cotton.

Alcohol.

Slides.

Reagents (anti A 1).

3.4.1.3 Procedure

Put in clean slide one drop from blood put anti-A1 used wood stick mix rotation of slide.

-See agglutination that is means antigen bound with corresponding antibodies.

-No agglutinate means missed of correspond.

2.5 Method

2.5.1 Collection of blood samples

Two and half ml of venous blood were collected in EDTA anticoagulant containers from any participant.

2.5.2 Particle immunodiffusion (ID)gel card technique (Dia Med – ID Micro Typing system).

(i) Principle

Blood sample was applied in a single purpose –micro tube filled with nutrient gel or agar gel containing specific antibody. The gel was a suspension of porous microspheres whose size and distribution were selected to produce settling of non agglutinated cells at the bottom of the micriosphere and retention of agglutinated cells in the top at various levels.

The retention of cells indicates a positive agglutination reaction where settling of cells indicates no reaction with the specific antibody and hence a negative result (Mollison, 1997).

2.3.4 Blood samples:

Five percent of red cells suspension was prepared was in ID-diluent 1 as follows:

The diluents was allowed to warm at room temperature before use .

500 l of ID-diluent 1 was dispensed into clean tubes.

Fifty l of whole blood was added.

The cell suspension was used after incubation for 10 minutes at room temperature.

2.5.2 Slide test procedure

1- A new glass slides were washed and labeled with patient's number.

2- The slides were pre- heated.

3-Fifty ul of the respective reagents was pipette onto the slide .

4-Fifty ul of red blood cells was added.

5-The mixture was mixed well with a clean mixing stick.

6-The slided were gently rotated for two minutes.

7-Macroscopic agglutinantion was observed .

2.5.3 Interpretation of the results

The results were as follows

Positive: Agglutination of + (fine visible granulary) to ++++ (visible large single agglutinate) is indicative of a reaction between the antibody and the red cells for respective antigens.

2.5.4 Interpretation:

Agglutination or Hemolysis constitutes appositive test.

Chapter Four

Results

Results

This descriptive prospective analytical cross sectional study conducted faculty of Medical Laboratory Sciences, in Shendi university during the period from March to August 2018 and aimed to determine frequency of sub group of A blood group phenotype (A1 and A2).

According to the table and figure (4-1) the common sub group of A was A1 with frequency of (92%), while A2 with frequency of (8%).

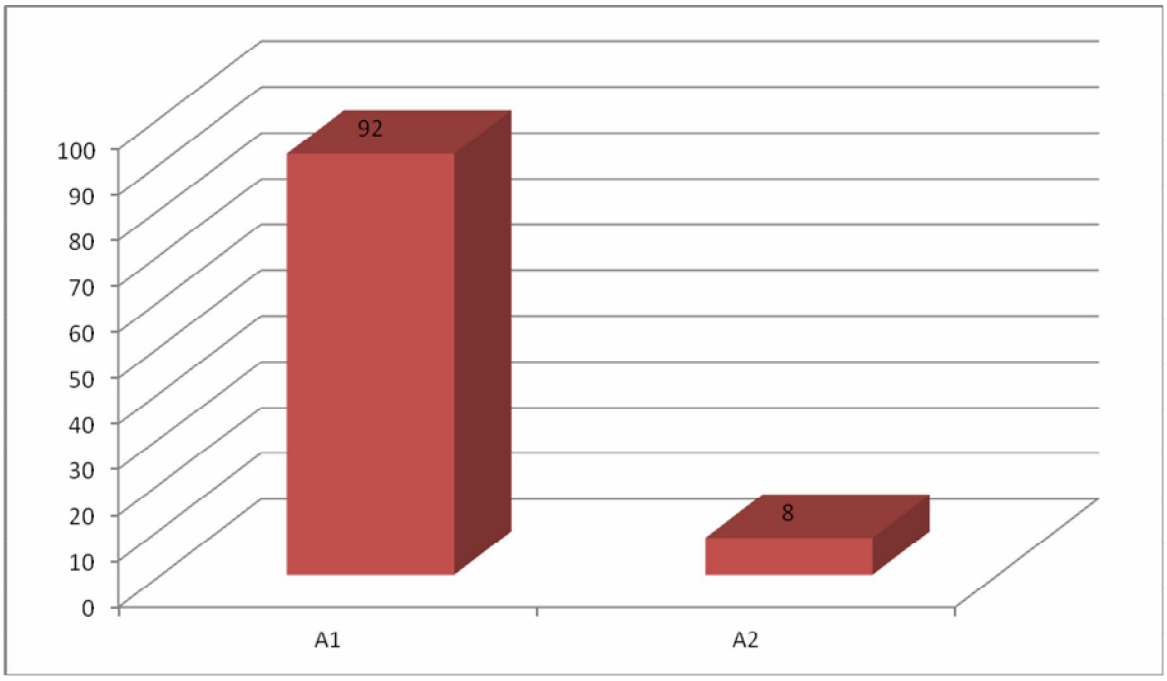
Also according to table and figure (4-2) the common sub group of A phenotype in male was A1 with frequency of (90%), while A2 phenotype was (10%).

Also according to table and figure (4-3) the common sub group of A phenotype in female was A1 with frequency of (92.5%), while A2 phenotype was (7.5%).

Table (4-1): Showed the frequency of sub group A phenotype (A_1 ,A_2):

Subgroup A	Frequency	Present
A1	92	92%
A2	8	8%
Total	100	100%

Figure (4-1): Showed the frequency of sub group A phenotype (A_1, A2):

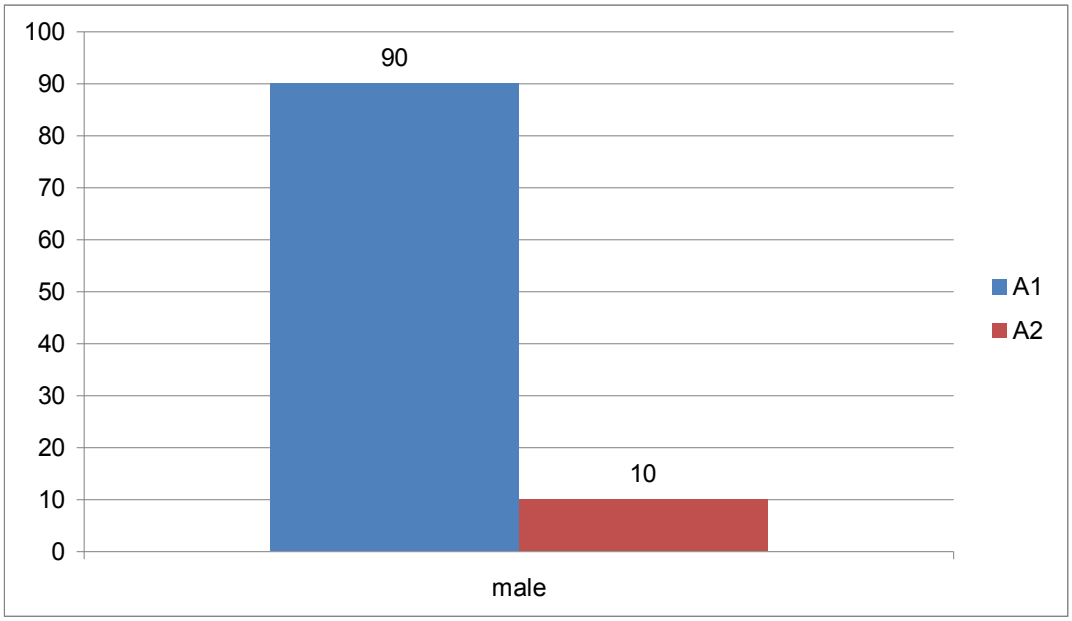


According to table and figure one it is clear that A1 is common (92%) for A1 while (8%) for A2.

Table (4-2): Showed the frequency of sub group of A phenotype (A_1,A_2) In Male:

Blood group	A1	A2
Male	90%	10%

Figure (4-2): Showed the frequency of sub group of A phenotype (A_1,A_2) In Male :

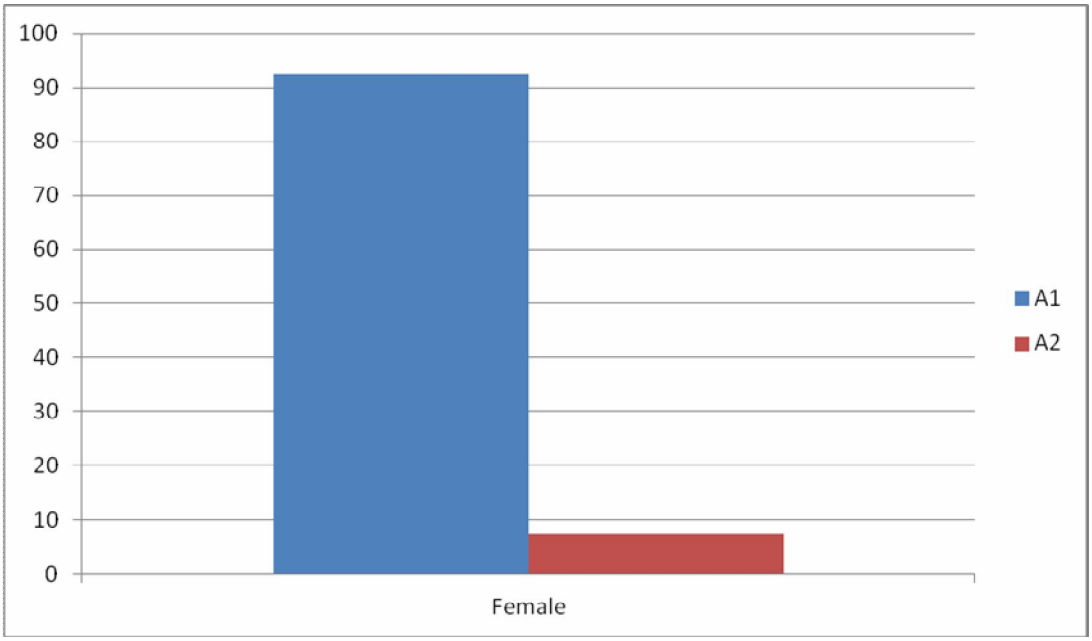


According to table and figure one it is clear that A1 is common (90%) for A1 while (10%) for A2.

Table (4-3): Showed the frequency of sub group of A phenotype (A_1,A_2) In Female:

Blood group	A1	A2
Female	92.5%	7.5%

Figure (4-3): Showed the frequency of sub group of A phenotype (A_1,A_2) In Female:



Chapter Five

Discussion
Conclusion
Recommendations

5-1 Discussion

This descriptive prospective analytical study which conducted Shendi university during the period from March to June 2014 and aimed to determine frequency of sub group of A blood group phenotype (A1 and A2).

Our results revealed that the common sub group of A was A1 with frequency of (92%) and, while A2 with frequency of (8%). This result was similar to previous study made in south India.

According to table and figure (4-2) A1 was the common sub group of A phenotype in male with frequency of (90%) and A2 was (10%).

Also according to table and figure (4-3) A1 was the common sub group of A phenotype with frequency of (92%) and A2 was (7.5%)

5-2 Conclusions

- A1 phenotype with frequency of (92%).
- A2 phenotype with frequency of (8%).

5.3 Recommendations

By the end of this study we recommended that:

- Further study should be done in this topic with increased sample size and study area to obtain accurate results.
- Subgroup of ABO blood group system should be performing routinely in blood bank.
- Studies in other blood group system should be carrying out to provide basic data about their prevalence.
- Uses of jell technique termination of sub A blood group.

Chapter Six

References

Appendix

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Appendix (1) Questionnaire

Shendi university

College of Graduate Studies

Frequency of Sub A Blood Group

- Name:
- Age:
- Sex:
- Tribe:
- Telephone number:
- Presence of inheritance disease
- Sub A blood group.

Ag	A1	A2
Result		

- Date:
- Signature:

Appendix II

بسم الله الرحمن الرحيم

جامعة شندي

كلية الدراسات العليا

بحث تكميلي لنيل درجة الماجستير

إقـــــــــرار بالموافقـــــــة

الاسم:----------

سوف يتم أخذ عينة من الدم بحجم 2.5 مل من الوريد بواسطة حقنة طعن معقمة وذلـــك بعد تعقيم منطقة أخذ العينة بمطهر.

كل الأدوات المستخدمة لأخذ العينة معقمة متبع فيها كل وسائل السلامة المعملية ولـــيس هنالك آثار جانبية للعملية.

أوافق أنا المذكور أعلاه على أخذ عينة دم لإجراء الدراسة.

الإمضاء : ----------

التاريخ :----------

Appendix (III):

Anti A1:-