

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**  
**College of Graduate Studies**

**Determination of ABO Blood Groups and Rh phenotypes  
Among Sudanese Patients Infected with Helicobacter pylori  
– Khartoum State**

تحديد مجموعات الزمر الدموية لدى السودانيين المصابين ببكتريا المعدة في ولاية  
الخرطوم

**A Dissertation Submitted in Partial Fulfillment for The Requirement of the M.sc  
degree in Medical Laboratory Science (hematology and Immunohematology)**

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# الآية

قال تعالى :

قَالُوا سُبْحَنَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم



سورة البقرة الآية

## *Dedication*

*` To my father who gave me confidence*

*To my mother who taught me the meaning of  
live and gave me love*

*To my brother who struggled a lot to educate  
me with smiling face*

*To my wife*

*To my daughters (Rawan–Mattab)*

*To my brothers and sisters*

*To my teachers*

*To my friends*

*To my country "Sudan"*

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*Primary may praise and thanks should be to Allah, the almighty most gracious and most merciful, who granted me the serenity, means of strength and patience to accomplish this work.*

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## **Abstract**

This is a descriptive cross-section study has been carried out in period from March to June 2014 to determine the frequencies of ABO blood group and Rh phenotype among Sudanese population infected with *H. pylori* in Omdurman Teaching Hospital. One hundred (100) blood samples were collected from unrelated individuals, 45 were males and 55 were females, whose age range between 11 to 75 years.

Samples were tested for *H. pylori* by using Rapid *H. pylori* Antibody test and also, tested for ABO blood group and Rh phenotype by using slide and tube methods. The frequencies of this study showed the blood group O (46%) followed by group B (29%), group A (23%) and the lowest proportion is noticed in AB blood group (2%). The possible phenotype found that cDe (39%) was highest observed percent frequency is followed by CcDe (27%), ce (11%), CDe (8%), cDEe (8%), CDe (3%), Cce (2%), cD (1%), and CcDEe (1%).

The result of the study showed there an association between ABO blood group and *H. pylori* infection. The results showed variation in infection with *H. pylori* according to gender that found females more prone to infection than males (55% respectively 45%).

## الملخص

هذه دراسة وصفية مقطعية آنية تحليلية اجريت في الفترة من مارس الى يونيو عام 2014م لقياس النسب المئوية للزمر الدموية (ABO blood group and Rh phenotype) لدى السودانيين المصابين ببكتيريا المعدة المترددون على مستشفى ام درمان التعليمي.

تم جمع مائة عينة دم من الافراد المصابين بعد اخذ موافقة كل منهم، 45 رجل و 55 امرأة وتراوحت اعمارهم ما بين 11 الى 75 سنة.

تم اختبار العينات لوجود بكتيريا المعدة عن طريق اختبار الاجسام المضادة في بلازما دم المريض باستخدام كرت التحليل، كما تم ايضا اختبار الزمر الدموية باستخدام طريقة الشريحة و انبواب الاختبار.

أظهرت الدراسة أن الزمرة الوظيفية (O) كانت الأعلى بنسبة 46 % وتليها الزمرة (B) بنسبة 29 % والزمرة (A) بنسبة 23 % والزمرة الوظيفية الأقل تردد وجدت هي (AB) بنسبة 2 %.

كما وجدت توزيع الانماط الظاهرية للعامل الريصي  $\bar{c}De$  هي الاعلى بنسبة 39 % تليها الانماط الظاهرية  $C\bar{c}D\bar{e}$  بنسبة 27 % و  $\bar{c}\bar{e}$  بنسبة 11 % و  $CD\bar{e}$  بنسبة 8 % و  $\bar{c}DE\bar{e}$  بنسبة 8 % و  $CDe$  بنسبة 3 % و  $C\bar{c}\bar{e}$  بنسبة 2 % و  $\bar{c}D$  بنسبة 1 % و  $C\bar{c}DE\bar{e}$  بنسبة 1 % .

اثبتت النتائج وجود علاقة ذات دلالة احصائية بين زمر الدم والإصابة ببكتيريا المعدة . كما وجدت فروق ذات دلالة احصائية في العدوى ببكتيريا المعدة حسب النوع ذلك أن النساء اكثر عرضة للإصابة بنسبة 55 % والرجال اقل عرضة للإصابة بنسبة 45 %.

## List of Contents

Subject	Page
الآية	I
Dedication	II
Acknowledgement	III
Abstract(English)	IV
Abstract(Arabic)	V
List of contents	VI
List of tables	X
List of abbreviation	XI
<b>Chapter one</b>	
Introduction and literature review	
	<i>1</i>
1.1 Introduction	<i>1</i>
1.1.1 blood group system	<i>3</i>
1.1.2 Discovery of ABO blood groups and Rh system	<i>5</i>
1.1.3 The ABO system	<i>5</i>
1.1.4 The inheritance of blood group	<i>6</i>
1.1.5 ABO Antigen Expression	<i>7</i>
1.1.6 bomby phenotype	<i>8</i>
1.1.7 Secreter and non-secreter	<i>8</i>
1.1.8 Cis AB	<i>9</i>
1.1.9 Development of the A, B and H antigen	<i>9</i>
1.1.10 The distribution of the A, B antigens	<i>9</i>
1.1.11 Biochemistry of ABH antigen	<i>10</i>
1.1.12 Antibodies of the ABO system	<i>10</i>
1.1.12.1 Anti-A and anti-B	<i>11</i>
1.1.12.2 Anti-A	

1.1.12.3 Anti-H	12
1.1.12.4 Crossreacting A and B	12
1.1.13 ABO system and disease	13
1.1.14 Rh blood group system	14
1.1.14.1 The antigen of Rh system	14
1.1.14.2 The Rh genotype	14
1.1.14.3 Rhesus antibodies	15
1.1.14.3.1 Rh Naturally occurring antibodies	15
1.1.14.3.2 Rh immune antibodies	15
1.1.15 Clinical significant of the blood group	16
1.1.15.1 Haemolytic Transfusion Reactions	16
1.1.15.2 Haemolytic disease of the newborn(HDN)	17
1.1.15.3 Universal donors and universal recipients	18
1.1.16 Blood group test ABO and Rhesus D	19
1.1.16.1 ABO grouping	19
1.1.16.2 Rh D grouping	20
1.1.16.3 Preparation of ABO reagents	21
1.1.16.4 Lectins	21
1.1.16.5 Techniques in blood group serology	22
1.1.16.6 Quality control of blood group serology	24
1.1.17 Helicobacter Pylori	24
1.1.17.1 General properties of H. pylori	25
1.1.17.2 Epidemiology of H. pylori	26
1.1.17.3 Genome of H. pylori	26
1.1.17.4 Transmission of H. pylori	27
1.1.17.5 Signs and symptoms of H. pylori	27
1.1.17.6 Helicobacter associated disease	27



1.1.17.7 Diagnosis of H. pylori infection	28
1.1.17.7.1 Rapid Urease Test	28
1.1.17.7.2 Polymerase Chain Reaction	29
1.1.17.7.3 Serodiagnosis	29
1.1.17.7.4 Urea Breath Test	29
1.1.17.7.5 Faecal antigen test	30
1.1.17.7.6 Endoscopy	30
1.1.17.8 Prevention of H. pylori	30
1.2 Literature review	32
1.3 Rationale	40
1.4 Objective	41
1.4.1 General objective	41
1.4.2 Specific objective	41
<b>Chapter two</b>	
2.1 Study design	42
2.2 Study population	42
2.2.1 Inclusion criteria	42
2.2.2 Exclusion criteria	42
2.3 Data collection	42
2.4 Sample collection	42
2.5 Ethical consideration	42
2.6 Laboratory requirement	43
2.7 Methods	43
2.7.1 Tube method	43
2.7.1.1 Principle	43
2.7.1.2 Reagents	43

2.7.7.3 Procedure	44
2.7.1.4 Interpretation	44
2.7.2 Slide method	45
2.7.2.1 Definition	45
2.7.2.2 Procedure	45
2.7.3 Rapid H. Pylori Antibody Test	45
2.7.3.1 Principle	45
2.7.3.2 Procedure	46
2.8 Data analysis	46
<b>Chapter three</b>	
Results	47
<b>Chapter four</b>	
4.0 Discussion, Concluts and Recommendation	51
4.1 Discussion	51
4.2 Conclusion	54
4.3 Recommendation	54
References	55
Appendix (1)	59
Appendix(2)	60
Appendix(3)	61

## List of tables

Table 1.1	The ABO system
Table 1.2	Glycosyltransferases produced by genes encoding for antigens with the ABO, H and lewis blood group system
Table 1.3	Secretors status
Table 1.4	ABO group-blood system
Table 1.5	ABO/Rh blood group distribution in patients
Table 1.6	Gender and blood group relation to Helicobacter pylori infected patients compared with general population
Table 1.7	Gender and blood group relation to Helicobacter pylori infection in seropositive
Table 3.1	Frequency of H. Pylori in infected patients according gender
Table 3.2	Mean values of IgG Ab of H. Pylori positive according to age group
Table3.3	Distribution of Rh(D) antigen among study population
Table 3.4	proportion of Rh(C) Antigen among study group
Table 3.5	Frequency of Rh (E) antigen among study population
Table 3.6	Distribution of Rh ( $\bar{c}$ ) antigen among study group
Table 3.7	Frequency of Rh ( $\bar{e}$ ) antigen among study population
Table3.8	Distribution of ABO blood group phenotype among study population
Table3.9	phenotype frequency distribution of Rh blood group among study group

## List of abbreviations

HDFN	Haemolytic disease of fatal and Newborn
GTRs	General Transfusion Reactions
Se	Secretor gene
Oh	Bombay phenotype
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IgG	Immunoglobulin G
H. pylori	Helicobacter pylori
HDN	Haemolytic Disease of the Newborn
RBCs	Red Blood Cells
EDTA	Ethylene Diamine Tetra Acidic acid
AHG	Antihuman globulin
Vac A	Vacuolating cytotoxin
PCR	Polymerase Chain Reaction
rRNA	ribosomal Ribonucleic acid
EIAs	Enzyme-linked Immunoassays
RUT	Rapid Urease Test
ELFA	Enzyme linked fluorescent Assay
ISR	Immune Status Ratio
RDT	Rapid Diagnostic Test
LISS	Low Ionic Strength Saline
ISBT	International Standardization of blood Transfusion

# **Chapter one**

**Introduction and Literature review**

# Chapter one

## **Introduction and Literature review**

### **1-1 Introduction**

#### **1-1-1 blood groups system**

Human red blood cells contain on their surface a series of glycoproteins and glycolipids which constitute the blood group antigens. The development of these antigens is genetically controlled; they appear early in fetal life and remain unchanged until death. On the basis of these antigens, at least 15 well-defined red cell blood group systems of wide distribution in most racial groups have been described (Frank et al.,1996). They are ABO, MNSs, P, Rh, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Yt, Xg, Li, Dombrock, and Colton systems; of these only two are of major importance in clinical practice the ABO and Rh systems(Frank et al.,1996)

ABO was the first blood group systems to be discovered. In 1900, Landsteiner mixed sera and RBCs from his colleagues and observed agglutination. On the basis of the agglutination pattern, he named the first two blood group antigens A and B, using the first two letters of the alphabet. RBCs not agglutinated by either sera were first called type C and finally called O (Christopher et al.,2009).

Rh blood group system was described by Landsteiner and Levine in 1940. The Rh system is very clinically important is being able to cause Hemolytic Disease of the Newborn (HDN) during pregnancy or a reaction following transfusion .Antibodies to the Rh system antigen can only be produced as result to of stimulation by red cells. These antibodies are called "immune" whereas the antibodies of the ABO system are

known as "natural" since they occur with stimulation by pregnancy (Phil learoyo et al.,1999) .

Other blood group system such as Kell, Duffy and Kidd occasionally cause problems during transfusion .Their " immunogenicity " is considerably less than ABO and Rh and matched antigens-identical blood is only needed when clinically significant " immune " antibodies have been formed .Lewis antigen ( $Le^a$  and  $Le^b$ ) are located on soluble glycosphingolipids found in saliva and plasma and are secondarily absorbed to the red cell membrane from the plasma. Lewis antibodies are naturally occurring and are usually IgM and complement binding. Kell blood group system is an interesting mix of high-and-low frequency antigen. The first one was discovered in 1946 shortly after the introduction of antiglobulin testing. It was defined by an antibody in the serum of Mr. Kelleher. An immune anti-K is the commonest antibody found outside the ABO and Rh system. It is commonly IgG and occasionally complement binding Duffy blood group system (FY) was discovered in 1950. It was defined by an antibody in the serum of Mr. Duffy who had been transfused for several times during preceding 20 years (Darcie and Lewis, 2001)

Kidd blood group system (JK) is defined by two alleles,  $JK^a$  and  $JK^b$  ,whose products were first identified with allo antibodies responsible for hemolytic disease of new born or transfusion . There are three common phenotype JK(a+b-), JK(a-b+), and JK(a+b+) and a rare null phenotype , JK(a-b-) (Daniel G, 1995).

Since red cells from JK(a-b-) individuals lack JK antigens exhibited an increase resistance to lyses in 2 M area transport (Frolich et al. ,1991),it was suggested that both phenotypes could be carried by an

single polypeptide ,this hypothesis was fully confirmed by the mother cloning of the urea transport expressed in human erythrocyte . The genes encoding the kidd/HUTII urea transport polypeptide has been assigned to chromosome 18q12-q21 by Sit hybridization (Olives.et al. ,1995) , where the Kidd blood group gene locus has been mapped (Greit et al. ,1987). The Kidd/urea transport protein is present on human red cell as well as in the kidney, particularly on the endothelial cells, of the vasa recta in the inner and outer medulla, but in not present in renal tubules (Olives, et al., 1997).

MNS, with a total of 46 antigens, is second only to Rh in complexity. These antigens are present on one or both of two red cell membrane glycoprotein, glycophorin A (GPA) and glycophorin B (GPB) they are encoded by tow homologous genes, GYPA and GYPB on chromosome 4. Anti-M and –N are not generally significant, though anti-M is occasionally hemolytic. Anti-S, the rare anti-s, and anti-U can cause HDFN and have been implicated in GTRs (Murphy and Pamphilon, 2009).

### **1.1.2 Discovery of ABO blood groups and Rh system**

Before 1901, the prevailing belief was that all human blood was the same. However, this changed in 1901 with Karl Landmark discovery of ABO blood groups. Landsteiner noticed that human blood mixed in test tubes with other specimens of human blood sometimes resulted in agglutination. By incubating red cells from some individuals with serum from others, he identified agglutination patterns, leading to the initial identification of three blood groups ,A,B, and C (C was later renamed O).(Christopher et al,2009). In 1902, Alfred Decastello and Adriano



Sturli, two of Landsteiner's former student, found the fourth blood group AB (Christopher et al.,2009).

Although a major discovery in transfusion medicine, ABO blood group typing was sufficient to prevent much fatal hemolytic transfusion reaction. In 1939 Philip Levine published a case report of post-transfusion hemolysis in a blood group O patient who received blood from her blood group O husband. Levine found that incubation of the patient serum with her husband's red cells resulted in agglutination. Additionally, the woman's serum was found to agglutinate 80 of 104 other sample of ABO-compatible blood. The name of the offending antibody came from parallel experiments conducted by Landsteiner and Alex Wiener in which antibody produced by immunization of rabbits and guinea pigs with blood from rhesus monkeys caused red cells agglutination of 85% of humans tested. Those individual whose red cells were agglutinated by those antibodies were classified as rhesus (Rh) positive (Christopher et al., 2009).

### 1.1.3 The ABO system

ABO is often referred to as histo-blood group system because, in addition to being expressed on red cells, ABO antigens are present on most tissues and in soluble form in secretions. Its most basic level, the ABO system consists of two antigens, A and B, indirectly encoded by two alleles, A and B of ABO gene. A third allele, O, produces neither A nor B. These three alleles combine to effect four phenotypes: A, B, AB and O (Michael and Derwood, 2009).

Table 1.1 The ABO systems (Michael and Derwood,2009).

		Frequency			
Phenotype	Genotypes	Europeans*	African ◊	Indians#	Antibodies present
O	O/O	43%	51%	31%	Anti-A,-B,-A,B
A <sub>1</sub>	A <sup>1</sup> /A <sup>1</sup> , A <sup>1</sup> /O, A <sup>1</sup> /A <sup>2</sup>	35%	18%	26%	Anti-B
A <sub>2</sub>	A <sup>2</sup> /A <sup>2</sup> , A <sup>2</sup> /O	10%	5%	3%	Sometimes anti-A <sub>1</sub>
B	B/B,B/O	9%	21%	30%	Anti-A
A <sub>1</sub> B	A <sup>1</sup> /B	3%	2%	9%	none
A <sub>2</sub> B	A <sup>2</sup> /B	1%	1%	1%	Sometimes anti-A <sub>1</sub>

\* English people

◊ Donors from Kinshasa, Congo.

# Maker from Mumbai.

### 1.1.4 The inheritance of blood group

The nucleus of each cell of the blood contains thread-link structures called chromosomes. In man, there are 46 chromosomes in 23 pairs. Each member of a pair contains the same number of genes and the gene loci are arranged in the same order (Kathleen, et al.,1988).

Agene is usually extremely stable and its replication at cell division is complete. Occasionally, however, it may undergo a change, called

mutation. A number of alternative genes occur at a particular locus. These are called alleles or allomorphic genes. Therefore, an individual possesses two and only two alleles are derived from each parent the identical is said to be homozygous for the particular allele, and if different alleles are derived from each parent the individual is heterozygous for alleles at the locus (Kathleen, et al,1988).

#### 1.1.5 ABO Antigen Expression

The expression of ABO antigen is controlled by three separate genetic loci: ABO located on chromosome 9, and *FUT<sub>1</sub>* (H) and *FUT<sub>2</sub>* (Se), both of which are located on chromosome 19. The genes from each locus are inherited in pair as Mendelian dominants. Each gene codes for different enzymes (glycosyltransferase) which attaches specific monosaccharides onto precursors disaccharide chain. There are four types of disaccharide chains known to occur on red cells, other tissues and in secretion. The type 1 disaccharide chain is found in plasma and secretion and is the substrate for the *FUT<sub>2</sub>* (Se) gene, whereas type 2, 3 and 4 chains are only found on red cells and are the substrate for the *FUT<sub>1</sub>*(H) gene. It is likely that the O and B genes are mutations of the A gene. The O gene does not encode for the productions of a functional enzyme; group O individual commonly have a deletion at nucleotide 261 (the *O<sub>1</sub>* allele) which result in a frame-shift, premature termination of translated polypeptide and the production of an enzyme with no catalytic activity. The B gene differs from A by consistent nucleotide substitution. The expression of A and B antigens is determined by H and Se genes, which both give rise to glycosyltransferases that add L-fucose, producing the H antigen. The presence of an A or B gene (or both) results in the production of further glycosyl transferases which convert H substance into A and B antigen by terminal addition of N-acetyl-D-

galactosamine and D-galactose respectively. Since the O gene produces an inactive transferase, H substance persists unchanged as group O (Dacie and Lewis, 2001).

Table 1.2 Glycosyltransferases produced by genes encoding for antigens within the ABO ,H and Lewis blood group systems(Dacie and Lewis,2001).

<b>Gene</b>	<b>Allele</b>	<b>Transferase</b>
FUT1	H	$\alpha$ -2-L-fucosyltransferase
	h	None
A	A	$\alpha$ -3-N-acetyl-D-galactosaminyltransferase
B	B	$\alpha$ -3 -D-galactosyltransferase
O	O	None
FUT2	Se	$\alpha$ -2-L-fucosyltransferase
	se	None
FUT3	Le	$\alpha$ -3/4-L-fucosyltransferase
	le	None

#### 1.1.6 Bombay phenotype (Oh)

The Bombay phenotype discovered by Bhende and Bhatia in 1952.Salient features of this phenotype is absence of A, B and H antigens on red cells and serum/plasma of these persons possess anti-A, anti-B and anti-H (Talib and Dutta,1995).

The Bombay phenotype (Oh) individual are therefor devoid of antigens present in ABO system which can be observed in vitro by lack of reaction of Oh phenotype red cells with antisera A, antisera B, antisera and antisera H whereas a person or individual with O group does not exhibit reaction with -A, -B, -AB but reacts very strongly with –H and this indicates clear differentiation between O and Oh individual .The importance of Oh lies in multiplicity of antibodies in serum and for

transfusion purpose a Bombay phenotype individual can receive blood from another Bombay phenotype individual only (Talib and Dutta,1995).

### 1.1.7 Secretor and non-secretor

The ability to secrete A, B and H substance in water-soluble form is controlled by FUT2 (dominant allele Se), in a causion population , about 80% are secretors(genotype Se Se or Se se) and 20% non-secretors (genotype se se).Secretors have H substance in saliva and other body fluids together with A and/or B substances depending on their blood group . Only traces of these substances are present in the secretions of non-secretors, although the antigens are expressed normally on their red cells and other tissues (Dacie and Lewis,2001).

Table 1.3 Secretor status (Dacie and Lewis,2001).

	Genes	Blood group of red cells	ABH substance present in saliva	Incidence(%)
Secretors	SeSe	A	A+H	80
		B	B+H	
	or			
	Sese	AB	A+B+H	
		O	H	
Non-sectors	sese	A, B, AB or O	None	20

### 1.1.8 Cis AB

In this group the members who are AB inherits both A and B from one parent, the other parent is usually O group .First described by Seyford et, al in 1964. In this phenotype B antigen is very weak. The condition is attributed to mutant gene producing a single enzyme (Talib and Dutta,1995).

### **1.1.9 Development of the A, B and H antigens**

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. The number of A and B antigens sites are less on cord red cells than on adult red cells. Similarly, the H antigen is less well developed at birth than in adult life. After birth, the expression of A, B and H antigens increases until about 3 years of age, and thereafter, in health, remain stable throughout life ( Hoffbrand, et al., 2001).

### **1.1.10 the distribution of the A and B antigens**

A and B antigens are not confined to red cells. They are present on white cells, platelets, epidermal and other tissue. They are also present in alcohol-soluble form in the plasma of people of suitable ABO groups, whether they are secretor or non-secretor of A , B or H , and in the saliva and secretion of ABH secretor ( Hoffbrand ,et al., 2001).

### **1.1.11 Biochemistry of ABH antigen**

A, B and H antigens can exist as glycoprotein in secretion of secretors but in cell membrane it exists as glycolipid. The basic difference in chemical reactivity of glycolipid and glycoprotein respectively shows that glycolipid is alcohol soluble and could be extracted by treatment with alcohol only. Subsequently it was observed that antigen are highly branched complex of glycosphingolipids-lipid part of which remain attached to red cell membrane whereas protein part exposed above is responsible for antigenic specificity. Glycoprotein is soluble antigen observed in at last 75% to 78% in individuals in their tissues and body secretion. Glycoprotein are water soluble and specificity corresponds to individual own blood group. Their presence was detected in almost all

body secretion except cerebrospinal fluid. They have been traced to saliva, urine, bile, sweat, tears, serum and gastric juice. The person in whom this antigens, called blood group substance are found are called secretors and whom it is not found are called non-secretors (Talib and Dutta,1995).

The polysaccharide portion of glycoprotein and glycosphingolipid are similar and the chemical constitution of two basic chain reveals presence of L-Fucose , N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-galactose (Talib and Dutta ,1995).

#### **1.1.12 Antibodies of the ABO system**

Naturally occurring antibodies occur in the plasma of subjects who lack the corresponding antigen and who have not been transfused or been pregnant. They are usually immunoglobulin M (IgM) and react optionally at called temperature,(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 possessing antigens which 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 . Only IgG antibodies are capable of transplacental passage from mother to fetus. The most important immune antibody is the Rh antibodies antibody anti-D (Hoffbrand, et al., 2001).

##### **1.1.12.1 Anti-A and anti-B**

ABO antibodies, in the absence of the corresponding antigens, appear during the first few months' after birth, probably as result of

exposure to ABH antigen –like substances in the diet or the environment (Dacie and Lewis, 2001).

The antibodies are a potential cause of dangerous hemolytic transfusion reactions if transfusions are given without regard to ABO compatibility. Anti-A and anti-B are always, to some extent, IgM (Dacie and Lewis, 2001).

Although they react best at low temperatures, they are nevertheless potentially lytic at  $37^{\circ}\text{C}$ . Hyper immune anti-A and anti-B occur less frequently, usually in response to transfusion or pregnancy, but they may also be formed following the injection of some toxoids and vaccines. They are predominantly of IgG class and are usually produced by group O and sometimes by group  $A_2$  individuals. Hyper immune IgG anti-A and/or anti-B from group O or group  $A_2$  mothers may cross the placenta and cause haemolytic disease of new born (HDN) (Dacie and Lewis, 2001).

#### 1.1.12.2 **Anti- $A_1$**

Anti- $A_1$ , reactive at room temperature ( $18-22^{\circ}\text{C}$ ), can be found in the serum of 1-8% of group  $A_2$  and 22-35% of group  $A_2B$  persons. These antibodies do not agglutinate  $A_1$  red cells at  $30^{\circ}\text{C}$  and above, and so are unlikely to result in increased in vivo red cell destruction. Very rarely, anti- $A_1$  able to agglutinate  $A_1$  red cells in vivo. The appropriate group  $A_2$  or  $A_2B$  red cells should be cross-matched in these rare instances (Hoffbrand et al., 2001).



#### 1.1.12.3 **Anti-H**

Anti-H occurs in the serum of persons with 'Bombay' blood and is very rare. When it occurs, it is very important from the point of view of selecting blood for transfusion; since the antibody is active at 37°C, only 'Bombay' blood can be transfused (Hoffbrand et al., 2001).

#### 1.1.12.4 **Cross reacting A and B**

It is found mainly in group 'O' sera possess both anti-A and anti-B agglutinins. The cross reacting antibody reacts with both A cells and B cells. However Rosenfield first stated the possible clinical significance of cross reacting antibody, that it is capable of crossing the placental barrier more readily than the nonspecific antibody may be due to smaller size of type immunoglobulin molecule. So this cross reacting antibody are more important from view point of hemolytic disease of new born and its possible etiology (Talib and Dutta, 1995).

Table 1.4 ABO group-blood system (Dacie and Lewis,2001).

Blood group	Sub-group	Antigens on red cells	Antibodies in plasma
A	$A_1$	$A+A_1$	Anti-B
	$A_2$	A	(Anti- $A_1$ )*
B	-	B	Anti-A, Anti- $A_1$
AB	$A_1B$	$A+A_1+B$	none
	$A_2B$	$A+B$	(Anti- $A_1$ )*
O	-	(H)***	Anti-A Anti- $A_1$ Anti-B Anti-A,B**

\* Anti- $A_1$  found in 1-2% of  $A_2$  subjects and 25-30% of  $A_2B$  subjects.

\*\* Cross-reacting with both A and B cells.

\*\*\*The amount of H antigen is influenced by the ABO group;

O cells contain most H and  $A_1B$  cells least .Anti-H me be found in occasional  $A_1$  and  $A_1B$  subjects.

### 1.1.13 ABO system and disease

Group A individual may rarely acquire B antigen as result of bacterial infection which results in the release of adeacetylase enzyme. This converts N-acetyL-D-balactosamine into  $\alpha$ -galactosamine which is similar to galactose, the immunodominant sugar of group B, thereby sometimes causing the red cells to be group AB. In the original report, five out of seven of the patients had carcinoma of the gastrointestinal tract. Case reports attest to the danger of individuals with an acquired B antigen being transfused with AB red cells, resulting in a fatal haemolytic transfusion reaction following the production of hyper immune anti-B (Dacie and Lewis,2001).

The inheritance of ABH antigens is also known to be weakly associated with predisposition to certain diseases. Group A individual

have 1.2 times the risk of developing carcinoma of the stomach than group O, or B, group O individual have 1.4 times the risk of developing peptic ulcer than non-group O and non-secretors of ABH have 1.5 times the risk of developing peptic ulcer than secretors. ABH antigens are also frequently more weakly expressed on the red cells of persons with leukemia (Dacie and Lewis, 2001).

#### **1.1.14 Rh blood group system**

The Rh system is more complex than ABO system. There are three closely linked loci with alternative antigens, Cc, D or, no D (termed, d, for which there is no antigen) and Ee ( Hoffbrand et al., 2001).

##### **1.1.14.1 The antigen of Rh system**

The antigens of Rh system are encoded by two genes, RHCE, with produce D and CcEe antigens, respectively (Murphy and Pamphilon, 2009).

The most significant Rh antigen from the clinical point of view is D. About 85% of white people are D+ (Rh- positive) and 15% are D- (Rh-negative). In Africans, only about 3-5% are D- and in the far East D- is rare (Murphy and Pamphilon, 2009).

##### **1.1.14.2 The Rh genotyping**

Rh genotyping is a useful means to determine the Rh phenotype of patients who have been recently transfused or whose RBCs are coated with IgG. Rh genotyping in the prenatal setting can be used to determine paternal RHD zygosity and to predict fetal D status to prevent invasive and expensive monitoring for possibility of HDN. The ethnic background of the parents is important to the design of assay, because the different molecular events responsible for D-negative phenotype must be

considered. Testing of samples from the parents limits the possibility of misinterpretation (Christopher et al., 2009).

Rh genotyping can aid resolution of D typing discrepancies. These often are the result of differences in manufacturer's reagent, but in the donor setting they can be FDA reportable. Molecular genotyping can aid in the selection of compatible blood for transfusion and ultimately long-term transfusion support (Christopher et al., 2009).

#### **1.1.14.3 Rhesus antibodies**

Rh antibodies directed against all Rh antigens, except Rh d, have been described: namely, anti-D, anti-C, anti-c, anti-E and anti-e (Dacie and Lewis, 2001).

##### **1.1.14.3.1 Rh Naturally occurring antibodies**

Anti-E is often naturally occurring; about half may occur without a history of pregnancy or transfusion. Occasionally, naturally occurring anti-D and anti- $C^w$  are found in patients and blood donors. Such antibodies react optionally with enzyme-treated cells (Hoffbrand et al., 2001).

##### **1.1.14.3.2 Rh immune antibodies**

The whole clinical importance of Rh system lies in the readiness with anti-D arises after stimulation by pregnancy or transfusion. Due to prophylaxis of Rh immunization with anti-D immunoglobulin, the incidence of anti-D has significantly decreased, but it still remains the most common a typical antibody of clinical relevance detected in a routine blood transfusion laboratory. D is considerably more immunogenic than the other Rh antigens, have the following order of immunogenicity:  $c > E > e > C$  ( Hoffbrand et al, 2001).

### **1.1.15 Clinical significant of the blood group.**

The importance of blood groups in clinical medicine lies in the fact that an antigens may, in certain circumstance, react with its corresponding antibody and cause harmful clinical effects (Frank et al.,1996).

Of the many red cell blood group systems, only two are of major clinical importance the ABO and Rh systems. The other systems are of much less clinical importance (Frank et al.,1996)

#### **1.1.15.1 Haemolytic Transfusion Reactions**

Blood transfusion involves the safe transfer of blood components from a donor to a recipient (Hoffbrand and Moss, 2010).

Haemolytic transfusion reaction may be immediate or delayed. Immediate life-threatening reactions associated with massive intravascular haemolysis are the result of complement-activating antibodies of IgM or IgG class, usually ABO specificity (Hoffbrand and Moss.,2010).Reaction associated extravascular haemolysis (e.g. Immune antibodies of the Rh system that are unable to activate complement) are generally less severe but may still be life-threatening. The cells become coated with IgG and are removed in the reticuloendothelial system (Hoffbrand and Moss, 2010).

Rare blood types can cause supply problems for blood banks and hospitals. For example Duffy-negative blood occurs much more frequently in people of African origin and the rarity of this blood type in the rest of the population can result in a shortage of Duffy-negative blood for these patients. Similarly for Rh D negative people, there is a risk associated with travelling to parts of the world where supplies of Rh D negative blood are rare, particularly East Asia, where blood services may

endeavor to encourage westerners to donate blood. (Nickel , Willadsen , Freidhoff , et al.1999).(Bruce , 2002).

#### **1.1.15.2 Hemolytic disease of the newborn (HDN)**

HDN is result of the passage of IgG antibodies from the maternal circulation across the placenta into the circulation of the fetus where they react with fetal red cells and lead to their destruction by the fetal reticuloendothelial system (Hoffbrand et al., 2001).

Before 1967, when the prophylactic use of anti-D IgG was introduced, anti-D Rh HDN was responsible for about 800 stillbirths and neonatal deaths each year in the UK. Anti-D was responsible for 94% of Rh HDN; other cases were usually caused by anti-c and anti-E, with range of antibodies found in occasional cases (Hoffbrand et al., 2001).

The most frequent causes of HDN are now immune antibodies of the ABO blood group system most commonly anti-A produced by group O mother against a group A fetus. However, this form of HDN is usually mild. Occasional cases of HDN are caused by antibodies of other blood group system, e.g. anti-kill.

The most common cases of HDN occur when an Rh-negative woman has a pregnancy with an Rh-positive fetus , Rh D-positive fetal red cells cross into the maternal circulation (usually at parturition) and sensitize the mother to form anti-D. Sensitization is more likely if the mother and fetus is ABO compatible. The mother could also be sensitized by a previous miscarriage, amniocentesis or other trauma to the placenta or by blood transfusion (Hoffbrand et al., 2001).

Anti-D cross the placenta to the fetus during the next pregnancy with on Rh-positive fetus, coats the fetal red cells with antibodies and anemia and jaundice. If the father is heterozygous for D antigen (D/d) there is a 50% probability that the fetus will be D positive (Hoffbrand et al., 2001).

#### **1.1.15.3 Universal donors and Universal recipients**

Good practice in transfusion testing requires compatibility testing, which consists of incubating the patient's serum with the donor's red cells. Group O red cells can be given to A, B or AB recipients and were formerly inappropriately called ,universal donor, red cells (Hoffbrand et al.,2001).

However, group O donors have anti-A, anti-B and anti-A,B in their plasma, which will react with the recipient's A or B cells. Normally, if group A,B or AB recipients are transfused with a relatively small number of group O units of whole blood, the anti-A or anti-B that is transfused will be diluted out and neutralized by the plasma of adult recipients, especially if plasma-reduced blood is used (Hoffbrand et al.,2001).

However, if the transfused units contain potent immune haemolytic antibodies, this neutralization and dilution effect may be insufficient and may lead to a marked destruction of the A or B red cells of the recipient, causing a severe haemolytic transfusion reaction. For this reason, the practice of transfusing group O blood to non-O recipients should be fusing strongly discouraged (Hoffbrand et al.,2001).

### **1.1.16 Blood group test ABO and Rhesus (D)**

Even today, with our detailed understanding of blood group antigen, we have no single technical procedure able to detect all known blood group antibodies. The hemagglutination technique is simple and inexpensive, does not require sophisticated equipment, and when done correctly is sensitive and specific in terms of clinical relevance (Christopher et al., 2009).

#### **1.1.16.1 ABO grouping**

Correct interpretation of the patient's ABO group require confirmation, whenever possible, by test on the patient's serum or plasma (except for newborn infants up to 4 month of age in whom naturally occurring anti-A and anti-B are normally absent). Ideally, cell and serum or plasma grouping should be carried out by different workers who then check each other's result. Where this is not feasible, the distinct tasks can be separated, e.g. separating the documentation of reaction patterns from the final interpretation (Dacie and Lewis, 2001).

Anti-A, anti-B and anti-A,B reagents were traditionally used for cell grouping tests. The anti-A,B reagent (group O serum) acted as an additional check on red cells which were agglutinated by anti-A or anti-B, and detected weaker A or B antigens. Conventional polyclonal reagents have been replaced by superior anti-A and anti-B monoclonal reagents, and there is now no requirement to continue to use anti-A,B or A+B in ABO grouping (Dacie and Lewis, 2001).

The monoclonal reagents should contain EDTA to prevent haemolysis they should be used in the presence of fresh patient's serum, as these potent IgM antibodies are strong haemolysins in the presence of complement (Dacie and Lewis, 2001). The method of ABO grouping may



be carried out by tube or slide methods or in micro plates. The reader is refer to the BCSH Guideline on Micro plate technology (Dacie and Lewis, 2001)

#### 1.1.16.2 Rh D grouping

This is usually performed at the same time as ABO grouping to minimize clerical errors that may arise through repeated handling of patient's samples. Each sample should be tested in duplicate, at least for first time patients, as there is no counterpart of 'reverse grouping', as in ABO grouping (Dacie and Lewis, 2001).

The availability of high-titre monoclonal IgM anti-D reagents has made it possible to use the same technique as for ABO grouping. These reagent work equally well at room temperature ( $20^{\circ}\text{C}$ ) at  $37^{\circ}\text{C}$  and are reliable for emergency D grouping by immediate spin-tube technique. The potentiated anti-D reagents must be used strictly according to the manufacturer's instruction an additional reagent or diluents control must be include. The diluents itself does not promote agglutination of the patient's cells , as might occur with red cells already coated with immunoglobulin owing to in vivo sensitization(Dacie and Lewis,2001).

All anti-D grouping reagents should be checked by the method used in the laboratory for specificity with positive ( $OR^1r$  or  $OR^2R^2$  and negative ( $Or^1r$  or  $Or^1r$ ) controls. Additional controls are necessary for polyclonal reagents to confirm the absorption of any contaminating anti-A(using  $A1rr$  cells) and anti-B (using  $Brr$  cells). Before a new batch of reagent is introduced, it should be evaluated in parallel with the reagent in current use( Dacie and Lewis, 2001).The method of Rh D grouping may be carried out by slide, tube and micro plate as for ABO grouping (Dacie and Lewis, 2001).

#### 1.1.16.3 Preparation of ABO reagents

These ABO reagents could be prepared from animal or human source and lectins. In the former source reagents processed are usually of polyclonal in nature viz anti-A, anti-Rh, until and unless it is further processed by absorption to separated them (Talib and Dutta,1995).

Reagents processed from lectins seeds of plants are mostly monoclonal in nature viz  $A_1$  from *Dolichos bilforus* of course rare exception of polyclonal are also found. Now-a-days most of reagent used of monoclonal nature and large scale production are done through hybridoma technique. In earlier days polyclonal antibody (responsive to different subgroups of one system say Rh D ,C,E etc. or  $A_1$  &  $A_2$  i.e.it has multiple specificity until and unless separated or processed further ) were produced by immunizing either animal or human being(Talib and Dutta,1995).

Anti-H human source can be obtained from persons having blood  $A_1$ ,  $B$  or  $A_1B$  groups or it can be obtained from Oh phenotype person. The former through commonly available the anti-H titre in them is not very high and as Bombay group is very rare other source viz animal (cattle and sheep), but the lectins which are available were the easiest and cheap source. (Talib and Dutt,1995).

#### 1.1.16.4 lectins

Lectins are sugar-binding proteins, extractable from plants and lower vertebrate animals, which are useful tools for routine and experimental blood group serology. They combine with simple sugars (e.g fucose, galactose, N-acetyl-galactosamine) present on the glycolipid and glycoprotein of cell membranes and body fluid. Extracts from several

thousand plant species have been investigated for red cell binding properties, but only a small minority can agglutinate human erythrocytes. Most of such lectins do so irrespective of target cell phenotype; hence only a handful is used with any regularity in blood group serology. The three most commonly used blood group specific lectins are from *Dolichos biflorus*, *vicia graminea* and *ulex europaeus*, which have anti- $A_1$  anti-N and anti-H specificities respectively. In addition to blood grouping, lectins are also used for determining ABH secretor status, separating mixture of red cells, and for partially purifying and identifying blood group active membrane glycoproteins (Hoffbrand et al., 2001).

Lectins are not immunoglobulin and are not produced as a result of a specific immune response. They do not possess the uniform molecular structure of immunoglobulin. The lectins used in blood grouping are high specific for example, *U.europaeus* and *D.biflorus* lectins recognize L.fucose and N.acetyl-galactosamine respectively.( Hoffbrand et al., 2001).

#### **1.1.16.5 Techniques in blood group serology**

The most important technique is based on the agglutination of red blood cells. Saline agglutination is important in detecting IgM antibodies, usually at room temperature and  $4^\circ\text{C}$ , e.g. anti-A, anti-B. Addition of colloid to incubation or proteolytic enzyme treatment of red cells increases the sensitivity of the indirect antiglobulin test, as does low ionic strength saline (LISS). These latter methods can detect arrange of IgG antibodies.(Hoffbrand et al.,2001)

The antiglobulin (coombs) test is a fundamental and widely used test in both blood group serology and general immunology. Antihuman globulin (AHG) is produced in animals following the injection of human

globulin, purified complement or specific immunoglobulin (e.g. IgG, IgA or IgM). Monoclonal preparations are also now available. When AHG is added to human red cells coated with immunoglobulin or complement, agglutination of the red cells indicates a positive test.(Hoffbrand et al., 2001).

The antiglobulin test may be either direct or indirect. The direct antiglobulin test is used for detecting antibody or complement on the red cell surface where sensitization has occurred in vivo. The AHG reagent is added to wash red cells and agglutination indicates a positive test. A test occurs in haemolytic disease of the newborn, autoimmune or drug-induced immune haemolytic anaemia and haemolytic transfusion reaction.(Hoffbrand et al., 2001).

The indirect antiglobulin test is used to detect antibodies that have coated the red cells in vitro. It is a two-stage procedure: the first involves the incubation of test red cells with serum; in the second step, the red cells are washed and the AHG reagent is added. Agglutination implies that the original serum contained antibody which has coated the red cells in vitro. This test used as part of the routine antibody screening of the recipient's serum prior to transfusion and detecting blood group antibodies in a pregnant woman(Hoffbrand et al.,2001).

Most of the above methods were originally developed for tube techniques but 96-well micro plates and gel-based spin columns are now widely used.(Hoffbrand et al.,2001).

#### 1.1.16.6 **Quality control of blood group serology**

The quality control of anti-sera reagents must always be carried out by the exact technique by which they are to be used. All reagents should be used according to manufacturer instructions, unless appropriately standardized for other methods (Dacie and Lewis, 2001).

An ISBT/ICSH free-dried reference reagent is available for evaluating either polyspecific antihuman globulin reagents or those containing their separate monospecific components. The validation of a new antiglobulin reagent should assess the specificity, potency of anti-IgG by serological titration and potency anti-complement antibodies( Dacie and Lewis, 2001).

The ISBT/ICSH antiglobulin reference reagent can be used to calibrate an, in-house, antiglobulin reagent for use as a routine standard (Dacie and Lewis,2001).

#### 1.1.17 **Helicobacter pylori**

This spiral-shaped organism have been observed in the stomachs of humans for over one hundred years, but it was not until Warren and Marshall ,in 1982, isolated a campylobacter-like bacterium from patients with gastritis, that a relationship between gastric disease and a bacterium was realized. The bacterium was initially classified as a campylobacter, but was placed in a new genus *Helicobacter*, on the basis on its ultrastructure and morphology, fatty acid composition, growth characteristics, respiratory quinines and enzymatic properties (Max Sussman,2002).

Since its discovery, *Helicobacter* has been cited as the most prevalent bacterial pathogen of humans. It is estimated that about half the world's

human population is affected and it is the subject of more than 10000 research papers. Helicobacter is implicated in one of the most common 'trivial' complaints-dysgenic-and is a major cause of death in gastric adenocarcinoma. Although it is mainly confined to the human population , it is also found in some domestic animals, possibly as an 'anthroponosis' ; it is virtually absent from the environment and its mode of transmission largely remain a mystery.(Max Sussman, 2002).

#### 1.1.17.1 General properties of *H. Pylori*

Helicobacter are curved gram-negative rods similar in appearance to campylobacters, but because they differ sufficiently in certain biochemical and flageller characteristics, they are classified as separate genus. In particular, helicobacters are strongly urease-positive, whereas campylobacters are urease-negative (Levinson and Jawetz, 2002). The urease is produced in amounts so great, that its action can be demonstrated within minutes of placing *H. pylori* in the presence of urea. Another secreted protein called the vacuolating cytotoxin (VacA) causes apoptosis in eukaryotic cells it enters generating multiple large cytoplasmic vacuoles. The vacuoles are left to be generated by the toxins formation of channels in lysosomal and endosomal membrane. (Kenneth and George Ray.2004).

Helicobacter consists many species isolated from human include *H. pylori* ,*H. cinaedi* , *H. fennelliae* , *H. heilmannii*, *H. westmeadii* , *H. canis* ,*H.canadensis* sp. ,*H.pullorum* and *H.rappini* , all most of species having strong urease activity. Because *H. pylori* , *H. cinaedi* , and *H. fennelliae* are significant human pathogens ,only these species are addressed.(Betty , et al., 2007).

#### 1.1.17.2 Epidemiology of *H. Pylori*

Infection with *H. pylori* is the most prevalent disease in the world. The organism is found in the stomach of 30% to 50% of adults in developed countries, and it is almost universal in developing countries. Molecular epidemiologic analysis indicates the strain themselves have strong linkages to ethnic origin that can be traced back to the earliest known patterns of human migration. *H. pylori* has been called an "accidental tourist" which was established in the stomach of human thousands of years ago and remained bound to the original population as it dispersed from continent to continent. (Kenneth and Geory Ray, 2010).

#### 1.1.17.3 Genome of *H. Pylori*

*H. pylori* has a single, circular chromosome that varies in size between strains. (Taylor et al., 1992) analyzed chromosomes of some 30 strains and found an average of 1.608-1.727 Mbp with a mean value of 1.67 Mbp. This is in the lower range of size reported for pathogenic bacteria and reflects the limited biochemical capacity of *H. pylori*. The guanine and cytosine composition is low at 36-38 mol %. Approximately 45% of strains of *H. pylori* harbor plasmids, though none has been found in the type strain NCTC 11637. Sizes of the plasmids are diverse, ranging from 1.8 to 63 kbp. The genome sequence data provide a dramatic insight into the potential molecular determinants of every aspect of physiology and molecular biology of the organism, based on prediction of gene function from homologies to known genes in other organisms (Max Sussman, 2002).

Jiang et al, (1996) showed that the genomic variability between strains was due to flexibility in gene order (macro diversity) as well as to allelic variations within genes (micro diversity). Chromosomal maps from five

unrelated strain of *H. pylori* were compared, but consistent clustering of genes was not observed. Indeed only four genes, katA, vacA, Hpa A and pfr, which respectively encode catalase, avacuolating cytotoxin, a putative adhesion subunit and bacterial ferritin, were consistently found in the same quarter of the chromosome and the order of event these genes was not constant (Max Sussman, 2002).

#### **1.1.17.4 Transmission of *H. pylori***

An exact mode of transmission is unknown but an oral-oral, fecal-oral, or a common environmental source have been proposed as possible routes of transmission with transmission of *H. pylori* primarily occurring in families. Recent findings suggest that mother-to-child transmission is the most probable cause of interfamilial spread (Betty., et al., 2007).

#### **1.1.17.5 Signs and symptoms of *H. pylori***

Primary infection with *H. pylori* is either silent or causes an illness with nausea and upper abdominal pain lasting up to 2 weeks. Year later, the finding of gastritis and peptic ulcer disease include nausea, anorexia, vomiting, epigastric pain, and even less specific symptoms such as belching. Many patients are asymptomatic for decades, even up to perforation of an ulcer. Perforation can lead to extensive bleeding and peritonitis due to the leakage of gastric contents into the peritoneal cavity (Kenneth and George Ray, 2010).

#### **1.1.17.6 Helicobacter associated disease**

*Helicobacter pylori* is a highly adapted organism that lives only on gastric mucosa. The gastric antrum is the most favored site, but other parts of the stomach may be colonized, especially in patients taking



an acid-lowering drug such as an  $H_2$  antagonist or proton pump inhibitor. The bacteria are present in the mucus overlying the mucosa. Although gastric acid is potentially destructive to *H. pylori* protection is provided by its powerful urease, which acts on the urea passing through the gastric mucosa to generate ammonia, which neutralizes acid around the bacteria. Colonization often extends into gastric glands, but the mucosa is not invaded by the bacteria (Greenwood, et al., 2002).

*Helicobacter pylori* cause gastric and peptic ulcers. Infection with *H. pylori* is a risk factor for gastric carcinoma (Warren Levinson and Ernest Jawetz, 2002).

#### **1.1.17.7 Diagnosis of *H. pylori* infection**

Diagnostic techniques may be direct and performed on gastric biopsies obtained at endoscopy. These include culture, histology, the rapid urease test and polymerase chain reaction (PCR), which may also provide molecular-type information. Indirect tests include serology and breath test (Max Sussman, 2002).

##### **1.1.17.7.1 Rapid Urease Test**

The biopsy urease test exploits the ability of *H. pylori* to produce urease. The most widely used commercially available test is the CLO test in which the biopsy is placed on agar containing urea and a pH indicator. A positive result is indicated by a change in color from yellow to red after two hours. The test has a specificity approaching 100% and a sensitivity of 80–85%, and it is a cheap and reliable workhorse, albeit that it is now being superseded by more informative molecular methods (Max Sussman, 2002).

#### 1.1.17.7.2 Polymerase Chain Reaction

Test based on the PCR allow as few as 10 bacterial cells to be detected, whilst maintaining a very high specificity and coccoid or non-viable organism may also be detected. Amongst others, primers based on the urease A gene sequence and the gene encoding 16s rRNA have been used. The successful amplification of *H. pylori* DNA sequences from faeces and dental plaque has also been reported. More recently, PCR has been used to detect cag A and vac A sequences and to obtain genotype data (Max Sussman, 2002).

#### 1.1.17.7.3 Serodiagnosis

Another approach to *H. pylori* diagnosis is serologic testing. Numerous serologic enzyme-linked immunoassays (EIAs) designed to detect immunoglobulin G (Ig G) and immunoglobulin A (Ig A) antibodies to *H. pylori* are commercially available. Reported performance of these assays varies as a result of the reference method used to confirm *H. pylori* infection, antigen source for the assays and the population studied. In addition to variability in assay performance, the clinical utility of these assays has not been defined and may not differentiate between active and past *H. pylori* infection in all cases (Betty, et al. ,2007).

In general, the rapid tests are not as sensitive as laboratory serology but they are reasonably specific. Serology remains a highly effective and non-invasive approach to initial diagnosis but it is not useful for follow-up after treatment.

#### 1.1.17.7.4 Urea Breath Test

This test detects bacterial urease activity in the stomach by measuring the output of  $CO_2$  resulting from the splitting of urea into  $CO_2$  and

ammonia. A capsule of urea labeled with an isotope of carbon (carbon -14 or -13) is fed to the patient, and the emission of the isotope in  $CO_2$  subsequently exhaled in the breath is measured. Patient infected with *H.pylori* give high readings of the isotope. The test has excellent sensitivity and specificity, but there are drawbacks. Carbon -14 is radioactive, albeit weakly, so it is not used in children. Carbon -13 is not radio-active, but a mass spectrometer is needed for its assay (Greenwood, et al., 2002).

#### 1.1.17.7.5 **Faecal antigen test**

This is a promising new test in which polyclonal antibodies are used to detect *H. pylori* antigen in faeces. It has the potential to supplant serology as a routine screening test (Greenwood, et al., 2002).

#### 1.1.17.7.6 **Endoscopy**

Ideally, patients for endoscopy should not have received antibodies or proton pump inhibitor for a month before the test. Mucosal biopsy specimens are taken from the gastric antrum within 5 cm of the pylorus, and preferably also from the body of the stomach. For maximum sensitivity, duplicate specimens are taken: one lot for histopathology and the other lot for culture (Greenwood, et al. ,2002).

#### 1.1.17.8 **Prevention of *H. pylori***

*H.pylori* is sensitive to most B-lactam antibiotics, macrolides, tetracyclines and nitroimidazoles, but resistant to trimethoprim. It is also sensitive to bismuth compounds and partially sensitive to the acid-lowering proton pump inhibitors omeprazole and lansoprazole. To eradicate *H.pylori* infection, at least two antimicrobial agents must be given in combination with an acid-lowering agent. The reduction of

acidity not only enhances antimicrobial action, but it makes the patient feel better and thereby improves compliance. A popular regimen is a 1-week course of the macrolide clarithromycin, plus amoxicillin (or metronidazole) and omeprazole(or lansoprazole). These regimens eliminate *H. pylori* in about 90% of patients.Social deprivation is the dominant factor governing the prevalence of *H. pylori* infection (Greenwood, et al., 2002).

## 1.2 Literature review

There is association between *H. Pylori* and ABO blood groups system:Saad Shaheen Hamadi in University of Basrah he found two hundred (200) patients with various gastrointestinal symptoms attending endoscopy unit at Teaching Hospital in Basrah during the period from March 2011 to October 2012 were studied after obtaining their consent. These consecutively chosen patients were 110 males and 90 females whose age ranged from 18-75 years. Patients were diagnosed as having gastritis, gastric ulcer, duodenal ulcer and gastric cancer using specimens from the antrum of the stomach of each patient. Diagnosis of gastric carcinoma was based on the WHO classification (Hamilton and Aaltouren,2000) while gastritis was characterized by chronic inflammatory cells infiltration with degeneration and detection of microorganisms. One of the specimens was used for the rapid urease test (RUT) immediately after collection. The other two gastric biopsy specimens were placed in transport medium, brain heart infusion broth, for bacterial culture and PCR. Three ml of venous blood sample was drawn from each patient, then 2ml of it transferred into plain tube and centrifuged at 5000 rpm for 5-10 minutes. The serum was used for rapid diagnostic test in screening for the detection of antibodies against *H. pylori* (IgG). The remaining 1 ml of the blood was emptied into disposable tube and used for determination of ABO blood group antigens by a standard hemagglutination test.

The ABO blood group was determined for each patient by the conventional hemagglutination test using the anti- A, anti-B and anti-D sera. The ABO blood grouping procedure is based on the principle of agglutination or clumping as the patient's blood is reacted with anti-A, anti- B and anti-Rh antibodies separately.

The distribution of the ABO blood groups of the patients was O (40.5 %) followed by A (30 %), B (21 %) and AB (8.5 %), while 186 ( 93% ) of patients were Rh-positive and only 14 (7% ) of patients are Rh-negative. The Rh+ distribution among blood groups was as follow: 57 ( 30.6% ), 37 (19.9%), 15 (8.1%) and 77 ( 41.4%) for blood groups A, B, AB, and O respectively. While Rh- distribution among blood groups was 3 (21.4%), 5 (35.7%), 2 (14.3%) and 4 (28.6%) for blood groups A, B, AB, and O respectively as shown in (Table. 1).

**Table 1: ABO/Rh blood group distribution in patients**

ABO system	Blood groups				Total
	A	B	AB	O	
	No. (%)	No. (%)	No. (%)	No. (%)	
<b>Rh+</b>	57 (30.6)	37 (19.9)	15 (8.1)	77 (41.4)	186
<b>Rh-</b>	3 (21.4)	5 (35.7)	2 (14.3)	4 (28.6)	14
<b>Total</b>	60 (30)	42 (21)	17 (8.5)	81 (40.5)	200

$$X^2=3.042, p>0.05$$

In study done in Kosovo evaluated 671 blood donors who underwent ABO/Rhesus blood typing and measurement of serum anti *H. pylori* IgG antibodies. Stored blood donor sera (450 males, mean age of 32.23 and range 18 to 65 years; 221 females, mean age of 28.18 and range 18 to 65 years; p is 0.0001) are collected from the March to April 2009 in Kosovo's Blood Transfusion Center. Age, gender, social class, educational level and ABO/RhD blood groups and IgG values are recorded. All donors are divided in two groups: voluntary donors and non-voluntary or familial donors who gave blood for their relatives. Blood donors are categorized into three groups according to the educational level: group I with primary education level, group II with secondary and group III with

high educational level. Also, they are divided according to age (group I: 18-19 years, n=164; group II: 20-29 years, n=216; group III: 30-39 years, n=108, group IV: 40-49 years, n=110; group V: 50-59 years, n=61, and group VI: 60-65 years, n=12. Beforehand, blood donors are screened and anyone taking anti-inflammatory drugs, antibiotics, or corticosteroids or who is found to have any problem with health is not allowed to donate blood. Serum antibodies are tested against *H. pylori* infection by blood groups, age, and gender, which are later analyzed by ELFA (Enzyme Linked Fluorescent Assay) test for *H. pylori* IgG with Biomerieux HPY-VIDAS. The non-invasive serological method is used for determining presence of *H. pylori* IgG antibody in serum. Ten milliliters of blood sample is taken from each donor and the sera stored at -20° C until required. The assay principle combines a 2 step enzyme immunoassay sandwich method with final fluorescent detection (ELFA). The commercial kit for detecting anti-*H. pylori* antibody is found to have a sensitivity of 98.10% (Confidence interval 93.12%-99.77%) and a specificity of 90.82 % (Confidence interval 93.12%-99.77%). Interpretation of the test result is done as follows: the result lower than 0.75 is interpreted as negative, the result between 0.75- <1.00 is interpreted equivocal, and the values higher than >1.00 is interpreted as positive. In this study it is found that 48 samples of blood donors were equivocal to *H. pylori* antibody, therefore they are excluded and not evaluated. Also it is evaluated mean value of IgG Antibody to *H. pylori* according to age group, gender and blood group. Blood grouping is performed by slide agglutination test using monoclonal anti- A, anti-B, anti-AB and anti-D (Rho) antibodies.

From the total number of 671 tested blood donors for *H. pylori* infection, 382 or 56.9%, required IgG antibody for *H. pylori*, but there is not found

difference between voluntary (57.4 %), and non-voluntary blood donors (56.3%) (OR=1.05, 95% CI 0.76 to 1.43, and p value 0.8) .The mean of seropositive donors are older 31.9 years, compared with seronegative ones 29.5 years, p is 0.02 . The rate of *H. pylori* infection is not significantly different in male and female with anti *H. pylori* IgG antibodies detected in 57.0% (126 of 221) of women compared with 56.9% (256 of 450) of men (OR=0.99; 95% CI 0.72 to 1.38; p=0.96).

In the study done by Mohamad Salih Jaff in Kurdistan Region, Iraq, From February 2010 to March 2011, a total of 1108 patients with the symptoms of dyspepsia or other symptoms referable to the proximal alimentary tract, from an enterology outpatient clinic, were referred for serologic diagnosis of *H. pylori* infection. The study was performed according to the local Ethical Committee of Medical Sciences. From each patient, a sample of 3 mL of peripheral blood was collected and centrifuged, and the sera were separated for use.

This study was a prospective study of patients attending the outpatient clinic for symptoms of dyspepsia for the first time, with no previous history of *H. pylori* infection and treatment. The study population was screened for *H. pylori* infection by SD Bioline *H. pylori*, a rapid HRB kit (MT Promedt Consulting GmbH, Ingbert, Germany) to receive treatment. For this research purpose, the positive SD Bioline *H. pylori* screening test results were confirmed, by estimating the serum levels of anti-*H. pylori* IgG, using the commercial ELISA (Trinity Biotech, Wicklow, Ireland).<sup>17</sup> The results by this method were obtained as immune status ratio (ISR), and values of  $\geq 1.1$  were considered positive. Those patients who were positive for *H. pylori* infection by both methods were included in the seropositive, those who were negative by both methods were regarded as seronegative, and the rest (n = 38) were not included within the total



study population (n = 1108). ABO and Rhesus (Rh) blood groups were determined for seropositive and seronegative patients, using standardized hemagglutination methods. The results of this study (seropositive) were compared with the seronegative patient group and with the author's previous study on the ABO blood group frequency in the region,<sup>19</sup> as controls for both age and sex, and blood groups.

The seropositivity for *H. pylori* infection was present in 718/1108 (64.8%) and absent in 390/1108 (35.2%) of these patients (by both methods). Only in 38 patients, there was disagreement between two tests (i.e., an agreement of 95%), and

these patients were not included in the study population. The mean age of seropositive patients was ( $37.99 \pm 14.6$ ) years (range, 18–82 years), with a median age of 38.4 years and no significant difference from the mean age of seronegatives

( $37.6 \pm 15.7$ ) years (range, 18–70 years). There was a significant increase in the incidence of seropositivity up to the age of 31 years ( $r = 0.91$ ,  $P = 0$ ) then a significant decline in the incidence above the age of 31 years ( $-r = -0.94$ ,  $P = 0$ ). Of these 718 seropositive patients, 37.7% (271/718) were male (M) and 62.3% (447/718) were female (F) (M/F ratio 0.61:1.0). A significant difference was observed when comparing gender in *H. pylori* infection ( $P$ , 0.0001) with that of the general population (M/F ratio, 1.14:1.0) (Table 1) and with seronegative patients (M/F ratio, 1.2:1.0) (Table 2) but to a lesser degree ( $P = 0.0148$ ). When the frequencies of blood group phenotypes were

analyzed separately in the seropositive patients, seronegative patients, and the general population, it was possible to verify that the frequency of blood group O in seropositive patients was higher and blood group B was

lower than in the general population and to a lesser degree in seronegative patients.

**Table 1** Gender and blood group relation to *Helicobacter pylori* infected patients compared with the general population

Characteristic	Seropositive N = 718/1108 (64.8%)		General population <sup>14</sup> N = 53,234		P-value
Sex (M/F)	293/425 M: 40.8% F: 59.2%	0.69:1.00	28379/24855 M: 53.3% F: 46.7%	1.14/1.00	0.0001
<b>Blood group</b>					
A	230	32.0%	17,283	32.47%	0.811
B	140	19.5%	12,693	23.84%	0.007
AB	48	6.7%	3475	6.53%	0.880
O	300	41.8%	19,783	37.16%	0.011
Rhesus positive	664	92.5%	48,833	91.73%	0.497
Rhesus negative	54	7.5%	4401	8.27	0.497
Total	718		53,234		

**Table 2** Gender and blood group relation to *Helicobacter pylori* infection in seropositive patients compared with seronegative patients

Characteristic	Seropositives N = 718/1108 (64.8%)		Seronegatives N = 390/1108 (35.2%)		P-value
Sex (M/F)	293/425 M: 40.8% F: 59.2%	0.69:1.00	213/177 M: 54.6% F: 45.4%	1.2/1.0	0.0148
<b>Blood group</b>					
A	230	32.0%	126	32.3%	0.936
B	140	19.5%	110	28.2%	0.0495
AB	48	6.7%	31	8.0%	0.663
O	300	41.8%	19,783	31.5%	0.0397
Rhesus positive	664	92.5%	349	89.5%	0.288
Rhesus negative	54	7.5%	41	10.5%	0.288
Total	718		390		

Abbreviations: F, female; M, male.

ABO and Rh blood group frequencies in seropositive and seronegative patients are shown in Tables 1 and 2. These differences between the higher prevalence of type O and the lower prevalence of blood group B in the seropositive patients compared with that in the general population were statistically significant, with *P*-values of 0.01 and 0.007 respectively and also when compared with that in seronegative patients with *P*-values of 0.0397 and 0.0495 respectively. No significant differences in the frequency of ABO and Rh blood groups were observed in different ages in seropositive patients. There was also no significant difference in Rh+ frequency between seropositive patients, seronegative patients, and the general population (Tables 1 and 2).

In the study done in the clinical center of Serbia and Belgrade university was the detection of helicobacter pylori (HP) infection and estimation of this infection relationship with age, gender, blood groups and Rhesus factor, as well as the assessment of the accuracy of the method. A total of 227 patients with gastritis were examined. Blood ABO groups and Rh positivity were determined using standard tests. Infection by HP was proved by  $^{14}\text{C}$ -urea breath test and gastric biopsy. Patients were aged 20-81 years ( $X=51.7$  years) and the presence of HP was not related to the age ( $P > 0.05$ ). From the total number of patients, 25/69 males and 68/158 females were HP positive. There was no significant difference between genders and HP infection ( $P > 0.05$ ). From the 227 investigated patients, 69 (30%) belonged to blood group O, 96 (42%) to A, 40 (18%) to B and 22 (10%) to AB. HP was detected in 27/69 patients with blood group O, 45/96 patients with blood group A, 16/40 patients with blood group B and 5/22 patients with blood group AB. There was no statistically significant difference ( $P > 0.05$ ) in the incidence of HP infection between these groups (proving that HP infection did not depend upon significant correlation between the presence of particular blood group in HP + patients related to the reported frequency of the blood groups in Serbian population (O- 38%, A-42%, B-15%, AB-5%). HP was found in 16/36 Rh- and in 77/191 Rh+ patients without statistical difference ( $P > 0.05$ ). Also, there was no significant correlation of the presence of the Rh factor in the HP positive patients to the frequency of the Rh factor in the Serbian population (84% Rh+ and 16% Rh-). The basic value of the HP+ test was slightly, but not significantly lower in comparison to the HP- patients ( $P > 0.05$ ). On the contrary, test values showed a highly significant difference ( $P < 0.01$ ) in HP+ and HP- patients. In conclusion, in adults HP infection does not depend upon the patient's age, gender, blood group

type or Rh factor. In clinical terms, there were 93 true positive (TP), 129 true negative (TN), 5 false negative (FN) and 0 false positive (FP) patients. Sensitivity of the method was 94.9%, specificity 100%, positive predictive value 100%, negative predictive value 96.3% and accuracy 97.8%.

### 1.3 Rationale

*H. pylori* infection is now accepted as the most common cause of gastritis, and is etiologically involved in gastric ulcer, duodenal ulcer, gastric adenocarcinoma and primary gastric B-cell lymphoma (Graham. 1999).

The distribution of the ABO blood groups varies in populations throughout the world (Garratty et al.,2004). In addition to clinical significance for transfusion and transplantation, it is becoming increasingly apparent that ABO antigens are of biological significance and may be associated with predisposition to, or protection from, many diseases (Reid and Bird,1990). Many authors reported an association between blood group O and *H. pylori* infection (Kanbay,et al.,2005). While others failed to find such an association (Niv et al.,1996).

The aim of this study is therefore to verify the association between ABO blood group and Rh phenotype with *H. pylori* infection by increasing the numbers of observations and the statistical power.

## **1.4 Objective**

### **1.4.1 General Objective**

To determine and association of ABO blood groups and Rh phenotypes among Sudanese population infected with *H. pylori*

### **1.4.2 Specific Objective**

.to determines the existence of statistically significance difference in the *H. pylori* infection according to ABO blood groups.

.to determine the frequency of Rh phenotypes in patients infected with *H. pylori*

.to determine the frequency of gender and age of *H. pylori* infected patients .

# **Chapter two**

## **Study design**

## Chapter two

### 2. 1 Study design

This was descriptive cross-section study design.

**2.2 Study Population:** One hundred (100) patients infected with H. Pylori taken from Sudanese population (Omdurman Teaching Hospital).

#### 2.2.1 Inclusion criteria

Patients with H. Pylori positive by screening test.

#### 2.2.2 Exclusion Criteria

Individuals with H. Pylori negative by screening test.

### 2.3 Data Collection

Data was collected using structural interviewing questionnaire for blood sampling and patient information.

### 2.4 Sample Collection

- EDTA blood was collected from each individual for determination of ABO blood group and Rh phenotype.
- 2 ml of whole blood collected in plain container and separated and centrifuge (1500 rpm for 3 min) to harvest patient's serum for detection of H. pylori antibodies.

### 2.5 Ethical Consideration

A verbal consent was taken from each individual before being enrolled in the study. Each patient was informed about aim of the study.



## **2.6 Laboratory requirement**

- Microscope
- Centrifuge
- Water bath ( $37^{\circ}\text{C}$ )
- Sterile disposable needle
- Tourniquet
- Tubes (75 x 12 mm).
- Timer.
- Specimen collection container.

## **2.7 Methods**

### **2.7.1 ABO blood grouping Tube method**

#### **2.7.1.1 Principle**

The antigen (washed red cells from the test sample) and antibody (antisera or serum from test sample) when mixed in an optimal proportion (cell: serum ratio) and incubated for a recommended period at a recommended temperature ( $25^{\circ}\text{C}$  for ABO) under proper biochemical environment (ionic strength and pH) will exhibit a proper reaction within the framework of law of mass action. Positive reaction is denoted by agglutination or haemolysis and negative reaction is denoted by absence of either (Talib and Dutta. ,1995).

#### **2.7.1.2 Reagents**

- Anti sera A
- Anti sera B
- Anti sera D
- AHG

- Anti sera C
- Anti sera E
- Anti sera  $\bar{e}$
- Anti sera  $\bar{c}$
- Physiological Saline ( $N/10$  sodium chloride) for preparing cell suspension.

\* TULIP DIAGNOSTICS (P) LTD. PLOT NOS . 92/96,  
PHASE11c, VERNA IND . EST. , VERNA, GOA-403-722,  
INDIA.

#### 2.7.1.3 Procedure

Seven volume of 5% cell suspension was taken in graduated Pasteur pipette and one volume of saline cell suspension was added to each of seven tubes in tubes marked for cell grouping and Rh phenotypes. Similarly one volume from each anti- sera is taken and added to each seven tubes. All tubes were shake gently and lefted for 15 minutes at room temperature ( $25\text{ }^{\circ}\text{C}$ ) for incubation and after that all tubes were centrifuged at 1500 revolution per minute in respect of each tubes were given and the results read-agglutination, haemolysis or negative (Talib and Dutta ., 1995).

#### 2.7.1.4 Interpretation

It is done on the basis of positive reaction (agglutination or haemolysis) or negative reaction (no agglutination or haemolysis). All doubtful results where interpretation is difficult or in case of negative reaction help of optical aid is essential (Talib and Dutta. , 1995).

## **2.7.2 Slide Method**

### **2.7.2.1 Definition**

Define as technique use to determining the ABO grouping base on antigen antibody reaction.

### **2.7.2.2 Procedure**

On clean dry slide was transferred one volume (0.03 ml is the standard volume) of anti-sera A, anti-sera B and anti-sera D then also, transferred one drop of 20% cell suspension of test sample in the first row near to anti-sera A, anti-sera B and anti-sera D and mixed the anti-sera with cell suspension and the slide rotated for at least 2 minutes at room temperature and looked for the result of reaction. Recorded interpretation of the result immediately (Talib and Dutta. , 1995).

## **2.7.3 Rapid *H. pylori* Antibody Test**

### **2.7.3.1 Principle**

Rapid *H. Pylori* Antibody Test employs chromatographic lateral flow test device in a cassette format. Colloidal *H. pylori* antigens (Au-Ag) are dry-immobilized at the end of nitrocellulose membrane strip. *H. pylori* antigens are bond at the Test Zone (T) when the sample is added; it migrates by capillary diffusion rehydrating the gold conjugate. If anti-*H. pylori* antibodies present in sample, Antibodies will bind with the gold conjugated antigens forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by *H. pylori* antigens generating a visible red line. If there are no anti-*H. pylori* antibodies in sample, no red line is formed in the Test Zone (T). A built-in control line will always appear in the Control Zone (C) when the

test has performed properly, regardless of the presence or absence of anti-H. pylori antibodies in the specimen.

#### **2.7.3.2 Procedure**

The strip and sample allowed to reach room temperature ( $25^{\circ}\text{C}$ ) then the pouch was opened, dip the strip into the specimen with the arrow marked end toward the sample until the sample move to the membrane or pipette 80-100ml (Two drops) of serum to the arrow marked end of the strip. Placed the strip on flat surface and the result is recorded at 10 minute.

\*BOSON Biotech. lotus Global Co. , Ltd. Middlesex London , United Kingdom.

#### **2.8 Data analysis**

Collected data analyzed by Statistical Package for the Social Sciences computerized program version 11.5.

# **Chapter three**

## **Results**

## Chapter Three

### 3.0 Results

The distribution of ABO blood group and Rh phenotypes among the patients infected with H. pylori as related to age, sex and gender are shown in the following tables:

**Table 3.1 Frequency of H. Pylori in infected patients according gender**

Tested patients for anti IgG H. Pylori	Males	Female	Total
Number	45	55	100
Percent	45.0	55.0	100.0

**Table 3.2 Mean values of IgG Ab of H. Pylori positive according to age group**

Age group							
Variable	10-19 (n=3)	20-29 (n=30)	30-39 (n=28)	40-49 (n=15)	50-59 (n=14)	60-69 (n=6)	70-79 (n=4)
Mean	14.67	25.13	34.86	44.33	53.29	64.17	72.25
Medium	16.00	26.00	35.50	44.00	53.00	64.00	72.00
St.Deviation	3.215	2.837	3.052	2.895	2.840	2.927	2.630
Range	6.00	9.00	9.00	9.00	8.00	9.00	5.00

**Table 3.3 Distribution of Rh (D) antigen among study population**

Phenotype	No. tested	%
Rh(D)+	87	87.0
Rh(D)-	13	13.0
Total	100	100

**Table 3.4 proportion of Rh(C) antigen among study group**

Phenotype	observed No.	Percent
Rh(C)+	37	37.0
Rh(C)-	63	63.0
Total	100	100

**Table 3.5 Frequency of Rh (E) antigen among study population**

Phenotype	Observed No.	%
Rh(E)+	12	12.0
Rh(E)-	88	88.0
Total	100	100

**Table 3.6 Distribution of Rh ( $\bar{c}$ ) antigen among study group**

Phenotype	Observed No.	Percent
Rh( $\bar{c}$ )+	92	92.0
Rh( $\bar{c}$ )-	8	8.0
Total	100	100

**Table 3.7 Frequency of Rh ( $\bar{e}$ ) antigen among study population**

Phenotype	Frequency	Percent
Rh( $\bar{e}$ )+	96	96.0
Rh( $\bar{e}$ )-	4	4.0
Total	100	100

**Table 3.8 Distribution of ABO blood group phenotype among study population**

Phenotype	percent
O	46.0
A	23.0
B	29.0
AB	02.0
Total	100.0

\*The results in table 3.8 showed the blood group O was highest (46%) and the lowest percent was seen in AB blood group(2%).



**Table 3.9 phenotype frequency distribution of Rh blood group among study group**

Rh phenotype	percent
$\bar{c}D\bar{e}$	39.0
$C\bar{c}D\bar{e}$	27.0
$\bar{c}\bar{e}$	11.0
$CD\bar{e}$	8.0
$\bar{c}DE\bar{e}$	8.0
$\bar{c}DE$	3.0
$C\bar{c}\bar{e}$	2.0
$\bar{c}D$	1.0
$C\bar{c}D\bar{e}$	1.0
Total	100.0

\*the results in table 3.9 showed the  $\bar{c}D\bar{e}$  phenotype was highest observed percent (39%) and the lowest percent phenotype was  $C\bar{c}D\bar{e}$  (1%) .

# **Chapter four**

**Discussion, Concluts and Recommendation**

## Chapter four

### 4.0 Discussion, Concluts and Recommendation

#### 4.1 Discussion

The ABO blood group antigens confer advantage of resistance against certain infectious disease (Reid and Bird, 1990). *H. pylori* infection has a relevant clinical importance and the testing for *H. pylori* Antibody helps in early detection of “silent” peptic ulcer (Vaira et al., 1994).

The topic of this study is to determine the frequency of ABO and Rh phenotype in hundred (100) unrelated individuals infected with *H. pylori* as related to gender, age and sex by the using Rapid *H. pylori* antibody test.

We found variation in infection with *H. pylori* in sample taken randomly from unrelated individual according to gender that found females more prone to infection than males (55% respectively 45%). That shown in table ( 3.1 ). Our present study agree with study done in Turkish women are more prone to *H. pylori* infection than man (60.8% respectively 42.9%) (Kanbay et al.;2005). But disagree with data reported by EUROGAST study group which investigates asymptomatic subject in 17 population showed no sex difference to *H. pylori* infection (Research article, 1993). The similar data with a lower frequency (16.8% in men and 13.6% in female) with no sex difference are found also, in Malaysian blood donors (male16.8% and female 13.6%) (Sasidharana et al. 2009), and in the Swedish blood donors (male 19% and female 16%) (Sorberg et al. 2003).

In this study we observe increase of *H. pylori* infection with age, 20 to 59 years and then decrease after 60 years table (3.2). This data agree with results from previous study have shown a steady rise of *H. pylori* carrier

rate from under 20 years to 40 years age (Ching et al.,1994). Also, the study by Ponzetto et al (Ponzetto et al, 2001), in Italian blood donor confirms that the seroprevalence was higher in older than in younger blood donors. But, there are some data from previous study, which did not match with ours. For example: The prevalence of *H. pylori* infection among asymptomatic healthy blood donors in Northern Peninsular Malaysia did not increase with age (Sasidarana et al, 2009).

The distribution of ABO blood group phenotypes frequencies among the patients in this study was shown in table (3.8). The blood group 'O' (46%) was predominantly occurring type followed by group 'B' (29%), group A (23%) and the lowest proportion is noticed in AB blood group (2%).

Our study is agree with previous studies done in Vishwakarma population of Mysore district in Karnataka is showed the blood group 'O' (37.76%) is predominantly occurring type and the lowest proportion is noticed in AB blood group (11.89%). But, disagree with some previous studies which demonstrated that the O blood group did not represent a risk factor for *H. pylori* infection ( Dickey et al.,1993).

The similar data with ours are presented by other Studies, for example: the study done in clinical center of Serbia and Belgrade University it was showed (A 27.0%; B 12.2%; AB 4.0% and O 56.8%) among *H. pylori* infected patients.

In contrast to previous mentioned data and our data, Kanibal et al in Turkey demonstrated that *H. pylori* infection can be related to ABO blood group (Kanbay et al., 2005). They found that blood groups O and A are more prone to *H. pylori* infection than other blood groups.

The frequency distribution of Rh blood group phenotype among the patients infected with *H. pylori* in our study was depicted in Table (3.9). That shown *cDe*( 39 %) has the highest observed percentile frequency

and *CcDEe* (1%) was the lowest percent. The present study showed different results when compared with the previous studies based on the study of phenotype and haplotype frequencies of the Rh (CcDdEe) system among the Rajput and the Varli populations of Dadra and Nagar Haveli. It is shown that *CDe* has the highest observed percentile frequency for both the Rajput and the Varli populations. Amongst the Rajputs it is followed by *cDe*, *cDE* and *CDE*. Amongst the Varli population also *CDe* frequency is followed by *CDE*, *cDe* and *cDE*.

## **4.2 Conclusion**

It can also be concluded that O blood group and cDe Rhesus phenotype individuals are more susceptible to *H. pylori* infection and AB blood group individuals are less susceptible to *H. pylori* infection. Also, it can be concluded that females and adolescents are more prone to *H. pylori* infection.

## **4.3 Recommendation**

- It recommended that study sample should be increased in future studies and covers all the country to obtain reliable and precious result.
- Multiple tests like RUT, RDT, culture and PCR were used to provide a more accurate diagnosis of *H. pylori* infection.
- Secretor and non-secretor should be tested beside ABO blood group in patients infected with *H. pylori* to verify association between O blood group and *H. pylori* infection.
- It can be recommended that *H. pylori* infection is an endemic problem, which should be dealt with by improving sanitation and purified water supply and also should be investigated for and eradicated.

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## Appendix (1)

Sudan University of science and Technology

Faculty of graduate studies

Department of Haematology and immunohaemtology

1. Name : ..... No. : .....
2. Age : .....years.
3. Gender:  
Male : ..... Female : .....
4. The disease : .....
5. Result:
  - Blood group : .....
  - Rh Phenotype  
D .....  
C .....  
c.....  
E.....  
e.....
6. Comment  
.....  
.....  
.....  
.....  
.....

## **Appendix (2)**

### **Rapid H. pylori Antibody Test**

The principle of Rapid H. Pylori Antibody Test employs chromatographic lateral flow test device in a cassette format. Colloidal H. pylori antigens (Au-Ag) are dry-immobilized at the end of nitrocellulose membrane strip. H. pylori antigens are bond at the Test Zone (T) when the sample is added; it migrates by capillary diffusion rehydrating the gold conjugate. If anti-H. pylori antibodies present in sample, Antibodies will bind with the gold conjugated antigens forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by H. pylori antigens generating a visible red line. If there are no anti-H. pylori antibodies in sample, no red line is formed in the Test Zone (T). A built-in control line will always appear in the Control Zone (C) when the test has performed properly, regardless of the presence or absence of anti-H. pylori antibodies in the specimen.

### **Appendix (3)**

#### **Ethical Consideration**

A verbal consent was taken from each individual before being enrolled in the study. Each patient was informed about aim of the study.