FREQUENCIES OF THE ABO BLOOD GROUP SYSTEM AND ITS RELATIONSHIP WITH *Helicobacter pylori* INFECTION AMONG PATIENTS WITH GASTROINTESTINAL COMPLAINTS IN ASELLA TEACHING AND REFERRAL HOSPITAL, ARSI ZONE, OROMIA, ETHIOPIA

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Frequencies of the ABO Blood Group Systems and its Relationship with *Helicobacter pylori* Infection among Patients with Gastrointestinal Complaints in Asella Teaching and Referral Hospital, Arsi Zone, Oromia, Ethiopia

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MASTER OF SCIENCE IN BIOLOGY

Ibrahim Furo Feyiso

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POSTGRADUATE PROGRAM DIRECTORATE

We hereby certify that we have read and evaluated this Thesis entitled "**Frequencies of the ABO Blood Group Systems and its Relationship With** *Helicobacter pylori* **Infection among Patients With Gastrointestinal Complaints in Asella Teaching and Referral Hospital, Arsi Zone, Oromia, Ethiopia**'' prepared, under our guidance by Ibrahim Furo we recommend that it to be submitted as fulfilling the thesis requirement.

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As a member of the board of the examiners of the M. Sc Thesis open defenses examination, we certify that we have read and evaluated the thesis prepared by Ibrahim Furo and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Biology.

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DEDICATION

I dedicate this Thesis manuscript to my mother Zelika Butta who laid the foundation to all my life since my early stage of childhood and devoted much in nursing me with special affection and love throughout my life.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation. This Thesis is submitted in partial fulfillment of the requirements for M.Sc. degree at the Haramaya University. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author Ibrahim Furo was born from his father Furo Feyiso and his mother Zelika Butta on August 30, 1984 in Arsi Zone Ziway Dugda district Ogolcho 02 kebele. He had completed his elementary and secondary education (grade1-10) at Ketar Fuafuate Elementary and Secondary School and grade (11-12) at Asella Comprehensive Secondary School in Asella town. In 2002 he joined higher education at Addis Ababa University. Then, he was employed in Oromia Region Education Bureau in Arsi Zone at Tiyo district, Ketar Preparatory School as Natural Science Teacher in 2006. In 2015, he joined the Postgraduate Program Directorate at Haramaya University, School of Biological Sciences and Biotechnology to pursue his M.Sc. in Biology study.

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ACRONYMS AND ABBREVIATIONS

AHTR Acute Hemolytic Transfusion Reaction

D U Duodenal Ulcer

G U Gastric Ulcer

ISBT International Society of Blood Transfusion

PMN Polymorphic Nuclear Lymphocytes

RBC Red Blood Cell

Rh Rhesus

WHO World Health Organization

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ABSTRACT

Helicobacter pylori are a small, spiral-shaped bacterium that lives on the surface of the stomach and duodenum. H. *pylorus* is *closely associated with onset of diseases such as gastric ulcers, duodenal ulcers, gastritis and gastric cancers. The aim of this study was to determine the frequencies of ABO blood group system and its relationship with H. pylori infection among patients with gastrointestinal complaints at the Asella Teaching and Referral Hospital. A total of 422 patients of whom, (233 males and 189 females), ranging from 18 to 79 years in age took part in this study. Stool samples were taken from all patients for detection of H. pylori antigen by chromatography immunoassay test. The ABO blood group system was determined by hem agglutination test. A total of 142(33.6%) of the participants were H. pylori positive. The phenotypic frequencies of ABO blood group is O-type202 (47.9%), A-type 121(28.7), B-type 69(16.4%) and AB-type 30(7.1%) and the allelic frequencies of ABO blood group of patients were* I^A *=0.1924,* I^B *= 0.1320 and* I^O *was 0.6756 and genotype frequencies were* I^AI^A *=0.0370,*I^AI^O*=0.2599,*I^BI^B*=0.0174,*I^BI^O*=0.1784,*I^AI^B*=0.0508,*I^OI^O*=0.4564.The prevalence in males and females respectively is 75(32 %) and 67(35%). Patients of blood type-A were more prone to H. pylori infection 47(38.8%) than patients in other blood type, and patients in the AB blood type were less prone to H. pylori infection 6 (20%) as compared with patients in other blood type at 0.05 significant level . The finding of this study indicates that people of blood type-A were more susceptible to infection with H. pylori as compared with other blood type. The results of this study also showed that there was a significant association between ABO blood type and H. pylori infection (p-value=0.027) at 0.05 significant level, in which type-A has a greater tendency towards infection and type -AB to non-infection.*

Key words: Agglutination test, Duodenal ulcer, Gastric ulcers, Gastrointestinal Complaints, Immunoassay test.

1. INTRODUCTION

The classification of blood is based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, Carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens that stem from one allele (or very closely linked genes) collectively form a blood group system. If an individual is exposed to a blood group antigen that is not recognized as self, the immune system produces antibodies that can specifically bind to that particular blood group antigen, on the surface of the red blood cells and leading to the destruction of those cells (agglutination), low blood pressure, and even death (Anthea *et al*., 1993). Blood types were inherited and they represented contributions from both parents. A total of 30 human blood group systems have now been recognized by the International Society of Blood Transfusion (ISBT, 2008).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).

The ABO blood group is a genetic polymorphism that leads to phenotypic polymorphism, due to antigenic differences on the surfaces of the red blood cells (RBCs), and is one of the classical genetic markers that have well been studied since its discovery around the beginning of the 20^{th} century(Rahman and Lodhi,2004). Based on serological agglutination tests of blood, individual humans can be categorized into four ABO blood phenotypes: A, B, AB, and O. The ABO blood groups consist of A, B and H carbohydrate antigens which can regulate protein activities during infection and antibodies against these antigens. The enzyme, Histo-blood group ABO system transferase, is a glycosyltransferase, which adds the carbohydrate antigens to the surfaces of RBCs is encoded by the ABO gene, which is located on chromosome 9 at the band 9q34.2 and contains 7 exons. Generally, there are three alleles: the A allele encodes for

the enzyme that adds α1,3-N-acetylgalactosamine to the H-antigen to produce the A-blood type; the B-allele encodes for an enzyme that adds α1,3-D-galactosamine to the H-antigen to produce B- blood type; and the O-allele does not code for any functional enzyme and no carbohydrate is added to the H-antigen, resulting in O-blood type (Reid *et al*., 2012).

The need for blood group prevalence studies is multipurpose; as besides their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine (Green *et al*., 1995). Various studies on ABO incompatibility have produced a very high frequency of prenatal death among incompatible mating. Red blood cells have a series of glycoproteins and glycolipids on their surfaces which constitute the blood group antigens (Srikumeri *et al*., 1987).

The study of blood grouping is very important as it plays an important role in genetics, blood transfusion, forensic study, blood bank, organ transplantation, paternity test and some groups may have association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, Rh incompatibility and ABO incompatibility of newborn (Rahman *et al*., 2004). It is, therefore, imperative to have information on the distribution of these blood groups in any population group that comprise different ethnic group, (Kumar *et al*., 2009).

Helicobacter pylori are spiral-shaped and gram-negative bacteria that grow in the digestive tract and have a tendency to attack the stomach lining. *H.pylor* infection is a high prevalence infection worldwide, and it is more common in developing countries than developed ones (Magalhaes and Luzza, 2006). *H. pylori* infect the gastric epithelium and are a cause of chronic gastritis and gastro duodenal ulcers. Chronic infection and inflammation can lead to intestinal metaplasia, gastric adenocarcinoma, and gastric lymphoma. In 1994 the World Health Organization (WHO) designated *H.pylori* a class I carcinogen (IARC, 1994; WHO, 1994).

A number of studies were conducted to investigate the association between ABO blood group systems and some disease conditions (Nakao *et al.,* 2011). Many authors

reported that there was an association between ABO blood group and *H. pylori* infection (Kanbay, *et al.*, 2005).

The ABO phenotype has been linked with duodenal ulcers, which were more common in group O individuals and gastric ulcer, which was more common in group A individuals (Iodice *et al.*, 2010).The association between blood group A and gastric cancer has been mentioned in the studies of several groups (Aird *et al*., 1970) and also individuals with blood group A were more susceptible to pernicious anemia, compared with non-A blood group individuals (Roberts, 1959).

Previous study indicated that the ABO blood groups in humans and *H. pylori* infection reveal a correlation between disease and ABO groups (Nakao *et al*., 2011). Many authors report an association between blood group O and *H. pylori* infection (Kanbay *et al*., 2005).

However, no previous study has been reported in the literature regarding the association of ABO blood group with infection of *H. pylori* among patients of Asella Teaching and Referral Hospital. Therefore, the aim of this study was to generate data on the association of frequencies of ABO blood group with *H.pylori* infection in Asella Teaching and Referral Hospital.

General Objective of the study

The general objective of this study was to determine the frequencies of ABO blood group system and to see its relationship with *H. pylori* infection among patients with gastrointestinal complaints in Asella Teaching and Referral Hospital, Arsi zone, Oromia Regional State.

Specific Objectives of the study

- To determine the frequencies of the ABO blood group phenotypes among patients with gastrointestinal complaints visiting Asella Teaching and Referral Hospital.

- To estimate allelic and genotypic frequencies from the phenotypic frequencies among patients with gastrointestinal complaints visiting Asella Teaching and Referral Hospital.
- To estimate the prevalence of *H. pylori* infection among patients with gastrointestinal complaints in Asella Teaching and Referral Hospital.
- To test the relationship between the ABO blood group system and *H. pylori* infection among the patients visiting the hospital.

2. LITERATURE REVIEW

2.1. History of ABO Blood Group System Study

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who found three different blood types in 1900; he was awarded the Nobel Prize in Physiology or Medicine in 1930 for his work. Landsteiner named the first two blood groups antigen A and antigen B, using the first two letters of alphabet while RBCs not reacting with anti A and anti B were called type C. Classification of the blood group was based on his observation of the agglutination reaction between an antigen on erythrocytes and antibodies present in the serum of individuals direct against these antigens. Where no agglutination had occurred either antigen or antibody was missing from the mixture in1902 Van Decastello and Sturil describe RBCs reacting with both anti- A and anti- B but did not these type a name, but continued calling RBCs that did not react with anti- A and anti-B type C (Garrattey *et al.,* 2000) .

In 1911,Van Dungern and Hirszfeld were the first to use the term O to describe RBCs not reacting with both anti- A and anti-B and the term AB for RBCs reacting with both anti-A and anti- B(Mollison 1994).Ludwik Hirsfeld and E.vondungern describe the heritability of ABO blood groups in 1910-1911.With Felix Bersteien demonstrating the correct ABO blood group inheritance pattern of multiple alleles at one locus in 1924.Watikins and Morgan,in England discovered that the ABO epitomes were conferred by sugars, specifically N-acetylgalactosamine for the A-type and galactosyl for the B type. After the published literature claiming that the substance were all attached to glycosphingolipides (Mollison, 1993).

Due to inadequate communication at the time, it was subsequently found that the Czech serologist Jansky had independently pioneered the classification of human blood into four groups, but Landsteiner's independent discovery had been accepted by the scientific world while Jansky remained then in relative obscurity. However, in 1921 an American medical commission acknowledged Jansky’s classification. Jansky is nowadays credited with the first classification of blood into the four types (A, B, AB

and O). Ludwik Hirszfeld and E. von Dungern discovered the heritability of ABO blood groups in 1910–1911. Felix Bernstein demonstrating the ABO correct blood group inheritance pattern of multiple alleles at one locus in 1924. Watkins and Morgan, in England, discovered that the ABO epitopes were conferred by sugars, to be specific, N-acetylgalactosamine for the A-type and galactose for the B-type.After much published literature claiming that the ABH substances were all attached to glycosphingolipids, (Finne *et al*., 1978).

2.2. Distribution of ABO Blood Group System in different populations

The O blood type is very common around the world; about 63% of humans share it. Type O is particularly high in frequency among the indigenous populations of central and South America where it approaches 100%. The lowest frequency of (O) is found in Eastern Europe and central Asia, where B is common (Khan *et al*, 2009). Of the Rhesus blood group system, the allele D which gives rhesus positive status is at its lowest in Europe. It increases in frequency east ward and south ward to approximately 80% over almost all of Africa south of the Sahara. In eastern Asia, Australia and Indonesia; it often attains 100%. The same holds for American indigenous population in many of whom the D frequency is 100 % (Neil, 2006).

Blood type-A is associated with high frequencies in Europe, especially in Scandinavia and Central Europe, although its highest frequencies occur in some Australian Aborigine populations and the Blackfoot Indians of Montana (Dean, 2005). Blood type-B has its highest frequency in Northern India and neighboring Central Asia, and its incidence diminishes both towards the west and the east, falling to single digit percentages in Spain. It is believed to have been entirely absent from Native American and Australian 7 Aboriginal populations prior to the arrival of Europeans in those areas (Encyclopedia Britannica, 2002).

2.3. Genetics of ABO Blood Groups

A and B are co-dominant, giving the AB phenotype. Blood groups are inherited from both parents. The ABO blood group is controlled by a single gene (the ABO gene)

with three types of alleles inferred from classical genetics: *i*, I^A and I^B. The gene encodes a glycosyltransferase that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens. The gene is located on the long arm of the ninth chromosome (9q34.2). The I^A allele gives type A, I^B gives type B, and *i* gives type O. As both I^A and I^B are dominant over *i*, only *ii* people have type O blood. Individuals with I^AI^A or I^Ai have type A blood, and individuals with I^BI^B or I^{Bi} have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship: co dominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi, I^Ai). The cis-AB phenotype has a single enzyme that creates both A and B antigens. The resulting red blood cells do not usually express A or B antigen at the same level that would be expected on common group A1 or B red blood cells, which can help solve the problem of an apparently genetically impossible blood group (Yazer *et al*., 2006).

A and B alleles have seven nucleotide substitutions. Four nucleotide substitutions are translated in to different amino acid substitutions. The antigen A, B, and their variants result from functional glycosyltransferase genes capable of transferring N-acetyl-D-galactosamine or D-galactose or both to non-reducing ends of suitable oligosaccaride chains found on red cell membrane glycoprotein and glycolipids. The red cell phenotype denoted O occurs because the glycosyltransferase gene that generates A or B or both antigens are inactive (Anstee, 2010).

2.4. Serology of ABO Blood Group System

For the half-century following Landsteiner's discovery, human blood groups were understood predominantly as patterns of inherited serological reactions. The ABO blood group is determined by the presence of A and B antigens on the surface of the red blood cells, and of anti -A or anti -B antibodies in the serum. Thus, the red blood cells of blood type A possess antigen A and the serum containing anti –B antibody. Similarly, blood type B has antigen B and anti -A antibody. Blood type AB contains both A and B antigens but no antibodies. Blood type O has no antigens but contains

both anti -A and anti –B antibodies. Anti -A and anti -B antibodies are usually IgM type, and not present in newborns, but appear in the first year of life. It is possible that the antibodies are produced against food and environmental antigens (bacterial, viral or plant anti gens), which are similar in structure to A and B antigens (Ogasawara *et al.*, 1998).

2.5. Importance of ABO Antigens in Transfusion Medicine

Blood groups are of great clinical importance in blood transfusion and in transplantation. In fact, the discovery of the blood group system was one of the most important factors in making the practice of blood transfusion possible (Pasha *et al.*, 2009).Transfusion is a specialized branch of hematology that is concerned with study of blood groups, along with the work of a blood bank to provide transfusion services for blood and other blood products. Across the world, blood products must be prescribed by a medical doctor in a similar way as medicines. Much of the routine work of blood bank involves testing blood from both donors and recipients to ensure that every individual recipient is given blood that is compatible and is as safe as possible. If a unit of incompatible blood is transfused between donor and recipient a severe acute hemolytic (RBCs destruction), renal failure and shock is likely to occur and death is a possibility. Antibodies can be highly active and can attack RBCs and bind components of the complement system to cause massive hemolytic of the transfused blood (Nickel *et al.*, 1999).

For a blood donor and recipient to be ABO-compatible for a transfusion, the recipient must not have Anti-A or Anti-B antibodies that correspond to the A or B antigens on the surface of the donor's red blood cells (since the red blood cells are isolated from whole blood before transfusion, it is unimportant whether the donor blood has antibodies in its plasma). If the antibodies of the recipient's blood and the antigens on the donor's red blood cells do correspond, the donor blood is rejected. On rejection, the recipient may experience acute hemolytic transfusion reaction (AHTR) (Mohammad *et al*., 2014).

In addition to the ABO system, the Rh blood group system can affect transfusion compatibility. An individual is either positive or negative for the Rh factor; this is denoted by a '+' or '−' after their ABO type. Blood that is Rh-negative can be transfused into a person who is Rh-positive, but an Rh-negative individual can create antibodies for Rh-positive RBCs. Because of this, the AB+ blood type is referred to as the "universal recipient", as it possesses neither Anti-B nor Anti-A antibodies in its plasma, and can receive both Rh-positive and Rh-negative blood. Similarly, the O− blood type is called the "universal donor"; since its red blood cells have no A or B antigens and are Rh-negative, no other blood type will reject it. Identification of ABO and Rh gene frequencies among human populations has various benefits in transfusion medicine, transplantation and disease risk (Akinnuga *et al.,* 2011).

2.6. The Hardy-Weinberg Principle

The Hardy-Weinberg model describes a mathematical relationship that allows the prediction of the frequency of offspring genotypes based on parental allele frequencies. It also predicts that allele frequencies will not change from one generation to the next, indicative of non-evolution (Klugand Cummings, 2002, Mayo, 2008).

In a large population where there is no genetic drift, and in the absence of selection, migration and mutation, the allelic frequencies remain constant from generation to generation. If mating is random, the genotypic frequencies are related to the allelic frequencies by the square expansion of allelic frequencies. Thus, for autosomal genes in diploid organisms in which there are two alleles with frequencies p and q, the frequencies of the three genotypes are predicted by the formula $(p+q)^2 = p^2 + 2pq + q^2$.

Furthermore, for autosomal genes the equilibrium genotypic frequencies at any given locus are attained in a single generation of random mating providing there is no overlapping of generations. With sex-linked genes the situation is rather more complex than with autosomal genes. The relationship between gene frequency and genotype frequency in the homogametic sex is the same as with an autosomal gene, but the heterogametic sex has only two genotypes and each individual carries only one

gene instead of two. The gene frequency in the population as a whole does not change, but its distribution between the two sexes oscillates as the population approaches equilibrium (Bryant, 1994).

Modified Hardy- Weinberg equation will be used to calculate both genotypic and allelic frequencies of ABO blood groups from phenotypic frequencies (Strickberger, 1976). When two alleles, for example, *p* and *q* are present at a locus, based on the Hardy-Weinberg principle at equilibrium the frequencies of the genotypes become $p^2 + 2pq + q^2=1$, which is the square of the allelic frequencies $(p + q)^2$.

This is a simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are inherited two at a time into a diploid zygote. The three alleles of ABO blood group which are I^A, I^B and I^O are represented as *p*, *q* and *r*, respectively in which *p* is the frequency of allele A, *q* is the frequency of allele B and *r* is the frequency of allele O. Therefore the genotypic frequencies were represented by trinomial expansion as $(P+q+r)^2= p^2 + 2pq + q^2 + 2pr + 2qr + r^2=1$(Hananiaet al.,2007).

Where: P^2 is the frequency of genotype I^AI^A

$q^2$is the frequency of genotype I^BI^B

2pq is frequency of genotype I^AI^B

2pr is frequency of genotype I^AI^O

2qr is the frequency of genotype I^BI^O

r^2 is the frequency of genotype I^OI^O

ABO allele frequencies were estimated according to a published method which yields results that are close to maximum likelihood estimates. Preliminary estimates were calculated as: $p = 1 - \sqrt{B+O}$, $q = 1 - \sqrt{A+O}$, $r = \sqrt{O}$ (*p*, *q*, and *r* denote allele frequencies and A, B, O denote observed frequencies of blood groups A, B and O). A correction factor (θ) will be calculated according to $\theta = 1 – p – q – r$. The final allele frequencies were then calculated as follows: $p^1 = p\ (1 +)$, $q^1 = q\ (1 +)$ and $r^1 = (r +)$ .Where p^1,q^1 and r^1 denote corrected allele frequencies.

Calculating the phenotype frequencies and Expected number

Expected number = genotype frequency x total sample.

For A blood type E_f = frequency of (AA + AO) X number of total sample

For B blood type E_f = frequency of (BB + BO) X number of total sample

For AB blood type E_f = frequency of AB X number of total sample

For O blood type E_f = frequency of OO X number of total sample

2.7. History of *H. Pylori* Study

In the early 1980s, Barry Marshall, a physician specialized in internal medicine, and the pathologist Robert Warren found spiral-shaped bacteria closely associated with the gastric mucosal surface (Marshall and Warren, 1984). The bacteria were obvious in about half of routine biopsies and were closely associated with mucosal inflammation. Attempts to culture the bacteria failed until a bacterial culture from a gastric specimen was accidentally incubated longer than planned, over an Easter holiday. The bacterium was named *Campylobacter pyloridis*. Warren and Marshall found *C. pyloridis* in all patients with duodenal ulcer and in the great majority of patients with gastric ulcer. They suggested that *C. pyloridis* could be an important factor in the development of peptic ulcer disease and that antibiotic therapy might be successful in healing peptic ulcers (Marshall and Warren, 1984).

Marshall himself managed to prove the link between gastritis and *C. pyloridis* infection by drinking a culture of *C. pyloridis*. He developed acute dyspeptic illness, and biopsies showed spiral-shaped bacteria in his inflamed gastric mucosa. Marshall then treated himself with antibiotics. After a series of clinical trials with antibiotic therapy and successful eradication of *C. pyloridis* infections and associated gastric disease, the scientific community finally accepted the causal role of C. *pyloridis* infection in peptic ulcer disease. In 1989, *C. pyloridis* was re-named *Helicobacter pylori* (Andersen and Wadstrom, 2001; Marshall and Warren, 2001). The discovery of the association of *H. pylori* with peptic ulcer disease was the starting point for the medical revolution of efficient treatment for peptic ulcer disease. *H.pylori* is a spiral-shaped, Gram-negative rod with 5-7 flagella at one end. The bacteria are sensitive to

oxygen, and require a microaerophilic atmosphere, i.e., about 5% O_2 and 5-10% CO_2 (Andersen and Wadstrom, 2001).

A unique feature of *H. pylori* is its colonization of the acidic gastric environment for the lifetime of the host. It shows a strict tissue and host tropism for the gastric mucosa of humans and primates. *H. pylori* is normally found in the less acidic atrium region (lower part) of the stomach, but colonizes the corpus (mid) region during conditions of low acid secretion *H. pylori* survive the low pH in the stomach by producing high levels of the enzyme urease, which hydrolyzes urea into ammonia (NH3) and carbonic acid. Ammonia buffers the cytosolic and periplasmic pH, as well as the microenvironment immediately surrounding the bacteria. Unlike the urease of most other bacterial species, the *H. pylori* enzyme can also be found associated with the bacterial surface or be shed into the medium. This appears to be due to release of urease by lysis of bacteria and subsequent adsorption of the protein onto the surface of living bacterial cells. The urease enzyme is an important colonization factor of *H. pylori*, since it is produced at high levels by all clinical isolates and is essential for growth in vivo (Montecucco and Rappuoli, 2001).

For many bacteria including *H. pylori*, flagellum-dependent movements are essential for infection. In *H. pylori*, each flagellum is approximately 3 μm long and covered by a sheath, which is a membranous layer continuous with the outer membrane (Goodwin *et al,* 1985). The sheath has been suggested to protect the polymeric filament core from dissociation by low pH, and from being recognized by the immune system. The *H. pylori* flagella are composed of three structural units: a basal body in the cell wall which contains the components required for flagellar rotation and chemotaxis, a flagellar filament that works as a propeller, and a hook that connects the flagellar filament with the basal body (Spohn and Scarlato, 2001).

More than 40 proteins of the *H. pylori* genome have been suggested to be involved in regulation, secretion and assembly of the flagellar structures (Tomb *et al.*, 1997). Though, the flagellar filaments consist mainly of two proteins: the major flagellin FlaA and the minor flagellin FlaB (Leying *et al*, 1992; Suerbaum *et al.*, 1993). FlaA

disrupted mutants are completely non-motile whereas flaB mutants are still motile (Suerbaum et al., 1993). Eaton and colleagues showed that full motility was necessary for persistent *H. pylori* colonization of piglet stomachs (Eaton *et al.*, 1989; Eaton *et al.*, 1996). *H. pylori* lives deep in the mucus layer where part of the bacterial population is attached to the gastric epithelial cells, but the great majority of bacteria are motile and are found within the viscous mucus layer (reviewed in Testerman *et al.*, 2001).

The mucus is composed of highly glycosylated proteins (mucins) and is continuously secreted by gastric glands and epithelial cells to form a layer that protects the gastric epithelial cells. It also plays an important role in maintaining a neutral pH at the epithelial cell surfaces through its ability to retain bicarbonate. The surface of the gastric mucus layer is continuously shed into the gastric lumen. To avoid being cleared by mucus turnover, *H. pylori* swims towards the gastric epithelial cells, guided by a chemical gradient in the mucus layer. Experimentally challenged Mongolian gerbils demonstrated that *H. pylori* uses mucus pH for chemotactic orientation. Elimination of the mucus pH gradient by simultaneous reduction of arterial pH and bicarbonate concentration caused bacteria to lose orientation and disseminate in the mucus layer (Schreiber *et al.*, 2004).

2.8. Association between ABO Blood Group and *H-Pylori* Infection

A number of studies were conducted to investigate the association between ABO blood group systems and some disease conditions (Nakao *et al.,* 2011). Many authors reported that there was an association between ABO blood group and *H. pylori* infection (Kanbay, *et al.,* 2005). Previous study indicated that the ABO blood groups in humans and *H. pylori* infection reveal a correlation between disease and ABO groups (Nakao *et al*., 2011).

The ABO phenotype has been linked with stomach ulcers, which are more common in group O individuals and gastric cancer, which is more common in group A individuals (Iodice et al., 2010). It has been known that individuals with blood group O phenotype have higher risk of developing duodenal ulcers. Similarly, gastric carcinoma was

found to be associated with blood group A, but no explanation for this condition was found. In 1993, Boren *et al*. reported that people with blood group O had more *H. pylori* receptors, and Lewis b antigens mediated the attachment of *H. pylori* to the gastric mucosa.

However, the findings of different epidemiological studies reported on the association between O blood group and *H. pylori* infection were controversial and inconsistent. While many authors reported statistically significant association between O blood group and *H. pylori* infection (Jaff, 2011; Mattos et al., 2010; Mattos et al., 2002).

2.9. *H. pylori* Infection and Gastric Diseases

2.9.1. Gastritis

The healthy, uninfected human stomach contains very few immune and inflammatory cells. Initial colonization by *H. pylori* results in an acute inflammatory response (acute gastritis), which is characterized by infiltration by polymorph nuclear lymphocytes (PMN cells) and neutrophils into the gastric mucosa. The acute infection is also accompanied by transient hypochlorhydria, i.e. reduced gastric acidity. If these initial responses fail to clear the infection, there is a gradual accumulation of neutrophils, T cells, B cells and macrophages into the gastric mucosa. After a few weeks, there is a massive invasion of the tissue by immune and inflammatory cells, which is a characteristic histological picture of chronic active gastritis. The continuous presence of *H.pylori* elicits a local mucosal IgA antibody response and a systemic humoral response, neither of which can eradicate the infection. Once established, the infection persists for the Antrum Duodenum Corpus Esophagus Cardia Marina Aspholm 18 lifetime of the host if not eradicated with antibiotics. In some cases, it progress to severe gastric diseases such as duodenal ulcer, gastric ulcer, gastric atrophy and gastric carcinoma. However, in the great majority of infected individuals the *H. pylori*-related chronic gastritis is asymptomatic (Dixon, 2001).

2.9.2. Duodenal ulcer (D U)

Helicobacter pylori is present in >95% of patients with duodenal ulcers and in >80% of patients with G U which is a significantly higher prevalence than among patients without ulcer disease (Walsh and Peterson, 1995). The most convincing evidence for a causal relationship between *H. pylori* and peptic ulcer disease, however, is healing of the ulcer following antibiotic therapy (Forbes *et al.,* 1994; Graham *et al.*, 1992). Duodenal ulcers are often associated with excess gastric acid secretion. Hypersecretion of acid into the duodenum promotes development of gastric metaplasia, i.e. the presence of gastric-type mucus secreting cells in the surface epithelium of the duodenum. The appearance of gastric epithelial cells in the duodenum allows colonization by *H. pylori*, which will establish a chronic inflammatory response. The inflammation process and bacterial effect on the epithelial cells renders the duodenal mucosa sensitive to gastric acidity, and thus predisposes it to ulceration (Dixon, 2001). Atrophic gastritis is defined as gradual loss of gastric glandular tissue as a consequence of long-term mucosal damage, in particular due to chronic inflammation. In fact, the tissue destruction may involve progressive loss of all specialized mucosal cells including the acid producing parietal cells, pepsinogen producing chief cells and mucus producing gland and foveolar cells. When these cell types have diminished, the protective mucus layer will gradually disappear and the acid secretion will cease. Such pathological changes increase the risk of gastric ulceration and development of gastric adenocarcinoma, but, somewhat contradictory, they are found protective against duodenal ulcers because acid secretion is lowered (Blaser and Atherton, 2004).

2.9.3. Gastric ulcer (G U)

In contrast to D U, G U are associated with low acid secretion in addition to *H. pylori* infection. In individuals with normal or high acid secretion, *H.pylori* does not normally colonize corpus because of the low pH, whereas in individuals with low acid secretion, the colonization will be more evenly spread throughout the stomach. Colonization by *H. pylori* leads to continuing inflammatory cell infiltration, epithelial degeneration, increased exfoliation of the epithelial cells and compensatory cell

proliferation by immature precursor cells. This leads to impaired mucin and bicarbonate production, which makes the mucus barrier compromised and the tissue more susceptible to ulceration (Dixon, 2001).

2.10. Epidemiology and Transmission

Table 11: Prevalence of *H. pylori* infection across the globe.

Country	Prevalence %	Refrance
Netherlands	46	Den Hollander *et al*.,2013
Portugal	84.2	Bastor *et al.*,2013
Canada	37.9	Sethi *et al.*,2013
Mexico	52.2	Alvarado-Esquives,2013
India	62	Sodhi *et al.*,2013
Morocco	75.5	Benajah *et al*.,2013
Ethiopia	65.7	Mathewos *et al*.,2013

Source: http://www.helicobacter.org/2014/

Helicobacter pylori colonize all human populations worldwide. The risk of being colonized by *H. pylori* depends on geographic area, socioeconomic status and age, and initial colonization is thought to occur during early childhood. In developing countries the infection can be almost ubiquitous, whereas in industrialized countries *H. pylori* infect 30–50% of adults. The decline in *H. pylori* infection incidence that relates to industrialization and improvements in socioeconomic levels may be explained by the frequent use of antibiotics, improvements in sanitation, and reduced crowding. The higher prevalence of *H. pylori* in individuals over 40 years of age is considered to be due to a birth cohort effect rather than a continuous risk of being infected, i.e. the incidence of infection was higher in the past (Cover *et al.*, 2001). Transmission may be related to the ability of *H. pylori* to form non culturable coccoid forms when exposed to unfavorable environmental conditions. However, controversy exists as to whether these coccoids are alive and important for transmission (discussed in O´Rourke and Bode, 2001). There is also a high probability that no significant reservoirs exist outside the human stomach, since *H. pylori* has a rather small genome

which does not support all necessary metabolic pathways for a nonparasitic life-style (Alm *et al.*, 1999; Tomb *et al.*, 1997). Thus, person-to-person contact involving ingestion of *H. pylori* from saliva, vomits, feces or recently contaminated foods or beverages would be the most likely modes of transmission. By use of serological and DNA fingerprinting analyses, several studies have suggested that person-to-person transmission occurs mainly within families i.e. vertical transmission instead of horizontal (epidemic) transmission, which is the most common for infectious diseases (Drumm *et al.*, 1990; Mitchell, 2001; Suerbaum *et al.*, 1998). In support of this, the same strain is frequently shared between mothers and their children, but less frequently between spouses. Interestingly, transmission is less common between fathers and their children (Han *et al.*, 2000; Kivi *et al.*, 2003)

2.11. Clinical Manifestation of *H. pylori*

The organism is a major cause of upper gastrointestinal diseases such as gastritis, peptic ulcer (D.U and G.U) and gastric cancer (Ahmed *et al.*, 2007; Tanih *et al.*, 2009). The primary disarray that follows initial colonization of the host is chronic active gastritis (Kusters *et al.*, 2006). It has been suggested that up to 95% of D.U and 70% of G.U are attributable to this infection and most cases occur in middle aged subjects (Rothenbacher, 2007). In the US, nearly all persons with duodenal ulcer are infected, and that persons without the infection will ever develop duodenal ulcer is highly unlikely. Although gastric ulcer is usually caused by these bacteria, about 30% of gastric ulcers in the US occur in persons without *H. pylori* and could be related to non steroidal anti-inflammatory drugs. Most gastric adenocarcinomas and lymphomas occur in persons with current or past infection with *H. pylori*. The clinical outcome of long-term infection is variable and is considered to relate both to bacterial virulence factors (Gatta *et al.*, 2003)

2.12. Colonization of *H. pylori*

Bacteria that colonize the stomach are discovered in the 1980's; their colonization causes peptic ulcers that used to be a major medical problem. The identification of *H. pylori* as the principal etiologic agent in peptic ulcer disease led to a search for

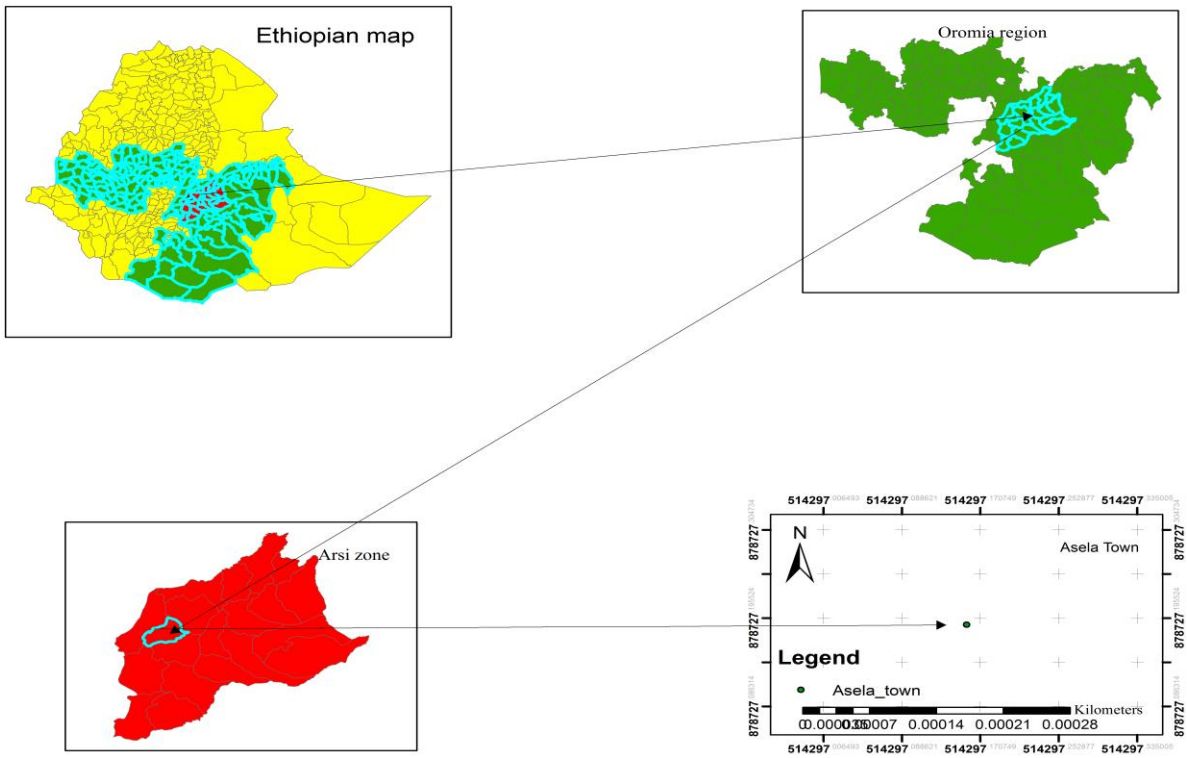
bacterial colonization factors able to overcome the hostile environment of the stomach. In microscopic studies, *H. pylori* are located within the mucin layer in close proximity to the foveolar gastric epithelium, with little or no binding to the deeper glandular epithelium (Moore *et al*., 2011). In contrast, the deep glandular epithelium expresses type 2 antigens (Le^X and Le^Y) on MUC6, including growth-inhibitory mucins capped with a terminal α1, 4-GlcNAc (Magalhaes and Reis, 2010).

A role for fucose in *H. pylori* binding is inferred from early inhibition studies with secretory IgA from human colostrum (Falk *et al*., 1993). Boren *et al*. later showed that colostrum rich in Le^b, but not Le^a, inhibited *H. pylori* binding to the gastric epithelium by 78% (Boren *et al*., 1993). This is confirmed by inhibition studies with anti-Le^b and commercial Le^b antigen. Moreover, *H. pylori* are shown to directly recognize Le^b and H-active GSLs by thin-layer chromatography analysis (Boren *et al*., 1993).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Asella town at Asella Teaching and Referral Hospital, Arsi Zone, Oromia Regional State. Asella town is one of the towns located in Arsi Zone. The town is located at about 175km to the south-east of the capital city of the country, Addis Ababa. The area has an elevation of about 2210m-2700m above sea level and located at $7^{o}5'55''$ and $8^{o}00'05''$ north with annual rainfall 823 mm on average. It had a total population of 160,000 according to Central Statistical Agency (2007) with growth rate of 2.9% per year consisting of 80,000 male and 79,550 females and the current population is 173,920 as per the mentioned growth rate is concerned. The town comprises different ethnic groups such as Oromo, Amhara, Gurage, Silte, etc. The duration of this study has taken a time period from march 2018 up to August 2018 G.C. The annual average temperature of the town is ranging from 10-23 ^{o}C. (Source: Asella Town Administration)



Source: Ethio. GIS www.ema.gov.et

Figure 1: Map of the study area

3.2. Study Design

A hospital-based cross-sectional study design was used in the present study to determine the prevalence of *H. pylori* infection and the frequencies of the ABO blood group system and to test relationship between them in patients of gastrointestinal complaints visiting the Asella Teaching and Referral Hospital.

3.3. Study Population

The study population consisted of patients of gastrointestinal complains visiting the Asella Teaching and Referral Hospital.

3.4. Sample Size Determination

The sample size for the present study was determined using the formula shown below based on the 95% confidence limits and 5% sampling error (Naing *et al*., 2004):

$$n = \frac{Z^2(P)(1-P)}{d^2}$$

Where:

n= required sample size.

Z=standard deviation which is= 1.96

P=prevalence of the issue under study= 0.5

d= confidence limit of prevalence which =0.05.

The formula gave us sample size three hundred eighty four (384) and this was used to calculate additional 10% contingency that made the final total sample size around four hundred twenty two (422) patients.

3.5. Selection of Participants

Patients visiting Asella Teaching and Referral Hospital were considered and it was drawn by using purposive selection of patients according to their serial registration identified by physician those who are expected to having gastrointestinal problems.

3.6. Stool Sample Collection and Testing of *H. pylori* Infection

The prevalence of *H. pylori* was determined by One Step *H. pylori* Test technique (www. Wondfo.com.cn). Wondfo One Step *H. pylori* Feces Test strip is a rapid chromatographic immunoassay for the qualitative detection of antigen to *H. pylori* in feces to aid in the diagnosis of *H. pylori* infection. One study using a stool antigen test found it to be a cost-effective and rapid method for initial screening of *H. pylori* infection (Kalach N., 2017).

3.6.1. Stool Sample Collection

Stool sample was collected by using the sample collection tube provided with sampling stick. The patients were instructed on how to collect stool sample using stool

sampling stick, a small portion of the fecal material was taken using a sterile applicator stick which are screwed on the collection tube and is placed the stick in the tube and tighten securely. The sample was diluted using the extraction buffer solution.

3.6.2. Testing of *H. pylori* Infection

The *H. pylori* kit device and the extracted feces were brought to room temperature. Then the sample solution was mixed well by gently shaking the collection tube. The test device were removed from the foil pouch by tearing at the notch and placed on level surface. A sample collector kept up right, the tip of the collector carefully broken off at the break point, three drop of sample solution will be squeezed to the sample pad below the mark line. After 15 minutes the results were recorded and read as positive if pink color appeared and negative if not.

3.7. Blood sample collection and Determination of Blood Type

3.7.1. Blood Sample Collection Method

Blood samples were collected by finger prick with sterile lancet using an open slide method of testing ABO blood types and were tested by the researcher and with a guidance of qualified laboratory technician during the collection of the sample. Blood samples were taken by scrubbing the middle finger with a piece of cotton saturated with alcohol and piercing it with a sterile packed lancet, using the standard clinical procedure and protocol.

3.7.2. Blood Type Determination Method

A drop of blood was placed on three glass slide on which anti-A, anti-B and anti-D were added and was mixed thoroughly with the anti sera and was rocked gently for 60 seconds using applicator stick to observe agglutination. The slide then tilted to detect for agglutination and the result was recorded accordingly. In case of doubt, the test was examined under a microscope, or the results confirmed by reverse grouping using known group A and B red cells. Blood type was determined on the bases of agglutination and was recorded as blood type A, B, AB, and O blood types and Rh^+ and Rh^- types (Daniels, 2002).

3.8. Data Analysis

Statistical package for social science (SPSS), Windows version 20 was used for data analysis. Descriptive statistics was applied to indicate the prevalence of *H. pylori* infection as frequencies and percentage. The association between *H. pylori* and ABO blood group systems was determined by using chi-square test with P value less than 0.05 was considered as statistically significant.

3.8.1. Determination of phenotypic frequencies

The phenotypic distribution of blood types among the study participants was expressed in simple percentages and frequencies.

3.8.2. Determination of Allelic Frequencies and Genotype Frequencies

Modified Hardy-Weinberg equation was used to calculate both genotypic and allelic frequencies of ABO blood groups from phenotypic frequencies,(Strickberger, 1976).When two alleles, for example, *P* and *q* are present at a locus, based on the Hardy-Weinberg principle at equilibrium the frequency of the genotypes become $p^2+2pq+q^2$,which is the square of allelic frequencies(p^2+q^2).This is a simple binomial expansion, and this principle of probability theory can be extended to any number of alleles that are inherited two at a time in to a diploid zygote(Daniel *et al* ,2007).The three alleles of ABO blood group which are A, B, and O are represented as *p, q, r,* respectively. In which *p* is the frequency of allele A, *q,* is the frequency of allele B, *r,* is the frequency of allele O. Therefore, the genotypic frequencies are represented as $(p+q+r)^2= p^2+2pq+q^2+2pr+2qr+r^2$

ABO alleles were estimated according to a published method which yields results that are closer to maximum likelihood estimate. Preliminary estimates will be calculated as: p=1-√B+O, q=1-√A+O, r=√O (p, q, r) denote allele frequencies and A, B, O denote observed frequencies blood groups A, B, and O and correction factor ($\theta = 1 - p - q - r$) can be used.

3.8.3. Chi-Square test for observed versus expected frequencies of ABO blood distribution

Chi-square test was used to see the Goodness-of-fit between the observed and the expected frequencies of blood types for ABO blood group systems. The Observed versus expected frequencies of phenotypes of the ABO system was statistically tested using the chi-squared formula as:

$$\chi 2 = \sum \frac{(O-E)^2}{E}$$

Where: χ^2 = Chi-Square

O = Observed frequency

E = Expected frequency

Expected phenotypic frequencies were calculated as:

E_f = Genotypic frequency X number of total sample

For A blood type E_f = frequency of (AA + AO) X number of total sample

For B blood type E_f = frequency of (BB + BO) X number of total sample

For AB blood type E_f = frequency of AB X number of total sample

For O blood type E_f = frequency of OO X number of total sample

3.8.4. Estimation of the prevalence of *H. pylori* infected individuals

The prevalence of *H. pylori* infection among study participants was determined from the results of antigen stool sample examination and expressed in simple percentages and frequencies.

3.8.5. Test of association between the ABO blood group and *H. pylori* infection

The degree of association between blood type and the diseases from the populations was subjected to Chi-square testes using the statistical package SPSS version 20.From the results of Chi-square test and P-value analyzed from SPSS, observed difference was considered to be significant at $P<0.05$ with 95% confidence interval (CI). The

relationship was considered to be statistically significant when the P-value obtained is less than 0.05 and if not association was considered to be insignificant.

3.10. Ethical Considerations

This study followed the ethical standards and requirements set by the country in general and those by Haramaya University. Therefore, authorization to carry out the study was obtained from the Health Office Management/ Ethical Committee of the Asella town using a Cooperation letter prepared by the Haramaya University, School of Biological Sciences and Biotechnology and after the objectives and procedures of the study were explained by the researcher.

4. RESULTS AND DISCUSSIONS

4.1. Frequencies of ABO Blood Group System Phenotypes

Blood type O was the most frequent and blood type AB was the least frequent blood type occurred in the sample study(Table 2).

Table 2: The frequencies of the ABO blood group phenotypes among patients with gastrointestinal complaints visiting Asella Teaching and Referral Hospital.

ABO blood group	Frequency	
	Number	Percent (%)
A	121	28.7
B	69	16.4
AB	30	7.1
O	202	47.9
Total	422	100

Among the four ABO blood types: type-A, type- B, type-AB and type-O the most frequent blood was blood type- O 202(47.9%) followed by blood type-A , type-B , type-AB that appeared 121(28.7%),69 (16.4%), and 30(7.1%) respectively that means O>A>B>AB (Table 2).

In population of south west Ethiopia (at Gilgel Gibe Field Research Center), the distribution of type- O, is 42%; type- A, is 31%; type- B, is 21%; and type- AB, is 6% (Abraham *et al.*, 2012). The possible explanation for the disparity in the frequency distribution of the present study from the one at Gilgel Gibe might be due to the different populations/ethnic groups tested and due to sampling from patients who might have different susceptibility to diseases. The distribution of ABO blood groups varies in populations throughout the world (Garatty *et al*., 2004).

4.2. Frequencies of Allele and Genotypes of ABO Blood Group System

The allelic frequencies of ABO blood group of patients were I^A =0.1924, I^B= 0.1320 and I^O was 0.6756 and genotype frequencies were I^AI^A =0.0370, I^AI^O=0.2599,

I^BI^B=0.0174, I^BI^O=0.1784, I^AI^B=0.0508, I^OI^O=0.4564(table 3).

Table 3: ABO blood Allele and Genotype frequencies among study sample

Allele	Allele frequency	Genotype	Genotype Frequency
I^A	0.1924	I^AI^A	0.0370
I^B	0.1320	I^AI^O	0.2599
I^O	0.6756	I^BI^B	0.0174
		I^BI^O	0.1784
		I^AI^B	0.0508
		I^OI^O	0.4564

Table3 presents the allele and genotype frequencies of ABO blood groups in sample population of gastrointestinal complaints which visited in Asella Teaching and Referral Hospital**.** The allelic frequencies of ABO blood group was occurred in the order $I^O > I^A > I^B$. It shows similar patterns of allelic frequencies with those documented from earlier studies among various segments of the world population including Ethiopia in which I^O (0.66) > I^A (0.1759) >I^B (0.1638) indicated (ISBT, 2006).

On the predominance of blood allele O over other blood alleles in the population sampled, the researcher agreed with the suggestion of Jonatan G., 2014 that these alleles do not appear with equal frequency in the gene pool. In plain terms there are so many more i alleles out there in the gene pool that the chance of getting ii are higher than AA or Ai.

For example, this finding shows that the frequency of $I^A I^A$ genotype was 0.0370 while $I^A I^O$ genotype was 0.2599. Thus, among those who are blood group A, 13.09 % were homozygous I^AI^A, while about 98.03% were heterozygous $I^A I^O$. Similar

deduction can be made for O allele to be carried silently in $I^B I^O$ heterozygous form in blood group B in the sample population.

4.3. The chi-square test of ABO phenotype frequencies

The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square.Table 4 presents the observed versus the expected values of ABO blood group phenotypes in the sample population.

Table 4: Observed versus expected frequency of ABO blood group phenotypes of patients in the total sample

ABO Blood Group	Observed	Expected	Difference	d^2	
A	121	125.3298	-4.3298	28.86	0.25
B	69	82.6192	-13.6192	258.4	3.04
AB	30	21.4351	8.5649	95.1	2.39
O	202	192.6177	9.3823	79.08	0.41
Total	422	422.0018	0.000		χ^2**=6.2739**

The distribution of the overall observed frequencies of ABO blood group phenotypes do not differ significantly from those expected under Hardy- Weinberg equilibrium. This shows that the population is at genetic equilibrium.

As indicated in Table 4 calculated chi-square test in the sample population, which have the P-value is 0.1116 at α= 0.05, with 3 degree freedom, the result is not significant. The result showed that the deference obtained between observed and an expected frequency of ABO blood group was not significant.

4.4. Prevalence of *H. pylori* Infection

The prevalence of *H. pylori* varies in different societies, geographical location and environmental sanitation. *H. pylori* infection were well known to be the most common human infection worldwide on the basis of the fact that approximately 50% of the world's populations are infected and that human beings are the main reservoir(Brown,2000). As indicated in Table 5 the overall positivity *H. pylori* infection among patients attending the Asella Teaching and Referral Hospital was 33.6%.

Table 5: The prevalence of *H. pylori* infection among gastrointestinal complaint Patients.

H. pylori	Frequency	Percentage (%)	χ^2
Positive(+ve)	142	33.6	
Negative(-ve)	280	66.4	
Total	422	100	χ^2***=0.571***

The pattern of infection was an early childhood acquisition of *H. pylori* (30%-50%) that reaches over 90% during adulthood in developing countries. This has been attributed to the poor socioeconomic status and overcrowded conditions (Cheng *et al*., 2009). The Observed prevalence of *H. pylori* infection (33.7%) was lower compared with the results reported from Ethiopia 85.6% in Gonder (Moges *et al.*, 2006),69-91% in Addis Ababa(Yohannes *et al*.,2005) and 49-70% in Bahirdar (Daniel *et al.*,2004).

The possible explanation for the disparity in the prevalence of the H-pylori infection (33.7%) from the one at Bahirdar (49-70%) might be due to the average daily sunshine time correlated with positivity with H-pylori infection. Average daily sunshine time correlated positively with H-pylori infection (Lu et al., 2018).

4.4.1. Prevalence of *H. pylori* infection by sex

The rate of *H. pylori* infection was 75(32%) in male and 67(35%) in female. As indicated

in table 6 female were more susceptible than male for *H. pylori* infection.

Table 6: Prevalence of *H. pylori* infection by sex

Sex of patients	*H. pylori*		Total	χ^2
	+ve	-ve		
Male	75	158	233	
Female	67	122	189	
Total	142	280	422	χ^2=0.571

Out of the four hundreds twenty two patients with various gastrointestinal complaints 233(55%) were male and 189(45%) were females (table 6). The Pearson chi-square χ^2=0.571, , df=1, p-value is 0.4498, the result is not significant at α= 0.05. Therefore, this study shows that sex and H-pylori infection is not associated among the patients visiting Asella Teaching and Referral Hospital.

In this present study, *H. pylori* colonization was higher in females than in males as seen in some other studies reported (Kanbay *et al.*, 2005; Lacy *et al.*, 2001) and (Alizadeh *et al*., 2009) reported that the prevalence of *H. pylori* was higher in females than males. The researcher think that such divergent observation in this study could be due to female frequently involve themselves fully in home activities that bring them in contact with the source of infection.

4.5. Association between ABO Blood Group and *H. pylori* Infection

The least percentage of *H. pylori* positive individual were found in AB(20%) and in type-B(21.7%) and the largest one was found in type A(38.8%) followed by type O(36.6%).

Table 7 presents the distribution of ABO blood group and *H. pylori* infection demonstrated that positivity rate of *H. pylori* infection was A>O>B>AB were 47(38.8%) in blood type-A, 74(36.6%) in blood type-O, 15(21.7%) in blood type-B and 6(20%) in blood type-AB.

Table *7*: Association of *H. pylori* and ABO blood groups systems.

ABO Blood group	*H. pylori* +ve	*H. pylori* -ve	Total	χ^2 +ve total %	
A	47	74	121	38.84	
B	15	54	69	21.74	
AB	6	24	30	25.0	
O	74	128	202	36.6	
Total	142	280	422		X^2=9.155

The χ2-value calculated at the df 3 produce a P-value of 0.027 which is less than 0.05 critical point. This shows that there is significant association between ABO blood group system and *H. pylori* infection.

Many authors reported that there was an association between ABO blood group and *H. pylori* infection (Kanbay, *et al.*, 2005).

4.6. Association of *H. pylori* and ABO Blood Group Systems and the Odds Ratio for Each Blood Group

Blood type O was the highest prone to H-pylori infection than all other type. As indicated in table 8 adds ratio for blood type A, B, AB, and O was 0.64, 0.28, 0.25, and 0.59 respectively. An odds ratio is a measure of an association between an exposure and an outcome and it is obtained by the ratio of exposure and an outcome (Magdalena S, 2010).

Table *8*: Association of *H. pylori* and ABO blood groups systems and odds ratio.

ABO Blood group	*H. pylori*		Total	Odd ratios
	+ve	-ve		
A	47	74	121	0.64
B	15	54	69	0.28
AB	6	24	30	0.25
O	74	128	202	0.58
Total	142	280	422	

Blood type A(odd ratio=0.64) and O(odd ratio=0.59) are more prone to *H-pylori* infection than blood type B(odd ratio=0.28) and AB(odd ratio=0.25). Therefore, blood type A was the highest prone to *H. pylori* infection while blood type AB was the least prone to *H. pylori* infection. Many authors observed that individuals with blood groups A and O were more prone to the *H. pylori* infection and those with AB blood group were less prone to *H. pylori* infection (Kanbay, et al., 2005).

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

This study aimed to investigate the allelic, phenotypic and genotypic frequencies of the ABO blood group system and to test relationship with *H. pylori* infection, among patients with gastrointestinal complaints in Asella Teaching and Referral Hospital.

A hospital-based cross- sectional study design was used in the present study to investigate the prevalence of *H. pylori* infection and the frequencies of the ABO blood *types of the ABO blood group systems and its relationship with H-pylori infection.*

Stool sample were collected by using the sample collection tube provided with sampling stick and one step *H. pylori* Faces Test strip was chromatographic immunoassay was used for the qualitative detection of antigen to *H. pylori* in feces. Blood samples were taken by Scrubbing the middle finger with a piece of cotton saturated with alcohol and piercing it with a sterile packed lancet, using the standard clinical procedure and protocol**.** Among these ABO blood groups the most commonly frequent blood type was O which occurred 202 (47.9%) followed by blood type-A, type-B and type-AB that appeared 121(28.7%), 69(16.4%), and 30(7.1%) respectively.

Allele frequencies showed a high frequencies of the allele I^O over I^A and I^B alleles of 0.6756, 0.1924 and 0.1320($I^O > I^A > I^B$) respectively and genotype frequencies were $I^A I^A$ =0.0370,$I^A I^O$=0.2599,$I^B I^B$=0.0174,$I^B I^O$=0.1784,$I^A I^B$=0.0508,$I^O I^O$=0.4564.

The distribution of ABO blood group system and *H. pylori* infection demonstrated that positivity rate of *H. pylori* was 47(38.8%) in blood group A, 74(36.6%) in blood group O, 15(21.7%) in blood group B and 6(20%) in blood group AB. Positivity rate of *H. pylori* infection was A>O>B>AB.

5.2. Conclusion

From the finding of the present study the phenotype frequencies of ABO blood group system the most commonly frequent blood type was blood type-O than the others blood type and followed by type-A, type-B and type-AB. From all the genotype and allele frequencies, the I^O alleles show higher frequency than allele I^A and I^B.

In the association between ABO blood types and *H. pylori* infection, the positivity rates of *H. pylori* were the highest in blood type-A, whereas the positivity rate of *H. pylori* were lowest in AB blood type. The results of the above finding shows that female were *H. pylori* positive than male, the frequency of *H. pylori* infection among the patients were highest in patients of blood type-A.

Generally the finding of this study has shown that people of blood type-A were more susceptible to infection with *H. pylori* as compared with other blood type. And there was a significant association between A and O blood type and *H. pylori* infection, in which type-A has a greater tendency towards infection and type -AB to non-infection.

5.3. Recommendation

- The data generated in this study would be helpful as a base for researchers who are interested to conducting similar type of study in the study area.
- The present study is the first study that generates Data on the phenotypic, genotypic and allelic frequencies of ABO blood group system and their relationship with *H. pylori* infections among gastrointestinal complaints patients in Asella Teaching and Referral Hospital and this is used as documented data in the study area.

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7. APPENDIX

Appendix Table 1.1Data collection form

S/N	Sample code	Sex	Age	Blood Type	*H.pylori* +ve or -ve	Clinicaldiagnosis
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Appendix Table2. Patients' Agreement Consent Form

I have been informed about the purpose and objectives of the study that plans to determine "Allelic, phenotypic and Genotypic frequencies of the ABO Blood Group system and its Association with Helicobacter pylori Infection among Patients with Gastrointestinal Complaint in Asella Teaching and Referral Hospital Arsi Zone, Oromia, Ethiopia" For the study I have been requested to participate in the study and give a drop of blood from finger.

They told me that the qualified and experienced laboratory technician would do the blood collection according to the established aseptic procedures by using sterile disposable lancet.

Based on this, I have agreed to participate in the study based on my interest.

I have been also informed that all laboratory results would be kept confidential.

I have been given enough time to think over before I signed this informed consent. It is therefore; with full understanding of the situation that I have gave my informed consent and cooperate at my will in the course of the conduct of the study.

No	**Name of the patients**	**Sex**	**Age**	**signature**	**Remark**

AppendixFigure1

Figure 1. Sample picture of the researcher and the laboratory technician while collecting and processing blood sample

Appendix Figure 2

Figure 2. Sample picture Showing Patients in to be seen by their physician

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ARSI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
Phone: 251-233-311-231
Fax: +251-223-313-533
✉ 04 or 396 Asella, Ethiopia

ቀን/ Date 24/12/2010
ቁጥር/ Ref A/U/H/S/C/ 36/1661/2010

Chief Clinical Director
Elubabor Buno (MD Assistant professor in Neuro Surgery

To Haramaya University

SUBJECT: INFORMING ABOUT IBRAHIM FURO

Ibrahim Furo who is MSc student at your college of Natural Science came to o... hospital with letter to collected data for a research project entitled "**phenoty... ,Allelic and Genotypic frequencies of the ABO Blood Group system and its relationship with Helicobacter pylori Infection** among patients with **gasteointestinal complaints in Asella Teaching and Referral Hospital , Arsi** ...

Therefore, the hospital want to inform you that our hospital Laboratory sup... him during his collection of data from 03/11/2010 to 21/12/2010.

C/C

CCD

Laboratory dep't

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Arsi University College of Health Science

ARSI UNIVERSITY
COLLEGE OF HEALTH SCIENC
Asella, Ethiopia

Ref No. A/u/HS/C/12/14
Date 3/11/2010

ETHICAL REVIEW COMMITTEE/ERC/ APPROVAL SHEET

TITLE OF THE PROJECT : Phenotypic, Allelic and Genotypic frequencies of the ABO Blog Group system and its relationship with Helicobactor paloryi Infection among pateints with gastrointestinal Compliants in Assela Teaching and Referal Hospital Arsi Zone Oromia, 2018"

Principal Investigator: Ibrahim Furo

Project protocol No. A/CHS/RC/43/18

Recommendation of the College of Health Sciences Arsi University Ethicals Review Committee

The request for an initial review on the above mentioned research project was duly considered and approved by the **College of Health Sciences Arsi University** ERC during its meeting dated **on June 25/2018** The investigators should submit final report upon completion. The investigators should also notify the ERC ahead of any amendments or modifications in the protocol or premature suspension or termination of the study.

Regards!

Hailu Fekadu
Demssie (MPH)

Vice Dean For Research and Community Service

Arsi University
College of Health Science

C/c

V/D for Research and community service