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Relationship between *Helicobacter pylori* infection and ABO/Lewis blood groups and secretor status in Palestine

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Thesis Approval

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Jerusalem - Palestine

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Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

Ola TalalAbdallahKarmi.

Date: 01-06-2013

Dedication:

I dedicate my work to my beloved parents who supported me all the way long, and to my lovely sisters Abeer, Areen, Ahd and Iman.

Also I dedicate this to all my teachers and supervisors who lightened the way with their wisdom, patience and knowledge.

Acknowledgments:

Abstract:

The red blood cell membrane antigens have been associated with several diseases as well as susceptibility for specific pathogens. Since *Helicobacter pylori (H.pylori)*infections have been noted to be associated with different red blood cell antigens, several reports have been conducted to identify potential host factors that may increase the risk for symptomatic infection with this pathogen.

This study aimed to investigate the relationship between symptomatic/successful *H. pylori* infections and ABO/Rh(D), Lewis antigens and secretor status in infected patients.

This is a case-control study. Eighty three patients who experienced symptomatic *H. pylori* infection and eighty five control subjects were analyzed for their gender, body mass index (BMI), smoking habits, drug intake, different environmental pollutants, water source and quantity of water intake, spicy food intake, ABO blood group, Rh(D) type, Lewis blood phenotype, and secretor status. The association of the different variables with *H. pylori* infection was analyzed statistically by Chi-square test.

Analysis of the control subjects for *H. pylori* specific IgG, revealed that 98% of them were positive, although they have never experienced symptoms of infection (asymptomatic). Comparison of the patients' and control groups, showed a statistically significant association between *H. pylori* infection and BMI, drug intake, amount of water intake, Lewis phenotype and secretor property. While comparison of the study groups, showed no statistically significant association between *H. pylori* infection and gender, drinking water source, spicy and chilly food intake, smoking, exposure to environmental pollution and ABO/Rh group.

In conclusion, there is a high rate of asymptomatic infection with *H. pylori* among Palestinian subjects. *H. pylori* infection is influenced by host and environmental factors.

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Abbreviations:

Abbreviation	Word
RBCs	Red Blood Cells
Rh	Rhesus
LW	Landsteiner-Wiener
K	Kell
Le	Lewis
Se	Secretor
OMPs	Outer Membrane Proteins
H. pylori	*Helicobacter pylori*
HRP	Horse Radish Peroxidase
TMB	Tetramethylbenzidine
χ^2	Pearson's Chi-Square
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization
ELISA	Enzyme Linked Immuno- Sorbent Assay

Chapter One:

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1. Introduction:

Cells are the basic structural units in all living organisms. Although cells may share many common characteristics, they may perform different functions due to the difference in their protein-synthesizing ability and their different surrounding environment which may affect their gene expression. The cell membrane is concerned with accurately and sensitively identifying molecules involved in cell compartmentalization especially controlling the interface between the cell and the environment (Gallagher, 2007, Erica et al., 2010).

Red blood cells (RBCs) are the primary cells in the human body and are essential in gas transportation process. Scientists grouped humans into four blood groups depending on the presence of one (A) or another (B) or both (AB) or none (O) of the antigens on their RBCs. The importance of most blood group antigens had been recognized by the immunological complications of blood transfusion or pregnancies. However; the molecular structure and function of the RBC antigens remained undefined for many decades. Recently due to the

advances in molecular genetics and cellular biochemistry resulted in an abundance of new information of the structure and function of the RBC surface molecules (Lichtman et al., 2007;Pourazar, 2007; Lin et al., 2009).

During the twentieth century many researchers including membrane biochemists and molecular geneticists showed interest in studying the membrane proteins expressed on the surface of the RBCs. These studies have revealed the structure and the function of some of these antigens. Additionally, several studies have revealed that some of the RBC antigens are used as cellular receptors for different pathogens and support their life cycles and pathogenesis. Viruses, bacteria and parasites were found using certain RBC antigens as receptors for these pathogens and support their cycle to cause different diseases such as examples of pathogens that bind RBCs include *Escherichia coli*, Parvovirus B19, *Helicobacter pylori* and *Plasmodium falciparum* (Pourazar, 2007; Anstee, 2010; Yamamoto et al., 2012).

1.1. Red blood cell antigens:

The RBC membrane is about 1% of the total weight of the cell. The membrane has a primary role in RBC integrity and together with its skeleton provides the RBCs with their flexibility, durability and tensile strength (Lichtman et al., 2007). RBCs have drawn the attention of scientists for hundreds of years; a major discovery regarding these cells was the elucidation of the RBC surface antigens and their role in blood transfusion (Pasini etal., 2010). Since the discovery of human blood groups by Landsteiner in 1901, the genetics, serology, and biochemistry of the RBCs and blood group antigens were extensively studied. Recently the biological functions of some of these antigens were discovered (Lewis et al., 2006).

The cell membrane of human cells contains a large assortment of molecules including specific receptors responsible for cell-cell signal transduction and the interaction of each cell with its surrounding environment (Leninger, 2003).Since 1980 efforts were directed towards gene cloning and sequencing of the genes encoding known blood groups and identifying proteins anchoring them in the cell membrane (Anstee, 2011). Many of the RBC membrane glycoproteins express certain blood group activity as a result of variation in their oligosaccharides or amino acid sequences. The clinical relevance of blood group antigens in the practice of blood transfusion has led to extensive analysis of the red cell surface. Additionally; most genes encoding the different blood group systems have been cloned (Burton et al., 2011).

There are about 30 blood antigen systems recognized on the RBCs (Anstee, 2011). A group of red cell antigens is determined either by a single genetic locus or closely linked loci, such loci are directly or indirectly responsible for forming protein or carbohydrate epitopes presented as glycoproteins or glycolipids (e.g. ABO, Lewis, P). Protein-defined antigens aredirectly encoded by genesthat follow Mendelian inheritance. These proteins are inserted into cell membrane in one of three ways; single pass, multipass, or linked to phosphatidylinesitol (GPI-linked). Antigens defined as blood group antigens are generally found on RBCs and many other tissues except for the erythroid specific antigens including Rhesus (Rh), Landsteiner-Wiener (LW), Kell (K) and MNSs (Lewis et. al., 2006).

Over the years RBC antigens were identified and blood group gene products can be schematically divided into five functional categories: i) receptors for exogenous ligands like viruses, bacteria and parasites e.g. P glycolipid is the receptor for Parvovirus B19 on the surface of the erythroid progenitors, also glycoconjugates carrying Lewis antigen (Le^a) specificity bind *Bordetella pertussis* and *Staphylococcus aureus* toxins; ii) adhesion molecules e.g. Knops antigens that are responsible for binding and transporting of C3B/C4b coated immunocomplexes; which is also a specific binding of IgG; iii) membrane transporters and channels e.g. the product of Diego blood group gene which is

the exchanger of HCO3-/Cl- and Kidd protein that mediates urea transport or the Colton/ aquaprorin-1 protein which mediates water transport; iv) enzymes e.g. Kell glycoprotein antigens that play a role in erythropoiesis, also have a part in Zn- metalloproteinases; and v) structural proteins e.g. the Diego blood group gene product have a role as a membrane skeleton linkage and Gerbich antigen that have a role in RBC shape and integrity (Pourazar, 2007; Lin et al., 2009).

1.1.1. ABO blood group system:

The ABO antigens are formed as a result of interaction of three independent loci (ABO, *Hh* and *Sese*). These genes code for specific glycosyltransferases that in turn add an immunodominant sugar to a precursor substance type 1 or type 2. Precursor substance type 1 refers to a β 1-3 linkage between galactoseand N-acetylglucosamine while it is in type 2, the bond between galactose and N-acetylglucosamine is β 1-4 linkage. Structures of ABO antigens are illustrated in Figure 1.1. In red cell precursors, the *Hh* gene (*FUT1*) is located on chromosome 19 and codes for the α-2-L fucosyltransferase which adds fucose to precursor substance type 2 generating the H antigen (Figure 2). While in the epithelial the *Sese*gene (*FUT2*) codes for α-2-L fucosyltransferase that adds fucose to precursor substance type 1 converting it to H substance (Figure 1.2) (Harmening, 2005; Lewis et al., 2006). The expression of A and B antigens (Figure1.3) is determined by the H gene that along with type 2 precursor give rise to the basic structure that is needed to form the A or B antigens. A and B genes are located onto chromosome 9, the A gene encodes for the enzyme α-3-N-acetyl-D-galactosamine which adds the N-acetylgalactoseamine onto the H antigen, while the B gene encodes for the enzyme α-3-D-galactosyltransferase which adds the D-galactose onto the H antigen. This modification converts the H antigen into A or B antigens or even A and B antigens together in a heterozygous individual; when both genes are inherited along with the *FUT1* gene. When both alleles are inactivated, this will give the O blood group phenotype marked by the expression of H antigen on the cells. Mutations in*FUT1* gene give rise to the Oh Bombay phenotype (Figure 1.2), in which the red cells show the O blood group phenotype and their plasma contains Anti-H antibodies.

On the other hand, this may prevent the expression of A or B antigens since there is no H precursor, consequently, anti-A and/or anti-B antibodies will be seen in the Bombay phenotype plasma (Harmening, 2005; Lewis et al., 2006).
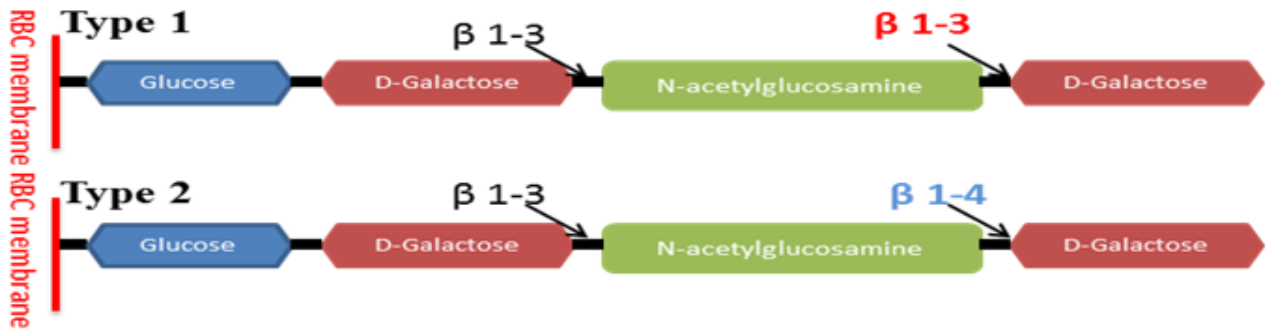
Figure 1.1: Type 1 and 2 H precursor chain.

Figure 1.2: The composition of O blood group on the RBC membrane.

Moreover, von Dungern (1911) described the presence of two different A antigens which are A1 and A2, later this classification into A1 and A2 was found to account for almost 99% of all A group individuals. A1 blood group accounts for about 80% of all group A while A2 accounts for around 19% of group A. Other weakAsubgroups are rare and contribute less than 1% of A group. The A1 and A2 groups differ in the extent to which their respective enzymes change the H antigen, which in turn is found in four different structures differing in their branching patterns. There are also different subgroups of the B antigen; but they are considered to be less important than the A subgroupsthe antigens can

be arranged according to their immunogenicity in the following order: O>A2>A2B>B>A1>A1B(Harmening, 2005; Lewis et al., 2006).
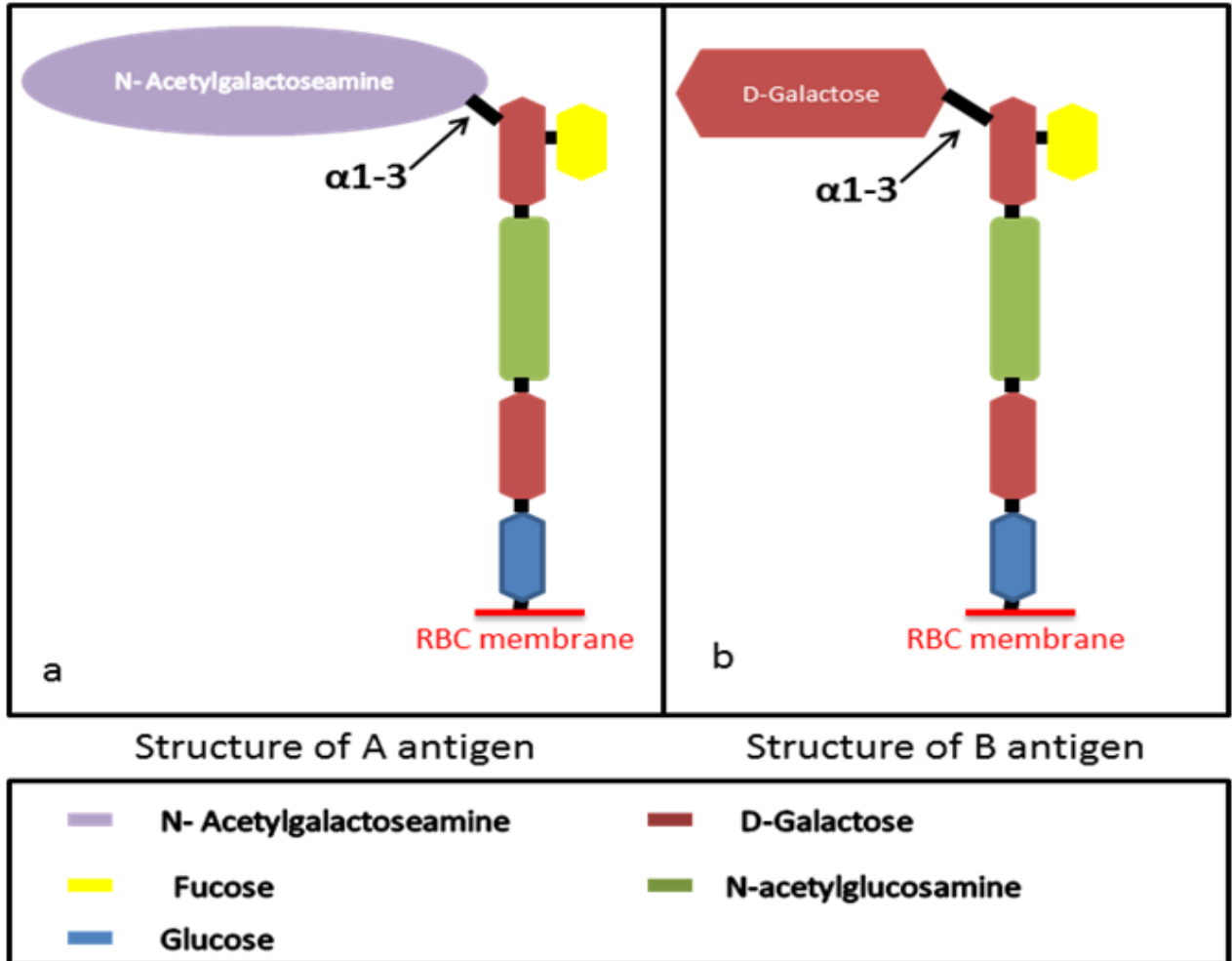
Figure 1.3: Formation of Blood Group A antigen in (a) and formation of Blood Group B in (b).

The A, B and H antigens are expressed in the early fetal development but they are not being fully developed at birth and they reach their adult levels one year postpartum and remain constant for life with slight reduction in the elderly (Harmening, 2005; Lewis et al., 2006).

Defining the nucleotide sequences of the cloned genes and predicting the amino acid sequences of the encoded proteins helped in determination of the three-dimensional structures which are vital for understanding the nature of the blood group epitopes and their cellular functions in cell-cell interaction (Burton et al., 2011).

1.1.2. Rh blood group system:

The Rhesus (Rh) blood system is encoded by three closely linked loci (*RHD, RHCE, RHAG*) present on chromosome 1. The *RHCE* gene encodes the Rh C/c and Rh E/e antigens and the *RHD* gene encodes the Rh D antigen. There is no "d" allele and the letter "d" is used to indicate the absence of Rh D antigen. Since the most immunogenic antigen is the D antigen, it is convenient and practical to classify individuals as D positive or D negative (Harmening, 2005; Lewis et al., 2006).

1.1.3. Lewis (Le) blood group system:

Another important blood group system is the Lewis (Le) system which is considered a unique system since it is the only one that is not manufactured by the red blood cells but manufactured by tissue cells. The Le gene is located on chromosome 19 at the *FUT3* locus; and it is linked to the *Hh* and Secretor (*Se*) genes. This Le gene encodes for α-4-L-fucosyltransferase, which transfers L-fucose to a special precursor chain oligosaccharide on glycoprotein or glycolipid structures. Le^a phenotype is produced when *Le* gene is segregated from *Se* gene. The Le^b phenotype is produced when both *Le* and *Se* genes are present. *Le* gene is required for the production of either Le^a or Le^b phenotypes. The inheritance of the *Le* gene acts in competition with ABO genes adding L-fucose to the special sugar precursor that is manufactured by tissue cells. The final structure which is a soluble antigen is secreted and adsorbed or acquired by RBCs, lymphocytes, and platelet membrane. The antigen is also found on cells of pancreas, stomach, intestine, skeletal muscles, renal cortex, and adrenal glands. The frequency of Lewis phenotypes is: (72%)

forLe(a-,b+), (22%) for Le(a+,b-) and (6%) for Le(a-,b-). Additionally, the phenotypeLe(a+,b+) is rarely produced as a result of a mutation in the *FUT2* gene which is *Se*wA385T. The phenotype associated with the *Se*wA385T mutation is partially secretor (Henry et al., 1996; Harmening, 2005; Lewis et al., 2006).

1.1.4. ABH soluble substances:

In 1930, the classification of individuals into secretors and non-secretors has been established according to the ability of an individual to secret soluble ABH blood group antigens. The ABH antigens and their precursor on blood cells and platelets are present on other tissue cells and are differentially found in secretor body fluids such as saliva, urine, tears, semen, breast milk and gastric juice. The *FUT2* or Secretor (*Se*) gene on chromosome 19 is used to classify individuals as secretors when they have the genotype (*SeSe* or *Sese*) or non-secretors when they have the genotype (*sese*). The *FUT2* gene codes for a α-1, 2-L-fucosyltransferase which adds L-fucose to a precursor substance to generate the H substance. The H substance is then modified by the A or B enzymes generating the A and B soluble antigens, respectively. The secretor enzyme is active in mucus membranes and glands and is responsible for the secretion of blood group antigens in the different body fluids. The majority of the population (80%) expresses the secretor phenotype, while the remaining 20% expresses the non-secretor phenotype (Harmening, 2005; Lewis et al., 2006; Jaff, 2010).

1.2. Frequency of blood group antigens:

Determining the frequency of each blood group antigen is very important for clinical aspects so there are many studies to determine them all over the world. In Palestine, the frequency of ABO blood groups in West Bank region were found to be as follows: O 41.2%, A 39.5%, B 11.4% and AB 7.9% (Al-Kawasmi, 2011). Similar results were reported in the West bank region by Shtayeh et al., (1988), and in Gaza Strip by Skaiket. al.

(2007). These results are closely similar to frequency of ABO blood groups in different countries worldwide with slight variation between the A and O blood groups (Bashwari et al., 2001; Hassan, 2010; Eweidah et al., 2011). However, the frequency of Rh(D) positive is about 80% and the frequency of Rh(D) negative is about 20% worldwide (Magen David Adom, 2013; Bashwari et al., 2001; Skaik et al., 2007;Eweidah et al., 2011).

1.3. Blood group antigens and disease association:

Many studies have found strong correlations between individual's susceptibility to some diseases and secretor status. There are clear relationship between infectious diseases and inherited polymorphism in genes encoding and regulating the expression of ABH and Lewis antigens in the body secretions. The most important pathogens that may have this correlation are *Helicobacter pylori (H. pylori)*, norvovirus, cholera infections, and malaria (Anstee, 2010; Yamamoto et al., 2012; Valliani et al., 2013). *H. pylori* is considered a major human pathogen, it is a gram negative spiral bacterium. It's estimated that *H. pylori* has infected one half of the world's population. *H. pylori* infections are most likely affected by host and environmental factors. Diseases described by *H. pylori* and related species include peptic ulcer, chronic gastritis, adenolymphoma, lymphoma, stomach malignancies, gastric, hepatic and colon cancers. Some of *H. pylori* strains are associated with gastroesophageal reflux disease (Appelmelk, 2000; Jonge et al., 2004; Barghouthi, 2009).

The clinical outcome of *H. pylori* infection is determined by the complex interaction between the bacterium and the host (Torres et al., 2008). Bacterial adherence to gastric epithelial cells may protect the bacterium against peristalsis and mucosal shedding and warrants access to nutrients that are released from damaged epithelia. Putative outer membrane proteins (OMPs; AlpA, AlpB, BabA, SabA, Oip A and HopZ) are associated with adhesion of the bacterium to host cells (Jonge et al., 2004; Torres et al., 2008). *H. pylori* developed its ability to adhere to different host receptors; some of its OMPs bind to

fucosylated blood group antigens e.g. BabA, while others may bind to sialytated Le blood group antigens (located on epithelial cells). There is evidence that the dynamics of mucosal glycosylation is a response to this bacterial infection that may cause a modulation in the expression of adhesion molecules as a response to the changes in the host mucosa (Mcguckin et al., 2007; Sheu et al., 2007; Linden et al., 2008; Torres et al., 2008; Yamamoto et al., 2012; Valliani et al., 2013). In the early stages of colonization, OMPs of the bacterium bind to certain cellular antigens in the mucosa, this is followed by changing the environment and antigen expression which may increase binding (Mcguckin et al., 2007; Torres et al., 2008).

The mechanism of persistent infection of *H. pylori* is not clearly understood. Molecular mimicry between bacteria and human gastric environment, adaptation to host, the induction of autoreactive antibodies, or gastric damage may all be involved in colonization. One of the most important antigens that are expressed by human cells and play a major role in the host-parasite interaction is the Le blood group antigens that are normally present on the human gastric mucosa cells and on the lipopolysaccharide chains of *H. pylori* (Sheu et al., 2007).

1.4. Literature Review:

Hirszfeld and Hirezfeld in 1919 were the first to point out the differences in the distribution of blood groups among different populations. The ABH antigens were shown to be widely expressed by all body cells including RBCs as described (Anstee, 2010).

One of the most obvious and significant associations between blood group type and diseases are A, B and AB groups which have a higher risk to arterial and venous thromboembolisms while the O-group population has a lower risk. These studies are supported by the finding that non-group O patients have higher levels of von Willebrand factor and coagulation factor VIII. The O blood group was noticed to have reduced

Plasmodium falciparum infections compared to non-O blood groups; on the other hand *Vibreocholerae* and *Escherichia coli* infections were found to be more severe in O blood group patients than non-O blood groups (Anstee, 2010). Although some diseases are more induced by secretors such as influenze viruses, rhinoviruses, respiratory syncytial virus and echoviruses (Raza et al., 1991), there are other pathogens that are more sensitive in non-secretor population such as *Haemophilusinfluenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae* and urinary tract infections caused by *Escherichia coli* (Sheinfled et al., 1989).

One of the first observed associations between blood group phenotypes with disease was blood group O and peptic ulceration. *H. pylori* which is considered to be the causative agent for peptic ulceration and gastric cancer was strongly associated with blood group O, Lewis antigens especially Le(a-b+) phenotype and secretor property, especially that there are evidence demonstrated that Le[b] antigen acts as a receptor for *H. pylori*, this antigen is most frequently found on O blood group compared to other groups (Anstee, 2010; Jaff et al., 2011; Yamamoto et al., 2012; Valliani et al., 2013). Another study showed a correlation between this infection and blood group O with a nonsecretor phenotype property (Jaff et al., 2011).

Worldwide,*H. pylori* cause the most common chronic infections; despite the advances in understanding its biology, the factors that determine the outcome of infection are still poorly understood. Host factors might seem to be very important in the outcome of these infections since only a proportion of infected subjects develop ulcers, while the bacterial factors have a role in influencing the inflammatory response and the development of a more severe infection (Wu et al., 2003; Jafarzadeh et al., 2007).

Several studies found that the lipopolysaccharides of certain strains of *H. pylori* outer membrane contains molecules that share molecular similarity with different antigens expressed on the human gastric mucosa. For example, *H. pylori* synthesize polysaccharides

that have close similarity to the Le blood group antigens especially Le^x and Le^y (Magdalena et al., 1998; Hynes et al., 2000; Monteiro et al., 2000).

When *H. pylori* colonize the human host it binds to the gastric mucin, rather than directly to muocosal epithelium protecting itself from luminal acidity and shedding. Le antigens expressed by *H. pylori* may mediate the adhesion and colonization of bacteria by suppressing the immune response against the bacterium so it may play a role in certain autoimmune aspects of pathogenesis. These similarities could potentially allow the bacterium to escape immunity and mediate the bacterial attachment by depending on the expression of Le antigens on the human gastric epithelium. Due to structural similarity of bacterial Le antigens and that of the host Le antigens, production of cross reacting immune response with human gastric mucosa and with different gastric proton pumps is manifested as an autoimmune disease (Magdalena et al., 1998; Hynes et al., 2000; Monteiro et al., 2000; Harvey et al., 2010) that may relate to carcinogenesis.

Alternatively, *H. pylori* may induce a humoral response in infected subjects, however this humoral response does not protect against the pathogen (Magdalena et al., 1998).

For more than four decades the association between *H. pylori* and blood antigens is known but received little attention (Harvey et al., 2010). Lewis associated antigens that may have a primary role in *H. pylori* adherence are carbohydrates related biochemically to the ABO blood groups and carried on both glycoproteins and glycolipids and comprise type 1 (Le^a, Le^b) and type 2 (Le^x, Le^y) carbohydrates. The determinants of Le antigens are oligosaccharides synthesized by the sequential addition of sugar units to oligosaccharide chains by fucosyltransferases that are synthesized by *Hh*, *Se*, and *Le* genes. Le^a and Le^b antigens synthesized on type 1 precursor's oligosaccharides chains that are expressed on the digestive and respiratory tracts and in secretions. Le^x and Le^y antigens synthesized on

type 2 are expressed on erythrocytes and vascular endothelial cells (Lee et al., 2006; Harvey et al., 2010; Yamamoto et al., 2012; Valliani et al., 2013).

This antigen expression in the digestive organs is biologically more important than the expression on the erythrocytes, due to its role in mediating bacterial adherence and attachment that is considered to be essential steps in the initiation, establishment and maintenance of the infection and monoclonal antibodies to Le[b] inhibit this adhesion (Lee et al., 2006; Valliani et al., 2013).

Martiuset al. (2006) observed a significant increase of blood group phenotypes O, A2 and Le(a-,b+) in Brazilian patients with *H. pylori* infections. Increased *H. pylori* infection was more observed among secretors compared to non-secretors. In contrast, Rio Preto et al. (2002) found that there is no correlation between *H. pylori* infection and O blood group, neither Lewis antigens nor secretor status among Brazilian patients.

Kanbay et al. (2005) found a strong correlation between A and O blood groups, and the Le(a-,b+) phenotype and *H. pylori*infection. The presence of Le(a-,b+) in A, B and AB blood groups was associated with failure of *H. pylori* to bind to the gastric mucosa, while the association of A blood group with the gastric ulcer and carcinoma was not explained. Furthermore patients with AB blood group were less positive for *H. pylori* infections and this was the first indication of a negative relationship between *H. pylori* infections and AB blood group. The authors also found a correlation between age and the seroprevalance infections of *H. pylori*. Additionally, *H. pylori* infection among females was higher compared to males. In addition smokers were found to be more susceptible than non-smokers (Kanbay et al., 2005). In another study by Jaff et al. (2011) female gender was also found to have more risk factor for this infection rather than males and B blood group have more protective effect than other groups, and age of infection is mainly during

childhood and early adolescence increasing to reach its peak at 31 years old (Jaff et al., 2011).

In Turkey, Bayan et al. (2009) reported a relationship between blood group O, Le(a-,b+) and adherence of *H. pylori* by using its adhesion protein BabA (Bayan et al., 2009). A study by Linden et al. (2008) was performed on Rhesus monkeys originating from south Asia found that monkeys were susceptible to *H. pylori*infection when their bloodgroup was B along with secretor gene status (Linden et al., 2008). These results were also confirmed by several other studies using different a methodologies (Loffeld et al., 1991; Clyne et al., 1997; Keller et al., 2002; Turkolmez et al., 2007).

On the other hand,some studies did not observe any relationship between blood group phenotype and *H. pylori* infections (Loffeledet al., 1991; Clyneet al., 1997; Keller et al., 2002; Seydaet al., 2007) and concluded that *H. pylori* infection neither depends on the expression of different blood groups nor on the expression of Le antigens on the RBCs or on epithelial cells of the gastric mucosa.

Several studies have investigated the association between ABO/ Rh blood groups, Lewis system and secretor status and *H. pylori* infection. The finding of these studies showed contradictory results in different countries and races. However, such information concerning the Palestinian patients are not yet available.

1.5. Objectives of the Study:

H. pylori infection is common among most populations. Several studies have been conducted to investigate the correlation between *H. pylori* infection and blood groups. However, these studies have reported controversial results and did not yield a conclusive

result about this issue, which may be attributed in part to the ethnicity of the studied population, the different frequencies of blood groups and other environmental factors. In Palestine, there is a lack of local studies on this issue although high prevalence of *H. pylori* infection was reported. The investigation of the relationship between *H. pylori* infection and certain blood groups may help to shed some light on the susceptibility of Palestinian population to this infectious agent and the severity of infection and consequently improve prevention and management of infection.

Therefore, the main objective of this study is:

To investigate the relationship between symptomatic/successful *H. pylori* infections on one hand and ABO/Rh(D), Lewis antigens, A1 and A2 subgroups, and secretor status in the Palestinian patients compared to other studies on the other hand.

Chapter Two:

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2. Materials and Methods:

2.1. Materials:

All materials used in this study are listed in Table 2.1.

Table 2.1: Materials used in this study.

Item	Catalog number	Manufacturer/ Country
Plain Tubes		
EDTA Tubes		
0.9% Saline		
Baxter- BAC II(Blood Bank) Centrifuge		
Plastic Test Tubes 75X12 mm		
Volumetric Pipettes		Thermo Finepipette
Human Anti-A	600010	Lorne Laboratories Limited
Human Anti-B	610010	Lorne Laboratories Limited
Human Anti-D	740010	Lorne Laboratories Limited
Human Anti-A1	116005	Lorne Laboratories Limited
Human Anti-H	115002	Lorne Laboratories Limited
Human Anti-Le[a]	630002	Lorne Laboratories Limited
Human Anti-Le[b]	631002	Lorne Laboratories Limited
Swinging Centrifuge (Kubota 5100)		
Clean Cups		
Washed RBCs A-cells		
Washed RBCs B-cells		
Washed RBCs O-cells		
Nova Tec Anti- IgG H. pylori ELISA Kit	HELG0220	ImmunodiagnosticaGmbh Germany
BioTech ELISA Reader		

2.2.Methods:

2.2.1. Questionnaire:

A questionnaire was prepared for this study and aimed to collect information from the study samples (patients and controls) concerning socioeconomic information, anthropometric indices, and medical history. The questionnaire form is shown in Appendix A. The questionnaire form also contained a consent form in Appendix B; each subject participating in the study was asked to read and sign this form.

2.2.2. Study Samples:

Subjects were grouped into two groups, the patients' group and control group.

Patients' group: The patients' group contained individuals who were diagnosed to have *Helicobacter pylori* infection due to symptomatic disease, and were receiving medication to treat their disease. In this group the diagnosis of *H. pylori* infection was confirmed by histopathology examination of gastric biopsy.

Patients presenting at the Gastroscopy clinics at Hebron and Ramallah Hospital/ Palestine Medical Complex in Ramallah, either for assessment or confirmation of *H. pylori* infection were asked for participation in the study and have given a written consent form. Gastric biopsies were sent to the Pathology department at BietJala Hospital in BietJala for Histopathology examination. Samples that tested positive for *H. pylori* infection by Histopathology examination were retained for further analysis (patient's group) while those which tested negative were excluded from the study. All subjects were non-relatives in order to eliminate other genetic correlations. The patients' group contained 83 subjects from different Palestinian cities. Samples of blood and saliva were collected between January 2012 and November 2012.

Control group: This group contained individuals who never contracted symptoms of *H. pylori* infection, never had stomachache for a long period of time, or never been tested for *H. pylori* infection.

These individuals were from different institutes and they were not related to each other or to the patients in order to eliminate any other genetic correlations. The control group contained 85 subjects from different Palestinian cities.Samples of blood and saliva were

collected during the same period as for the patients' group. All subjects who accepted to participate in this study were asked to give a written consent.

2.2.3. Blood and Saliva Samples:

All subjects (patients' and controls) who accepted to participate in this study were asked to fill the study questionnaire, sign the consent form and donate a blood and saliva samples. Blood was collected into one EDTA and one plain tube, and saliva into clean cups. To enhance secretion of saliva, subjects were given a sugarless gum (Orbit) and asked to chew it for 3 minutes before collecting the saliva sample. Saliva was collected into wide mouth clean cups, kept at 4ºC and was processed within 24 hours. EDTA whole blood was kept at 4ºC and used for blood grouping within 24 hours. Blood samples collected in plain tubes, were allowed to stand at room temperature for 1 hour and then centrifuged at 5000 rpm for 5 minutes in a fixed-angle centrifuge; serum was harvested and stored at -25ºC until use.

2.2.4. Blood Grouping:

2.2.4.1. Procedure:

Typing for the ABO, Rh(D) and Lewis antigens was performed using the tube method. Four drops of EDTA whole blood were washed 3X in sterile saline (0.09% NaCl), then washed RBCs were resuspended in sterile saline to give 2-5% RBCs suspension. Five test tubes were labeled with A, B, D, Le^a and Le^b and one drop of blood grouping sera (anti-A, anti-B, anti-D, anti-Le^a and anti-Le^b) was placed in the appropriately labeled tubes then an equal volume of 2-5% RBCs suspension was added to each tube. Contents were mixed gently and for Lewis blood antigens typing, the respective test tubes (anti-Le^a and anti-Le^b) were incubated at room temperature for 15 minutes. All tubes were centrifuged using the

immediate spin program (1000 rpm for 20 seconds, Baxter, BAC II blood bank centrifuge). Every individual that was positive for anti-A was retyped with Anti-A1 to differentiate A1 antigens from A2 antigens.

2.2.4.2.Interpretation of results:

1. Positive: Agglutination of RBCs within the accepted limitations of the test procedure indicated the presence of the particular antigen on the tested RBCs.

2. Negative: No agglutination of test RBCs constitutes a negative result and within the accepted limitations of the test procedure, this will indicates the absence of the particular antigen on the tested RBCs.

2.2.4.3.Stability of the Reactions:

1. RBCs were tested for antigens within 24 hours in order to have good results, as long storage may cause poor detection of certain antigens especially Le^a and Le^b.

2. Testing of blood group antigens was done using the recommendations of the manufacturer of the typing sera.

3. Tests were read immediately after centrifugation because delays result in dissociation of antigen- antibody complexes leading to false negative or weak positive reactions.

4. Every negative macroscopically result of the Rh testing was confirmed microscopically, because certain antigens show very weak reaction that couldn't be identified macroscopically.

2.2.5. Determination of the Secretor Property:

2.2.5.1. Procedure:

The secretor property was determined using the Hem-agglutination method. Human polyclonal ani-A, anti-B and anti-H sera were diluted by titration against appropriate cells at immediate spin using saline. The appropriate dilution that gave +2 agglutination was selected for further use in this procedure. For anti-A and anti-B the appropriate dilution was 1:40, while for anti-H it was 1:2. Two to three mL Saliva sample was placed into a clean test tube. Saliva tubes were centrifuged at 900-1000 rpm for 8-10 minutes.The supernatantwas transferred into a clean glass test tube; cap was closed properly, and then placed into a boiling water bath for 10 minutes. Boiling is used to inactivate enzymes that might destroy blood group substances.Then all tubes were re-centrifuged at 900-1000 rpm for 8-10 minutes and the supernatant was collected into a clean tube.Then the supernatant was then diluted 1:5 with saline. Undiluted saliva contains nonspecific glycoproteins that can inhibit antisera and leads to incorrect results.One drop of diluted saliva was mixed with one drop of the diluted antisera (anti-A, anti-B and anti-H) in the appropriately labeled tube. Tubes were mixed well and incubated at room temperature for 8-10 minutes.To the saliva-antisera mixture, one drop of the appropriate indicator cells (2-5% washed cell suspension of A, B or O cells) was added to the properly labeled tube.All tubes were mixed well and incubated at room temperature for 30- 60 minutes, after this tubes were centrifuged using immediate spin program (1000 rpm for 20 seconds) using the blood bank centrifuge, and all agglutination results were examined macroscopically.

2.2.5.2. Interpretation:

1- Non-Secretor: Agglutination of RBCs by antiserum- saliva mixture in all three tubes (Table 2.2.).

2- Secretor: No agglutination of RBCs by antiserum and saliva mixture. The antiserum has been neutralized by the soluble blood group substances or antigens in the saliva. Therefore, no free antibody is available to react with the antigens on the reagent RBCs used in the testing. This negative reaction is a positive test for the presence of ABH-soluble antigens and indicates that the individual is a secretor (Table 2.2.).

Table 2.2: ABH Substances in Saliva.

ABH substances in saliva			
ABO Group	**A**	**B**	**H**
Secretors			
A	Much	None	Some
B	None	Much	Some
O	None	None	Much
AB	Much	Much	Some
Non-Secretors			
A, B, O and AB	None	None	None

2.2.6. *H. pylori*IgG ELISA:

The IgG antibody against *H. pylori* was determined in the serum of control group using the Nova Tec kit (ImmunodiagnosticaGmbh), a commercially available ELISA (Enzyme Linked Immuno-Sorbent Assay) kit, was used for the determination of the IgG class antibodies against *H. pylori* in serum samples. This kit can be used for qualitative and quantitative determination of the IgG class against this bacterium. In the present study it

was used for quantitative determination of the antibody.The anti-*H.pylori*IgG was determined in the serum of control subjects only to test for their previous asymptomatic exposure to the bacterium.

2.2.6.1.Principle of the assay:

The quantitative determination of the *H. pylori*IgG antibody was performed according to the manufacturer's instructions. In the Nova Tec ELISA kit the *H. pylori* antigens are coated on the microtiter wells and are used to bind the anti- *H. pylori*IgG antibodies in the serum. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) labeled anti-human IgG conjugate is added. This conjugate binds to the captured *Helicobacter* specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of *Helicobacter* specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using ELISA microwell plate reader.

All reagents and materials required for the ELISA wereprovided with the kit and were prepared per the manufacturer recommendations.

2.2.6.2.Assay Procedure:

2.2.6.2.1. ELISA testing:

Serum samples of control group were thawed at room temperature, diluted 1+100 with IgG diluent and mixed using a vortex mixer.Substrate Blank well was kept as a reference for the assay.One-hundred ul of each standard (A, B, C and D) and diluted samples were dispensed into the respective wells. Wells were covered with foil and incubated at 37°C for one hour. After incubation the foil was removed and the fluid in the wells were aspirated and discarded.Each well was washed three times with 300 ul of washing solution and soaking of wells each time for more than 5 seconds. Then one-hundred ul of *H. pylori* anti IgG conjugate was dispensed into all wells except the blank well. Wells were covered with foil and incubated at room temperature for 30 minutes. Wells were washed three times as described in the previous step. One-hundred ul of TMB substrate solution was dispensed into all wells and microtiter plate was incubated for 15 minutes at room temperature in the dark. To stop the reaction, 100ul of the stop solution (sulphuric acid) was added onto the wells in the same order and rate of the TMB substrate solution. The absorbance of the specimens was measured at 450/620 nm within 30 minutes after addition of the stop solution. The absorbance was read using the BioTech ELISA reader.

2.2.6.2.2. Assay Validation:

In order to consider the assay valid, the following criteria were applied per the manufacturer's instructions:

- Substrate Blank absorbance < 0.100.
- Standard A concentration= 0 NTU/mL, has absorbance < 0.200.
- Standard B concentration= 15 NTU/mL, has absorbance > 0.200.
- Standard C concentration= 75 NTU/mL, has absorbance > 0.500.
- Standard D concentration= 150 NTU/mL, has absorbance > 1.100.

Standard A< Standard B< Standard C< Standard D.

2.2.6.2.3. Calculation of IgG titer:

To obtain a quantitative result measured by NTU/mL, the mean of standards' absorbance (A, B, C and D) was blotted on a Linear/ Linear graph, the standard calibration curve (Figure 2.1).
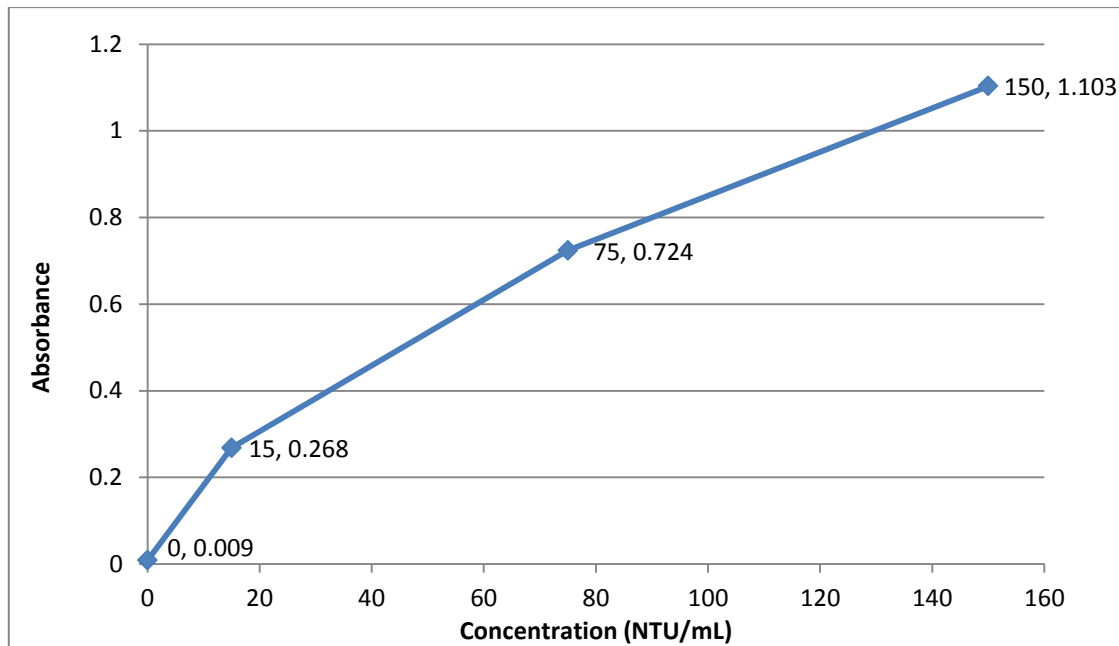
Figure 2.1: *H. pylori*IgG ELISA Calibration Curve.

2.2.6.2.4. Interpretation of Results:

Values provided by the kit as guidelines:

Reactive: >20 NTU/mL.

Gray zone (equivocal): 15- 20 NTU/mL.

Non-reactive: < 15 NTU/mL.

2.2.6.2.5. Specific Performance Characteristics:

2.2.6.2.5.1. Precision:

a- Interassya: CV%= 5.1%.

b- Intraassay: CV%= 6.5%.

2.2.6.2.5.2. Diagnostic Specificity:

The diagnostic specificity is defined as the probability of the assay to score negative in the absence of the specific analyte. For this kit it is 92.0% (95% confidence interval 0.84-0.99).

2.2.6.2.5.3. Diagnostic Sensitivity:

The diagnostic sensitivity is defined as the probability of the assay to score positive in the presence of the specific analyte. For this kit it is 94.4% (95% confidence interval 0.87- 1).

2.2.6.2.5.4. Analytical Sensitivity:

The analytical sensitivity is defined as the apparent concentration of the analyte that can be distinguished from the Zero calibration. For this kit it is 3 NTU/mL.

2.2.6.2.5.5.Interferences:

Interferences with hemolytic, lipemic or icteric serum are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL Triglycerides and 0.2 mg/mL bilirubin. These interferences were considered while running the assay for the control group.

2.2.7. Statistical analysis:

All data are shown as mean ± SD. Statistical analysis was performed using the SPSS software (version IBM SPSS statistics 20.Ink).

All variables were tested by Pearson's Chi-square (χ^2) test which calculates the goodness of fit and independency between variables, and were evaluated using the *P-value*. *P-value* less than 0.05 was considered statistically significant.

Chapter Three:

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3. Results:

3.1.Study samples:

Several host and environmental factors influence the outcome of *Helicobacter pylori*infection. Some of the complex factors of interaction between the bacterium and the host (Torres et al., 2008) were targeted in this study to show their correlation and interaction with the bacterial colonization and disease complications. These factors were tested statistically for their significant association with *H. pylori* infection. Patients and control groups were analyzed for their age, gender, body mass index (BMI), smoking habits, chronic drug intake, different environmental pollutants, water source and quantity of water intake, spicy food intake, ABO blood group, Rh system type, Lewis blood phenotype, and secretor status. Two groups of patients (symptomatic) and control (asymptomatic), were compared for different variables, statistical correlation of these

variables were tested. Data analyses were performed using Pearson's Chi-Square test to study the association between the study variables and *H. pylori* infection.

Subjects (n=83) of the patients' group were diagnosed to have the infection mainly by histopathological examination of stomach biopsies(60 samples, 72.3%), testing for serum *H. pylori* specific IgA (20 samples, 24.1%) or bytesting for *H. pylori* specific antigens in stool (3 samples, 3.6%). Patients are thoseexperienced symptomatic *H. pylori* infection and have received or were receiving the triple antibiotic therapy that contains tetracycline, metronidazole and bismuth subsalicylate as a treatment at the time of sample collection (Graham et al., 1992). On the other hand, controls were selected from different institutes and had volunteered to participate in the study; they were healthy and did not experience symptoms of *H. pylori* infection such as abdominal pain or continuous burning abdominal pain(Hawkey et al., 2005). This group was tested for asymptomatic exposure to *H. pylori* by serological testing for anti-*H. pylori*IgG antibodies in their sera.None of the control group subjects had been previously tested positive for *H. pylori* nor did they receive any treatment for this purpose.

Both study groups were collected from different Palestinian geographic areas (Jenin, Nablus, Qalqelia, Ramallah, Jerusalem, Bethlehem and Hebron. These subjects were categorized into three geographic groups; north, central and south regions of West Bank, Palestine, as shown in Table 3.1. Patients and control groups were non-kin (not related) in order to exclude genetic variables.

Table 3.1: Distribution of patient and control subjects by place of residence.

Residence place	Patients' group		Control group	
	No.	%	No.	%
North region (Jenin, Nablus, Qalqelia)	13	15.7	6	7.1
Central region (Ramallah, Jerusalem)	38	45.8	46	54.1
South region (Bethlehem, Hebron)	32	38.6	33	38.8
Total	83	100	85	100

3.1. Age:

The average age of the patients' group was 37.6 years (SD= 14.4), while that of the control group was 27.3 years (SD= 10.5). The main deviation in the age mean was due to the low response rate in older age subjects to participate in this study. Although, this difference was statistically significant (P=0.000), it had no association with the level of exposure to *H. pylori* (*H. pylori* infection). This difference in age between the whole study groups has no effect on all variable investigated in this study except for the amount of water intake and source of water. Statistical analysis of a subset of 43 subjects matched for age and gender showed no difference among analyzed variables except for water intake (amount and source) see Appendix C.

The age distribution for patient and control subjects is illustrated in Figure 3.1.
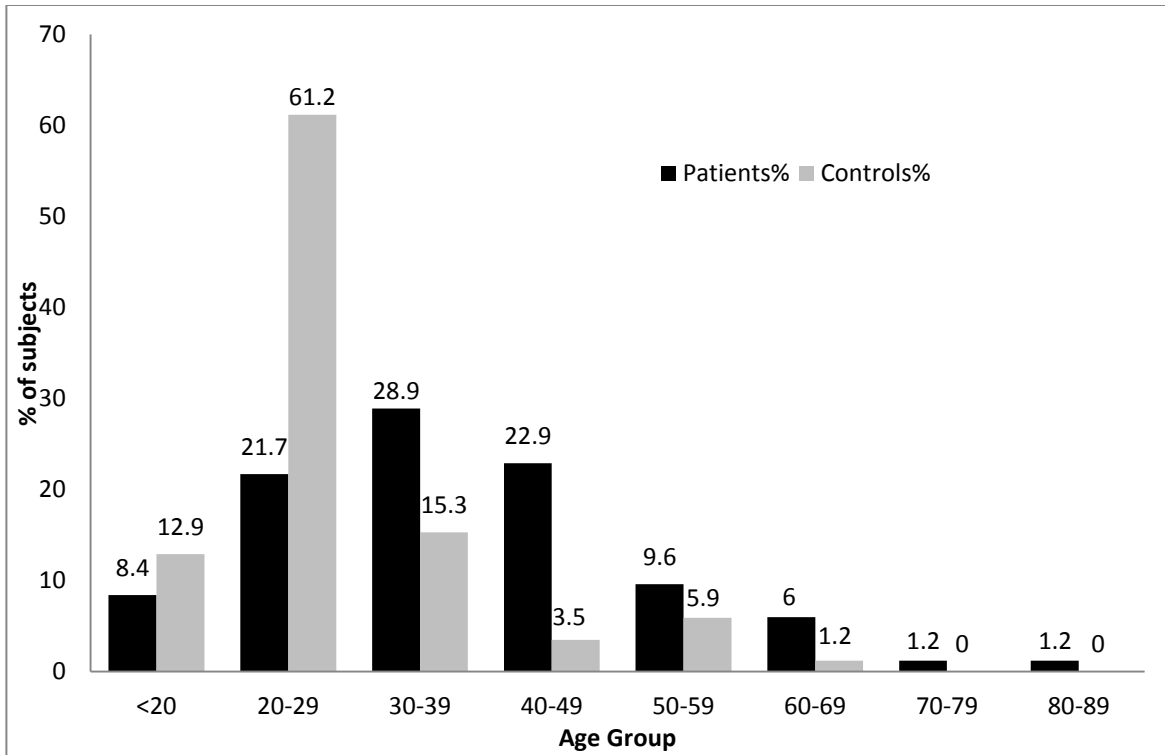
Figure 3.1: Age distribution for patients' and control groups.

3.2. Gender:

The patients' group (83 subjects) included 47 (56.6%) males and 36 (43.4%) females, while the control group (85 subjects) included 53 (62.4%) males and 32 (37.6%) females.

Figure 3.2 shows the gender distribution between the two groups. There was no significant difference concerning gender distribution between the patients' and control groups.
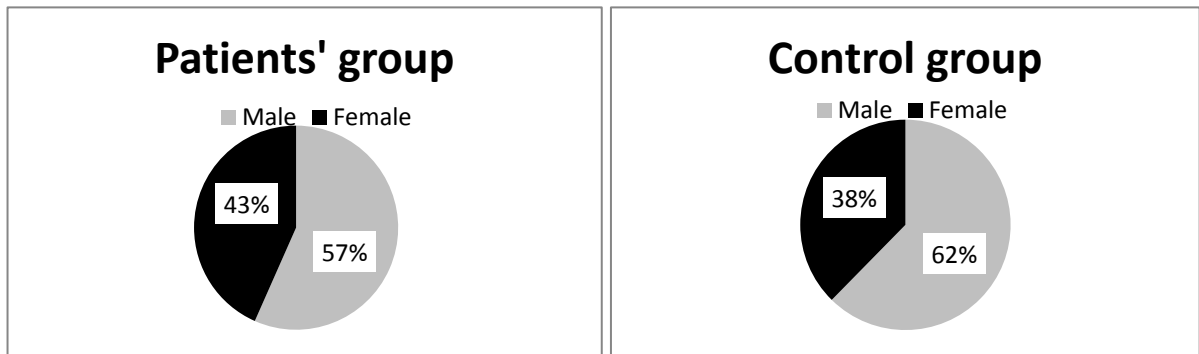
Figure 3.2: Gender distribution for patients and control groups.

3.3. Body Mass Index (BMI):

Body mass index (BMI) is the main indicator used to describe the weight problems in humans, overweight and obesity may reflect certain life style for individuals and may increase risk for certain factors to induce diseases and health problems. To calculate the BMI for subjects in this study, height and weight measures were collected for each contributor then BMI was calculated according to the following Rowland's formula (Rowland et al., 1990):

BMI (kg/m^2) = weight (Kg)/ [Height (m) X Height (m)].

Values of BMI were grouped as recommended by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA and the World Health Organization (WHO) as reproduced in Table 3.2 (WHO, 2004; CDC, 2013; WHO, 2013). The BMI for the patients' and control group is shown in Table 3.3.

Table 3.2: Correlation of BMI values with weight status. (WHO, 2004; CDC, 2013; WHO, 2013).

BMI Values	Weight Status
≤ 18.4	Underweight
18.5- 24.9	Normal
25.0- 29.9	Overweight
≥30	Obese

Table 3.3: Patients and control subjects distributed according to their BMI groups.

BMI Values	Weight Status	Patient No. (%)	Control No. (%)	*P*- value
≤ 18.4	Underweight	3 (3.7%)	6(7.1%)	>0.05
18.0- 24.9	Normal	30 (36.6%)	49 (57.6%)	0.033
25.0- 29.9	Overweight	28 (34.1%)	19 (22.4%)	>0.05
≥30	Obese	21 (25.6%)	11 (12.9%)	>0.05

When comparing both groups, using Pearson's Chi-Square test, a significant difference was observed (P= 0.033), specifically in the normal weight category. Indeed, although the mean BMI (27.2 kg/m^2) of the patients' group was in the overweight category, while the mean BMI (24.7 kg/m^2) of the control group was in the normal weight category; this reflects the fact that more than half (57.6%) of the control subjects are in the normal weight category,there was no significant difference obtained from the patients versus control groups. Figure 3.3 shows the different percentages of both groups distributed according to BMI.

The relative reduction in the number of patients from normal BMI category in favor of overweight and obese categories reflects the increased risk of *H. pylori* infecting among overweight and obese subjects.
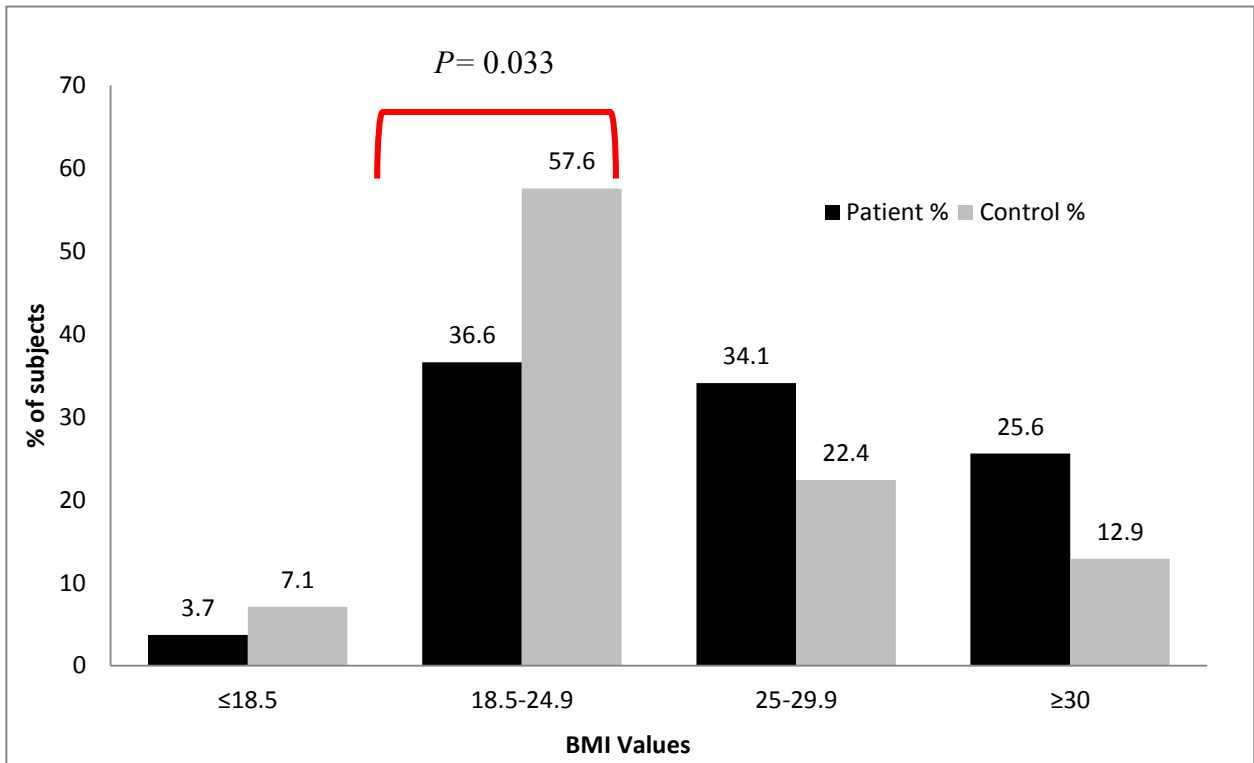
Figure 3.3: Patients' group versus control group percentages distributed according to BMI (Kg/m²) values.

3.4.Smoking Habits:

Every subject participated in this study was categorized as smoker or non-smoker. A smoker was defined as one who smoked any type of smoking materials for more than two months within the last two years. Among the patients' group 26 (31%) subjects were smokers and 57 (69%) subjects were non-smokers. While in the control group25 (29.4%) subjects were smokers and 60 (70.6%) subjects were non-smokers (Figure 3.4). Using

Person's Chi-square, no statistically significant difference was observed between the patients' and control groups when compared for smoking habits.
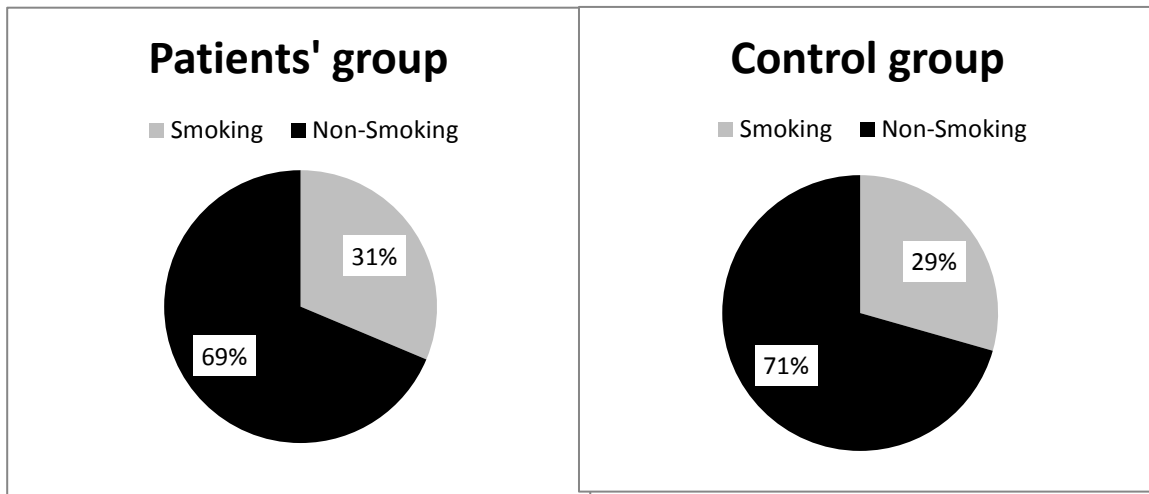
Figure 3.4: Distribution of patients' and control groups by smoking habits.

3.5.Drugs intake:

Each subject in this study was evaluated for different drug intake other than the triple-antibiotic therapy for *H. pylori*. Drugs that were considered in the questionnaire include those that were used by the study subjects for more than 2 months before the appearance of symptoms of *H. pylori* infection such as blood pressure controlling drugs, diabetes drugs, and others. From the patients' group, 45 subjects (54.2%) were taking drugs and 38 subjects (45.8%) were not. In the control group only 5 subjects (5.9%) were using drugs and 80 (94.1%) were not (Figure 3.5).
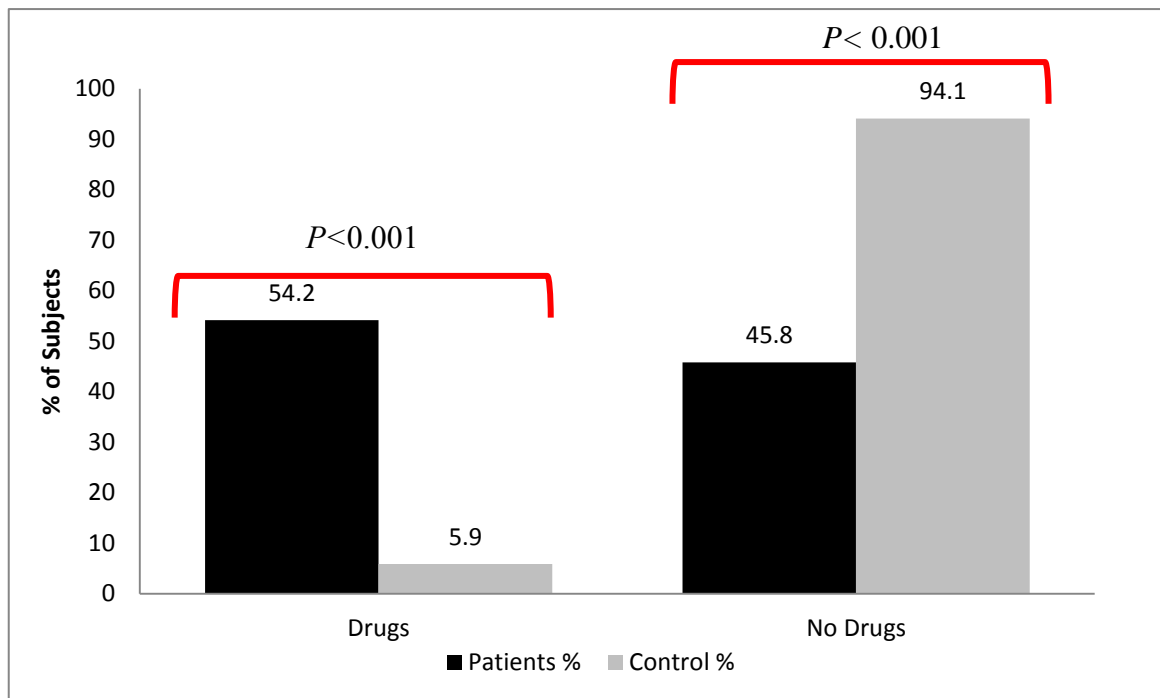
Figure 3.5: Distribution of patients and control groups based on their drug intake.

Comparison of the patients' and control groups for their drug intake using Person's Chi-Square analysis showed a statistically significant difference ($P< 0.001$) between the two groups.When patients on drugs were further sub-grouped based onto their BMI, ABO blood group, Lewis phenotype and secretor status, and analyzed statistically, no statistical differences among the subgroups were observed (data not shown).

3.6.Exposure to pollution:

Exposure to pollution in the living or working environment e.g. factories, waste incinerator, microbiology laboratories, chemical institutes is a serious health problem.

Polluted environment may expose subjects to different elements that predisposes or promotes infectious and non-infectious diseases by increasing exposure to microbial pathogens, immunosuppressing agents or toxic chemicals (i.e. pesticides). Analysis of the questionnaire data revealed that the patients' group included 16 subjects (19.3%) who were exposed to environmental pollution, and 67subjects (80.7%) were not. On the other hand, 16 subjects of the control group (18.8%) were exposed to environmental pollution and 69 (81.2%) were not exposed (Figure 3.6). Comparison of the study groups for exposure to environmental pollution revealed no statistically significant difference between the two groups ($P> 0.05$).
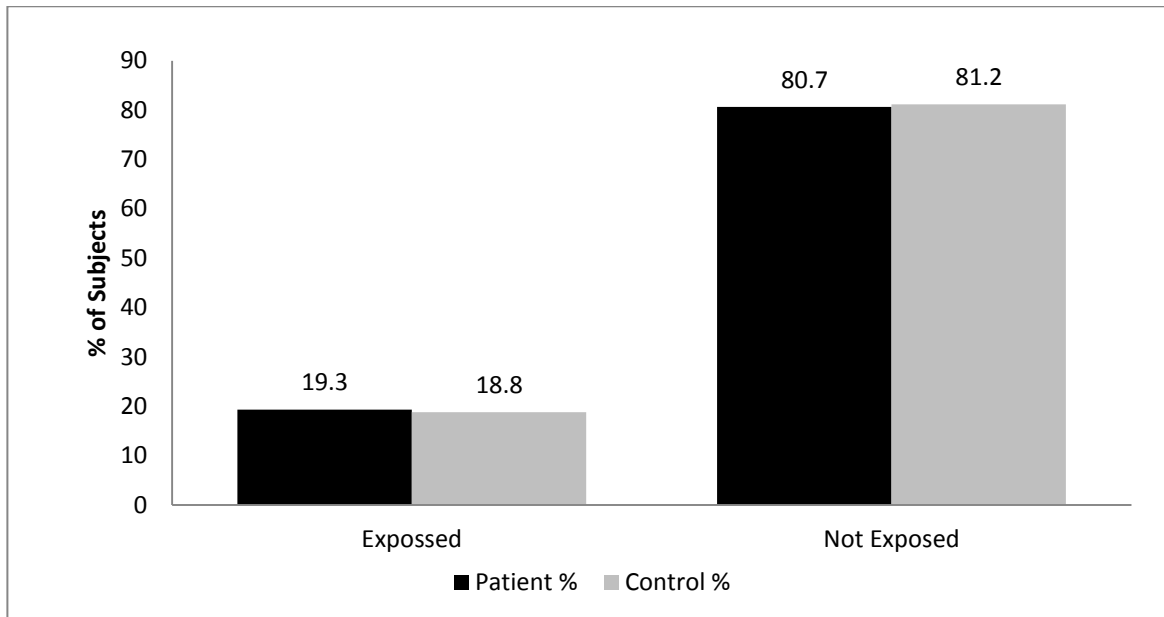
Figure 3.6: Exposure of patients' and control groups (%) to environmental pollution.

3.7.Water source and intake:

Water may be one of the primary sources by which the bacterium is transmitted. Intake of *H. pylori* contaminated water may contribute to establishing the infection. Water intake was evaluated in the questionnaire as "**Low intake"** when subjects were taking less than

one liter a day, "**Moderate**" when the intake was between 1-2 liters a day, and "**Adequate**" when water intake exceeded two liters a day (Robinson et al., 1989). The intakes of other fluids like juice or tea were also included in calculating water intake. The distribution of study subjects according to water intake is shown in table 3.4. Analysis of data concerning water intake, showed that more than half of the patients (56.6%) fell in the moderate water intake category, while more than half (52.9%) of the control group fell in the adequate water intake category. A statistically significant difference was observed between the patients' and control group concerning the water intakein the low category ($P= 0.003$) and adequate category ($P=0.005$).

The source of drinking water (well or municipality tap water) was also recorded for the study subjects. From the patients' group, 26 subjects (31.3%) were drinking well water and 57 subjects (68.7%) were drinking municipality tap water. While from the control group 23 subjects (27.1%) were drinking well water and 62 subjects (72.9%) were drinking municipality tap water. No statistically significant difference was observed between the two study groups concerning the source of drinking water ($P> 0.05$).

Table 3.4: Water intake patients and control groups.

Water intake quantity	Patients' group, No. (%)	Control group, No. (%)	*P*- value
Low (<1L/day)	14 (16.9%)	2 (2.4%)	0.003
Moderate (1-2L/day)	47 (56.6%)	38 (44.7%)	>0.05
Adequate (>2L/day)	22 (26.5%)	45 (52.9%)	0.005

3.8.Consumption of spicy Food:

Spicy and chilly foods were also evaluated to establish their role in mediating or predisposing to infection probably by irritating the intestinal tract. All study subjects were

asked to describe their intake of spicy and chilly food. For this purpose the study subjects were grouped into three categories: "**none**" for subjects who do not eat any spicy food or hot pepper at all; "**moderate**" for individuals who eat spicy food but cannot handle the hot pepper and "**high**" for individuals who consume spicy and hot pepper food. Table 3.5 shows the distribution of patients' and control groups by their consumption of spicy food. No statistically significant difference was observed between the two study groups concerning this factor ($P > 0.05$).

Table 3.5: Consumption of spicy food by patients' and control groups.

Amount of spicy food consumption	Patients' Group No. (%)	Control Group No. (%)	*P*- value
None	21 (25.3%)	13 (15.3%)	>0.05
Moderate	41 (49.4%)	46 (54.1%)	>0.05
High	21 (25.3%)	26 (30.6%)	>0.05

3.9. Correlation of ABO and Rh(D) phenotypes to *H. pylori* infection:

Blood samples from each subject were tested to determine the ABO and Rh phenotypes of patients and control subjects. For this purpose, RBCs of the study subjects were tested with anti-A, anti-A1, anti-B and anti-D antibodies.

Table 3.6 shows the ABO and Rh(D) phenotypes of the study subjects. The data showed that the prevalence of ABO blood groups among patients and controls was as follows: A>O>B>AB. The distribution of blood groups was similar among patients and controls. Indeed no statistically significant difference was observed among the patients' group and control group concerning the distribution of ABO blood groups.

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Table 3.7 shows the distribution of Rh (D) phenotype among patients' and control groups. The results showed that the prevalence of Rh (D) positivity is similar among the two groups. No significant difference was observed among the patients' and control group concerning the prevalence of Rh (D) phenotype.

Table 3.6: Prevalence of ABO and Rh (D) phenotypes among patients' and control groups.

Blood Group Ag	Patients' group		Control group	
	No.	%	No.	%
O blood group	27	32.5	29	34.0
ORh+ve	24	28.9	28	32.9
O Rh-ve	3	3.6	1	1.1
A blood group	34	41.0	34	40.0
A1 Rh+ve	31	37.3	27	31.8
A2 Rh+ve	1	1.2	3	3.5
A1 Rh-ve	2	2.4	3	3.5
A2 Rh-ve	0	0	1	1.2
B blood group	12	14.5	14	16.5
B Rh+ve	9	10.8	10	11.8
B Rh-ve	3	3.6	4	4.7
AB blood group	10	12.1	8	9.5
A1B Rh+ve	7	8.4	6	7.1
A2B Rh+ve	1	1.2	0	0
A1B Rh-ve	2	2.4	1	1.2
A2B Rh-ve	0	0	1	1.2
Total	83	100	85	100

Table 3.7:Prevalence of Rh (D) phenotypes among patients' and control groups.

Rh (D) type	Patients' group		Control group	
	No.	%	No.	%
Positive	73	88 %	74	87 %
Negative	10	12 %	11	13 %
Total	83	100	85	100

3.10. Lewis Antigens and Secretor status:

3.10.1. Inheritance of Lewis Antigens:

Phenotyping of Lewis antigens was determined using anti-Le^a and anti-Le^b. Subjects were tested and categorized in one of the following phenotypes: Le(a-,b-), Le(a-,b+), Le(a+,b-) and Le(a+,b+).

The results showed that Le^a was positive in 16 patients (19.3%) and negative in 67 patients (80.7%), while it was positive in 30 (35.3%) control subjects and negative in 55 (64.7%) control subjects (Figure 3.7). This difference was statistically significant ($P=0.020$) as determined by Person's Chi-Squire test, with control group subjects showing Le^a on their RBCs than patients. Suggesting that Le^a to be more resistance marker.

Le^b was positive in 43 patients (51.8%) and negative in 40 patients (48.2%), while it was positive in 36 (42.4%) of the control subjects and negative in 49 (57.5%) of the control subjects (Figure 3.7). This difference was not significant using the Person's Chi-Squire ($P=0.220$).

Regarding the inheritance of the Lewis phenotypes, individuals were categorized as follows: Le(a-,b-) in 24 (28.9%) patients and in 19 (22.4%) control subjects, Le(a-,b+) in 43 (51.8%) patients and in 36 (42.4%) control subjects and Le(a+,b-) in 16 (19.2%) patients and in 30 (35.3%) control subjects. None of the study subjects showed the Le(a+,b+) phenotype (Figure 3.7). A statistically significant difference ($P= 0.039$) was observed between the patients' group and control group regarding the Le(a+,b-) phenotype.
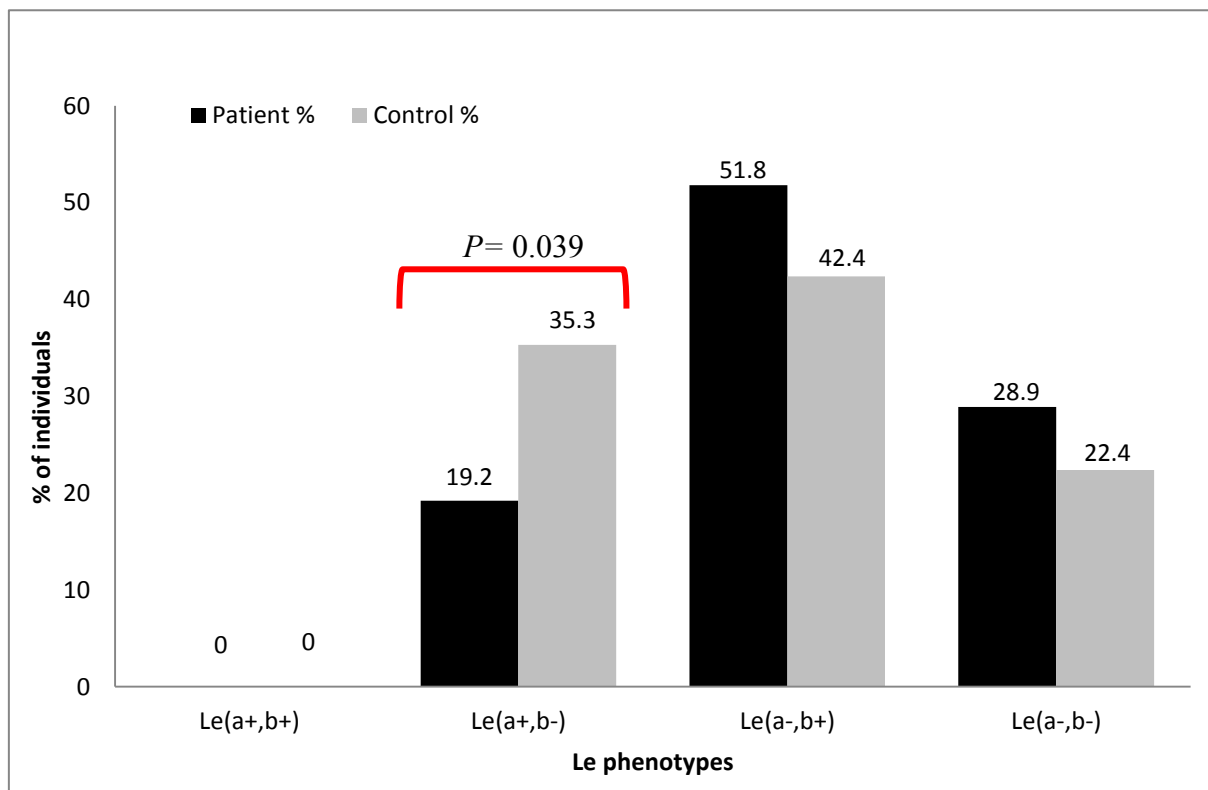
Figure 3.7: Distribution of Lewis system phenotypes for patients' and control groups.

3.10.2. Inheritance of secretor gene:

Inheritance of the Se gene was determined by testing saliva samples of study subjects for ABH soluble substances using the hemagglutination inhibition method. The results of the secretor status obtained from saliva study were also compared to the Lewis phenotypes to validate and confirm saliva testing. The secretor status was positive in 61 (73.5%) of the

patients and 47 (55.3%) of the control group, and the non-secretor status was found in 22 (26.5%) of patients and in 38 (44.7%) of control subjects (Figure 3.8). A statistically significant difference ($P= 0.039$) was observed between the patients' and control group concerning the non-secretor status, while no significant difference was observed between the study groups when compared for the secretor status ($P> 0.05$).
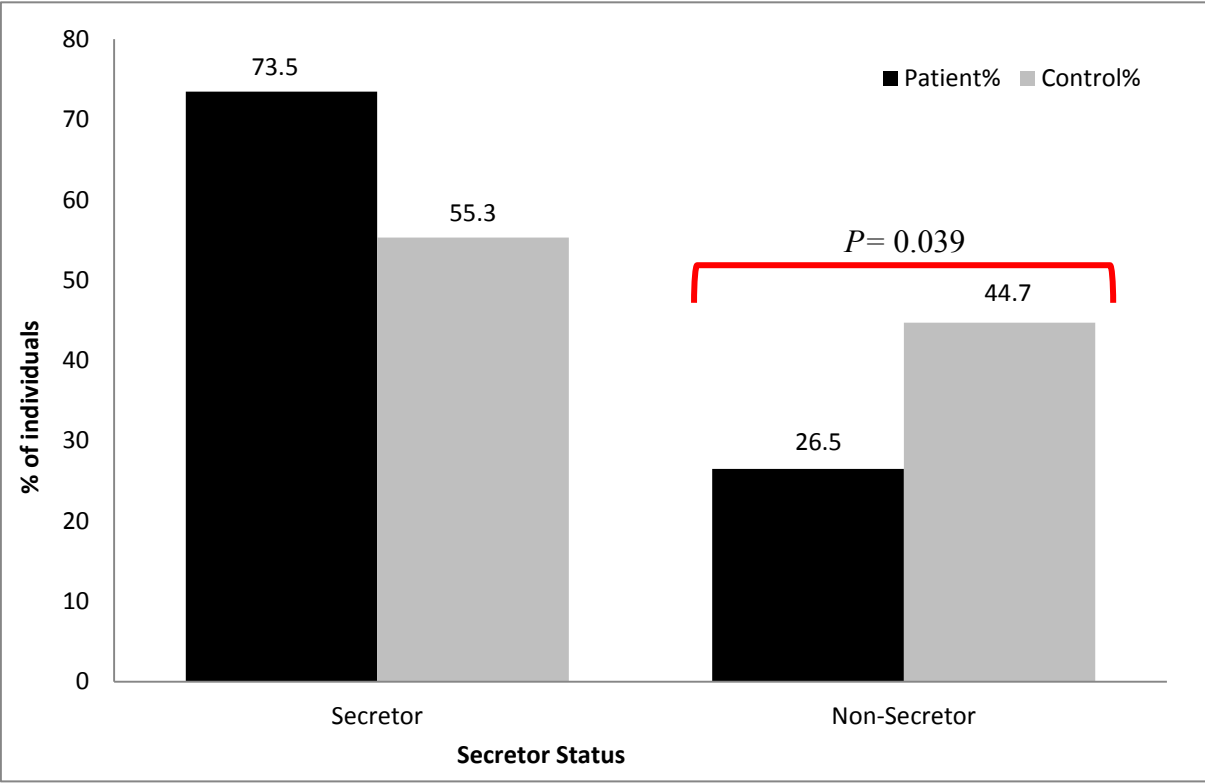
Figure 3.8: Comparison of the secretor status between patients' and control groups.

Figure 3.9 shows a comparison between the patients' and control groups for secretor status versus ABO/Rh(D) phenotypes.

	A1+	A1-	A1B+	A1B-	O+	O-	B+	B-	A2+	A2-	A2B+	A2B-
■ Secretor	19	2	5	1	19	3	8	3	0	0	0	0
■ Non-sec	12	0	2	1	5	0	1	0	1	0	1	0
■ No. of Patients	31	2	7	2	24	3	9	3	0	0	0	0
■ Secretor	15	3	2	0	18	1	5	3	0	0	0	1
■ Non-sec	12	0	4	1	10	0	5	1	2	1	0	0
■ No. of Controls	27	3	6	1	28	1	10	4	3	1	0	1
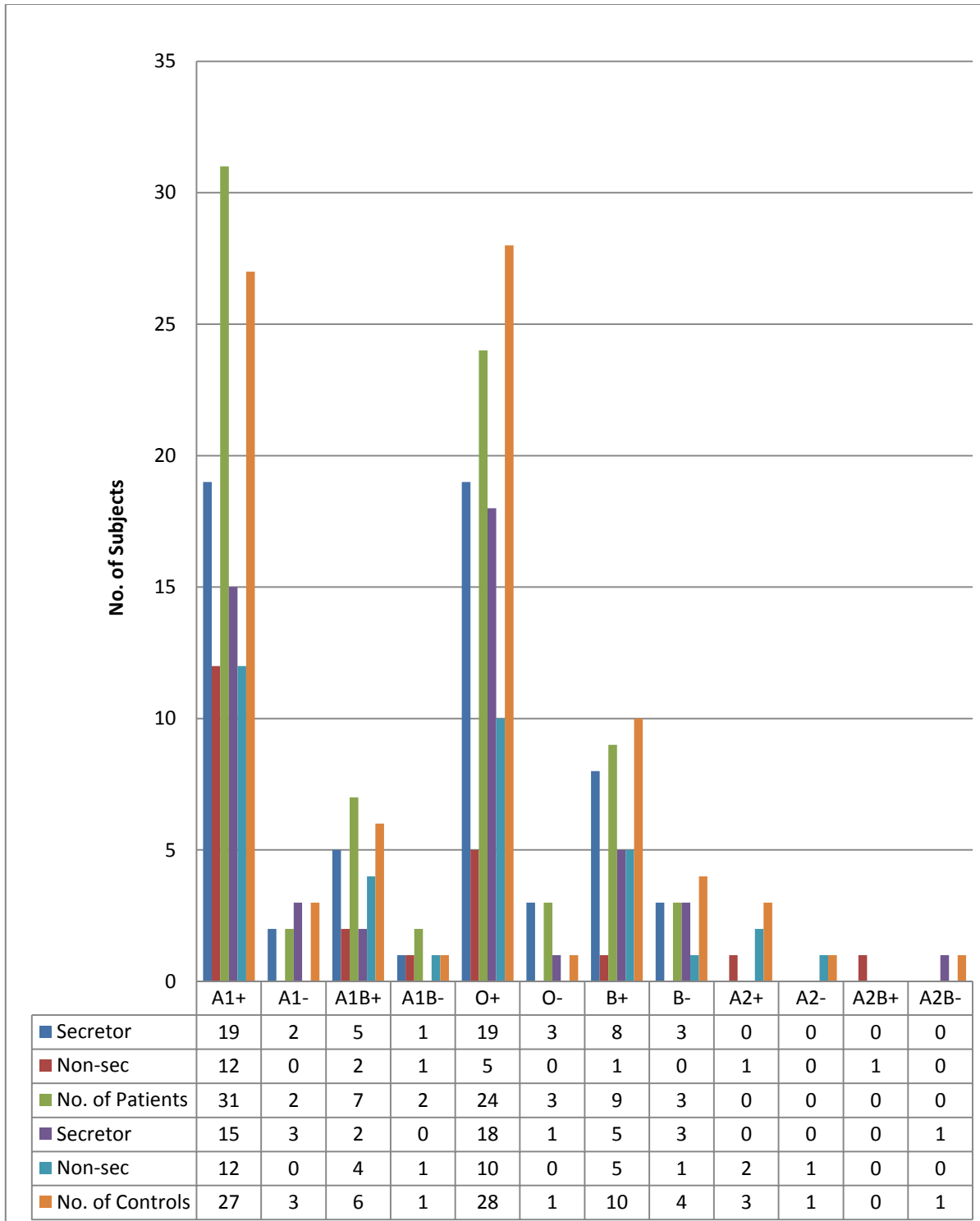
Figure 3.9: Secretor status versus ABO/Rh(D) groups of patients' and control groups.

3.11. *H. pylori*IgG:

The *H. pylori* specific IgG was determined by ELISA for the control group to assess previous asymptomatic exposure to *H. pylori*. Since the patients' group was already evaluated for these bacteria it was necessary to determine whether the asymptomatic control group individuals had been previously exposed to *H. pylori* or healthy subjects who were not exposed to the bacterium at all. However, tolerant individuals will produce a negative result to that particular antigen as well and will show negative IgG results.

Serum samples were tested twice (two independent assays) for the *H. pylori*IgG. The results showed that 83 (97.6%) of the control subjects were positive and only 2 subjects (2.4%) were negative for *H. pylori* IgG. As a result, the control group was considered to be asymptomatic, except for the two true negative subjects, who may be true negative or immune-tolerant.

Chapter Four:

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4. Discussion:

Helicobacter pylori infection may lead to ulceration of the gastric mucosa and duodenum; it is strongly associated with the development of epithelial and lymphoid malignancies of the stomach. The prevalence of *H. pylori* infection varies geographically. In addition several factors may contribute to its pathogenesis (Li et al., 2003; Martins et al., 2006; Torres et al., 2008;Barghouthi, 2009; Muhsenet al., 2012; Tonkic et al., 2012; Yamamoto et al., 2012).

Treatment of *H. pylori* aims to eradicate 90% of the bacterium, so patients are given a combination of two antibiotics plus a proton pump inhibitor and this is considered as a first line treatment. However; frequent use of antibiotics during childhood is associated with a decline in eradication rates of the bacterium(Ertem, 2012) reports of increasing bacterial resistance have been reported (Barghouthi, 2009).

The study samples were collected between January 2012 and January 2013. The study was performed at Al-Quds University Research Laboratory and Augusta Victoria Hospital Laboratory.

After detecting the IgG of *H. pylori* in the control group, the results revealed an astonishing high rate (98%) of asymptomatic infection with *H. pylori* among the control group. This was an indicator to consider the control group to consist of asymptomatic subjects.While the patients' group showed the clinical symptoms of *H. pylori* infection that was confirmed by laboratory findings of the bacteria in stomach biopsies or bacterial antigens in stool or *H. pylori* specific IgA in serum.Comparison between the two groups was performed using different predictive factors.Both host and bacterial factors may contribute to differences in *H. pylori* pathogenicity. Half of the world population is reported to be infected with this bacterium (Barghouthi, 2009). Although the prevalence of *H. pylori* infection is declining in some developed countries, the prevalence of infection in Asia and other developing countries is still high. Human host is considered to be the principle reservoir of the bacterium and transmission is believed to occur via person to person. Also several studies observed that children acquire *H. pylori*frequently from their mothers. On the other hand, unclean water has been implicated in the transmission of*H. pylori* infection (Wu et al., 2003; Ertem,2012; Valliani et al., 2013).

The subjects of both study groups were from different Palestinian cities in order to have contributors with different exposing factors, such as pollution, water sources, culture habits, and different genetic elements.

4.1.Serological Detection of anti- *H. pylori*IgG:

Testing for *H. pylori*specific IgGindicated bacterial exposure, asymptomatic subjects showedthat about 98% of the control group were positive for this antibody. Therefore, this

test cannot be employed as a diagnostic tool for this infection, this can be useful if only accompanied with clinical symptoms and/or verified by other means. The test is useful in showing equal exposure and differentiating between symptomatic and asymptomatic subjects. The mean IgG concentration for the asymptomatic group was about 109.9NTU/mL (SD= 60.1). Comparison of the IgG titer of symptomatic patients (determined for few random samples) with the mean titer of the control group (asymptomatic subjects)revealed no significant difference

Although serologic testing for *H. pylori* by detectingIgG antibodies have been proven to be useful in supporting the clinical diagnosis of infection with this bacterium. Serum immunoglobulin detection are considered good indicators to evaluate and diagnose the infection and treatment of *H. pylori* (Jaskowski et al., 1997; Li et al., 2003), this study showed that physicians must not relay on the IgG blood testing to evaluate patients for *H. pylori* infections because exposure to bacteria does not mean to suffer from symptoms, so there should be other ways to evaluate patients with symptoms in order to be confirmed of having problems due to *H. pylori*infection or there are other reasons for these troubles.

Prevalence of *H. pylori* asymptomatic infections in the Middle East was determined to be increasing with age (Novis et al., 1998; Muhsen et al., 2012; Tonkic et al., 2012). A recent study conducted on Jewish and Arab patients, showed that *H. pylori* infection was low among children, reached 39% in the second and third decades and further increased to 79% in those over 60 years old (Muhsen et al., 2012). Infection prevalence rates are higher in patients of Mediterranean and Asian origin (around 89%) compared to Western European/ North American origins (57%). The latter findings reflects the important role of socio-economic effect on the prevalence of *H. pylori* infection (Novis et al., 1998; Hussein, 2010), suggesting that socioeconomics variables including hygienic standards play major roles in the transmission of the pathogen.

4.2.Age:

The mean age for symptomatic patients was higher than that of the control group (asymptomatic subjects) due to the low response rate among older individuals to participate in the study (in the control group). Therefore, the study groups were not matched concerning the age parameter. All the study subjects are adults and were older than 15 years, thus it is unlikely that the age may pose a risk for contacting the infection. A recent study from Iran, a developing country, investigated 458 children and found that the rate of *H. pylori* positivity was increasing with age and rose from 43.6% by 4 months of age to 90% by 15 years of age (Soltani et al., 2013). In our study, testing of the control group revealed that about 98% of the subjects were positive for *H. pylori* specific IgG in serum, which means that the majority of the control subjects were previously exposed to *H. pylori* but remained asymptomatic. The use of this asymptomatic control group for comparison with symptomatic patients' group provides an advantage that both groups were exposed to the bacterium but certain host factors have probably influenced the course of infection, to remain asymptomatic or to develop into a symptomatic infection. However, in comparison with other studies that showed a lower rate of *H. pylori* infection 45-60% regarding the age of individual in Muhsenet al., 2012 the age range was 0- 77 years. . (Li et al., 2003; Martins et al., 2006;Torres et al., 2008;Ertem, 2012; Muhsenet al., 2012; Tonkic et al., 2012).

This difference in age between the whole study groups has no effect on all variable investigated in this study except for the amount of water intake and source of water, as shown in Appendix C by statistical analysis of a subset of 43 subjects matched for age and gender.

4.3.Gender:

It was essential to compare both groups with similar gender distribution in order to determine whether there was a significant correlation between the symptoms of infection and gender of individuals. In this study no significant difference was observed between both genders concerning *H. pylori* infection. This result is in accordance with previous studies (Fiedoreket al., 1991; Graham et al., 1991; Turkolmezet al., 2007; Petrovic et al., 2011). Yet, our result disagrees with other studies from Turkey and Iraq (Kanbay et al., 2005; Jaff et al., 2011) which showed that male gender as a protective factor for infection while females were more susceptible to *H. pylori* complications.

4.4.Source of drinking water:

Water sources are limited in Palestine and they were categorized into tap water and rain water collected by water wells. There was no significant difference (*P-value*>0.05) between the source of drinking water and symptomatic infection with *H. pylori*. Although the role of drinking water in transmission of *H. pylori* is not confirmed, recent studies reported that, water contamination may play a role in transmission of the bacterium (Ertem; 2012). However, further studies that include young children and a controlled and characterized source of drinking water is needed to determine the role of water in transmission of *H. pylori* and whether it contributes to the high seropositive asymptomatic individuals among Palestinians.

4.5.Water intake:

High water intake habits are necessary to maintain body hydration levels and cellular integrity. Water has several advantages and benefits for body tissues. After comparing the two study groups, for amounts of water intake, it was observed that the patients' group

were drinking less water ($P<0.05$) than control group, indicating that water intake may have a protective effect against symptomatic infection. A possible explanation include that adequate water intake increases the hydration of mucous membranes and enhances the effect of innate immunity in the mucous or secretions surrounding the mucous membranes of the digestive tract. Further investigation of this issue at the cellular level may provide more accurate explanations for this point.

4.6.Pollution and Smoking Habits:

The environmental contamination may play a primary role in bacterial transmission through contaminating food and water sources, individuals who carry *H. pylori* may transmit it to others by contaminating the environment leading to infecting others. No significant difference between the two study groups concerning environmental pollution was observed in this study. Palestine is not considered as an industrialized country so the chemical remnants are probably limited and not polluted. The level of exposure to environmental contamination is difficult to measure. Thus it is better to investigate the effect of this factor on *H. pylori* infection using a larger sample and to limit the study to specific regions with clear differences in environmental contamination or to measure certain indicators of pollution.

Concerning the effect of smoking on *H. pylori* infection, no statistically significant difference was observed between the study groups concerning this factor, this finding is consistent with the finding by Valliani et al. (2013) from Pakistan. The latter finding was in contrast with a study in Turkey that found smoking as a protective factor against infection with *H. pylori* (Kanbay et al., 2005).

4.7.Consumption of Spicy food:

Since food is considered one of the primary factors affecting and influencing gastrointestinal tract diseases (Singh et al., 2006; Yassibas et al., 2012); spicy and chilly food was evaluated for its contribution for the development of symptomatic infection with *H. pylori*. In our study there was no significant correlation between consumption of spicy food and *H. pylori* infection.

The general public usually relates gastritis as a temporary reaction to spicy/ chilly- foods. Indeed some reports indicated that spicy foodinfluences gastrointestinal diseases (Singh et al., 2006; Yassibas et al., 2012). In this study, there was no correlation between spicy food and symptomatic infection with *H. pylori*.

4.8.Drug intake:

Drugs are composed of different chemicals, many of which when taken in excess doses, in combination or for a period longer than indicated for such drugs, may cause adverse effects on the stomach tissues (Kuhn et al., 2010). In this study, there was a significant difference between the study groups, concerning the intake of drugs ($P < 0.001$).

It seems that individuals who are using drugs for a long time may be at risk of developing symptomatic *H. pylori* infection. It also may indicate that individuals with underlying infections or disorders may be predisposed to developing symptomatic infections of *H. pylori*.

4.9.Body Mass Index (BMI):

Body mass index (BMI) may reflect the food intake habits and physical activities of participating subjects. It was found that the patients' group showed significantly higher BMI values than the control group. This evidence supports the view that feeding habits (quantities and frequencies of feeding) may impact the function of the stomach and influence the interaction of the stomach cells with invading pathogens. It may also contribute to intake of higher infectious doses of the bacterium *H. pylori*. Whether obesity is directly or indirectly responsible for this association still awaits further investigation.

A statistically significant difference (*P*= 0.033) was observed between the patients' and control groups when compared for the BMI. Indeed the mean BMI for the control group being in the normal category and the mean BMI for the patients group being in the overweight category. This finding suggests that overweight may be predisposing for *H. pylori* infection.

4.10.   ABO and Rh:

Many studies found a strong relationship between ABO and Rh antigens and *H. pylori* infection, this correlation is due to the different glycosylation patterns of RBC antigens. Several pathogens have been demonstrated to use certain RBC antigens as receptors that facilitate adherence of the pathogen. Analysis of ABO blood groups and Rh (D) antigen distribution among the study subjects, showed no significant difference between the study groups. The primary results showed that the blood group A1 Rh+ve was slightly higher in the patients' group and the blood group O Rh+ve was slightly higher in the control group, but this difference was not statistically significant. The distribution of ABO blood groups was close to that reported among the Palestinian population (Al-Kawasmi, 2011).

No significant difference was observed between the study groups when compared for the ABO and Rh antigen, which is consistent with previous reports (Jaff et al., 2011; Petrovic et al., 2011).While other studies have found a significant correlation between blood groups and *H. pylori* infection. In certain patients a significant association was found between infection and O blood grouping with Le[b] and secretor inheritance (Kanbay et al., 2005; Martius et al., 2006; Bayan et al., 2009;Anstee, 2010; Yamamoto et al., 2012; Valliani et al., 2013).

The variations between different studies and correlations found within certain blood groups may be explained by the difference in epidemiological distribution and prevalence of particular blood groups. Therefore, it is noticed that blood group antigen effect is not that significant and the increase in certain antigen is due to the degree of dominance of ABO antigens in the population. In the present study, the finding that 98% of the control group were asymptomatic and previously have been exposed to *H. pylori*, suggests that, at least among our study subjects, the ABO blood group is not associated with *H. pylori* infection.

4.11. Lewis Antigens and Secretor Status:

Lewis antigens are described to resemble some *H. pylori* antigens that are carried by this bacterium and helps in its adhesion and interaction with host cells. The Lewis antigens (Le[a] and Le[b]) are produced through interaction of Lewis (*FUT3*) gene and Secretor (*FUT2*) gene. (Magdalena et al., 1998; Hynes et al., 2000; Monteiro et al., 2000; Mcguckin et al., 2007; Torres et al., 2008; Anstee, 2010; Yamamoto et al., 2012). In this study, the Le[a] antigen which correlates with the non-secretor phenotype was observed in a significantly (*P-value*= 0.020) less frequency in the patients' group compared to the control group. Previous studies have shown Le[b] antigen acts as a receptor for *H. pylori* and correlates with the secretor phenotype (Valliani et al., 2013).There was trend toward slightly a higher frequency in patients compared to the control group. This finding was further supported by the frequency of the secretor phenotype among the study groups discussed in the next

paragraph. These findings are consistent with previous reports that reported a role for Lewis antigens in the binding of *H. pylori* to the stomach mucosal cells (Hynes, 2000; Magdalena, 1998; Monteiro, 2000; Anstee, 2010; Yamamoto et al., 2012; Valliani et al., 2013).

An important finding of this study is the different distribution of the secretor phenotype among the study groups. Secretor phenotype showed similar expression in the patients' group compared to the control group (P>0.05) and the non-secretor phenotype was found in a significantly lowerfrequency (*P-value*= 0.014) in the patients' group compared to the control group. Inheritance of Le (a+,b-) phenotype is associated with the non-secretor phenotype while the inheritance of Le(a-b+) is associated with the secretor phenotype. Additionally, the number of Le^b antigens per red cell is higher than Le^a antigens (Harmening, 2005). Taken together, individuals with Le(a-b+) phenotype/ secretor phenotype has more Lewis antigens per cell compared to individuals with the Le(a+b-) phenotype/non-secretor phenotype. Since the *H. pylori* has been reported to use the Lewis antigens for attachment or adherence to the stomach mucosal cells, it can be deduced that individuals with the Le(a-b+) phenotype/secretor phenotype may facilitate infection with this bacterium. This finding is consistent with the findings reported by earlier reports which suggested that *H. pylori* needs high Le^b antigen concentration on the host cells to facilitate its attachment and colonization on host cells (Magdalena et al., 1998; Lee et al., 2006; Harvey et al., 2010; Yamamoto et al., 2012). Alternatively, the non-secretors express only Le^a which is usually found in a lower concentration compared to Le^b, which in turn may reduce the potential attachment sites for *H. pylori.*

4.12. Conclusion:

This study was a case-control study. The patients' group has experienced symptomatic *H. pylori* infection. In the control group or asymptomatic group, the majority of the subjects (98%) was exposed to *H. pylori* pathogen but did not show any symptoms of infection. To

the best of our knowledge, this study is the first study of its kind in Palestine. Comparison of the study groups, showed significant association between *H. pylori* infection and BMI, drug intake, amount of water intake, Lewis phenotype and secretor property. While comparison of the study groups, showed no statistically significant association between *H. pylori* infection and gender, drinking water source, spicy and chilly food intake, smoking, exposure to environmental pollution and ABO/Rh group.

4.13.   Future Work:

Future testing for the *H. pylori* behavior and infection mediating factors is still recommended especially to study the *H. pylori* exposure status among age groups, and to use certain genetic studies to determine the blood antigens affecting the infection and bacterial antigens chemical contents responsible for the adhesion and attachments to be blocked by certain reactions. On the other hand the analysis of drugs and their chemical components mediating the infection and improving its pathological characteristics. In addition, further investigations are needed to examine the effect of BMI and water intake on *H. pylori*infection.

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Appendices

Appendix A: Study Questionnaire

استمارة بحث علمي

جامعة القدس/ كلية الدراسات العليا/ ماجستير علوم طبية مخبرية
اسم الباحث: علا كرمي

السيدة /السيد:

تقوم الباحثة علا كرمي – جامعة القدسبإشراف الدكتور محمود سرور و الدكتور سمير البرغوثي-بإعداد دراسة حول "
بكتيريا الهليكوباكتر بيلوري و فصيلة الدم في الضفة الغربية ـ فلسطين".
و لذلك نرجو من حضرتك التعاون مع الباحثة بتعبئة الاستبيان المرفق و الموافقة على إعطاء عينات دم و لعاب
لاستخدامها في هذا البحث.

و سوف يتم التعامل مع العينات و نتائج البحث بسرية و خصوصية كاملة، أما نتائج البحث فسوف تستعمل لأغراض
البحث العلمي فقط.
و سيتم إعلامكم بنتائج البحث عند الانتهاء من الدراسة.

أقدم لكم شكري و امتنانيعلى تعاونكملإنجاح هذا البحث،،،،،

معلومات عامة:

1. رقم المشارك:
2. العمر:
3. الجنس: أ- ذكر   ب- أنثى
4. الطول:
5. الوزن:
(لحساب Body mass index):
6. ما هي فصيلة دمك:
7. هل تدخن: أ- نعم   ب- لا

إذا كنت تدخن فاذكر العمر الذي بدأت به التدخين:

كم الفترة التي دخنت بها من دون انقطاع:

8. منطقة السكن:

المدينة: القرية:

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9. هل تقطن قريبا من منطقة فيها تلوث (مكب نفايات، تعمل في مصنع ....الخ): أ- نعم   ب- لا

حدد:

10. هل تشرب من مياه أبار الجمع (الأمطار): أ- نعم   ب- لا   حدد:

11. كيف تصف شربك للماء: أ- قليلا (أقل من لتر)   ب- متوسط (1-2 لتر)   ج- أتناولها في كثير من الأحيان (لتران من الماء)

12. كيف تصف تناولك للطعام الحار و الفلفل: أ- لا أتناولها نهائيا   ب- قليلا   ج- أتناولها في كثير من الأحيان

❖ *إذا لم تكن مصابا بأعراض القرحة في حياتك الرجاء الذهاب للسؤال رقم(15):*

13. ما هي الطريقة التي كانت متبعة في تشخيص مرض القرحة:
أ- فحص دم   ب- خزعة من المعدة   ج- فحص تنفس   د- لا أعرف   هـ- غير ذلك، حدد:

_____

14. أذكر أمور أخرى تزيد أعراض المرض لديك و غير موجودة في الأسئلة السابقة: --------------------------------

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15. **هل هناك أشخاص في عائلتك مصابين بأمراض في الجهاز الهضمي أو القرحة: أ- لا   ب- نعم**

حدد نوع المرض: حدد صلة القرابة:

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Appendix B: Consent Form

جامعة القدس/ كلية الدراسات العليا/ ماجستير علوم طبية مخبرية   اسم الباحث: علا كرمي

أنا: الموقع أدناه، أوافق على المشاركة بالبحث العلمي الذي تقوم به الباحثة

علا كرمي- جامعة القدس بإشراف الدكتور محمود سرور و الدكتور سمير البرغوثي عن بكتيريا الهليكوباكتر

بايلوري و علاقتها بفصيلة الدم، و أوافق على استخدام المعلومات التي يطلبها الباحث في أغراض البحث العلمي، و

أوافق على إعطاء عينات دم و عينات لعاب لاستخدامها في هذا البحث.

التوقيع:

التاريخ:

توقيع الباحث:

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لاستعمال الباحثة فقط.

رقم الاستبيان :..................

راجع المعلومات:...............
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Appendix C: Comparison of an age-matched subset of the patients' and control groups

Analysis of the patients' and control groups with regard to age, revealed a statistical significant difference (P=0.000). In order to determine if this difference has affected the findings of this study with regard to other variables studied in this study, we have matched 43 patients and 43 control subjects with regard to age and gender. Statistical analysis of this matched subset revealed that the factors that have significant difference were the same as the whole group, which were: high body mass index, increased drugs intake, no inheritance to Le^a antigen and inheritance of Se gene (i.e., having the secretor phenotype). While factors that did not show any significant difference between the patients' and control groups were also the same as the whole group, namely: spicy and chilly food intake, smoking, exposure to environmental pollution and ABO/Rh grouping.

However, the statistical analysis of the age-matched subset was contradictory to the findings observed with the whole groups with regard to the amount of water intake and the source of water. Analysis of the patients' and control groups (whole groups) revealed a significant difference between groups when compared with regard to the amount of water intake, while no significant difference was observed between groups when compared with regard to the source of water. In contrast, analysis of the age-matched subset, did not reveal a significant difference between groups when compared with regard to amount of water intake, and revealed a significant difference between groups when compared with regard to the source of water. Therefore, we believe that further investigations are needed using a larger sample to study the association between the amount of water intake and source of water and *H. pylori* infection.

Statistical analysis was performed using the same methods used to analyze the whole study groups namely the Pearson's Chi- square test.

العلاقة بين بكتيريا الهليكوباكتر بيلوري و نوع فصيلة الدم و قدرة الشخص الإفرازية في فلسطين

إعداد: علا طلال عبد الله كرمي.

إشراف: د. محمود سرور، و د. سمير البرغوثي.

الملخص:

الغلاف الخلوي لكريات الدم الحمراء و ما يرتبط به من أنتيجينات وجد أن له علاقة مع عدة أنواع من الأمراض، كما أن له حساسية لعدة أنواع من الكائنات الدقيقة الممرضة. و بملاحظة أن بكتيريا المعدة الحلزونية المسببة للقرحة و الالتهابات المرافقة لها مرتبطة مع الأنتيجينات المختلفة التي تحملها خلايا الدم الحمراء كان هناك دراسات متعددة على تحديد العوامل التي تزيد خطورة إحداث أعراض مرضية لدى الأشخاص الذين يصابون بهذه الجرثومة.

الهدف الأساسي لهذه الدراسة هو البحث في العلاقة بين الإصابة ببكتيريا القرحة مع نجاح البكتيريا في إحداث أعراض و مضاعفات المرض من جهة و نوع فصيلة الدم و نوع الأنتيجينات التي تحملها من (ABO/ Rh (D) و Lewis و نوع الأنتيجين الخاص بـ A سواء كان A1 أو A2، و من ناحية قابلية الشخص لإفراز الأنتيجينات في المرضى الفلسطينيين.

اشتملت الدراسة على مجموعتين: مجموعة المرضى (83 مريض) الذين ظهرت عليهم أعراض الإصابة بجرثومة المعدة والمجموعة الضابطة (85 شخص) الذين لم تظهر عليهم أي من أعراض الإصابة بالجرثومة، وقد تم دراسة الفرق بينهما من ناحية الجنس و كثافة الجسم و عادات التدخين و تعاطي الشخص للأدوية المختلفة وتعرضهم للملوثات البيئية وكمية الماء ومصدرها ومقدار التعرض

للطعام الحار بالإضافة لنوع الدم وأنواع الأنتجينات المختلفة التي تحملها كريات الدم الحمراء وقابلية الشخص الإفرازية. هذه العوامل تمت دراستها إحصائيا لملاحظة وجود ارتباط بينها وبين أعراض الإصابة ببكتيريا القرحة.

أظهرت النتائج أن نسبة عالية من العينة الضابطة (98%) قد تعرضوا لجرثومة المعدة حيث تم كشف الأجسام المضادة للجرثومة في مصل الدم، مع العلم أنهم لم يسبق لهم التعرض لأعراض الالتهاب ببكتيريا القرحة. وبالمقارنة بين مجموعة المرضى والمجموعة الضابطة لوحظ وجود علاقة ذات دلالة إحصائية بين الإصابة بالجرثومة وعدة عوامل تشمل: كثافة الجسم، وتناول الأدوية، وكمية الماء التي يتناولها الشخص، وحمله لنوع الأنتجين Lewis على كريات دمه الحمراء و خلايا جسمه، و قابليته الإفرازية للأنتيجينات المختلفة. كما ظهر أثناء المقارنة أنه لا يوجد علاقة ذات دلالة إحصائية بين الإصابة بالجرثومة والعوامل التالية: الجنس، ومصدر الماء الذي يتم تناوله، وتناول الطعام الحار والحاد، والتدخين، والتعرض للملوثات البيئية المختلفة، ونوع دم الشخص وما يحمله من أنتيجينات ABO/ Rh (D).

وكنتيجة لهذا البحث ظهر هناك نسبة عالية من الحاملين لجرثومة بكتيريا القرحة في المجتمع الفلسطيني، ظهر أيضا أن ظهور أعراض المرض عند الاصابة بجرثومة المعدة يرتبط بعوامل يحملها الإنسان وأخرى من البيئة المحيطة.