Helicobacter pylori, a potential causative agent of vitamin B12 deficiency.

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أمكانية نقص فيتامين ب12 الناتجه عن الاصابه بالبكتريا الحلزونيه

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II

Dedication

I dedicate this work to my parents who gave me the power to continue my graduate studies.

I also dedicate this work to my wife, and my children who provided me with the proper atmosphere and gave me the incentive to finish.

AAS

III

Acknowledgments

I would like to acknowledge the founder of the Master Program Clinical Laboratory Science Dr.Tamer Essawi. We are indebted to his tremendous efforts to provide us with the opportunity to obtain graduate education. I also acknowledge Dr.Mohammad Farraj for his continuous efforts, support and supervision during this study. ***Dr.Wail Hammoudeh*** **was a great help in providing me with all the assistance to interview and select the patients and to obtain specimens .Additional thanks to all my colleagues and Mr.Shadi Rifai for his assistance.**

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إمكانية نقص فيتامين ب 12 الناتجه عن الاصابه بالبكتريا الحلزونيه

ملخص الدراسه:

تعتبر البكتريا الحلزونيه " والتى تعيش تحت الطبقه المخاطيه من بطانة المعده المسبب الرئيسي لالتهاب وتقرح المعده والاثنى عشر الاكثر انتشارا في العالم . حيث ان نسبة الاصابه في هذه البكتريا في دول العالم الثالث تصل من 70- 90 % بينما في الدول المتطوره تصل ما بين 20-50%.كما ان هذه البكتريا تزيد من مخاطر الاصابه بسرطان المعده.
ان نسبة حدوث نقص فيتامين ب12(كوبالمين) عند كبار السن يبلغ اكثر من 20 % ويعود سبب ذلك النقص الى عدة عوامل اهمها سوء الامتصاص وسوء التغذيه والتى تبلغ نسبته 60 % من هذه الحالات اما فقر الدم الخبيث تبلغ نسبته من 15-20% من هذه الحالات.
ان التهاب المعده الناتج عن الاصابه بعدوى البكتريا الحلزونيه والتى بدورها تؤدي الى تدمير الخلايا الجداريه للمعده (البرتيال) المسؤوله عن انتاج العامل الداخلي الذي يساعد على امتصاص فيتامين ب12 في الجسم .

هذا وقد وجدت بعض الدراسات ان هناك علاقه بين نقص فيتامين ب12 عند الاشخاص المصابين بالتهابات المعده الناتحه عن العدوى بالبكتريا الحلزونيه وقد وجد ان القضاء على هذه البكتريا يؤدي الى ارتفاع في نسبة فيتامين ب12 في الدم.

ان الهدف الرئيسي من هذه الدراسه هو: تحديد العلاقه بين التهاب المعده الناتجه عن الاصابه بالبكتريا الحلزونيه ونقص فيتامين ب12 في الدم بالاضافه الى عمل مقارنه بين نتائج الفحوصات المختلفه التي تستخدم في تشخيص الاصابه بهذه البكتريا.
وقد تمت الدراسه على 60 مريض ممن يعانون من الام في المعده تم اختيارهم من اصل 76 مريض تم استثناءهم بناءا على عدة عوامل هي:
1- المعرفه بوجود فقر الدم الخبيث.
2- اجراء عملية استئصال للمعده او جزء منها.
3- التداوي بالفيتامين ب12 .

4-التداوي بالمضادات الحيويه
5- الحمل.

تشير النتائج المستخلصه من هذه الدراسه الى:
1- ان نسبة الاصابه بالبكتريا الحلزونيه في مجموعة البحث تصل الى 72% وهي من النتائج العالميه.
2-ان نسبة حدوث نقص في فيتامين ب12 في هذه المجموعه تصل ال 62% عند المرضى المصابين بالبكتريا الحلزونيه.
3-ان هناك علاقه بين نقص فيتامين ب12 وارتفاع متوسط حجم الخلايا الحمراء.
4- لقد بينت الدراسه ان التدخين و الجنس لا يلعبان دورا في زيادة الاصابه بهذه البكتريا و لا في زيادة نسبة حدوث نقص فيتامين ب.12
5- كما ان هذه الدراسه اتاحت الفرصه لمقارنه تشخيص الاصابه بالبكتريا الحلزونيه بواسطة اجراء تنظير المعده مع الوسائل الاخرى والتي تعتمد الفحوصات المخبريه.

Helicobacter pylori, a potential causative agent

of vitamin B12 deficiency.

Abstract:

Helicobacter pylori is considered to be the most common human pathogen colonizing the gastric mucosa of almost all people worldwide. It has been implicated in increasing the risk of developing gastric cancer which is the second most frequent cause of cancer-related deaths. The prevalence of *Helicobacter pylori* in the developing countries is 70 to 90% as compared to 20 to 50% in developed countries. The infection is believed to be acquired during childhood.

Vitamin B12 or cobalamin deficiency occurs frequently (> 20%) among elderly people. Causes of the deficiency include food cobalamin malabsorption syndrome (> 60% of all cases), pernicious anemia (15%–20% of all cases), insufficient dietary intake and malabsorption. *Helicobacter pylori* has been implicated as the potential causative agent of vitamin B12 deficiency and pernicious

anemia. Gastritis caused by *Helicobacter pylori* leads to destruction of parietal cells *Helicobacter pylori* producing the intrinsic factor needed for vitamin B12 absorption. It is well established that eradication of *H. pylori* led to improved blood levels of vitamin B12 in 40% of the cases.

The aim of this study was to evaluate the association between vitamin B12 deficiency, and *Helicobacter pylori* infection. In addition we compared invasive and non invasive methods routinely used to diagnose *Helicobacter pylori* infection. Our finding indicates that *Helicobacter pylori* infection was detected in 71.66% of those patients tested. Vitamin B12 deficiency was evident in 72.1% of those cases. These result correlated well with elevated mean cell volume (MCV) 62.8%. Smoking as well as gender does not seem to increase the risk of developing of vitamin B12 deficiency.

Our results indicate that *Helicobacter pylori* is implicated as a causative agent in the development of adult vitamin B12 deficiency in adult.

Introduction

Helicobacter pylori:

Helicobacter pylori is one of humanity's oldest and closest companions, and yet it took scientists more than a century to recognize it. As early as 1875, German anatomists found spiral bacteria colonizing the mucus layer of the human stomach [1]. At that time the organism could not be grown in culture and this significant finding was overlooked for a long time. Over a century later, in 1982, two Australian physicians Barry J. Marshall and J. Robin Warren were able to isolate *Helicobacter pylori* from the stomach of patients and discovered its role in causing gastritis and peptic ulcers [2, 3]. They have been awarded the Nobel Prize, (2005) in physiology and medicine for their pioneer work on this organism. In order to prove that this organism follows Robert Koch's Postulate, Marshall went to the point of swallowing a culture of this organism.

Helicobacter pylori is a fastidious Gram negative microorganism that has a spiral or helical shape. It has five to seven sheathed uni-polar

flagella. It requires high humidity, microaerophilic environment (5% O_2 and 5 to10% CO_2) and incubation temperature of 37^o C for growth. Although growth of *Helicobacter pylori* may appear after three to five days, the primary growth may take up to 7 days to appear [(4)]. The colonial morphology is described as translucent, small pinpoint colonies [(5)].

Helicobacter pylori is considered to be the most common human pathogen colonizing the gastric mucosa of almost all people [(6)]*Helicobacter pylori* causes chronic gastritis [(7)] and peptic ulcer disease [(8)]. *Helicobacter pylori* has been classified as a type I (definite) carcinogen since 1994. *Helicobacter pylori* has been implicated in increasing the risk of developing gastric cancer which is the second most frequent cause of cancer-related death [(9,10)]. Furthermore, *Helicobacter pylori* infection has also been considered to increase the risk of B-cell lymphoma of gastric mucosa-associated-lymphoid-tissue (MALT –lymphoma) [(21)]. It was shown that 72% to 98% of patients with gastric MALT-lymphoma are infected with *Helicobacter*

pylori ([11]) and treatment of *Helicobacter pylori* induced regression of MALT lymphoma in most cases [(12)].

Epidemiology and Transmission:

It is well accepted in the scientific community that infections with Helicobacter pylori occur worldwide. The prevalence of *Helicobacter pylori* varies depending on age, race, ethnicity, geographical location, household crowding and socioeconomic class [(13)] .Although more than 50% of the world population carries *Helicobacter pylori*, the prevalence in the developing countries reaches a high proportion of 70 to 90% [(14)].

In developed countries such as Japan, the United States and Europe, the rate of infection was found to be below 20% in adolescents and increased slowly afterwards [(15)]. It was also reported that 40% of infants and children of developing countries are already infected and the prevalence increases to reach 80% among adults [(15)]. Many epidemiological studies on the prevalence of *Helicobacter pylori* among infants and children have been conducted in the developing

and developed countries. In Vietnamese children aged 0-4 years, the incidence of *Helicobacter pylori* infection was found to be 14%, as compared to 15% incidence among Egyptian children of similar age [16].

The infection is believed to be acquired before 10 years of age [17]. This is apparent in several developing countries where children have high rates of sero-positivity [18]. Although most studies reported an equal incidence rate between males and females, one study reported male sex to be a risk factor [19]. The socioeconomic conditions are strongly correlated to *Helicobacter pylori* infections [20].

The mode of transmission of *Helicobacter pylori* remains undetermined until now. Theories support the notion that humans are the primary reservoir for *Helicobacter pylori* [21]. The person-to-person mode of transmission is supported by the higher incidence of infection among institutionalized children and adults and the clustering of *Helicobacter pylori* infection within families [13]. Also

lending support to this concept is the detection of *Helicobacter pylori* DNA in vomitus, saliva, dental plaque, gastric juice, and feces.

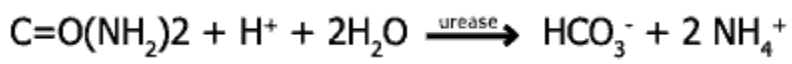
Waterborne transmission, probably due to fecal contamination, may be an important source of infection, especially in parts of the world in which untreated water is common [(13)]. Studies have been also conducted on fresh and well water to determine the possibility to incriminate water as a source of transmission of this organism. One study concluded that *Helicobacter pylori* is waterborne and represent public hazard. In this study, it was suggested that *Helicobacter pylori* entered a morphological transition from rods to cocci. Although the bacteria was viable but non-culturable [(22)]. Another study used a selective medium (HP selective medium) supplemented with growth factors, polymyxin B, amphotericin B and phenol red indicator [(23)]. *Helicobacter pylori* was isolated from well water on this selective medium within 7 days. Other reservoirs suggested include domestic cats [(24)] and houseflies [(25)].

Since *Helicobacter pylori* has been isolated from the feces of young children [26], or direct detection of stool antigens [27, 28], it is appropriate that transmission occurs via fecal-oral route within families during childhood [29, 30]. The oral-oral route of transmission was reported in African women who chew the food before feeding it to their children [30]. A possible route of transmission in developing countries is fecally contaminated water [31, 69]. It can also be transmitted from one person to another by vomit and saliva [32, 33].

***Helicobacter pylori* Virulence:**

It is apparent that *Helicobacter pylori* have tropism to the gastric epithelial cells. It is the only bacterium that is adapted to colonize the hostile and highly acidic environment of the stomach (pH about 1-2). Upon entering the stomach, *Helicobacter pylori* can survive the deleterious effects of the gastric acid and dwell in the gastric mucus. This is achieved by the production of copious amounts of the urease enzyme. The urease enzyme (EC 3.5.1.5), is the most abundant protein in *Helicobacter pylori* constituting about 10% of the total

protein content of the cell. Urease raises the pH in the microenvironment surrounding the bacteria by hydrolyzing urea producing ammonia and carbon dioxide [34]. The pathogenecity of *Helicobacter pylori* is definitely associated with the urease enzyme, since urease negative mutants fail to colonize mice [35,68].



The active urease enzyme consists of two structural subunits, UreA and UreB, six subunits each. Nickel ions are required as cofactor for the catalytic function of the enzyme [36]. In addition to the structural genes (ureA and ureB), the urease gene cluster carries ureI, ureE, ureF and ureH genes that must be co-expressed with the structural genes [36] These additional genes are translated to proteins required for the synthesis of the catalytically active urease enzyme. The specific function of these accessory proteins is not well characterized except for ureI which can form a urea specific pore in the bacterial membrane.

The spiral shape of *Helicobacter pylori* and its uni-polar flagella are considered to be virulence factors that contribute to the pathogenecity of the organism. Both the spiral shape and the flagella cause a corkscrew-like motion enhancing the motility through the gastric mucus [37]. Flagellar motility is essential for colonization and protects the bacterial removal from the stomach by gastric peristalsis. Several researchers have studied the contribution of flagella and motility on the pathogenicity of *Helicobacter pylori* [38, 39]. Motility is considered to be a virulence factor since non-motile mutants are either destroyed by the acidic environment of the stomach, or have great difficulty colonizing the gastric mucosa [38]. *Helicobacter pylori* sheath is composed of a phospholipids bi-layer that functions specifically to protect the flagella from depolymerization in the acid environment of the stomach [40]. Genetic analysis of *Helicobacter pylori* genome revealed the presence of about 40 proteins that control the secretion and assembly of the flagellar structure. The flagellar filaments consist of two protein subunits: a major flagellin (FLaA), and a minor flagellin (FLaB). The intricate relationship of the structure, function

and assembly of the flagella and the genes controlling their activities is reviewed by Armelle et al [40].

The lipopolysaccharide layer (LPS) of *Helicobacter pylori* is also considered to be a virulence factor contributing to the pathogenecity of the microorganism. Strains lacking the LPS layer (O, somatic antigen) loose their ability to colonize marine stomach [37]. In addition to virulence, the LPS allow *Helicobacter pylori* to evade the immune system and colonize the host for a very long time [38]. This is due to the fact that O antigen is composed of N-acetyl-D-glucosamine, L-fucose , and D-galactose [41]. These structures were proved to be identical to human blood group antigens Lewis X, Y, and b [37,67]. Recent research had demonstrated for the first time a correlation between *Helicobacter pylori* internalization and lewis antigen expression by this organism [42]. This significant finding was confirmed by testing *Helicobacter pylori* strains expressing Lewis antigens and strains with low level expression of the antigen. The strains expressing Lewis antigens were internalized at much greater levels than those with low level or non-expressing strains.

Helicobacter pylori live inside the mucus layer of the gastric mucosa. Studies have shown that *Helicobacter pylori* stimulate endothelial cells to upregulate adhesion molecule expression [43]. Attachment to the gastric epithelial cells is mediated by outer membrane proteins (Hop proteins). There are about 32 of these outer membrane proteins discovered. Several of these outer membrane proteins function as porin protein while others contribute to adhesion [44]. At least five different *Helicobacter pylori* adhesions are designated as BabA, SabA, AlpA, AlpB, and HopZ have been identified [45, 66, 70]. BabA adhesion has been thoroughly investigated and well characterized. BabA adhesion is 78KD protein that mediates binding of *Helicobacter pylori* to the fucosylated Lewis b histo-blood group antigen present on the surface of gastric epithelial cells [46, 71]. Studies on animal models revealed that Lewis b-dependent attachment of *Helicobacter pylori* to gastric epithelial cells are accompanied by increased severity of inflammation, development of parietal cell auto-antibodies, and parietal cell loss.

Helicobacter pylori infection is definitely the causative agent of gastritis and peptic ulcer disease [7, 8]. In addition, it is involved in the development of gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [10]. Cure of this type of cancer by eradicating *Helicobacter pylori* has established the association between this organism's virulence factors and MALT formation [47].

Several *Helicobacter pylori* virulence associated genes that may play a role in its pathogenicity have been identified. The most important virulence associated genes are vacA and cagA. Clinical isolates of *Helicobacter pylori* have been classified as type I and type II strains depending on the presence or absence of these two genes. Type I strains carry both genes while Type II strains do not [48]. vacA gene is translated to produce a 95 KD protein known as vacuolating cytotoxin A (VacA) [49]. VacA is an enterotoxin that induces vaculation in a wide variety of epithelial cells, cell death and destruction of epithelial integrity. Theories about the mechanism of action of VacA indicate that it is internalized by the gastric epithelial cells. Once inside the cell, VacA has two functions: one function is to form an anion

channel in the cell membrane where bicarbonate and other organic anions pass out of the cell and utilized by the bacteria. The other function is to cause damage to the mitochondria releasing cytochrome C and initiating apoptosis [(49)]. vacA gene contributes significantly to the diversity of *Helicobacter pylori* strains. vacA is considered to be a mosaic gene containing both conserved as well as diverse regions[(50)]. Within the diverse region two regions have been identified: the mosaic combination of signal region, (s-region) and the middle region (m-region) [(51)]. In the s-region, there are three s type alleles referred to as s1a, s1b and s2 and two m type alleles, m1 and m2 in the m-region [(50, 51)]. Genotypes containing s1a are always associated with severe inflammation while m1 strains are associated with severe epithelial injury [(52)].

Another major virulence factor of *Helicobacter pylori* is the cag pathogenicity island (PAI). PAI constitutes a 37-kb fragment on the DNA and contains 29 genes [(49)]. Several of these genes encode a type IV secretion system (T4SS) which is responsible for transporting the translated cagA protein inside the host gastric epithelial cells. CagA

protein is 120-kD that becomes phosphorylated by the action of a tyrosine kinase inside the host cell, inducing it to produce cytokines. It is commonly found in patients with severe gastro- duodenal diseases, including peptic ulcers and gastric adenocarcinomas [53].

Studies have revealed an area of *Helicobacter pylori* chromosome that is distinct from the cag Pathogenicity Island (PAI) and with different G+C content. This was referred to as the plasticity region. Recent research revealed the existence of JHP940 and JHP947 genes. Both genes were detected in *Helicobacter pylori* strains from patients with gastric cancer. JHP947 on the other hand, was more frequently detected (64.7%) in strains from patients with gastric cancer [54].

Gastroduodenal Pathology and Immunity:

Helicobacter pylori causes active chronic gastritis [7], peptic ulcer disease [8], gastric cancer and increase the risk for developing B-cell lymphoma of gastric mucosa-associated-lymphoid-tissue (MALT –

lymphoma) [11]. The pathogenesis of *Helicobacter pylori* -associated gastrointestinal disease remains poorly understood. Available information confirms that *Helicobacter pylori* infections never resolve spontaneously. The chronic gastritis usually contributes to the development of peptic ulcer disease and gastric cancer. Known virulence factors of this organism can either determine the course of the disease, colonization of gastric mucosa or the pathogenesis of the disease.

After infection, *Helicobacter pylori* causes acute gastritis accompanied by epithelial degeneration of the apical mucosa and localized erosion. Acute gastritis is characterized with infiltration of neutrophiles into the lamina propria underlying the mucosal surface. Acute phase is replaced by a chronic phase which is characterized by persistent infiltration to the lmina propria of neutrophiles accompanied by infiltration of mononuclear cells mainly lymphocytes and plasma cells [55]. The function of neutrophiles is to engulf the bacteria and destroy it by oxygen burst creating highly reactive radicals such as hydrogen peroxide, superoxide anions and hydroxyl

radicals. *Helicobacter pylori* can defend it self against these deleterious effects. It has the enzymes catalase and superoxide dismutase. The catalase enzyme degrades the hydrogen peroxide into water and oxygen while the superoxide dismutase catalyzes the superoxide anions to hydrogen peroxide and oxygen. In addition, *Helicobacter pylori* colonize the mucus layers of the gastric epithelium. This area is out of reach of the neutrophiles. When the neutrophiles reach this area they become exposed to the gastric acid, lyse and their reactive molecules cause damage to the gastric epithelium.

Exposure of *Helicobacter pylori* to the immune system is minimal. The inflammatory response to the presence of this organism is not capable of eliminating it. *Helicobacter pylori* cause three different types of immune reactions acute, chronic active, and the atrophic stages of gastritis. These are all stages of inflammation caused by the immune system. The infected mucosal cells secrete interleukin-8 (IL-8), a neutrophile activating chemokine that actually initiates the inflammatory cascade and eventually results in the development of

chronic active gastritis. *Helicobacter pylori* strains carrying the vacA pathogenicity island tend to secrete factors that increase the production of IL-8, leukotrienes and complement factors which represent the chemo attractants for the neutrophiles and lymphocytes.

As the pH in the stomach returns to normal (pH=2), the chronic active gastric phase begins. During this stage, the immune system cells will encounter *Helicobacter pylori* antigens and mount an immune response releasing IgG and IgA antibodies. The immune response can not eliminate the bacterium, but amplify damage to gastric epithelial cells. This stage may last for many years and results in development of ulcers in most patients.

Chronic infections with *Helicobacter pylori* results in the development of multifocal atrophic gastritis. There is a loss of gastric glands, transmural inflammation, and intestinal metaplasia. The presence of the intestinal metaplasia is of great significance because it is considered as the first step in malignant transformation [(56)].

Studies on experimental animals show a strong relationship between the presence of *Helicobacter pylori* and the development of gastric cancer. There are two possible theories on how *Helicobacter pylori* cause cancer. Cancer is either a result of years of inflammation causing damage and regeneration of the gastric epithelial cells or a result of an increased pH in the stomach allowing bacteria that produce reactive nitrogen compounds, to colonize the stomach. These bacteria could produce cancer-causing compounds that induce mutations in the gastric epithelium [(57)].

Helicobacter pylori is capable of antigenically stimulating the immune system leading to MALT lymphoma. These types of cancer develop from the continual activation of T lymphocytes to increase the proliferation of B-lymphocytes. Evidence shows that MALT lymphomas of the B-cell type, contain many tumor infiltrating T-cells that are antigenically stimulated by *Helicobacter pylori*. These T-cells stimulate the growth of the low-grade MALT lymphoma [(57)].

Removing the *Helicobacter pylori* antigenic stimulus causes the lymphoma to degenerate. This is some of the most important evidence in the relationship between *Helicobacter pylori* and malignant tumors.

Vitamin B12 (cobalamin) Deficiency and Pernicious Anemia:

Vitamin B12 is a large molecule composed of a heme-like compound kmown as corrin that holds an atom of cobalt. The corrin and the cobalt atom make up the cobalamin portion of vitamin B12. Several

cobalamines exist and are identified according to their attachments. Methylcobalamin for example, is a cobalamin with a methyl group attached. There are two active forms of cobalamins in the human body: adenosylcobalamin and methylcobalamin. Other cobalamines commonly found in food and supplements such as cyanocobalamin and hydroxycobalamin. These forms can be converted inside the human body into one of the two active forms.

Vitamin B12 (cobalamin) is an important water-soluble vitamin. In contrast to other water-soluble vitamins it is not excreted quickly in the urine, but rather accumulates and is stored in the liver, kidney and other body tissues. As a result, a vitamin B12 deficiency may not manifest itself until after 5 or 6 years of a diet supplying inadequate amounts [(41)]. Vitamin B12 functions as a methyl donor and works with folic acid in the synthesis of DNA and red blood cells and is vitally important in maintaining the health of the insulation sheath (myelin sheath) that surrounds nerve cells [(73)]. The classical vitamin B12 deficiency disease is pernicious anemia, a serious disease characterized by large, immature red blood cells. It is now clear

though, that a vitamin B12 deficiency can have serious consequences long before anemia is evident. The reference levels in blood of vitamin B_{12} ranges between 200 and 600 picogram/milliliter (148-443 picomol/liter) [74].

A deficiency often manifests itself first in the development of neurological dysfunction that is almost indistinguishable from senile dementia and Alzheimer's disease. There is little question that many patients exhibiting symptoms of Alzheimer's actually suffer from a vitamin B12 deficiency. Their symptoms are totally reversible through effective supplementation. A low level of vitamin B12 has also been associated with asthma, depression, AIDS, multiple sclerosis, tinnitus, diabetic neuropathy and low sperm counts. Clearly, it is very important to maintain adequate body stores of this crucial vitamin [74].

The amount of vitamin B12 actually needed by the body is very small, probably only about 2 micrograms or 2 millionth of a gram/day. Unfortunately, vitamin B12 is not absorbed very well so much larger amounts need to be supplied through the diet or supplementation. The

richest dietary sources of vitamin B12 are liver, especially lamb's liver, and kidneys. Eggs, cheese and some species of fish also supply small amounts, but vegetables and fruits are very poor sources. Several surveys have shown that most strict, long-term vegetarians are vitamin B12 deficient. Many elderly people are also deficient because their production of the intrinsic factor needed to absorb the vitamin from the small intestine decline rapidly with age [(74)].

Fortunately, oral supplementation with vitamin B12 is safe, efficient and inexpensive. Most multi-vitamin pills contain 100-200 microgram of the cyanocobalamin form of B-12[(74)]. This must be converted to methylcobalamin or adenosylcobalamin before it can be used by the body. The actual absorption of B12 is also a problem with supplements [(74)]. Swallowing 500 micrograms of cyanocobalamin can result in absorption of as little as 1.8 microgram so most multivitamins do not provide an adequate daily intake. The best approach is to dissolve a sublingual tablet of methylcobalamin (1000 micrograms) under the tongue every day. That will be sufficient to maintain adequate body stores. However, if a deficiency is actually

present then 2000 microgram/day for one month is recommended followed by 1000 microgram/day. Some physicians still maintain that monthly injections of vitamin B12 is required to maintain adequate levels in the elderly and in patients with a diagnosed deficiency. There is however, no scientific evidence supporting the notion that injections are more effective than sublingual supplementation [(74)].

Helicobacter pylori has been implicated as the causative agent of vitamin B12 deficiency and pernicious anemia [(48)]. *Helicobacter pylori* is a common cause of gastritis and ulcers and it destroys the parietal cells that produce the intrinsic factor needed for vitamin B12 absorption. Successful eradication of *Helicobacter pylori* led to improved blood levels of B12 in 40% of those infected [(48)]. *Helicobacter pylori* was found in 56% of patients with pernicious anemia [(63)]. It was also found that *Helicobacter pylori* -induced vitamin B12 deficiency occurs in the absence of atrophic gastric mucosa [(48)].

Pernicious Anemia

Pernicious anemia (PA) is the most common cause of vitamin B_{12} deficiency. B_{12} deficiency actually has many causes however pernicious anemia applies only to the condition associated with chronic atrophic gastritis [74].

Pernicious anemia was first described by Addison in 1849 and associated with the stomach by Austin Flint in 1860. Pernicious anemia was later successfully treated with cooked liver and subsequent theories on the pathogenesis of Pernicious anemia involved the loss of an extrinsic factor from the liver and an intrinsic factor (IF) from the stomach [74].

A recent population survey found that approximately 2% of persons over 60 years of age have undiagnosed Pernicious anemia. The disease was previously thought to occur only in those of Northern European extraction; however subsequent studies have noted Pernicious anemia to occur in Hispanic and African-American patients [74].

Pathology

Gross pathology – The stomach has three regions: the fundus and the body, which contain acid secreting parietal cells and pepsinogen secreting zymogen cells, and the antrum which contain gastrin secreting G-cells. Chronic atrophic gastritis is recognized grossly by the loss of gastric mucosal folds and thinning of the gastric mucosa.

There are two types based on whether the lesion affects the antrum[58].

Type A (autoimmune) Chronic Gastritis	Type B (nonautoimmune) Chronic Gastritis
Affects fundus and body, spares antrum	Affects antrum as well as fundus and body
Associated with PA, antibodies vs. IF, parietal cells	No autoimmunity, *Helicobacter pylori* infection common

Hypergastrinemia secondary G-cell hyperplasia and low serum pepsinogen-I	Hypogastrinemia secondary G-cell destruction with antral gastritis
Achlorohydria, gastric carcinoids	

Histopathology – Gastric biopsy specimens from patients with early Pernicious anemia demonstrate a mononuclear cellular infiltrate in the submucosa extending into the lamina propria between the gastric glands; the infiltrate consists of plasma cells containing autoantibodies to parietal cells and intrinsic factor. Extension of the cellular infiltrate into the mucosa is accompanied by degenerative changes in parietal and zymogenic cells. In the fully established lesion, there is marked reduction in the number of gastric glands and the parietal and zymogenic cells disappear, and replaced with mucus containing cells which resemble intestinal cells (intestinal metaplasia) [(74)].

Natural History – The progression of Type A chronic atrophic gastritis to gastric atrophy and anemia is estimated to be 20-30 years.

The presence of parietal cell antibodies is predictive of the presence of autoimmune gastritis, being found in 90% of patients with Pernicious anemia (50% of those with gastric atrophy without Pernicious anemia); anti-intrinsic factor antibodies are found in 60% of Pernicious anemia patients, anti-parietal cell antibodies are found in 10-15% of the general population whereas anti-Intrinsic factor antibodies are typically seen only in those with Pernicious anemia [(46)].

Immunopathogenesis – The pathologic process of type A gastritis appears to be directed against the gastric parietal cell. The pathology is restricted to the parietal cell containing gastric fundus and body, parietal cells are lost, and autoantibodies against parietal cells and their product, IF, are present in the serum and gastric juice. It was recently determined that antigen for the anti-parietal antibodies is the gastric H^+/K^+-ATPase. Although these antibodies fix complement and lyse parietal cells in vitro, it is unlikely that they are pathogenic in vivo as the H^+/K^+-ATPase is not accessible to circulating antibodies. Murine studies suggest that the lesion of autoimmune gastritis is initiated by CD4 cells that recognize the b subunit of gastric H^+/K^+-

ATPase. However the mechanism of activation of the CD4 cells is not known, nor is the pathway by which these cells produce chronic gastritis [74].

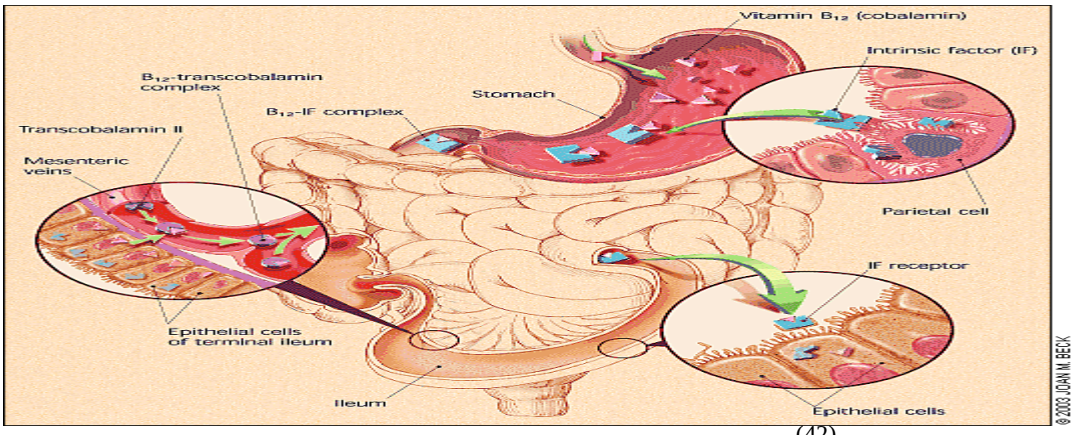
Genetics – A genetic predisposition to Pernicious anemia is suggested by the clustering of the disease and of gastric antibodies in families and the autoimmune endocrinopathies. About 20% of relatives of patients with Pernicious anemia have Pernicious anemia themselves. However there is no evidence of an association of Pernicious anemia with certain Human Leukocytes Antigen (HLA) molecules [74].

Associated diseases – Pernicious anemia may be associated with many other autoimmune endocrinopathies, including Hashimoto's thyroiditis, Addison's disease, primary gonadal failure, primary hypoparathyroidism, Graves' disease, vitiligo, myasthenia gravis, and the Eaton-Lambert syndrome [74].

Vitamin B_{12} absorption, malabsorption, and metabolism:

B_{12} is a complex molecule that cannot be synthesized in the human body and must be supplied in the diet (MDR=2.5mg) from meat and dairy foods primarily. During gastric digestion B_{12} is released from food and complexes with gastric R binder; in the duodenum the B_{12}-R binder complex is digested and the B_{12} then binds to intrinsic factor (IF). The B_{12}-Intristic factor complex then travels to the distal ileum where specific receptors bind and absorb the complex. In the mucosal cell the IF is degraded and the B_{12} associates with another transport protein, transcobalamin II (TC II) which is secreted into the circulation where it is rapidly taken up by the liver, bone marrow, and other cells. [(42)]. B_{12} malabsorption in PA is due to lack of IF due to loss of gastric parietal cell as well blocking antibodies present in the gastric that can bind and block the B_{12} binding site on IF[(42)]. Normally 2mg of B_{12} is stored in the liver and 2mg elsewhere in the body, in view of the MDR it would take 3-6 years to become B_{12} deficient if absorption were to cease [(42)]. B_{12} is metabolically active in 2 forms – methylcobalamin which is an essential cofactor in the conversion of homocysteine to methionine and adenosylcobalamin which is required

for the conversion on methylmalonyl CoA to succinyl CoA. Therefore with B_{12} deficiency one would expect to see elevated serum levels of homocysteine and methylmalonic acid [42].



Picture(1) show the mechanism of vitamin B12 absorption[42].

Clinical Presentations

Anemia with symptoms that include fatigue, weakness, light-headedness, vertigo, tinnitus, palpitations, angina, decreased exercise capacity with pallor, slight icterus, tachycardia, and systolic flow murmur on exam.

Gastrointestinal complications of B_{12} deficiency include atrophic glossitis with a smooth beefy red tongue and megaloblastosis of the small bowel with diarrhea and malabsorption. In the stomach

intestinal metaplasia is a risk factor for adenocarcinoma – population based studies demonstrated that the risk of gastric adenocarcinoma was increased three times and the risk of gastric carcinoid (felt to be due to the trophic effects of gastrin) was increased 13 times in patients with Pernicious anemia and one study recommended endoscope surveillance of patients with Pernicious anemia.

Neurologic complications include peripheral neuropathy, most frequently seen as numbness and paresthesias, lesions of the dorsal columns (with loss of vibration and position sense and ataxia) and lateral columns (with limb weakness, spasticity, and extensor plantar reflexes) of the spinal cord (subacute combined degeneration) and cerebrum (with changes ranging from mild personality defects and memory loss to frank psychosis (megaloblastic madness)). These lesions begin as demyelination and progress to axonal degeneration and eventually neuronal death; these complications may not be reversed by treatment.

Laboratory Diagnosis

The finding of macrocytosis suggests the presence of a megaloblastic anemia; an MCV <95fL implies a less than 0.1% chance of B_{12} (or folate) deficiency; an MCV of 100-110fL is most likely due to ethanol, stem cell disorders, liver disease, or the use of antineoplastics; as the MCV rises so does the chance of B_{12} or folate deficiency (MCV>130fL is associated with B_{12} or folate deficiency in nearly 100%).

The peripheral blood smear may also show hypersegmented neutrophils (about 91% sensitive, defined as the presence of at least neutrophil with 6 lobes, the presence of ³ 5% of 5-lobe neutrophils, or an increased neutrophil lobe average (normally <3.4lobes/PMN), RBCs, in addition to macrocytosis, demonstrate anisocytosis and poikilocytosis, decreased reticulocyte count as well as possible leukopenia and thrombocytopenia.

Megaloblastic anemias are characterized by ineffective erythropoiesis with enhanced intramedullary destruction of erythroblasts as evidenced an increase in indirect bilirubin and LDH_1.

Once a megaloblastic anemia has been identified, one should then determine whether a specific vitamin deficiency is responsible by measuring serum cobalamin (Cbl) and folate levels. However there has been some recent controversy regarding Cbl levels, with some feeling that the true lower limit of normal for B_{12} should be 300pg/mL (and not 193 as at UNCH).

There are several other tests for diagnosing Cbl deficiency and PA. These include serum methylmalonic acid (MMA) and homocysteine (Hcy) levels, both of which should be elevated in B_{12} deficiency whereas only Hcy should be increased in folate deficiency; a recent study found that elevations of both MMA and Hcy had a 98% sensitivity for B_{12} deficiency. Additional tests include serum transcobalamin II, which delivers B_{12} to cells and whose concentration falls before that of B_{12}, the deoxyuridine suppression test (dUST) which measures thymidine incorporation into DNA by marrow cells and their response on addition of Cbl and folate (not widely used due to need for bone marrow biopsy), MMA 24 hour urinary excretion, IF and parietal cell antibodies, type A chronic atrophic gastritis on

gastric biopsy, achlorohydria (very sensitive as Pernicious anemia is the only gastric lesion that results in total achlorohydria); One may also obtain serum gastrin and pepsinogen levels, which are increased and decreased respectively. Once B_{12} deficiency has been established, its pathogenesis can be established by means of a Schilling test. The classic test is conducted by first giving the patient 1000 m g of cold B_{12} intramuscularly then giving 1 mg of ^{57}Co-cyanocobalamin orally; a 24 hour urine is then obtained with normal subjects excreting more than 8% of the oral dose; subsequent steps entail the administration of the labeled B_{12} with IF (which should normalize the urinary excretion in Pernicious anemia patients), pancreatic extract for patients with suspected pancreatic insufficiency, and following antibiotic administration for those with suspected bacterial overgrowth. In patients with suspected Pernicious anemia usually only steps one and two are performed [(74)].

Helicobacter pylori and vitamin B12 deficiency

A Turkish study showed a patient population consisted of 102 men and 208 women with a mean age of 43 ± 12 (range16 to 75) years. The mean serum vitamin B12 concentration for 310 patients was 216 pg/mL. The percentages of patients with concentrations < 250 pg/mL, < 200 pg/mL and ≤100 pg/mL were 67.4%, 46.8%, and 6.5% respectively. Patients with <200 pg/mL of serum vitamin B12 concentration were older than those with >200 pg/mL ($p<0.01$). Sex and other hematology parameters of patients with or without vitamin

B12 deficiency did not show any significant difference by univariate analysis [48].

Other study showed that patients with good oral hygiene had less gastric recurrence of *Helicobacter pylori* after treatment, and eradication of the organism increased serum vitamin B12 [49]. Other research has given further support to a suggested link between *Helicobacter pylori* infection and vitamin B12 deficiency [43]. Investigators previously found that both pernicious anemia and food-cobalamin malabsorption the most common causes of vitamin B12 deficiency, especially in older people - were associated with chronic gastritis and might be associated with *Helicobacter pylori* infection [43]. To test this in more detail, gastroenterologists prospectively studied 138 patients with macrocytic anemia and vitamin B12 deficiency that did not have a classic cause, such as pernicious anemia or postgastrectomy state, and who had *Helicobacter pylori* confirmed by gastric biopsies [50].

Helicobacter pylori infection was eradicated with a triple-therapy regimen and eradication was confirmed by further gastric biopsies

after four weeks. All patients were followed up at three-month intervals for up to five years [50].None of the patients infected with H pylori received vitamin B12 replacement unless the *Helicobacter pylori* could not be eradicated [50].

A total of 77 (55.8 %) patients were infected with H pylori. Eradication was successful in only 31 cases (40.3%). In all patients with H pylori, serum vitamin B12 levels, macrocytosis and anaemia were normal within six months of the infection being vanquished [50] Although multi-stage Schilling and auto-antibody tests were not performed, and the histological findings of the gastric biopsy were not reported, the findings did support a relationship between H pylori infection and vitamin B12 deficiency, the authors concluded [50]. They suggested that eradicating *Helicobacter pylori* infection might correct vitamin B12 levels and improve anemia by reversing gastric atrophy related to the infection [50]. *Helicobacter pylori* In a new study, 34 individuals with dysmotility-like dyspepsia, *Helicobacter pylori* infection, and low vitamin B12 levels were assessed before and after a three-month course of vitamin B12 therapy. Each person received

daily intramuscular injection of vitamin B12 (1,000mcg) for 10 days, followed by 1,000 mcg per day orally for 80 days. The rate of gastric emptying and the severity of symptoms were determined before and at the end of treatment period. The average time required to empty the stomach contents decreased from 230 minutes at the start of the study to 98 minutes after vitamin B12 therapy. In addition, the severity of the intestinal symptoms improved by an average of 78%. These results suggest that correction of vitamin B12 deficiency can improve delay gastric empting and relieve symptoms in this particular subset of individuals with dyspepsia [(51).] Another new study on 133 adults how have a history of *Helicobacter pylori* infection, tested for complete blood count ,vitamin B12 level, gastrin, folic acid, and h.pylori IgG antibodies, the study show that 51% of subjects seropositive to *Helicobacter pylori* had vitamin B12 >250 pg/ml which indicated a relationship between *Helicobacter pylori* infection and vitamin B12 level [(51).]

Hypothesis and specific aims

Hypothesis:

We hypothesize that *Helicobacter pylori* is implicated as a causative agent for vitamin B12 deficiency by causing stomach ulcer and destroys the parietal cells which impair vitamin B12 absorption regardless of age.

Specific aims:

Our specific aims in this study are the following:

To investigate the existence of a possible association between *Helicobacter pylori* infection and vitamin B12 deficiency.

To evaluate and compare invasive and non-invasive methods used for detecting *Helicobacter pylori* infection. The outcome of such evaluation may establish a correlation between the reference invasive method (Gastric biopsy) and other non-invasive methods. Such correlation may be used to adopt those non-invasive techniques in diagnosing *Helicobacter pylori* infection.

Materials and Methods

This study was done on 60 adult patients (32 males, 28 females) selected for this study from 86 patients, 26 patients were excluded from this studies (12 patient have renal or liver disease, 14 patient received *Helicobacter pylori* eradication therapy), the duration of the study was between november/2003 and november/2004. All patients had symptoms of NUD (Non Ulcer Dyspepsia) or GERD (Gastroesophageal Reflux Disease).Gastroscopy was performed in the endoscopy unit of Arabcare Hospital in Rammallah by Dr. Wail Hammoudeh, FACP. Three to four antral gastric mucosa biopsies were collected by endoscopy. If the patient had previous treatment with PPI (Proton Pump Inhibitor), another biopsy was taken from the

corpus. Distal esophageal mucosa biopsies were taken when observing an esophageal abnormality, the Esophageal Gatro-duodenal finding were documented. One biopsy was examined immediately by rapid urease test (CLO test) for *Helicobacter pylori* infection and other for histolopathology. Prior to gastroscopy blood sample were obtained, and patient information was collected regarding age, sex, duration, of symptoms, previous treatment with PPI (yes or no) and smoke (yes or no). The blood sample was aseptically collected from each patient in an EDTA and plain vacutainer tubes for Complete Blood Count (CBC) to determine the MCV and for vitamin B12 assay. Complete Blood Count was performed using Sysmex analyzer (automated counter, Sysmex-Japan) to obtain the MCV for each specimen and vitamin B12 was measured using an Immulite analyzer (DPX, USA).

Selection criteria for enrollment in the study were:

Patients enrolled in this study were selected according to the following criteria:

The patients do not have any classic cause of cobalamin deficiency, such as pernicious anemia or the postgastrectomy state. And had no evidence of renal failure or liver disease. Female patients were not pregnant. The patients had not received previous *Helicobacter pylori* eradication therapy.

Patients were interviewed to role out the existence of medical problem that may affect cobalamin status and to determine that they had received cyanocobalamin treatment parenteral.

Rapid Urease Test (CLO-Test)

The CLO-Test (manufacture by Biomerux Company) is a qualitative test for the detection of the unease enzyme produced by H. pylori. If *Helicobacter pylori* is present in the sample, unease will hydrolyze the urea causing the formation of positive ammonium ions. This causes a rise in pH which is detected by the phenol red indicator that changes color from yellow to red/magenta. A solution of 10% urea broth containing 0.1% phenol red is used as outlined by Eugina [(46)].

The biopsy specimens of each patient were examined by this rapid urease test by placing a biopsy in the broth. The development of a red color within 2 hours indicated a positive result.

Impression biopsy slides

One biopsy was firmly pressed on several places on a clean sterilized microscope slide. The slide was air-dried and stained by gram stain. The presence of Gram negative spiral shaped bacteria indicating the presence of *Helicobacter pylori*.

Histopathology

Biopsies (several pieces 2-4) were placed in 10% buffered-formalin and sent to the pathology department at Medicare laboratories in Nablus. The biopsies were dehydrated and then embedded in paraffin. Sections were cut at 5um thick and stained with hematoxylin and eosin. Examination for the presence of *Helicobacter pylori* was made

by the Pathologist Dr.Husni Magbool (Medicare Nablus). The presence of *Helicobacter pylori* can be seen colonizing the gastric mucosa, indicating positive histopathology test for *Helicobacter pylori*.

Serological testing

Poly Stat *Helicobacter pylori* test kit (Polymedco Inc. USA) was used for the qualitative detection of anti-*Helicobacter pylori* IgG in human serum was used. This is a chromatographic immunoassay. The test cartridge contains a membrane coated with H. pylori antigens conjugated with colloidal gold on the test band and *Helicobacter pylori* specific monoclonal antibody on the control band. The formation of a visible line in the test region indicates the presence of IgG antibodies specific for *Helicobacter pylori*. Another band will always appear in the control region. The appearance of the control band indicates that enough samples have been added and proper flow is obtained.

Vitamin B12 Assay

Vitamin B12 (cobalamin) was assayed using a solid-phase chemiluminescent competitive binding assay (Immulite, DPC, USA). Specimens to be assayed (serum or plasma), were heated for 15 minutes in a water bath at 100^{o} C in the presence of dithioereitol and potassium cyanide. This pretreatment was used to release vitamin B12 from its binding proteins. Mix of 100 ul of the pretreated sample in a reaction vessel with hog intrinsic factor (HIF) and an anti-HIF monoclonal antibody. This anti-HIF monoclonal antibody was labeled with alkaline-phosphatase. An aliquot of this mixture was introduced into a reaction tube containing vitamin B12-coated beads. The chemiluminescent substrate was then added and undergoes hydrolysis in the presence of the enzyme label. This reaction yields a result that relates inversely to the vitamin B12 concentration in the patient sample. The anti-HIF MAb used in this assay was specifically produced and selected to bind HIF without interfering with the latter's ability to bind vitamin B12. Unbound materials are removed by a patented centrifugal wash technique. The chemiluminescent substrate,

a phosphate ester of adamantyl dioxetane, yields an unstable intermediate in the presence of the alkaline phosphatase label. This intermediate is continuously produced, resulting in a sustained emission of photons, and allowing multiple readings for enhanced precision by IMMULITE analyzer.

Results

This study was conducted between November 2003 and November 2004. There were 60 patients (29 females and 31 males) randomly selected from patients with symptoms suggestive of *Helicobacter pylori* infection. Several tests were performed on the specimens collected from these patients. These tests included CBC, vitamin B12, CLO, pathology and serology. A summary of all the patients, symptoms, age, smoking status as well as the laboratory and pathology test results obtained for each specimen is outlined in Table (1).The age distribution of the patients was between 20 and 82 years as shown in Figure 1. The mean age was 44.1 $\pm$ 17.5 years. Most of the patients tested were 20 to 40 years old. With respect to age, we

divided the population into three age groups, group 1 (20 – 40 years old), group 2 (41-60 years old) and group 3 (> 61 years old). Our results indicated that *Helicobacter pylori* was present in 56% in age group 1, 19% in age group 2 and 26% in age group 3. The results are shown in Table 2 and depicted in Figure 1.

Table 1. Summary of all patients and results obtained. Demographic information with respect to age and gender as well as smoking is shown. Other information shown, include symptoms, serology results, CLO test results, MCV and vitamin B12 levels.

PATIENT NO.	**AGE**	**SEX**	**SMOKING**	**RAPIDSIGNAL TEST**	**CLO TEST**	**PATHOLOGY RESULT(PRESENCE OF *H.PYLORI*)**	**PATHOLOGY REPORT**	**MCV**	**VITAMINB12 LEVEL**
1	65	f	no	positive	positive	positive	Chronic sup.gadtritis	93	146
2	75	f	no	negative	negative	negative	Esophageal Adenocarcinoma	85	242
3	32	m	Heavy smoker	positive	positive	positive	Sever gastritis	97	206
4	82	m	no	positive	positive	positive	Chronic gastritis	103	174
5	73	f	no	positive	positive	positive	Chronic gastritis	98	172
6	68	m	s	positive	positive	positive	Sup.Gastritis	88	493
7	20	m	no	positive	positive	positive	Mild esophegitis chronic anturm gastritis	83	340
8	61	m	no	positive	positive	positive	Mild esophegitis chronic gastritis	107	103
9	26	m	s	positive	positive	positive	Mild esophegitis chronic gastritis	83	422
10	35	f	no	positive	positive	positive	Epithelial hyperplasia chronic gastritis	108	>100
11	24	m	no	positive	positive	positive	Mild esophegitis chronic gastritis	92	242
12	41	m	s	positive	positive	positive	Esophageal hyperplasia chronic gastritis	89	116
13	57	m	s	positive	positive	positive	Esophageal ulceration	103	>100
14	37	f	no	negative	negative	negative	Epithelial hyperplasia mild chronic gastritis	79	342
15	36	m	s	positive	positive	positive	Epithelial hyperplasia chronic gastritis	105	162

16	58	f	no	positive	positive	positive	Epithelial hyperplasia active chronic gastritis	98	139
17	34	m	s	positive	positive	positive	Epithelial hyperplasia chronic gastritis	89	249
18	27	f	no	positive	positive	positive	Moderate gastritis	78	802
19	33	m	no	positive	positive	positive	Epithelial hyperplasia chronic gastritis	88	109
20	49	f	no	negative	negative	negative	Non –hodgkins lymphoma	83	321
21	32	m	s	negative	negative	negative	Mild gastritis	85	378
22	27	m	s	negative	negative	negative	Chronic gastritis	80	380
23	24	f	no	negative	negative	negative	Mild gastritis	78	447
24	57	f	no	negative	negative	negative	Mild gastritis	83	328
25	38	m	s	positive	positive	positive	Epithelial hyperplasia chronic gastritis	89	177
26	38	f	no	positive	positive	positive	Active gastritis	93	135
27	33	m	s	negative	negative	negative	Esophageal hyperplasia chronic gastritis	78	791
28	80	m	no	negative	negative	negative	adenocarcinoma	73	1185
29	80	f	no	positive	positive	positive	Esophageal hyperplasia chronic gastritis	101	127
30	26	m	no	positive	positive	positive	Esophageal peptic ulceration	99	>100
31	30	m	no	negative	negative	negative	Mild gastritis	75	375
32	32	f	no	positive	positive	positive	chronic gastritis	93	120
33	81	m	s	positive	positive	positive	Active peptic ulcer	96	120
34	61	f	no	positive	positive	positive	Mild Epithelial hyperplasia chronic gastritis	91	173
35	56	m	s	negative	negative	negative	Mild gastritis	78	664
36	32	m	s	positive	positive	positive	Mild Epithelial hyperplasia chronic gastritis	102	138
37	38	f	no	positive	positive	positive	Severe active chronic gastritis	93	299
38	32	m	s	positive	positive	positive	Severe active chronic gastritis	83	1103
39	28	f	no	negative	negative	negative	Severe active chronic gastritis	80	451
40	42	f	no	positive	positive	positive	Severe active chronic gastritis	99	126
41	28	f	no	negative	negative	negative	Mild chronic gastritis	76	465
42	28	f	no	negative	negative	negative	normal	81	401
43	48	m	no	positive	positive	positive	Severe chronic gastritis	98	164
44	45	f	no	positive	positive	positive	Severe active chronic gastritis	100	161
45	62	f	no	positive	positive	positive	Severe active chronic gastritis	101	136
46	38	m	no	positive	positive	positive	Severe active chronic gastritis	96	155
47	38	f	no	positive	positive	positive	Severe active chronic gastritis	99	178

48	71	f	no	positive	positive	positive	Acute ulceration Severe chronic gastritis	89	377
49	60	f	no	positive	positive	positive	Severe active chronic gastritis	88	362
50	42	m	no	positive	positive	positive	Severe active chronic gastritis	98	162
51	30	m	no	positive	positive	positive	Severe active chronic gastritis	81	>1200
52	52	m	s	negative	negative	negative	Mild esophagitis Severe chronic gastritis	80	566
53	77	f	no	positive	positive	positive	Severe active chronic gastritis	101	<100
54	27	m	s	positive	positive	positive	Severe active chronic gastritis	99	120
55	37	m	no	positive	positive	positive	Severe active chronic gastritis	75	1088
56	18	f	no	positive	positive	positive	mild chronic gastritis	83	378
57	42	f	no	negative	negative	negative	Severe active chronic gastritis	81	264
58	41	f	no	negative	negative	negative	Severe active chronic gastritis	80	261
59	28	m	s	positive	positive	positive	Severe active chronic gastritis	103	100
60	35	f	no	positive	positive	positive	Severe active chronic gastritis	95	152

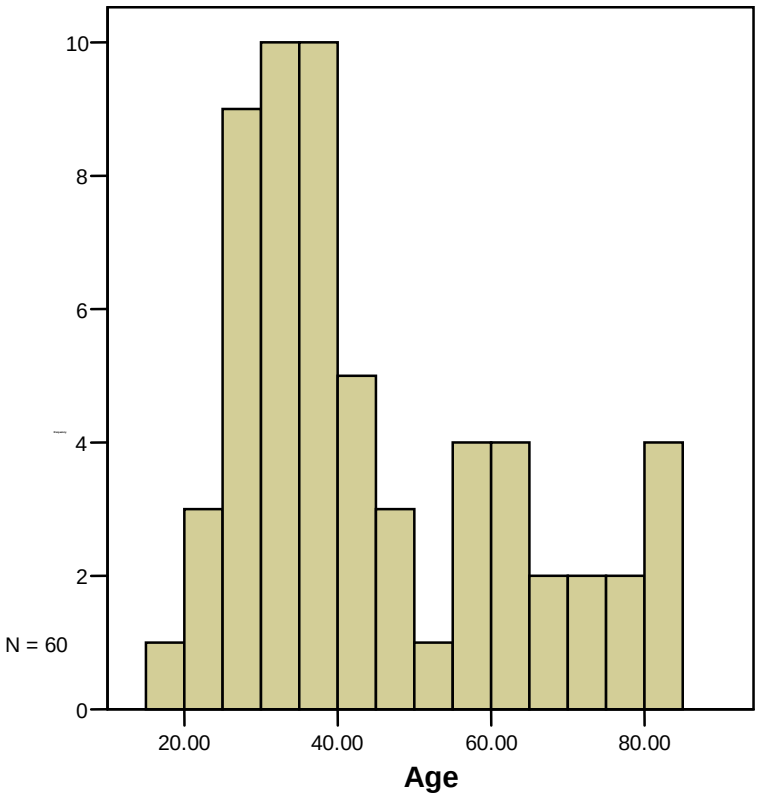


Figure 1. Histogram showing the frequency distribution for age in all patients screened for *Helicobacter pylori*.

Out of the total patients enrolled in this study tests, 43 were positive for *Helicobacter pylori* giving a percentage of 72%. The results indicated that 25 males (25/31) were positive for *Helicobacter pylori*

giving a percentage of 81%. Among the female population there was 18 (18/29) positive for *Helicobacter pylori* giving a percentage of 62%. With reference to the total population tested, there were 25 males (25/43) positive for *Helicobacter pylori* giving a percentage of 58%. Furthermore, there were 18 females (18/43) out of the positive population positive for *Helicobacter pylori* giving a percentage of 42%. With respect to smoking, the number of smokers was 11 (11/43) out of the total number of patients positive for *Helicobacter pylori*, giving a percentage of 26%. These results are shown in Table 2

Parameter Evaluated	**Total Number Positive**	**Number Positive**	**Number Negative**	**Percent Positive (%)**
Male	**43**	**25**	**6**	**58%**

Female	**43**	**18**	**11**	**42%**
Age: **Grp 1 (20-40)** **Grp 2 (40-60)** **Grp 3 (>60)**	**43**	**24** **8** **11**	**8** **6** **3**	**56%** **19%** **26%**
Smoker	**43**	**11**	**49**	**18%**

Table 2. Distribution of the positive patients for *Helicobacter pylori* infection with respect to gender, age and smoking.

The relationship between MCV and vitamin B12 was evaluated in this study. In megaloblastic anemia, MCV was determined to be >90 fl and vitamin B12 <250 pg/ml. Our results revealed that 29 patients out of the total positive patients (29/43) have MCV above 90 fl, giving a percentage of 67%. With respect to vitamin B12, there were 31 patients out of the positive ones (31/43) have levels <250 pg/ml, giving a percentage of 72%. These results are shown in Table 3.

Number of patients	**MCV (>90 fl)**	**Vitamin B12 <250 pg/ml**
Positive	**29**	**31**

Total Positive	**43**	**43**
Percentage Positive	**63%**	**72%**

Table 3. Relationship between vitamin B12 deficiency and elevated MCV in patients with *Helicobacter pylori* infection.

In this study we compared the test results for invasive methods (CLO test and pathology) with non invasive methods (serology). All the results obtained were actually identical. All patients with *Helicobacter pylori* infection were positive with CLO test, rapid signal serology test and the histopathology results. The results are summarized in Table 4.

Test	**Total Number of Patients**	**Positive**	**Negative**	**Percent Positive (%)**
CLO	**60**	**43**	**17**	**100%**

Rapid Signal Test (Serology)	**60**	**43**	**17**	**100%**
Histopathology	**60**	**43**	**17**	**100%**

Table 4. Comparison between the different testing methods with respect to *Helicobacter pylori* infection.

Discussions

Helicobacter pylori infection in developing countries was reported to be (70%-90%) [9]. our results fall within this range. *Replogle* reported that male sex can be considered as a risk factor [10]. Our results

showed that 25 male patients (58%) were positive for *Helicobacter pylori* but our study results may be suggestive that male sex could be a potential risk factor for *Helicobacter pylori* infection. On the other hand, male sex can not be a conclusive risk factor (P=**0.606**).

It is apparent from the results obtained that smoking has no effect on the acquisition of *Helicobacter pylori*. There were 30 non-smoking patients (70%) out of all those diagnosed with *Helicobacter pylori* infection Table (2). This is a reliable conclusion, since the acquisition of *Helicobacter pylori* occurs at childhood where the great majority of the population is non-smokers. Comparing the different methods for diagnosing *Helicobacter pylori*, there was complete agreement between histopathology, CLO-test and serology. There was no significant difference between CLO test and histpathological results when paired t-test was applied **(P <0.05**) Table (4).

Vitamin B12 deficiency or pernicious anemia is characterized by enlarged red blood cells and elevated MCV [(44)]. Vitamin B12 or cobalamin deficiency occurs frequently (> 20%) among elderly people

[44]. The causes of such deficiency are attributed to malabsorption, pernicious anemia and insufficient dietary intake [44]. Therefore, the choice of patients for our study was restricted to those who are symptomatic with *Helicobacter pylori* infection only. Patients with malabsorption syndrome, pernicious anemia without *Helicobacter pylori* infection and patients suffering from malnutrition were excluded from our study. Our results showed that 62.8% of all positive cases have elevated MCV and 72.1% have Vitamin B12 deficiency.

Our study as well as other studies focuses on the changes that takes place in the gastric environment. The tropism of *Helicobacter pylori* to survive the highly acidic environment of the stomach significantly contributes to the pathological changes of the gastric mucosa [36]. The first line of defense for this organism is to neutralize the acidity of the stomach by the production of ammonia [35]. In the absence of chronicity of the infection, neutralizing the acid environment in the stomach will definitely impair the binding of the intrinsic factor to its epitope on vitamin B12-R [44]. Consequently, the subsequent

absorption of vitamin B12 can be affected by lowering the levels of this vitamin without the development of deficient state [44].

Chronic active gastritis causes the destruction of the parietal cells that produces the intrinsic factor. In the absence of the intrinsic factor, vitamin B12 deficiency will ensue once the storage pool of this vitamin is depleted [51].

Other theories to explain the development of vitamin B12 deficiency takes advantage of the existence of Lewis blood group antigens on *Helicobacter pylori*. The immune system produces antibodies (IgG) against these antigens. Lewis blood group is also expressed on the parietal cells. This may lead to the evolution of autoimmunity against parietal cells. Eventually, they will be destroyed and consequently the mal-absorption syndrome will develop [64].

Patients suffering from heart burn due to increased acidity of the stomach, tend to take medications such as ranitidine and others to alleviate the symptoms. The potential danger of such action is the reduction of acidity of the stomach. As mentioned previously, this

may have deleterious effects on the binding of the intrinsic factor to vitamin B12-R. The outcome of this will be a reduction of vitamin B12 levels. This condition will amplified by the presence of *Helicobacter pylori* infection. Both will rapidly cause the development of vitamin B12 deficiency. Diagnosis of *Helicobacter pylori* by serology is not helpful. It cannot differentiate between recently acquired infection and a chronic one. Considering the high rate of infection by *Helicobacter pylori* in developed countries[50]., and further higher rates in developing countries, the results of serological tests will not contribute much to the diagnosis of active infection [51] .

Reviewing the complete blood count (CBC), the MCV was found to be elevated in all cases of *Helicobacter pylori*. This means that the patient is suspected of having megaloblastic anemia. Following the MCV results here will be logical to evaluate vitamin B12. Now in our study we followed strict criteria to select our patients. All our patients have no inherent problems related to vitamin B12 absorption and they are not suffering from malnutrition. This will lead us to consider the

possibility of *Helicobacter pylori* infection. Here, in addition to the gastritis and other related symptoms, the physician will not feel pressured to perform the ultimate invasive procedures, gastric and duodenal biopsies. The number of patients in our study is not enough to conclusively extract this conclusion, but it definitely points to it.

Histopathology is considered to be the gold standard for diagnosing *Helicobacter pylori* infections. Our study addressed the significance of this important test by performing histopathology on all patients of the study. In addition, vitamin B12 was determined for all the patients 29(67%) of positive *Helicobacter pylori* infection 43, (72.%) had vitamin B12 level <200). Statistical analysis using SPSS paired t-test (2 tailed and **<0.05** level of significance) revealed no significant difference between those two important parameters for infection with *Helicobacter pylori*. Considering that our variables in the study were restricted only to patients with *Helicobacter pylori* infection, and considering the reduction in vitamin B12 values, we can assume that there must be a correlation between the two. Our hypothesis is built on the assumption that *Helicobacter pylori* can be a potential

causative agent for vitamin B12 deficiency. It is a plausible theory with suggestive results. The only risk to adopt this conclusion in my opinion is the sample size. Our recommendation for future studies will be the following:

-To increase the sample size

-To determine the reference levels for vitamin B12 for the Palestinian population. This will make our interpretation of the results obtained more accurate.

-Our results indicate that 19% of the patients have vitamin B12 levels between 200 – 250 pg/ml. Follow up on these patients may prove the possibility of developing deficiencies for vitamin B12.

-Coproantigens for *Helicobacter pylori* can be detected in the stool of patients. It is not an invasive technique as compared to gastric biopsies. This test can be used to augment or clarify the results of serological tests.

A study like this should not be left without follow up. All *Helicobacter pylori* infected patients are usually treated with triple therapy according to predetermined therapeutic protocols. It will add a great value to this study to follow these patients and evaluate their vitamin B12 and MCV at least 6 months post therapy.

The outcome of this study is in accordance with our theory. In the absence of other interfering factors, such as old age, mal-absorption, malnutrition, pregnancy, liver disease, *Helicobacter pylori* can be considered as potential causative agent of vitamin B12 deficiency.

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