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### Thesis Approval

Association between *Helicobacter pylori* Infection and Vitamin B12 Level  
among Palestinian People in Hebron Palestine

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## Abstract

### Association between *Helicobacter pylori* Infection and Vitamin B 12 level among Palestinian People in Hebron-Palestine.

*Helicobacter pylori* are spiral-shaped gram-negative bacteria that colonize and live near the surface of the human gastric mucosa. *H. pylori* is widespread throughout the world and is present in about 50% of the global human population, and the incidents increase in developing countries. They have evolved intricate mechanisms to avoid the bactericidal acid in the gastric lumen and to survive near, to attach to, and to communicate with the human gastric epithelium and host immune system. This interaction sometimes results in severe gastric pathology. *H. pylori* is associated with an increased risk for development of duodenal ulcer, dyspepsia and gastritis, gastric ulcer disease, gastric adenocarcinoma, and gastric lymphoma. *H. pylori* classified as class I carcinogen according to the World Health Organization. Recently, there is a possibility of an association between *H. pylori* infection and Vitamin B12 deficiency. The main objective of this study is to investigate and to understand the association between *H. pylori* infection and decrease Vitamin B12 level, in young patients (18-50) among Palestinian people in Hebron.

Blood samples were collected from 133 patients. All had Vitamin B12 deficiency, and 105 healthy volunteers (42 male, 63 female) with no disorders in the stomach and were included in the study with the same criteria; a mechanism for exclusion criteria was developed. All study groups were selected from Governmental Alia Hospital in Hebron. Interview and questioner (attached) were completed from all patients and healthy volunteers. All study groups underwent the following tests : *Helicobacter* IgA, Vitamin B12, Anti Intrinsic Factor, and Parietal Cell Antibodies.

The results showed that the mean Vitamin B12 level in patients was 149.0 pg/ml with a standard deviation 32.8, while in healthy control the mean Vitamin B12 was 352.49 pg/ml with a standard deviation 90.8. The mean *H. pylori* IgA titer in patients was 58.75 NTU/ml with a standard deviation 45.49, while the mean of *H. pylori* IgA titer in the

Healthy control was 7.35 NTU/ml with a standard deviation of 6.68. The results showed that there is a significant negative correlation ( $p \leq 0.05$ ) between *H. pylori* infection and Vitamin B12 level in patients and healthy volunteers, as the value of the correlation coefficient between *H. pylori* and Vitamin B12 deficiency patients -0.417 at the level of significance 0.001. The value of the correlation coefficient between *H. pylori* and Vitamin B12 level of the healthy volunteers was -0.207 at the level of significance 0.034, while the results showed the total score and a correlation coefficient is high, as the value of the correlation coefficient on all individuals (133 patients and 105 healthy) was -0.862. It has been found that the mean ratio of Vitamin B12 level/ *H. pylori* titer for healthy was 100.2, while the mean ratio of Vitamin B12 level/ *H. pylori* titer for patients was 5.08, and T calculated was -10.379 at the level of significance 0.000. There are 108 patients have reactive *H. pylori*, that is equivalent to 81.2% of the study sample, and there was 15 non-infected with *H. pylori*, that is equivalent to 11.3% of the study sample, and also there was 10 equivocal to *H. pylori*, (suspected of having *H. pylori*) that is equivalent to 7.5 % of the study sample. The study showed high negative correlation between *H. pylori* infection and decreased Vitamin B12 level among the study group, and this study is considered supportive of previous studies done in the same area. However, the mechanism of this correlation remains unknown and requires more investigation.



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## Chapter One: Back ground

### 1.1 Introduction

*Helicobacter pylori* is pathogenic bacteria found in the lining of the gastric system that can infect the stomach causing inflammation, it is considered as the dominant microbiota colonizing human stomach, first isolated by Warren and Marshall in 1983 (Cover and Blaser, 2009; Kusters et al., 2006). *H. pylori* is associated with an increased risk for development of duodenal ulcer disease, dyspepsia and gastritis, gastric ulcer disease, gastric adenocarcinoma, and gastric lymphoma (Barghouthi, 2009). The strong association of gastric cancer with *H. pylori* infection led to the classification of *H. pylori* as class I carcinogen depending on the World Health Organization/ International Agency for Research on Cancer (IARC) in 1994 (Kusters et al., 2006; Barghouthi, 2009). *H. pylori* may be linked to a variety of extragastric disorders this may be due to a series of factors linked to the epidemiological features of the studies and to the diseases investigated. These disorders include coronary heart disease, dermatological disorders such as rosacea and idiopathic urticaria, autoimmune thyroid disease and thrombocytopenic purpura, iron deficiency anemia, Raynaud's phenomenon, scleroderma, migraine, and Guillain-Barre' syndrome (Pellicano et al., 2009). *H. pylori* is widespread throughout the world and is present in about 50% of the global human population. The prevalence of *H. pylori* in industrialized countries under 40% while; it is high in various developing countries where more than 80% of the populations were *H. pylori* positive. It is considerably lower in children and adolescents than in adults and elderly people (Kusters et al., 2006; Perez-Perez et al., 2004). The most common symptom can occur with *H. pylori* infection include An ache or burning pain in abdomen, nausea, vomiting, frequent burping, bloating, weight loss. But most cases of *H. pylori* infection do not cause signs or symptoms in most infected persons (Kusters et al., 2006). Recently, there is a possibility of an association between *H. pylori* infection and Vitamin B12 deficiency. Several studies showed that *H. pylori* may associate with development of adult cobalamin deficiency (Akcem, 2010). Food Cobalamin malabsorption, a major cause of Vitamin B12 deficiency could be due to several factors include pernicious anemia, aging, gastrectomy, drugs such as (H2-receptor



antagonists, proton pump inhibitors, metformin), alcohol abuse, lymphomas or tuberculosis, celiac disease, Crohn's disease, and as it has been suggested *H. pylori* infection (Dali-Youcef and Andrès, 2009). *H. pylori* may directly damage the surface epithelial layer leading to impair in the gastric acid and Pepsin secretion which are critical to the splitting of the Cbl from food binders (AL-Alami et al., 2009). In the present study investigated the relationship of these bacteria and the extent and influence in the level of Vitamin B12 in patients living in West Bank city of Hebron, Palestine.

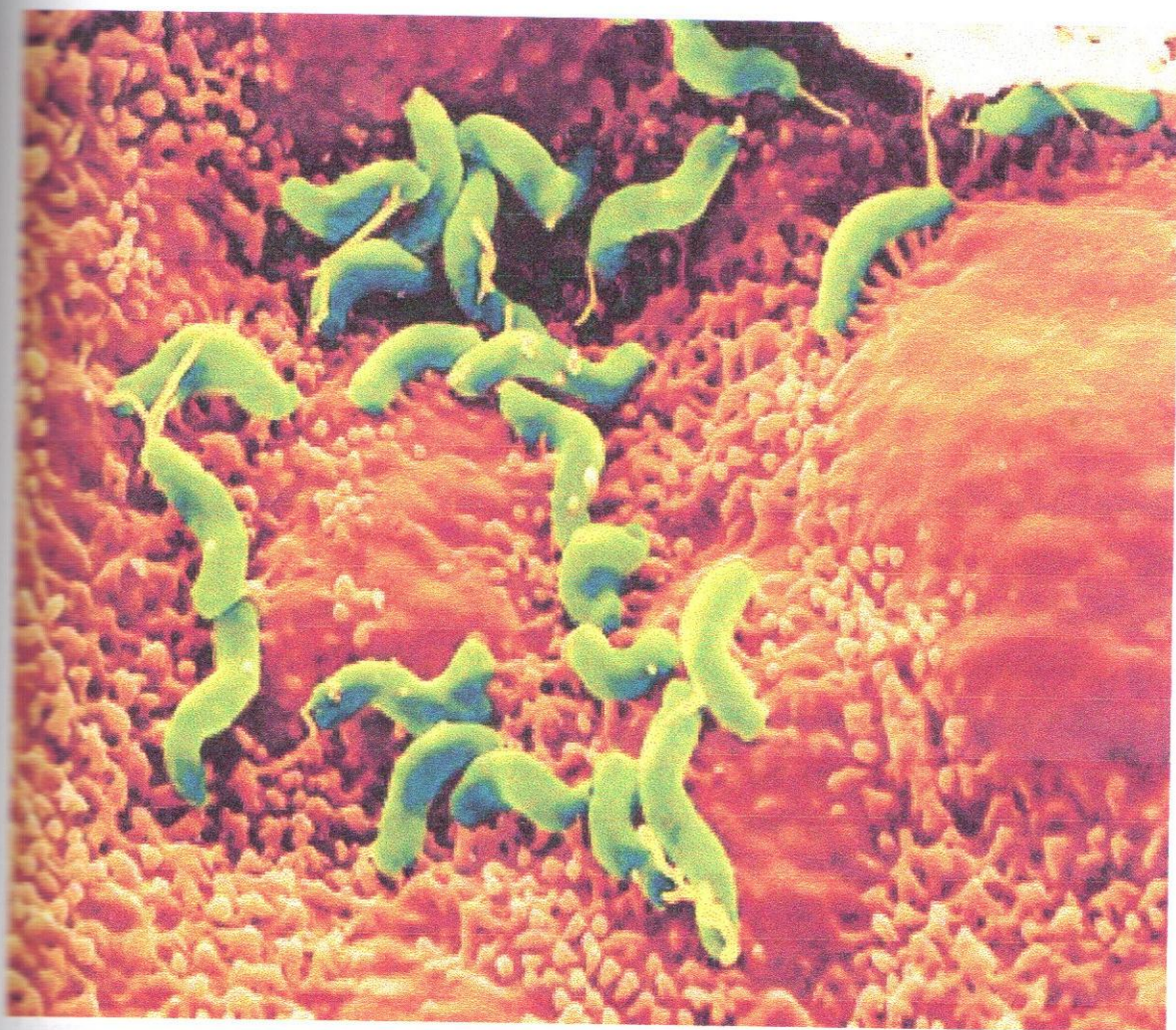


Figure 1.1: Colored scanning electron micrograph of *H. pylori* on surface of gastric cell (<http://www.diasource-diagnostics.com/en/Products/Rapid-Tests/InfectiousDiseases/Helicobacter-Pylori-Hpylori>).



## 1.2 Statement of the problem

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*H. pylori* infection and Vitamin B12 deficiency show high prevalence rates among Palestinians. It has been observed that most people who suffered from Vitamin B12 deficiency, were positive for *H. pylori* during my work as medical laboratories in Palestine and Jordan for the last thirteen years. In this study we investigated the relationship between the decline in Vitamin B12 and the infection with *H. pylori*, and whether *H. pylori* infection is the reason for the deficiency in this vitamin. This observation has been supported by previous studies (Kaptan et al., 2000; Shuval-Sudai and Granot, 2003). Therefore, this study was planned to investigate if there is an association between *H. pylori* infection and loss of Vitamin B12 (deficiency) among Palestinians in Hebron.

## 1.3 Study justification

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The importance of this study was the choice of study group, which consisted of young adult patients whom suffered from Vitamin B12 deficiency and healthy volunteers whom do not suffer from any problem in the stomach. This preliminary study is considered an introduction to pave the way for understanding the real mechanism that works by these bacteria which leads to Vitamin B12 deficiency. It was the first study of its kind carried out on Palestinian population of Hebron whom has a high rate of *H. pylori* infection similar to other developing countries. Previous studies have shown that *H. pylori* is present in about half of the human population, it is the main cause of several diseases of the digestive system such as acute and chronic gastritis, peptic ulcer disease, intestinal metaplasia, and gastric cancer, as well as it is believed that *H. pylori* infection is a catalyst for some extragastric manifestations such as Atherosclerotic disease, Arrhythmias, Idiopathic Thrombocytopenic Purpura, Iron-deficiency Anemia, Hepatobiliary Diseases and Alzheimer's disease (Cover and Blaser, 2009, Pellicano et al., 2009 ). Some recent studies have been shown that these bacteria may affect some Micronutrient, especially Ferritin levels And Vitamin B12 levels (Akcem, 2010).

## 1.4 Study Hypotheses

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**Hypothesis:** There is statistical significance at the level of ( $\alpha \leq 0.05$ ) between *H. pylori* infection and Vitamin B12 deficiency.

## 1.5 Aims and Objectives

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- 1- To investigate young adult patients suffer from Vitamin B12 deficiency, and try to investigate and understand its association with *H. pylori* infection.
- 2- To highlight the real situation of *H. pylori* infection and Vitamin B12 deficiency among Palestinian population of Hebron.



## ملخص بالعربية

بكتيريا المعدة اللولبية هي بكتيريا عسوية لولبية سالبة غرام ، وتوجد على شكل مستعمرات تعيش بالقرب من سطح الغشاء المخاطي في المعدة الإنسان. وتقدر الدراسات أن جرثومة المعدة المسببة للقرحة يحملها حوالي نصف سكان العالم وتزيد هذه النسبة في الدول النامية. لقد طورت بكتيريا المعدة اللولبية آليات معقدة لتجنب تأثير الحمض في تجويف المعدة لكي تبقى على قيد الحياة بجانبه، و تتوافق وتتواصل مع الخلايا الطلانية في معدة الإنسان و الجهاز المناعي، وهذا التفاعل النتائج يسبب امراض شديدة في الجهاز الهضمي. تعد هذه البكتيريا أقوى عوامل الخطر في ظهور القرحة المعدية، وكذلك تؤدي الى التهاب المعدة ،وسرطان المعدة. لقد وجد ان هناك علاقة محتملة بين التهاب البكتيريا اللولبية ونقص مستوى فيتامين ب ١٢. تهدف الدراسة الحالية الى فهم العلاقة بين وجود التهاب البكتيريا اللولبية و نقص فيتامين ب ١٢ في المرضى من عمر الشباب (١٨- ٥٠ ) المصابين بنقص فيتامين ب ١٢ في محافظة الخليل - فلسطين.

تم جمع عينات من ١٣٣ مريض يعانون من نقص فيتامين ب ١٢ و ١٠٥ (٤٢ ذكور، ٦٣ اناث) متطوع كمجموعة ضابطة لا يعانون من اي اضطرابات في المعدة ، طبقت على جميعهم نفس الشروط، ووضعت آليه للقبول والرفض، تم اختيار جميع المبحوثين من مستشفى عاليه الحكومي في محافظة الخليل ،و تم تحضير استبيان(ملحق) ، واجريت مقابلات مع جميع المبحوثين. واجريت لجميع المبحوثين الاختبارات التالية:

Helicobacter IgA titer, Vitamin B12, Anti Intrinsic Factor, Parietal Cell Antibodies.

اظهرت النتائج ان المتوسط الحسابي لمستوى فيتامين ب ١٢ عند المرضى 149.0 pg/ml مع انحراف معياري ٣٢.٨١ ، بينما عند الاصحاء بلغ المتوسط الحسابي لفيتامين ب ١٢ 352.49 pg/ml مع انحراف معياري قدره ٩٠.٨٢ . بلغ المتوسط الحسابي لدرجة الإصابة ببكتيريا القرحة اللولبية عند المرضى 58.75 NTU/ml مع انحراف معياري 45.49. بينما بلغ المتوسط الحسابي لدرجة الإصابة ببكتيريا القرحة اللولبية عند الاصحاء 7.35 NTU/ml مع انحراف معياري 6.68 . اظهرت النتائج وجود علاقة سلبية ذات دلالة إحصائية عند المستوى بين بكتيريا القرحة اللولبية وبين نقص

فيتامين ب ١٢ لدى المرضى والأصحاء، حيث بلغت قيمة معامل الارتباط بين بكتيريا القرحة اللولبية ومستوى فيتامين ب ١٢ للمرضى ٠.٤١٧- عند مستوى دلالة ٠.٠٠٠٠، كما بلغت قيمة معامل الارتباط بين بكتيريا القرحة اللولبية ونقص فيتامين ب ١٢ للأصحاء ٠.٢٠٧- عند مستوى دلالة ٠.٠٠٣٤، في حين اظهرت النتائج على الدرجة الكلية (١٣٣ مريض و ١٠٥ اصحاء) وجود معامل ارتباط مرتفع، حيث بلغت قيمة معامل الارتباط على الدرجة الكلية ٠.٨٦٢-.

اظهرت النتائج وجود فروق ذات دلالة إحصائية بنسبة نقص فيتامين ب ١٢ والإصابة ببكتيريا القرحة اللولبية مقسوم لصالح الأصحاء، فقد بلغ المتوسط الحسابي للأصحاء 100.20 في حين بلغ المتوسط الحسابي للمرضى 5.08 كما بلغت قيمت المحسوبة ١٠.٣٧٩- عند مستوى دلالة ٠.٠٠٠٠. اظهرت النتائج إن هناك ١٠٨ مبحثين من المرضى لديهم إصابة شديدة ببكتيريا القرحة اللولبية، أي ما يعادل ٨١.٢% من عينة الدراسة، و هناك ١٥ مبحث ليسوا مصابين ببكتيريا القرحة اللولبية، أي ما يعادل ١١.٣% من عينة الدراسة، وهناك ١٠ مبحثين لديهم إصابة مشكوك باصابتهم ببكتيريا القرحة اللولبية، أي ما يعادل ٧.٥% من عينة الدراسة. اظهرت الدراسة وجود علاقة سلبية عالية بين التهاب البكتيريا اللولبية ونقص مستوى فيتامين ب ١٢ بين المبحثين، وتعتبر هذه الدراسة داعمه لدراسات سابقة في نفس المجال. ومع ذلك تبقى الآلية الحقيقية لهذه العلاقة غير معروفة وتحتاج الى مزيد من البحث.



## 5.2 Conclusion

The study showed a strong negative correlation between *H. pylori* infection and Vitamin B12 deficiency among young adult patients, this study is in agreement with previous studies conducted elsewhere. The exact mechanism behind this correlation is not clear more investigations are needed to elucidate this connection; whether it is direct or indirect type of cause-effect relationship.

## 5.3 Recommendations

Based on our results, it is recommended that:

1. There could be a clinical value to evaluate the serum Vitamin B12 in every *H. pylori* patient.
2. Treating *H. pylori* infection even when asymptomatic.
3. Further research is needed to find the appropriate vaccination to reduce *H. pylori* infections.
4. Further extensive research is required to determine the mechanism by which *H. pylori* influences Vitamin B12 levels.
5. Further community education (hygiene) on *H. pylori*.
6. Increase of Vitamin C uptake which has adverse effect on *H. pylori*.