The Libyan Academy-Misurata Branch

School of Basic Sciences

Department of Biological Science

Division of Microbiology

The *Helicobacter pylori* Infection in Diabetes Mellitus Patients Type 2

Misurata –Libya

A Thesis Submitted to the School of Basic Science Partial Fulfillment of the Requirements for the Degree of Master of Science in Microbiology

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Spring 2017

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ ۝ الرَّحْمَٰنِ الرَّحِيمِ ۝ مَالِكِ يَوْمِ الدِّينِ ۝

إِيَّاكَ نَعْبُدُ وَإِيَّاكَ نَسْتَعِينُ ۝ اهْدِنَا الصِّرَاطَ الْمُسْتَقِيمَ ۝ صِرَاطَ الَّذِينَ

أَنْعَمْتَ عَلَيْهِمْ ۝ غَيْرِ الْمَغْضُوبِ عَلَيْهِمْ وَلَا الضَّالِّينَ ۝ صدق

الله العظيم

سورة الفاتحة

الاهداء

الى أحب الناس الى قلبي، الى من أفنيا حياتهما من أجلي، الي والدي

اللذين ضحيا من اجل ان يوفرا لي التعليم الصحيح .

إلى إخوتي وأخواتي . . . الذين كانوا أكبر تشجيع لي .

أهدي هذا المجهود المتواضع راجية الله ان ينتفع به وان يجعله خالصا

لوجهه الكريم .

الشكر والتقدير

أتوجه بالشكر والحمد لله وحده حق حمده، وكما ينبغي لجلال وجهه وعظيم سلطانه وشمول رحمته وسبوغ نعمائه الذي هدانا سواء السبيل ويسر لي كتابة هذا البحث والامر له عز وجل من قبل ومن بعد فهو العاصم من الزلل والموفق للحق والهادي الى الصواب .

أتوجه بالشكر للذين أسهموا في هذا البحث بالتشجيع والتفاعل الخلاق واخص بالذكر الرجل الذي كان لعنايته المستمرة وملاحظاته الهامة وتوجهه السديد الأثر الكبير في استكمال الكثير من نواحي البحث والتغلب على العديد من الصعوبات وذلك هو الدكتور سالم رمضان السريتي .

كما أقدم جزيل شكري الى الأطباء وكل العاملين في مختبر المركز المتخصص لتنظيم وعلاج السكري والغدد الصماء على مساعدتهم المعملية والعلمية وتجاوبهم وتعاونهم .

واضيف شكرا خاصا الى الأكاديمية الليبية وخاصه قسم علوم الحياة على اتاحتها فرصة مواصلة الدراسة وفتح مجالات وافاق للطلاب والمستفيدين فهي رمز لما يمكن ان يحققه التعاون العلمي في تحقيق طموحاتنا .

سدد الله الخطى ومنّ على الجميع بالتوفيق

الملـــخـص

بكتريا المعدة الحلزونية هي بكتريا ممرضه، لها شكل لولبي، سالبة لصبغة جرام، تستعمر الخلايا الظهارية في المعدة، تسبب في التهاب حاد في المعدة، وقرحة الاثني عشر، واحيانا أورام خبيثة في المعدة. معدل انتشار البكتريا يكون أعلى لدى كبار السن والتي من المحتمل تم اكتسابها منذ الطفولة، حيث تحدث العدوى عن طريق الاتصال المباشر بين البشر، من قيء وبراز المصاب.

ان الهدف من هذه الدراسة هو تحديد نسبة الانتشار وتقدير العلاقة بين عدوى بكتريا المعدة الحلزونية وداء السكري من النوع الثاني، وأيضا المقارنة بين طرق التشخيص المصلية واختبار البراز (الانتجين)، ومن الأهداف الأخرى لهذه الدراسة تحديد مستويات ارتفاع السكري في الدم واليوريا والامونيا وتحليل ناقلة الأمين (ALT) في مصل الدم للأشخاص المصابين بالسكري النوع الثاني.

أجريت هذه الدراسة في مركز السكري والغدد الصماء الواقع في مدينة مصراته- ليبيا خلال الفترة من شهر يوليو الى شهر أكتوبر 2016، وتضمنت الدراسة 195مريض يعانون من مرض السكري النوع الثاني، و17 شخصا من الاصحاء (مجموعة ضابطه).

طرق العمل : تم استخدام نوعين من الاختبارات لتشخيص العدوى ببكتريا المعدة الحلزونية، الاختبار المصلي للبحث عن الاجسام المضادة من نوع IgG ، واختبار البراز للبحث عن المستضد، وأيضا تقييم السكر في الدم واليوريا والامونيا وتحليل ناقلة الأمين عن طريق جهاز Vitros 350 .

وأظهرت نتائج هذه الاختبارات ان نسبة انتشار الاجسام المضادة من النوع IgG في مرضى السكري 60.51% مقارنه بنسبة 41.18% في المجموعة الضابطة، كما أظهرت الاختبارات ان نسبة انتشار المستضد في مرضى السكري 50.77% مقارنة 52.94% لدى المجموعة الضابطة، وقد أظهرت النتائج ان هناك اختلاف احصائي هام بين النتائج الموجبة والسالبة بين طريقتي التشخيص P-value=0.006 ، علاوة على ذلك كان هناك معدل عالي لانتشار بكتريا المعدة الحلزونية في مرضى السكري من النوع الثاني 71.28%، أعلى من المجموعة الضابطة 58.82%، P-value= 0.0295 .

ومن خلال النتائج اظهر اختبار السكر في الدم اختلاف احصائي هام ما بين مرضى السكري من النوع الثاني والمجموعة الضابطة P-value=0.00 ، لكن وجدت اختبارات ليست ذات دلالة إحصائية في نتائج اختبار اليوريا في مرضي النوع الثاني من السكري والمجموعة الضابطة.

من ناحية أخرى أظهر اختبار الامونيا اختلاف ظاهر ما بين مرضى السكري من النوع الثاني والمجموعة الضابطة P-value=0.005، وكذلك أظهر اختبار ناقلة الأمين ALT أظهر اختلاف بليغ مـــا بين مرضى السكري من النوع الثاني والمجموعة الضـــابطة P- value=0.0248

الاستنتاج: أظهرت النتائج الاجمالية للدراسة ان انتشار عدوى بكتريا المعدة الحلزونية يحدث بنسبة عالية جدا في مرضى السكري من النوع الثاني وإن الاختبارات المصلية للأجسام المضادة IgG لا يجب ان تستعمل منفردة للكشف عن عدوى البكتريا.

Abstract

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral-shaped pathogenic bacterium that specifically colonizes the gastric epithelium causing chronic gastritis, peptic ulcer disease, and/or gastric malignancy.
The prevalence of *H.pylori* is high in the older population – presumably acquired in their childhood, mainly by fecal-oral, oral-oral or gastro-route.
Type 2 diabetes mellitus (T2DM) is an emerging pandemic. The pathogenesis of T2DM is complex, with risk factors associated with lifestyle, genetic background, and socioeconomic factors. When affected by T2DM, the pancreas can no longer produce enough insulin to overcome the cellular loss of sensitivity, resulting in the accumulation of sugar in the bloodstream
The aim of this study is essentially to determine the prevalence and evaluate the relationship between *Helicobacter pylori* infection and type 2 diabetes mellitus patients in Misurata Libya. Furthermore, the research aims at comparing two non-invasive diagnostic methods of *H. pylori* infection, serological test, and stool antigen test. Another objective of this study is to determine the fasting blood sugar, urea, ammonia and ALT level of patients' serum connected with the *H. pylori* infection.

The study was conducted at the Diabetic and Endocrine Center in Misurata-Libya from July to October 2016. It comprised 195 type 2 diabetic patients and 17 non-diabetic subjects (control group).

Helicobacter pylori is assessed by the use of rapid chromatographic immunoassay for the qualitative detection of *H. pylori* antibody (IgG) in serum, and the detection of *H. pylori* antigen in feces by rapid chromatographic immunoassay among T2DM patients and control group.

The study also determined FBS, urea, ammonia, and Alanine aminotransferase (ALT) by Vitros 350 system device.

A positive antibody for *H. pylori* infection in T2DM patients was found in 60.51% compared to 41.18% of the control group subjects. In contrast, positive *H. pylori* test antigen in T2DM patients was found in 50.77% compared to 52.94% of the control group. However, there was a significant statistical difference between the positive and the negative results of the two diagnostic methods p-value= 0.006. Moreover, there was a higher prevalence of *H. pylori* infection in T2DM patients than the control group (71.28% vs. 58.82%) p-value= 0.0295.

The fasting blood sugar test showed a significant statistical difference between T2DM patients and the control group (p-value=0.000). However, there was a non-significant statistical difference between the results of urea test of T2DM patients and the control group.

Furthermore, the ammonia test showed a significant difference between T2DM and the control group in p-value= 0.005.

Alanine aminotransferase (ALT) test results showed a significant difference between T2DM patients and the control group, p-value=0.0248.

The overall study findings indicate that *H. pylori* prevalence was significantly higher in T2DM patients. In addition, the results of this research reveal that IgG serological tests should not be used alone to detect *H. pylori* infection.

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List of Abbreviations

Abbreviation	Full Form
H. pylori	*Helicobacter pylori*
T2DM	Type 2 Diabetes Mellitus
ALT	Alanine Transferase
IgG	Immunoglobulin G
IgA	Immunoglobulin A
IgM	Immunoglobulin M
FBS	Fasting Blood Sugar
PPIs	Proton Pump Inhibitors
SAT	Stool Antigen Test
PPV	Positive Protective Value
NPV	Negative Protective Value
IR	Insulin Resistance
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steato Hepatitis
HOMA-IR	Homeostatic Model Assessment-Insulin Resistance
GDM	Gestational Diabetes Mellitus
LADA	Latent Autoimmune Diabetes in Adult
DM	Diabetes Mellitus
HbA-C1	glycosylated hemoglobin
TC	Triglycerides
AST	Aspartate aminotransferase

CHAPTER I

INTRODUCTION

1-Introduction:

1-1.The bacteria: *Helicobacter pylori*

Helicobacter pylori (H .pylori) is a spiral-shaped gram-negative rod. It is associated with antral gastritis, duodenal (peptic) ulcer disease, and gastric carcinoma. Other *Helicobacter* species that infect the gastric mucosa exist but rare.

H .pylori is present on gastric mucosa of less than 20% of persons under the age of 30. However, it increases in prevalence to 40-60% of persons after the age of 60, including individuals who are asymptomatic. In the developing countries, the prevalence of infection among adults may be 80% or higher. Person-to-person transmission of *H .pylori* is likely because intrafamilial clustering of infection occurs. Acute epidemics of gastritis suggest a common source for *H .pylori (*Brooks *et al,* 2004).

Diagnostic methods to detect *H. pylori* infection are diverse, and the choice of one method or another depends on several factors, such as the availability of the diagnostic tests. This also needs performing an endoscopy, examining the cost, accessibility and the advantages or disadvantages of each method, and determining patient's age.

Despite the fact that triple therapy is the standard treatment for *H. pylori*, triple therapy offers acceptable cure rates. Quadruple therapies that use various combinations of drugs, sequential therapies, and concomitant therapies have been introduced as effective alternatives for *H. pylori* treatment (González *et al*, 2014).

1-1-1. Classification:

Domain: Bacteria

Phylum: Proteobacteria

Class: Epsilonproteobacteria

Order: Campylobacterales

Family: Helicobacteraceae

Genus: Helicobacter

Species: *Helicobacter pylori (*Fox*,* 2002*).*

The first helicobacter culture was actually *H muridarum,* a commensal organism colonizing the crypts of the mouse caecum (Lee *et al*, 1992). New *helicobacters* are discovered regularly, and some are now regarded as a human pathogen (Windsor &Orourke, 2000).

According to observations from human and animal helicobacter infections, the genus has the capacity to colonize and cause inflammation in the stomach (*H. pylori, H. heilmannii, H. mustelae),* colon (*H. fennelliae),* and in mice liver (*H. bilis, H. hepaticus). H. pylori* colonies in the mucus layer which line the human stomach and its relatives occupy similar ecological niches in the gastrointestinal tracts of animals (Tomb *et al*, 1997).

1-1-2 Morphology:

H. pylori is named because of its spiral or helical shape (Fig 1). The organism is approximately 0.6μm thick, taking the shape of a flat spiral with 1.5 wavelengths. The organism has up to seven sheathed flagella (Westblom *et al*, 1991). They are microaerophilic, and produce oxidase, catalase, and urease enzymes (Stark *et al*, 1999). They have a rapid, corkscrew motility from multiple polar flagella (Harvey *et al*, 2001). Two complete genomes of *H. pylori* organisms have been known, as well as a detailed comparison of the two (Marshall, 2002). The function of about 40% of *H. pylori* genes is unknown, but presumably relates in some way to survival in acid-secreting human stomach, prior to the genomic study of *H. pylori* (Salama *et al*, 2000).

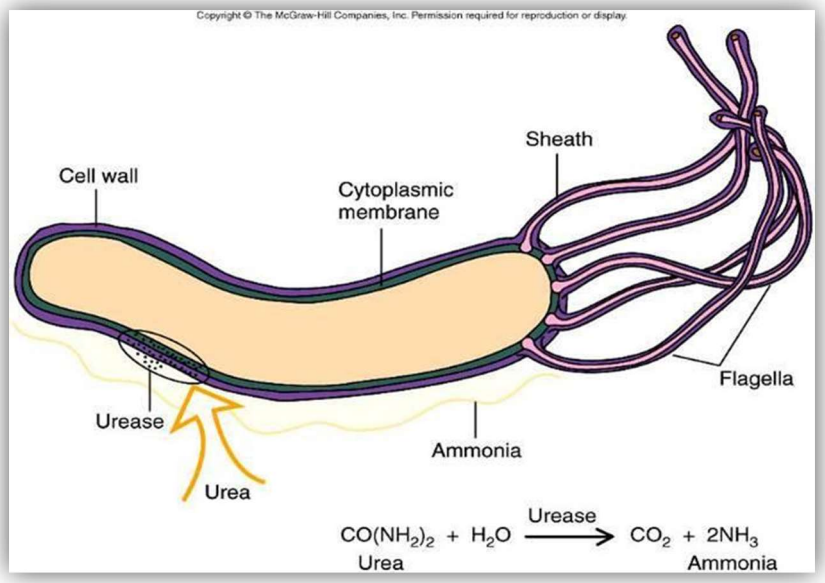

Fig. 1: Morphology of *H. pylori*

from: Ib-hl-bio_wikia.com

Restriction enzyme analyses had shown that the species is extremely diverse- i.e. nearly every person on earth has a specific restriction pattern for organism. In fact, this diversity has been exaggerated because most of these changes are synonymous. They are random mutations in the third base of a codon which do not necessarily alter the resulting protein. In addition, *H. pylori* can take up pieces of DNA from its environment and shuffle large portions of its genome from time to time. Nevertheless, most of its genes are expressed identically (Salama *et al*, 2000).

1-1-3. Transmission

H. pylori is mainly acquired in childhood by the faecal-oral route, or oral-oral within families. It has been recognized as a worldwide public health problem to be more prevalent in developing countries (Gonzalez *et al*, 2014).

1-1-4. Pathogenesis and virulence factor:

Man appears to be the only reservoir and source of *H. pylori* (Hawtin *et al,* 1990). By virtue of its rapid motility (by flagellae), *H. pylori* penetrates the mucus layer lining the epithelium and attach to the mucous-secreting cell deep in the gastric mucosa, near the epithelial surface (by adhesins), away from the acidity of the stomach. It synthesizes urease, an enzyme catalyzing the hydrolysis of urea to ammonia (Hawtin *et al,* 1990). Furthermore, it produces superoxide dismutase (Spigelhalder *et al*, 1993), and catalase which protects it from phagocytosis and killing by phagocytic cell (Hanzell *et al*, 1991). When coupled with an inflammatory response, it causes damage to the mucosa and loss of protective mucous coating predisposing to gastritis and peptic ulcer. The ammonia produced neutralizes gastric acidity allowing the organism to survive causing persistent colonization which may lead to chronic gastritis, gastric atrophy, peptic ulcer and gastric carcinoma (fig.2) (Marshall, 2002).

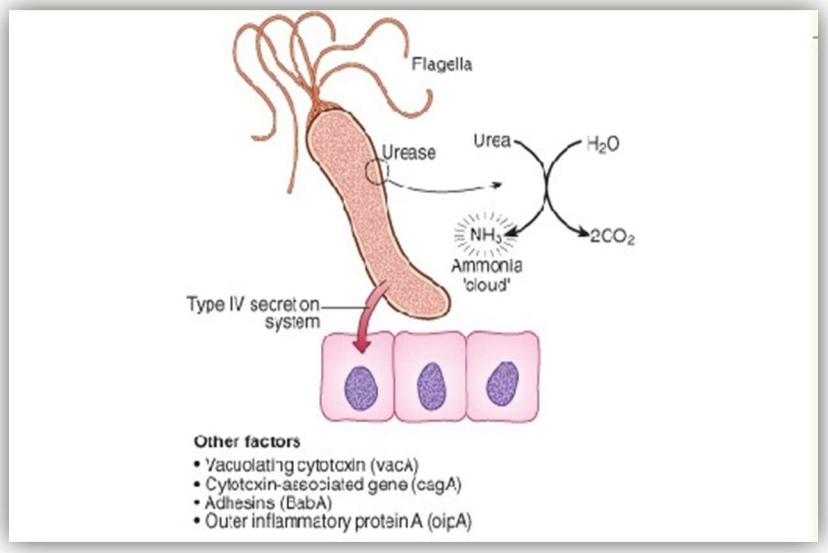

Fig. 2: Pathogenesis and Virulence of *H. pylori*

From www.studentconsult.com

Virulence factors which operate to damage the mucosa produced by some strains include; the maculating cytotoxin (VacA), cytotoxin-associated protein (CagA) coded by a gene within the Cag pathogenicity island, a cluster of genes coding cytotoxins and associated proteins (see fig. 3). These strains cause severe forms of gastritis, peptic ulcer, and malignancy (Gonzalez *et al*, 2014).

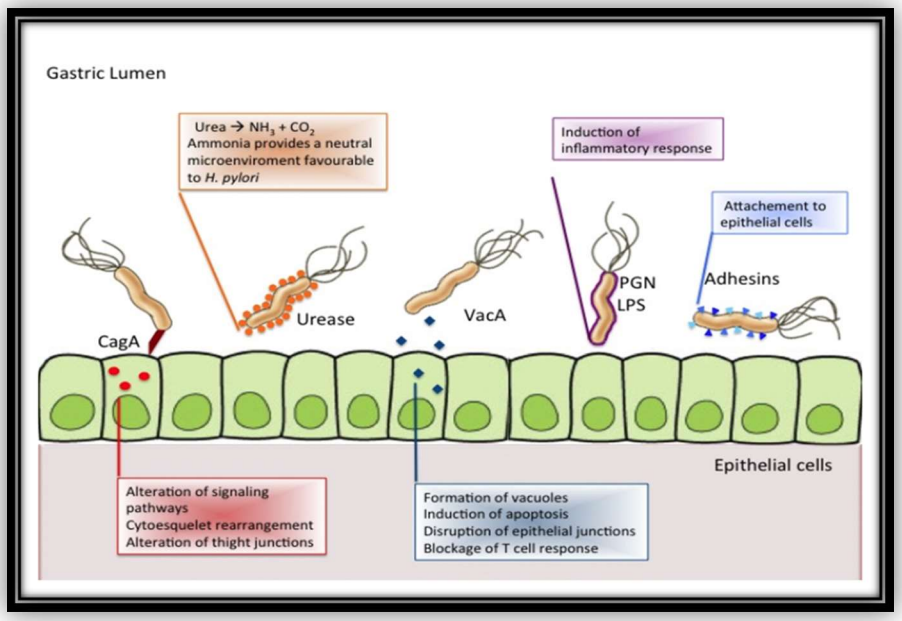

Fig.3: Pathogenesis and virulence of *H. pylori*

From: imgarcade.com

H. pylori grows optimally in the pH (6-7) where it would be killed or would not grow in the pH within the gastric lumen. Gastric mucus is impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1-2) while on the epithelial side the pH is about 7.4. *H. pylori* is found deep in the mucous layer near the epithelial surface where its physiology present. *H. pylori* produces a protease modifying the gastric mucus and further reducing the ability of an acid to diffuse through the mucus. *H. pylori* produces potent urease activity which yields production

of ammonia and buffering of acid. It is quite motile, even in mucus and is able to find its way to the epithelial surface. It overlies a gastric type but not an intestinal-type epithelial cell (Brook *et al*, 2004).

The mechanism by which *H. pylori* causes mucosal inflammation and damage is not well defined but probably involves both bacteria and host factors. The bacteria invade the epithelial cell surface to a limited degree. Toxic and lipopolysaccharide may damage the mucosal cell while the ammonia produced by the urease activity may directly damage the cell (Brook et al, 2004).

Histologically, gastritis is characterized by chronic and active inflammation. Polymorph nuclear and mononuclear cell infiltrates are seen within the epithelium and lamina propria. Vacuoles within the cell are often pronounced. Destruction of the epithelium is common, and glandular atrophy may occur. *H. pylori* thus may be a major risk factor for gastric cancer (Brooks *et al,* 2004).

1-1-5. Clinical significance *of H. pylori*:

Initial infection with *H. pylori* causes acute gastritis and sometimes results in diarrhea lasting for about one week. The infection usually becomes chronic with diffuse superficial gastritis which may be associated with epigastric discomfort. Both duodenal ulcer and gastric ulcer are closely correlated with infection by *H. pylori*. That infection appears to be a risk factor to the development of gastric carcinoma, gastric B-cell lymphoma mucosa and associated lymphoid tumour (Harvey *et al,* 2001).

1-1-6.Diagnosis of *Helicobacter pylori* infection:

1-1-6-A: Non-invasive Methods:

1-1-6-A1: C^{13} C^{14} Urea Breath Test:

This is a quick and easy way of detecting the presence of *H. pylori* used as a screening test. The measurement of $^{13}CO_2$ or $^{14}Co_2$ in the breath, after ingestion of C^{13} or C^{14} urea, requires a mass spectrometer, which is expensive. Nonetheless, the test is 98% sensitive and 95% specific. The breath test is also used to demonstrate eradication of the organism following treatment (Kumar & Clark, 2004).

1-1-6-A2: Serological test:

Detection of IgG antibody is reasonably 80% sensitive and specific. It is used in the diagnosis and epidemiological studies. IgG titers may take up to 1 year to fall by 50% after eradication therapy, hence; they are not useful for confirming eradication or the presence of a current infection. Antibodies can also be found in the saliva, but tests are currently not as sensitive or specific as serology (Kumar & Clark, 2004).

1-1-6-A3: Stool Test:

A specific immunoassay for the qualitative detection of *H. pylori* antigen is now widely available. The overall sensitivity is approximately 90% with a specificity of 95%. It is useful for the diagnosis of *H. pylori* infection and for the monitoring efficacy of eradication therapy. Patients should be off Proton pump inhibitors (PPIs) for one week but can continue with H2 blockers) (Kumar & Clark, 2004).

1-1-6-B: Invasive Methods (Endoscopy):

1-1-6-B1. Rapid Urease Test:

Gastric biopsies are added to a urea solution containing phenol red. If *H. pylori* is present, the urease enzyme splits the urea to release ammonia which raises the pH of the solution leading to a rapid color change (Kumar & Clark, 2004).

1-1-6-B2. Culture:

To suppress the growth of endogenous or exogenous contaminating bacteria, selective media are required to improve the isolation of *H. pylori* from biopsy samples. Several selective culture media have been developed for optimal isolation of *H. pylori.* In a large comparative study, BHI Agar supplemented with 10% of sheep blood, polymyxin B, Vancomycin, Trimethoprim and amphotericin B. This yields the highest isolation rate *(*99%*)* and showed to be superior to Skirrow's selective medium (isolation rate 71%) for primary isolation of *H. pylori* from gastric biopsy specimens. However, a combination of at least one selective and one non-selective culture medium is generally advocated, since no single culture medium allows a 100% recovery rate of *H. pylori* and because culture contamination occurs in about 25% of the cases (Glupczynski, 1998).
Finally, failure to detect *H. pylori* by culture may be due to the insufficient duration of incubation. Incubation periods of up to 10 days are usually recommended in order to optimize the culture isolation rates especially in a post-treatment setting (Glupczynski, 1998).

1-1-6-B3: Histology:

H. pylori can be detected histologically on routine (Giemsa) stained sections of gastric mucosa obtained at endoscopy (Kumar & Clark, 2004).

1-1-6-B4: Polymerase Chain Reaction:

PCR allows researchers and clinicians to identify *H. pylori* in small samples which contain few bacteria (Whitmire & Merrell, 2012). This does not require any special processing supplies or transportation, and it can be performed on samples obtained by both invasive and non-invasive methods (Rimbara *et al,* 2013). Moreover, PCR can be performed faster than many other diagnostic methods used to identify diverse bacterial genotypes, and employed in epidemiological studies. A considerable drawback of PCR is

that of its detection of DNA segments of the dead bacteria in the gastric mucosa of patients after treatment; consequently, it can produce false-positive results (Dus *et al*, 2013). Molecular detection of *H. pylori* using PCR is possible in materials obtained by non- or minimally invasive procedures, such as gastric juice, gastric content, saliva, stool, etc. Thus, molecular methods can be easily applied to specimens obtained by string tests or orogastric brushes. Molecular testing may be of a particular value for a sample that can no longer be successfully cultured because of prolonged transport or in case isolation of *H. pylori* is not feasible as a result of contamination (Owen, 2002).

In gastric biopsy specimens, molecular methods such as PCR have proved their worth in detecting pathogens and testing for clarithromycin resistance, which is attributable to a mutation in the 23S rRNA gene (Owen, 2002). Because of the increasing prevalence of antibiotic resistance in some population with a high prevalence of *H. pylori*, molecular tests may have important implication as relevant alternatives for *H. pylori* diagnostics. Efflux pumps have an importance in the resistance to antibiotics (Schweizer, 2012).

1-1-7: Treatments:

Triple therapy with metronidazole and either bismuth sub-salicylate or bismuth substrate plus either amoxicillin or tetracycline for 14 days eradicates 70-95% *H. pylori*-infected patients. An acid-suppressing agent given for 4-6 weeks enhances ulcer healing. Proton pump inhibitors directly inhibit *H. Pylori* and appear to be potent urease inhibitors. Either one week of a proton pump inhibitor plus amoxicillin and clarithromycin or of amoxicillin plus metronidazole is also highly effective (Brooks *et al,* 2004).

However, there is an increasing problem of *H. Pylori* antibiotic resistance. Antibiotics cannot be used to eradicate the infection of the whole population particularly in the developing countries where their indiscriminate use has led to the emergence of *H. Pylori* resistant strains. It is predictable that although antibiotics are useful, the long-term consequence of large-scale eradication programs will be a reduction efficacy of antibiotic therapy (Megraud, 1997).

1-2. Diabetes Mellitus:

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and relative insulin deficiency, resistance, or both. It affects more than 120 million people worldwide while around 220 million others are predicted to be affected by the year 2020 (Kumar & Clark, 2004).

Diabetes is usually irreversible and, although patients can have a reasonably normal lifestyle, its late complication results in reducing life expectancy and major health costs. These include macrovascular diseases, leading to an increased prevalence of coronary artery diseases, peripheral vascular diseases, strokes, and microvascular damages causing diabetic retinopathy and nephropathy, and contributing to diabetic neuropathy (Kumar & Clark, 2004).

1-2-1. Insulin Secretion:

Insulin is the key hormone involved in the storage and controlled release within the body of the chemical energy available from food. It is coded on chromosome 11 and synthesized in the beta-cell (β-cell) of the pancreatic islets (Kumar & Clark, 2004). The synthesis, intracellular processing and secretion of insulin by the beta-cell are typical of the way that the body produces and manipulates many peptide hormones (Kumar & Clark,2004).

After secretion, insulin enters the portal circulation and is carried to the liver, its prime target organ.

About 50% of secreted insulin is extracted and degraded in the liver. The residue is then broken down by the kidneys. C-peptide is only partially extracted by the liver (and hence provides a useful index of the rate of insulin secretion) but is mainly degraded by the kidneys (Kumar & Clark, 2004).

1-2-2: Types of Diabetes:

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. For example, a person with gestational DM may continue to be hyperglycemic after delivery and may be determined to have type 2 DM. Alternatively, a person who acquires DM because of exogenous steroids may become normoglycemic once these steroids discontinued, but then may develop many years later after recurrent episodes of pancreatitis (WHO, 1999).

Another example would be a person who was treated with thiazides and developed diabetes years later. Since thiazides themselves seldom cause severe hyperglycemia, such individuals probably have type 2 diabetes exacerbated by the drug. Thus, for the clinician and patient, it is less important to label the particular type of diabetes than it is to understand the pathogenesis of the hyperglycemia and to treat it effectively (WHO, 1999).

1-2-2-A1. Diabetes Mellitus TypeI: *(*Autoimmune Diabetes Mellitus)

This form of diabetes, previously encompassed by the terms; insulin-dependent diabetes, type-1 diabetes, or juvenile-onset diabetes, is caused

by the autoimmune-mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others. The rapidly progressive form is commonly observed in children, but may possibly occur in adults. The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may show symptoms of ketoacidosis as the first manifestation of the disease (WHO, 1999).

Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stresses. Still others, particularly adults, may retain residual beta–cell function, sufficient to prevent ketoacidosis, for many years (WHO, 1999).

Individuals with this form of type-1 diabetes often become dependent on insulin for survival eventually and are at risk of ketoacidosis. At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma C–peptide (WHO, 1999).

The peak incidence of type-1 diabetes occurs in childhood and adolescence, but the onset may occur at any age, ranging from childhood to the ninth decade of life. There is a genetic predisposition to autoimmune destruction of beta cells, and it is also related to poorly-defined environmental factors.

Although patients are usually not obese when they develop this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients may also have other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, and Addison's disease (WHO, 1999).

1-2-2-A2. Diabetes Mellitus TypeΠ:

Type-2 diabetes ranges from predominantly insulin resistance with a relative insulin deficiency to predominantly insulin secretory with a defect in the insulin resistance.

This form of diabetes, previously referred to as non-insulin-dependent diabetes, accounts for 90–95% of diabetic people. Type-2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency at least initially. Often throughout their lifetime, these individuals do not need insulin treatment to survive (American Diabetes Association, 2012).

There are probably many different causes for the development of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur and patients who do not have any of the other causes for diabetes.

Most patients of this form of diabetes are obese leading to some degree of insulin resistance. Patients who are not obese based on the traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (American Diabetes Association, 2012).

Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (American Diabetes Association, 2012).

While type-2-diabetes patients may have normal or elevated insulin levels, the higher blood glucose levels cause higher insulin values especially if the β-cell function is normal. The problem here is insulin resistance in the cells due to insulin receptors defect or destruction.

More frequently, it afflicts women with prior (gestational diabetes mellitus) GMD and individuals with hypertension or dyslipidemia. However, its frequency varies in different racial/ethnic subgroups (American Diabetes Association, 2012).

1-2-2-A3. Gestational Diabetes:

GDM has been defined as any degree of glucose intolerance with an onset or a first recognition during pregnancy.

Although most cases resolve with delivery, the definition is applied whether the condition is persisted or not after pregnancy .Not excluding the possibility of unrecognizing glucose intolerance which may have antedated or have begun concomitantly with the pregnancy. This definition facilitated a uniform strategy for the detection and classification of GDM, but its limitations were recognized for many years.

As the ongoing epidemic of obesity and diabetes has led to cases of type-2 diabetes among women of childbearing age, the number of pregnant women with undiagnosed type-2 diabetes has increased (American Diabetes Association, 2012).

1-2-2-A4. Other Genetic Syndromes Sometimes Associated with Diabetes:

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by

insulin–deficient diabetes and the absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness (WHO, 1999).

1-3. The Relationship Between *H. pylori* and T2DM:

A growing body of evidence has linked *H. pylori* infection with insulin resistance IR (Eshraghian *et al*, 2009), which is defined as a case when insulin can no longer effectively induce glucose disposal in skeletal muscle or suppress endogenous glucose in the liver (Dinneen *et al,* 1992). Insulin resistance and abnormal insulin secretion are central to developing T2DM, and most studies support the view that IR precedes defects in insulin secretion (Moller & Flier, 1991). The first direct evidence for an association between chronic *H. pylori* infection and IR primarily introduced in a study by Aydemir and his group showing higher homeostatic model assessment insulin (HOMA-IR) scores in *H. pylori* – positive individuals (Aydemir *et al*, 2005). Another study in Japan in 2009 which included a large population of 1107 asymptomatic subject showed that *H. pylori is* significant and independent from IR (Gunji *et al*, 2009).

Studies regarding *H. pylori* infection rate in patients with T2DM are still scarce. A hospital-based case-control study from Pakistan enrolling 74 patients with T2DM and 74 non-diabetic controls suggests that diabetic patients have a higher infection rate 73% vs. 51% (Devrajani *et al*, 2010). Similarly, a higher infection rate is observed in 210 patients with T2DM , vs. 210 controls in a study in the United Arab Emirates (Bener *et al,* 2007).

Preidt, study in 2010 showed that there may be a link between *H. pylori* bacteria and T2DM in adults. Research on participants in two US National Health and Nutrition Surveys revealed that the presence of *H. pylori*

bacteria was consistently associated with levels of glycosylated haemoglobin (HbA1c), an indicator of blood glucose levels and diabetes mellitus. The association was noticeably stronger in obese people (Preidt, 2012).

According to AL baker's study in Saudi Arabia, the association of Metabolic (MS) and *H. pylori* is still controversial with emphasis on the possible linkage between them. However, the high prevalence of both MS and *H. pylori* infection might explain the coincidence (AL baker, 2011).

In the Medical Services Clinics in Gaza Strip, the study was done randomly selected on 129 T2DM; it showed a strong relationship between *H.pylori* infection and insulin resistance risk factors. More than 70.0% of the sample found to have positive *H.pylori* (Mazan *et al,* 2013).

1-4: Alanine amino transferase ALT

This enzyme (often referred to as transaminase) is present in hepatocytes and leaks into the blood leading to considerable cell damage. One of two enzymes (ALT & AST) is measured: Alanine transferase (ALT) is a cytosol enzyme, more specific to the liver so that a rise only occurs with liver disease (kumar & clark, 2004).

Current upper limits of normal ALT level were set, on an average of 30 to 50 U/L. Nonalcoholic fatty liver disease (NAFLD) is now recognized as a frequent cause of chronic liver disease in obese adults, most commonly causing elevated serum aminotransferase levels among adults, and eventually, liver fibrosis and cirrhosis. NAFLD in adults is associated with obesity, insulin resistance; similarly, elevated ALT in overweight. Furthermore, obesity among adolescents is associated with increased age, rising glycosylated hemoglobin (HbA-1c), elevated triglycerides (TG) and

insulin resistance. In contrast, fatty liver disease may be more common in adults with type-2 diabetes (T2DM) (Nadeau *et al*, 2005).

Importance of the Study

Statement of Purpose:

Awareness of the danger of the *H. pylori* and serological follow-up during diabetes mellitus are of great importance in the prevention of the disease. In addition, understanding the infection rate and the risk of *H. pylori* in type-2 diabetes-mellitus patients in Misurata, Libya, is essential for the development of treatment and further biological and medical studies.

Infection by *H. pylori* is affects approximately 50% of the world population and is recognized as the major acquired factor in the pathogenesis of chronic antral gastritis, peptic ulcer disease, and gastric cancer.

Identification of the risk group is very important in this respect. Impairment of immune system is thought to be responsible for more frequent and severe infections among diabetic patients. *H. pylori* has an important role in delaying glucose and lipid absorption resulting in altered lipid metabolism. There are contradictory reports that *H. pylori* prevalence among diabetic patients can be high, low, or normal if compared to control subject. The relationship between *H. pylori* infection (as a communicable disease) and late complications of DM (as a non-communicable disease) is also not obvious. The aim of this study is to determine the *H. pylori* infection prevalence in type-2-DM patients and to evaluate the relationship between *H. pylori* infection and glycemic control (fasting blood glucose). The late complications of diabetes mellitus such as diabetic nephropathy, retinopathy, and peripheral neuropathy, assist DM type2 patients in treating *H. pylori* infections.

Considering that there is no much work has been done to connect the risk factors of the non-communicable and communicable disease. This study mainly aims to determine the prevalence of *H. pylori* infection within T2DM patients. Another goal of this study is to evaluate the *H. pylori*

infection and its relations to T2DM as an important disease. This study is conducted at Misurata Diabetic and Endocrine Center to enhance the understanding of the *H. pylori* infection among T2DM patients.

Aim of the Study

1- To determine the prevalence of *H. pylori* infection among type-2 diabetic patients.

2- To evaluate the relationship between *H. pylori* infection and type-2 Diabetic Mellitus.

3- To compare between serological test and stool test in the detection of *H. pylori*

4- To determine the patient's serum urea, ammonia, fasting blood glucose level and ALT level.

CHAPTER II

MATERIALS & METHODS

2. Materials and Methods:

2.1. Place of the Study and Subjects:

The study is conducted in the Diabetic and Endocrine Center in Misurata, Libya.

2-2. The Study Population:

The study involved 212 individual, 195 of them were diagnosed with type 2 diabetes and 17 non-diabetic subjects (control group). It took place from the beginning of July to the end of October in 2016.

2-3. Equipment:

- Sterile needles.
- Sterile syringes.
- Ethyl alcohol 70%.
- Cotton & blaster.
- Pipette (50µL, 100µL).
- Pipette tips.
- Refrigerator.
- Bio tubes.
- EDTA tube.
- Sterile gloves.
- Centrifuge.
- Vitros 350 system.
- Stool container.
- Rapid kit test Antibody from (ABON – Hangzhou- China).
- Rapid kit test antigen kit from (ABON – Hangzhou- China).

2-4. Collection of Samples:

From each individual, blood sample and stool sample have been collected as follows:

2-4-1: Blood Sample:

The blood samples were collected from venous blood (5ml) in special bio tube. Three millilitre were left to clot at room temperature for around 15-30 minutes. The samples underwent a centrifugation process at 2500 rpm⁄5 min. The other 2ml blood were transferred into EDTA tube for ammonia test. Following a centrifugation step the plasma is separated and transferred into a new tube to be frozen.

2-4-2: Stool Sample:

Stool specimens are collected in a clean wide-mouthed dry container.

In addition, a questionnaire has been filled up by each patient about some personal information and illness history.

2-5: Procedures of the Sample:

2-5-1. Rapid serological test:

This test required 3 serum drops (approx. 100µL) to the reagent well on the test device. After 10 minutes, the result was clear.

Interpretation of the Result:

Positive: Two distinct coloured lines appeared.

Negative: One coloured line appeared in the control line region.

2-5-2. Antigen Stool Test:

Sufficient quantity of faeces (1-2ml or 1-2g) was collected in a clean and dry specimen container. The antigen stool test supplied with a specimen collection tube for each individual test to obtain the maximum antigen amount.

For solid specimen: the cap of the test specimen collection tube was unscrewed, then it was randomly stabbed by the specimen collection applicator. The faecal specimen was in at least 3 different sites to collect, approximately 50 mg of faeces.

For liquid specimens: the dropper was held vertically, faecal specimens were aspirated, and 2 drops (approximately 80μL) were transferred into the test specimen collection tube containing the extraction buffer. The cap onto the test specimen collection tube was tightened, and then it was shook vigorously to mix the specimen and extraction buffer. The tube was left for 2 minutes. Two full drops of the extraction specimen (approximately 80μL) were transferred to specimen well of the test device. The results were read after 10 minutes from dispensing the specimen.

Interpretation of the Result:

Positive: Two distinct coloured lines appeared.

Negative: One coloured line appeared in the control line region.

2-5-3. Blood Urea Test, blood Sugar Test and ALT Test:

The serum specimen was examined by Vitros 350 system device.

Normal value for fast glucose sugar: (80-120 Mg/dl).

Normal value for urea: Adult (10-20Mg/dl), children (5-18Mg/dl).

Normal value for ALT: (9-72 U/L).

2-5-5. Blood Ammonia Test:

The frozen plasma samples were melted at room temperature to determine the ammonia level using Vitrus 350 system device.

Normal value for ammonia: Adult (9.5-49Mg/dl), children (40-80Mg/dl).

2-6. Sample Size:

In this study, 212 samples were determined to be examined, 195 samples of which were taken from type-2 diabetic patients, and the other 17 samples, referred to as the control group, have been taken from non-diabetic individuals.

The sample size has been determined by using the following equation:

Sample size = $\frac{4PQ}{L^2 \, of \, P}$ (Ertug *et al*. 2005)

Where

P: is a positive character or the prevalence rate of a disease.

Q = 100 – P **L**= allowable error

P= 70% (Sariti, non-published data)

Q= 100 – 70 = 30%

L= 10%

$$\frac{4PQ}{L^2 \, of \, P}$$

Sample size =195

Control sample= 17

195 + 17 = 212 sample.

2-7. Data analysis:

2-7-1. statistical analysis:

All the obtained date were subjected and statistically analyzed by using Minitab 16 program.

The results have been considered meaningful and statistically significant if p-value≤0.05 by using Chi-square test, two proportion test, Fisher's test, Mann-Whitney test and pears on correlation test to determine the degree of correlation which measures the degree of linear correlation between two variables and the direction of this link.

2-7-2. Data analysis to compare between the methods:

Reliability is the degree to which an assessment tool produces stable and consistent results. It depends on two parameters, sensitivity, and specificity (Phelan et al, 2005).

Sensitivity: Sensitivity is the ability of a test to correctly identify individuals who suffer from a disease or condition. If a person has a disease, how often will the test be positive (true positive rate)?. In other words, if the test is highly sensitive and the test result is negative you can be nearly certain that they don't have the disease. A Sensitive test helps rule out disease (when the result is negative). Sensitivity rule out or "Snout"

$$sensitvity = \frac{true\ postive}{true\ postive \times false\ negative} \times 100$$

True positive: a positive result obtained in the presence of the disease.

False negative: a negative result obtained in the presence of the disease.

Specificity: Specificity is the ability of a test to correctly exclude individuals who do not have a given disease or condition. If a person does not suffer from the disease, then, how often will the test be negative (true negative rate)?

In other terms, if the test result for a highly specific test is positive you can be nearly certain that the person actually has the disease.

A very specific test rules in disease with a high degree of confidence. Specificity rule in or "Spin".

$$specificity \quad \frac{true\ negative}{true\ negative \times false\ postive} \times 100$$

True negative: a negative result obtained in the absence of disease.

False positive: a positive result obtained in the absence of disease.

Accuracy: Accuracy refers to the closeness of a measured value to a standard or known value. It could be calculated according to the following formula:

$$\text{Accuracy} = \frac{\boldsymbol{true\ postive + true\ negative}}{\boldsymbol{postive + negati}}$$

(AACC, 2015)

The confidence intervals had been calculated for the two tests by using Cloopper-person confidence intervals. All the calculations have been conferred by using the Medcalc calculator from the website (http://www.medcalc.org/calc/diagnostic_test.php) as will the calculation of positive and negative predictive values (PPV & NPV).

CHAPTER III

RESULTS

3-Results:

In this study, 195 subjects had been screened to determine the prevalence of *H. pylori* in T2DM using two different methods; IgG in serum and *H. pylori* antigen in stool. In addition, 17 non-diabetic subjects, the control group, were selected randomly. This study was conducted within four months (July - October 2016) in Misurata Diabetic and Endocrine Center.

3-1. Detection of *H. pylori* IgG among diabetic and control subjects:

In this test, serum or plasma has been used in a rapid chromatographic immunoassay for the qualitative detection of *H. pylori* antibody in the human body.

The positive cases of *H. pylori* IgG among T2DM patients were 118 out of 195 (60.52%), whereas 77 (39.48%) were negative cases, with significant statistical differences (p-value=0.003), (see table 1 and figure 4). While in non-diabetic subjects (control group), 7 out of 17 were positive (41.18%), whereas 10 samples were negative (58.82%) with non-significant statistical differences as show in table (1) and illustrated in figure (5):

Table 1- Result of *H. pylori* IgG in T2DM and control subject:

H. pylori	**T2DM patient. n=195(%)**	**control subjects n=17%**	**p-value**
positive	118 (60.52%)	7 (41.18%)	**NS***
Negative	77 (39.48%)	10 (58.82%)	**NS***
p -value	0.003	NS*	

NS* = Non Significant

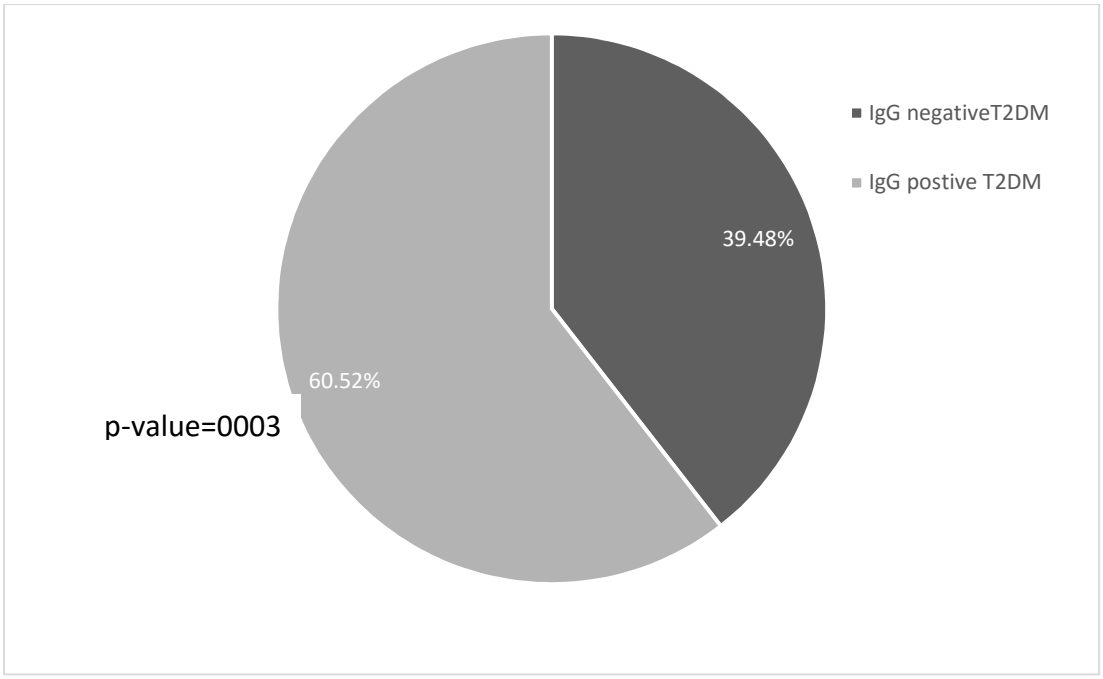

Fig 4. IgG in T2DM

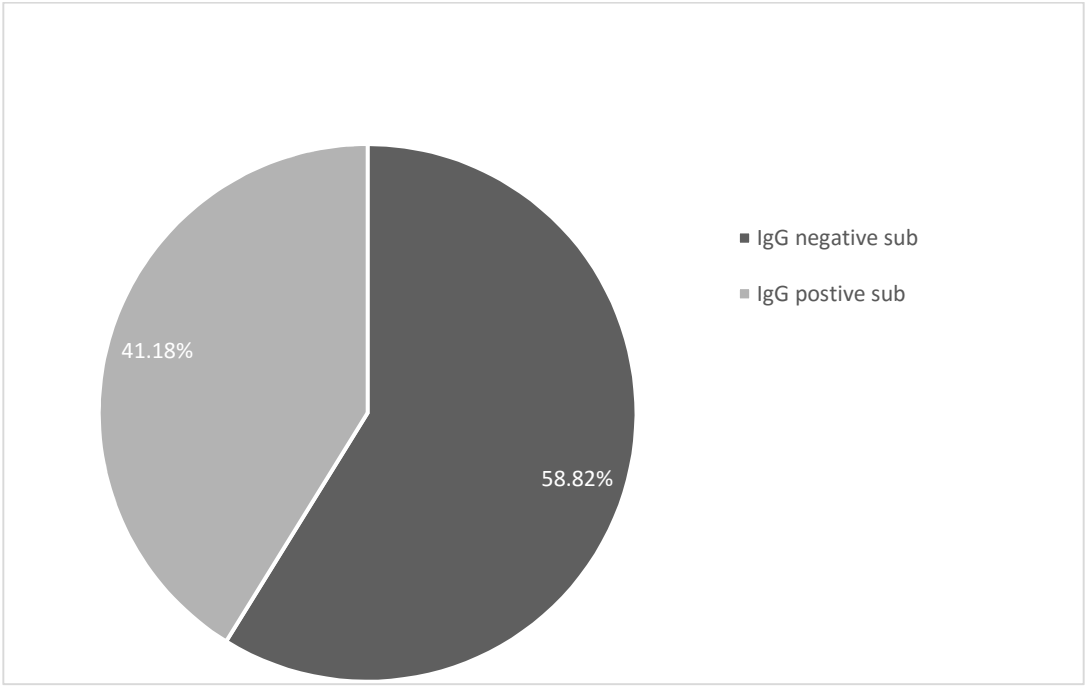

Fig 5. IgG in control group

3-2- Detection of *H. pylori* antigen among diabetic and control subjects

The testing method is a rapid chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human feces specimen. The positive cases by using *H. pylori* antigen method among T2DM patients were 96 out of 195 (49.23%), whereas 99 (50.77%) were negative, with non-significant statistical differences, (see table 2 and figure 6).

In contrast, 9 out of 17 control subjects were positive (52.94%), While 8 samples were negative (47.06%), with non-significant statistical differences, (see table 2 and figure 7).

Table 2- Results of *H. pylori* antigen in T2DM and control subjects:

H. pylori antigen	T2DM patient n=195 (%)	Control subject n=17 (%)	p-value
positive	96 (49.23%)	9 (52.94%)	NS*
negative	99 (50.77%)	8 (47.06%)	NS*
P value	NS*	NS*	

*NS= Non-Significant

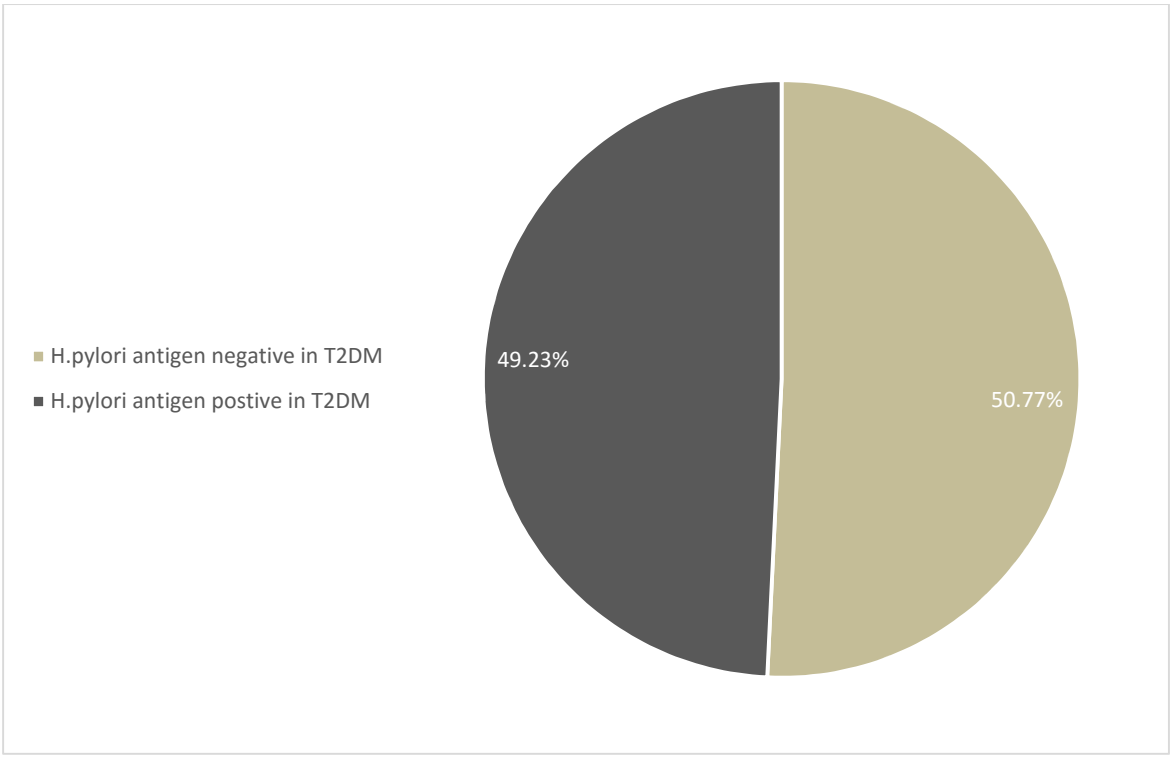

Fig 6. *H. pylori* antigen in T2DM

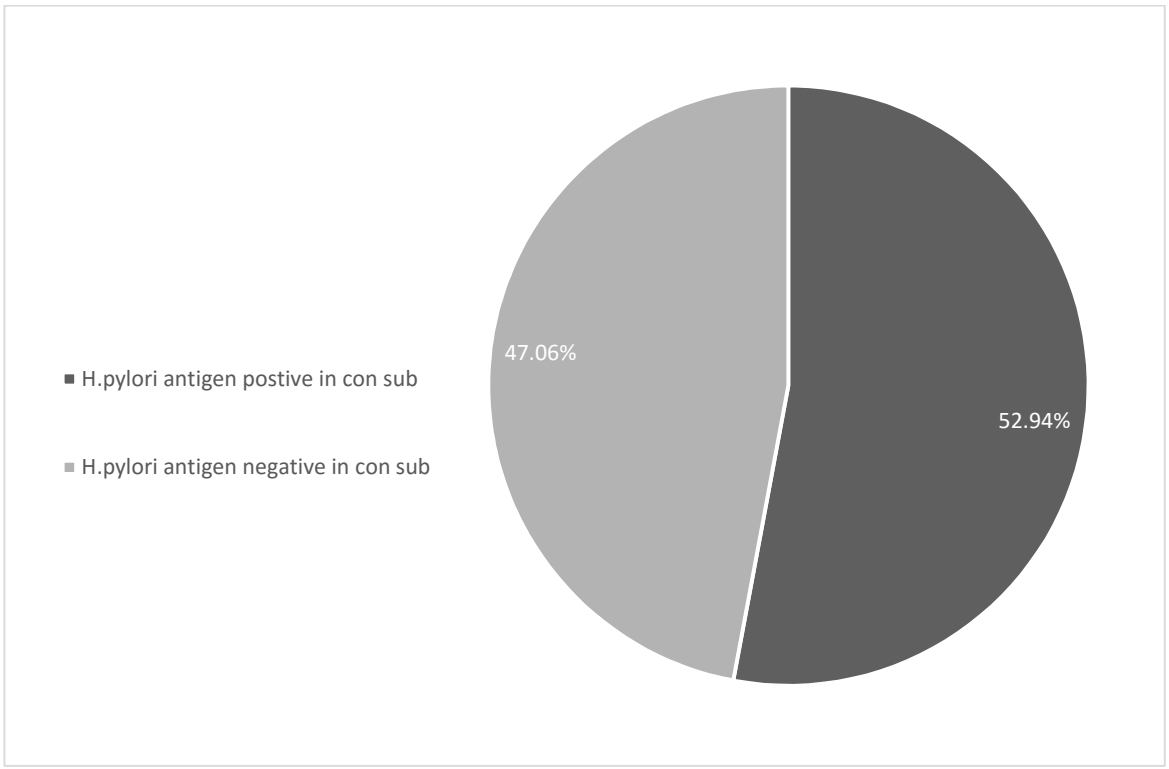

Fig 7. *H. pylori* antigen in control group

3-3- Comparative results between the two used methods:

In principle, *H. pylori* IgG test device uses serum or plasma. In this method, the antibody in blood, can be detected whereas the *H. pylori* antigen test device uses feces samples. So, the obtained result by using *H. pylori* IgG test shows 118 positive cases (60.51%), and 77 negative cases (39.49%), with a significant statistical difference (p-value=0.003). On the other hand the obtained result after using *H. pylori* antigen shows 96 (49.23%) positive cases, and 99 (50.77%) negative cases, with a non-significant statistical difference. The difference in positive results between the two methods (IgG test & antigen test) shows significant statistical differences (p-value=0.032), where as in negative results (p-value= 0.032). This significant statistical differences between the positive and the negative results lead to a significant statistical difference (p-value=0.006) in whole obtained results by using the two deferent methods, as shown in table (3) figure (8) considering the advantages and disadvantages of each method.

Table 3- comparison between the results of two methods in T2DM:

T2DM n=195	IgG (%)	Antigen (%)	p-value
Positive subjects	118 (60.51%)	96 (49.23%)	0.032
Negative subjects	77 (39.49%)	99 (50.77%)	0.032
p-value	0.003	NS*	0.006

*NS= Non-Significant

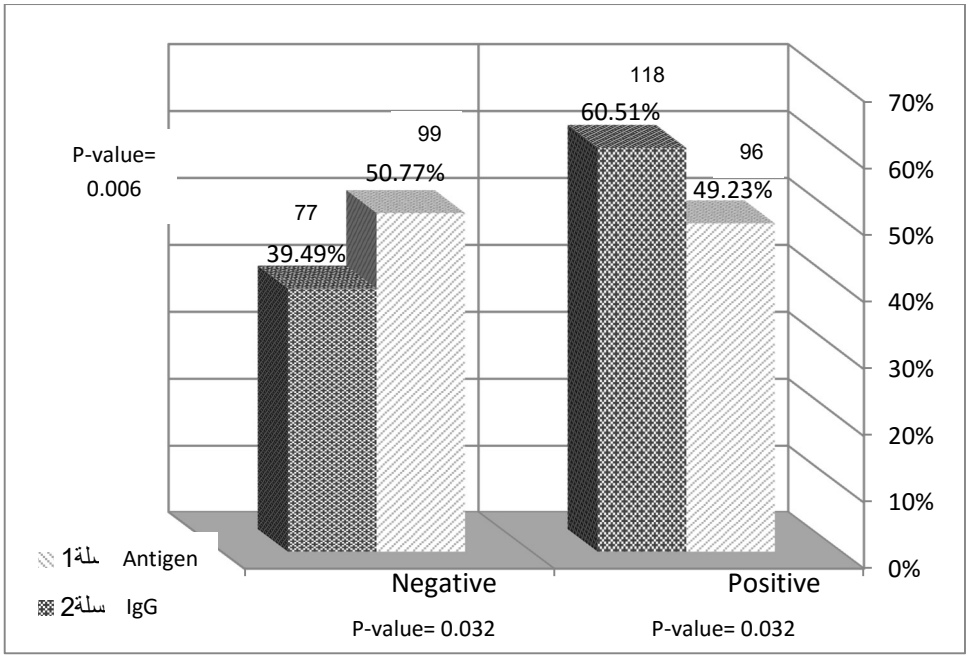

Fig 8. Comparative results between the two methods

3-4-The Reliability of the Two Used Methods for the T2DM Group:

The reliability will reflect two main phrases, meanwhile, the sensitivity indicates each method's ability to detect the real positive cases among the total positive. However, the specificity indicates its ability to detect the real negative cases among the total negative.

The test's sensitivity and specificity have been calculated according to the following variables: the number of positive cases obtained by each method, negative cases obtained by each method, cases which can be positive or negative depending on the method used, positive cases by the use of both methods, and negative cases using both methods. (See table 4 and 5.)

Table No. 4: A contingency table relating the positive and negative results of the IgG method and Ag method for the T2DM group:

	The Method	
	IgG Method n=195	Ag Method n=195
Positive by both	75	75
Positive by one	43	21
Total positive	118	96
Negative by both	56	56
Negative by one	21	43
Total negative	77	99
Total	195	195

Table No 5: A Contingency Table Relating the Positive and Negative Results of the IgG Method and Ag method for the Control Group.

	The Method	
	IgG Method n=17	Ag Method n=17
Positive by both	5	5
Positive by one	2	3
Total positive	7	8
Negative by both	7	7
Negative by one	3	2
Total negative	10	9
Total samples	17	17

3-4-1-Sensitivity and specificity of the IgG method

The concentration of serum IgG is shown to have sensitivity 78.12% (95% Cl 68.53% to 85.92%), specificity of 56.57% (95% Cl 46.23% to 66.50%), PPV (positive predictive value) of 63.56% (95% Cl 54.20% to 72.22%), NPV (Negative predictive value) of 72.73% (95% Cl 61.38% to 82.26%)

and accuracy of 67.17% for the diagnosis of *H. pylori* infection, as shown in table 6.

3-4-2-Sensitivity and specificity of the antigen method:

The concentration of antigen is shown to have a sensitivity of 36.56% (95% Cl 54.20% to 72.22%), a specificity of 72.73% (95% Cl 61.38% to 82.26%), PPV of 78.12% (95% Cl 68.53% to 85.92%) and NPV of 56.57% (95% Cl 46.23% to 66.50%), and an accuracy of 67.17% for the diagnosis of *H. pylori* infection, as shown in table 6.

Table No. 6: A contingency table relating the true positive, false positive, true negative and false negative results of the IgG method and Ag method for the T2DM group:

	IgG method n=195	Ag method n=195	P-value
Positive samples	118(60.51%)	96(49.23%)	0.032
Negative samples	77 (39.48%)	99(50.77%)	0.032
Total no. of samples	195 (100%)	195 (100%)	--
No. true positive	75	75	--
No. false positive	21	43	0.004
No. true negative	43	21	0.004
No. false negative	56	56	--
Sensitivity	78.12%	63.56%	0.029
Specificity	56.57%	72.73%	0.027
Positive predictive value	63.56%	78.12%	0.029
Negative predictive value	72.73%	56.57%	0.027
Accuracy	67.17%	67.17%	NS**

*** Total positive: 139 (71.28%),** **Non-significant.

3-5. The reliability of the two used methods for the control group:

3-5-1-Sensitivity and specificity of the IgG method

The concentration of serum IgG is shown to have sensitivity 62.50% (95% Cl 24.49% to 91.48%), specificity of 77.78% (95% Cl 39.99% to 97.19%), PPV of 71.43% (95% Cl 39.68% to 90.48%), NPV of 70.00% (95% Cl 47.18% to 85.91%) and accuracy of 70.59% for the diagnosis of *H. pylori* infection as shown in table 7.

3-5-2-Sensitivity and specificity of the antigen method:

The concentration of antigen is shown of have sensitivity 71.34% (95% Cl 29.04% to 96.33 %), specificity of 70.00% (95% Cl 34.75% to 93.33 %), PPV OF 62.50% (95% Cl 36.69% to 82.74 %) and NPV of 77.78% (95% Cl 50.33% to 92.36%), and accuracy of 70.59% for the diagnosis of *H. pylori* infection, as shown in table 7.

Table No 7: A contingency table relating the true positive, false positive, true negative and false negative results of the IgG method and Ag method for the control group:

	IgG method n=17	Ag method n=17	P-value
Positive samples	7 (41.18%)*	8 (47.06%)*	0.046
Negative samples	10 (58.82%)	9 (52.94%)	0.015
Total no. of samples	17 (100%)	17 (100%)	--
No. true positive	5	5	--
No. false positive	2	3	NS**
No. true negative	7	7	--
No. false negative	3	2	NS**
Sensitivity	62.50%	71.43%	0.005
Specificity	77.78%	70%	0.004
Positive predictive value	71.43%	62.50%	0.005
Negative predictive value	70%	77.78%	0.004
Accuracy	70.59%	70.59%	NS**

*** Total positive in the control group: 10 (58.82%).**

**NS=Non-significant.

3-6- The prevalence of *H. pylori* infection among T2DM patients and control subjects:

The study outcome shows a positive correlation between T2DM and *H. pylori* infection. The study included 195 T2DM patients and 17 control subjects. It showed that participants with T2DM have a higher prevalence of *H. pylori* infection 139 out of 195 (71.28%) (95% Cl 64.38% to 77.52%) when compared with the control participants 10 out of 17 (58.82%) (95% Cl 32.92 to 81.56), with a significant statistical difference (p-value=0.0295). As shown in table 8 figure 9.

Table No 8: The Prevalence of *H. pylori* infection in T2DM Patients and Control Group:

	T2DM	Control group	p-value
Number	195	17	
H. pylori prevalence	71.28%	58.82%	0.0295

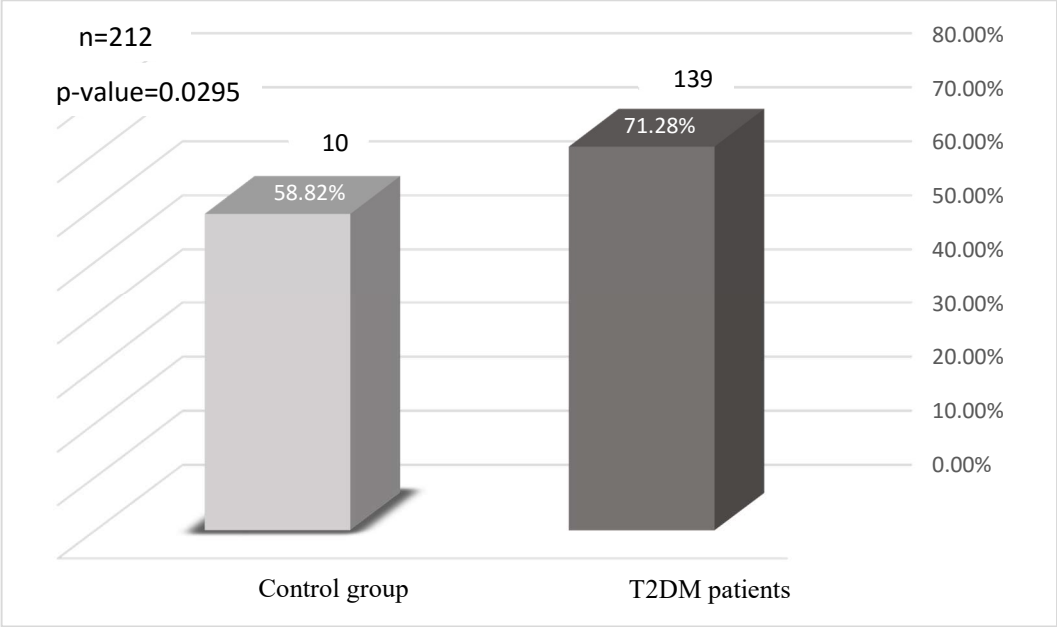

Fig 9.The Prevalence of *H. pylori* infection in T2DM Patients and Control Group

3-6- Laboratory Investigations of Diabetic Patients and Control Subjects:

3-7-1-FBS Test: The fasting blood sugar is a test used to diagnose diabetes mellitus by taking venous blood (serum) from the target subjects.

The normal glucose level in blood during fasting is 75-115 mg/dl.

The highest obtained result of T2DM subjects was 417mg/dl, whereas the lowest was 54mg/dl. With a mean of 184.54, median 176, and a standard error Mean (SEM= ±4.95).

Contrastively, when examining the results of the control group, the highest one was 114 mg/dl and the lowest was 64mg/dl. There was an exceptional result presenting an abnormal level of 136 mg /dl. However, the control group recorded a Mean of 93, median 90 and SEM±3.99.

Table (9) shows the results of FBS for both groups which are presented in mean, median and SEM, (see figure 10).

These results showed a clear significant statistical difference between T2DM group and the control group (p-value=0.000).

Table 9: The mean Results of FBS in T2DM and Control Group.

		FBS		
		Mean	SE Mean	Median
Patient	n=195	184.5	4.9	176
Control	n=17	93.0	3.99	90
P-value		0.000		0.000

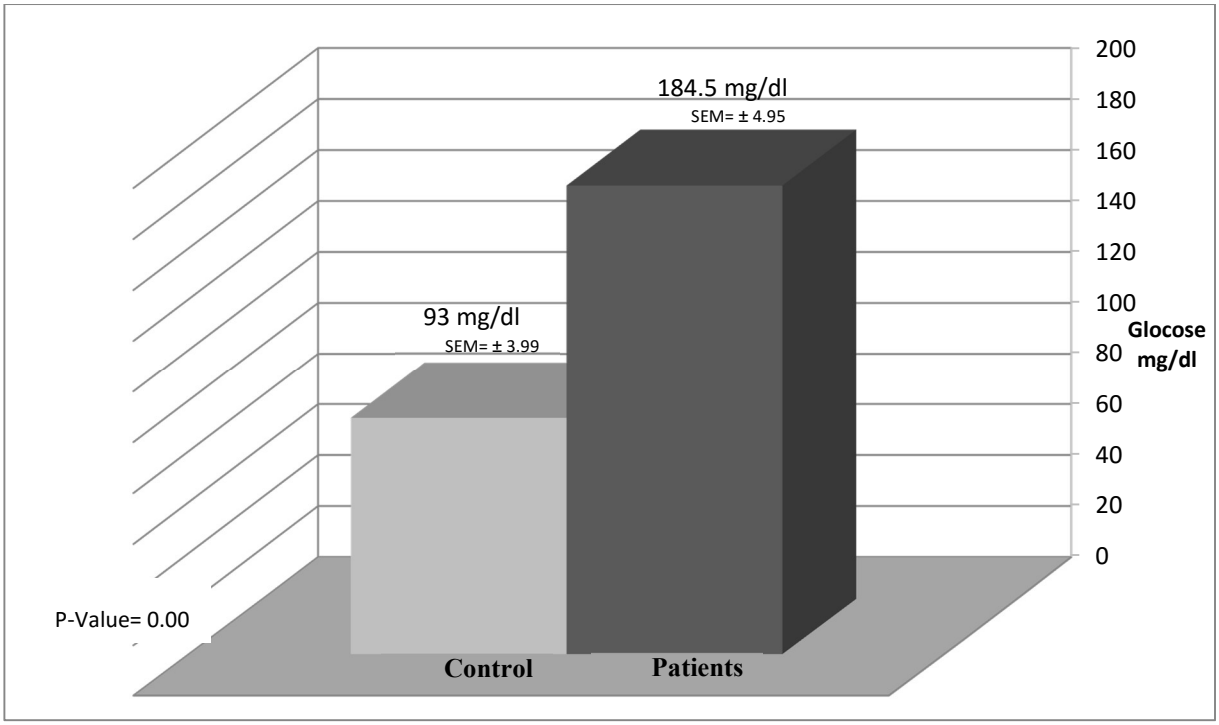

Fig 10. Results of FBS in T2DM and control subjects

3-7-2- Urea test: Blood samples had been taken from the veins converted into the serum to measure the nitrogen amount in the blood. The normal value is 9-20mg/dl.

The obtained result from T2DM subjects ranged from 4.2 to 48.4 mg/dl with a mean of 16.58, median 15 and SEM (±0.46).

In comparison, results of the control group ranged from 6.6 to 22.2 mg/ dl with a mean of 14.33, median 13, and SEM ±1.05

There were non-significant statistical differences between the results of the two groups.

Table (10) shows the urea results for both groups in terms of mean, median and SEM which have been drawn in fig (11).

27 T2DM patients (subgroup) are diagnosed with *H. pylori* positive and high urea elevation (79.41%), compared to 7 T2DM patients without infection who had a high urea of level (20.59%). There was a relationship

of correlation between *H. pylori* positive in T2DM and urea elevation in blood with significant statistical relation between the two results p-value=0.000.

Table 10. The mean Results of Urea in T2DM and Control Subjects

	N	Urea		
		Mean	SE Mean	Median
PATIENT	195	16.58	0.46	15
CONTROL	17	14.33	1.05	13
p-value		0.061		0.1514

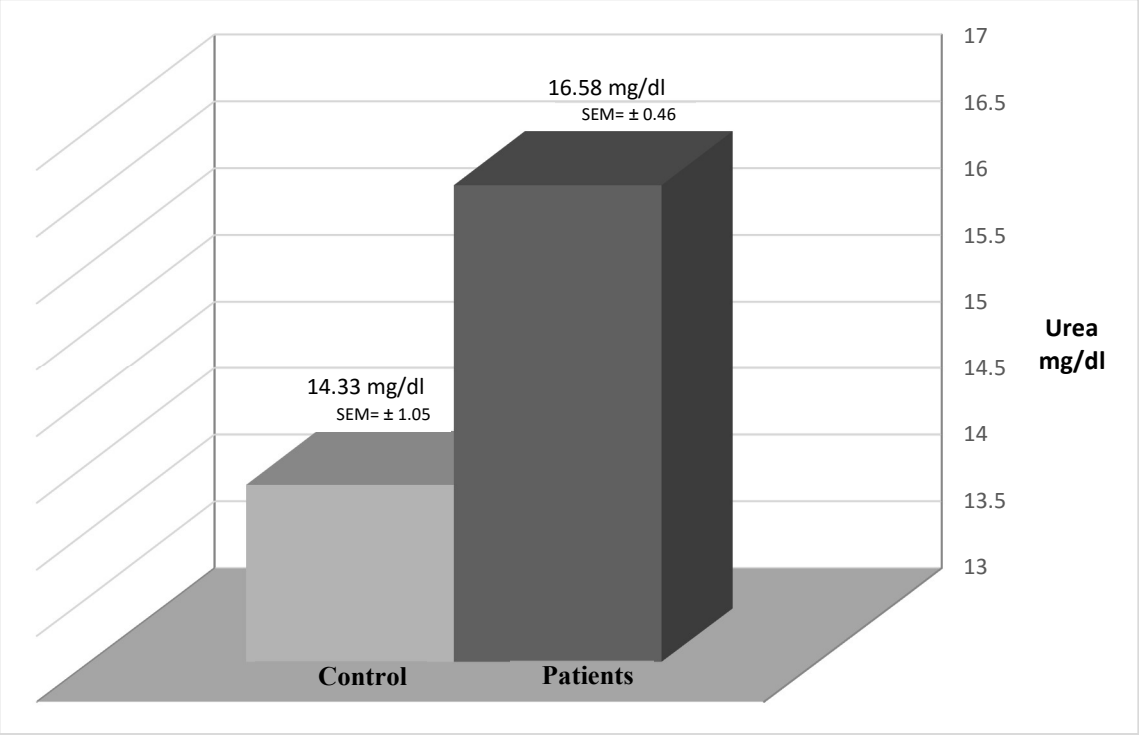

Fig 11. Results of urea in T2DM and control subjects.

3-7-3-Ammonia Test: This test measures the ammonia in circulating blood using plasma. The normal blood ammonia level is (9-33umol/L). On one hand, the ammonia results of the T2DM group have been confined between 6 and 79 umol/ L with a mean of 24.19, median 23 and SEM

±0.82. On the other hand, the ammonia results of the control group confined in between 8 and 28umol/ L with a mean 18.59, and SEM ±1.6, median 18.

The result showed that there is a significant statistical difference between T2DM and the control group (p=0.005).

Ammonia results of both groups are presented in mean, median and SEM as shown in table (11) and illustrated in fig (12). The study included 18 T2DM patients, a subgroup, diagnosed with *H. pylori* positive and high ammonia elevation at a percentage of (66.67%). Compared to 9 T2DM patients without infection who had high ammonia of level (33.33%). Therefore, a relationship of correlation existed between *H.pylori* and ammonia elevation in blood with a significant statistical relation between the two results p-value=0.001.

Table 11. The Mean Results of Ammonia in T2DM and control subjects

		Ammonia		
		Mean	SE Mean	Median
Patient	n=195	24.2	0.82	23
Control	n=17	18.59	1.62	18
p-value		0.005		0.0396

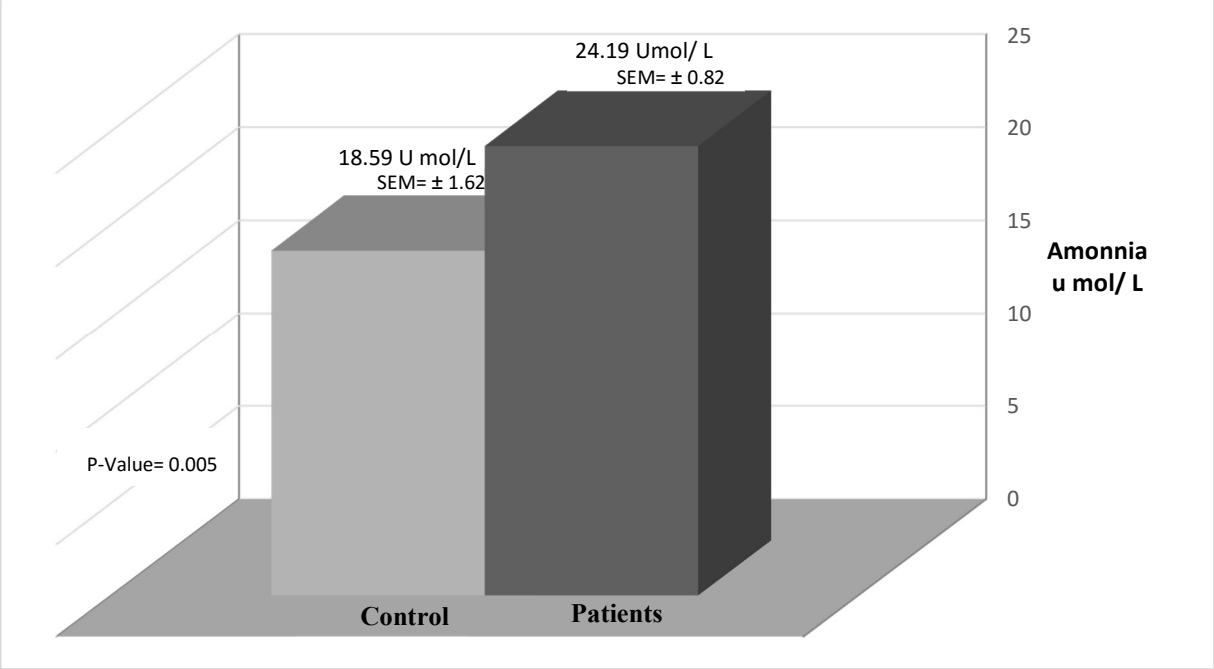

Fig 12. Results of ammonia in T2DM and control subjects.

3-7-4- ALT test: The Alanine aminotransferase is one of the enzymes that reflects the liver function and activity.
The ALT test will measure the amount of this enzyme in the circulating blood, the normal result ranged between 9 and 72
u/L.

The obtained results from the T2DM group gave minimum value of 6u/L whereas the maximum was 124u/ L with a mean of 27.85 and SEM ±1.41, median 21.50.

The control group ALT results gave the minimum and the maximum value of 6 and 43.6 u/L, respectively, with mean 19.72, SEM ±4.05, and median 13.10. The above ALT results showed insignificant statistical differences when comparing the median between T2DM patients group and the control group p-value=0.0248 regarding ALT and its correlation to the liver's function.

The results obtained via the ALT test for both groups are summed in mean, median and SEM as shown in table (12) and fig (13).

Table 12. The Mean Results of ALT in T2DM and Control Subjects.

		ALT		
		Mean	SE Mean	Median
Patient	195	27.85	1.41	21.50
Control	17	19.72	4.05	13.10
P-value		0.072		0.0248

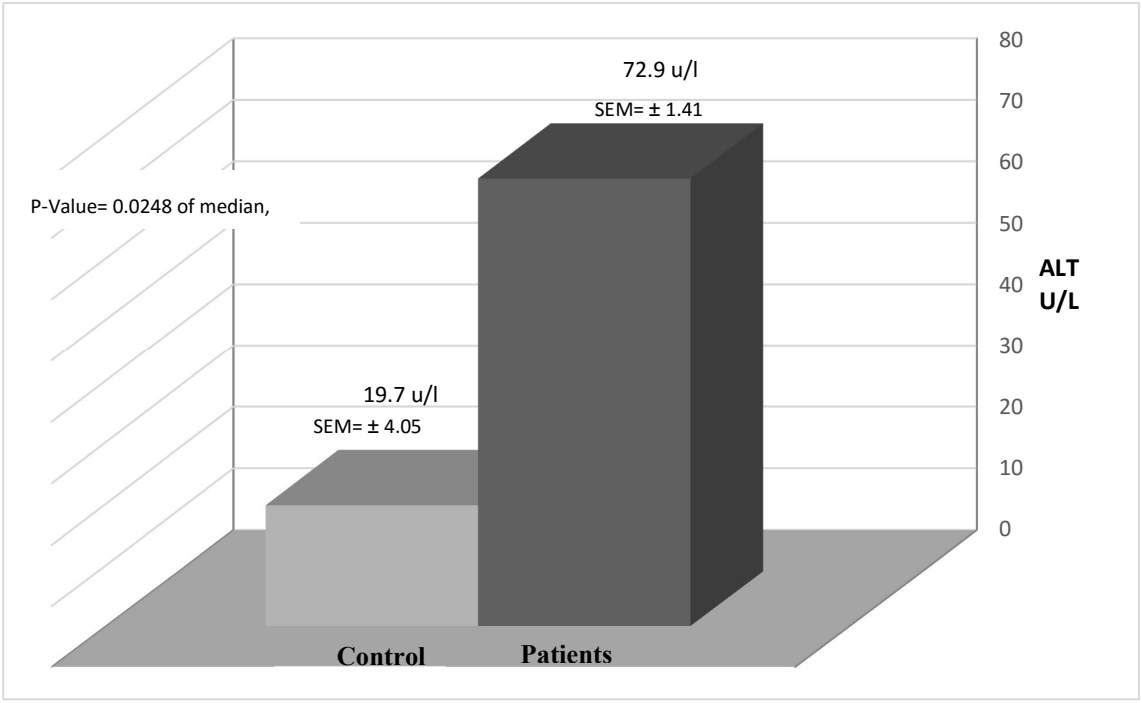

Fig 13. Result of ALT in T2DM and control subjects.

CHAPTER IV
DISCUSSION

4- Discussion of the Results:

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral-shaped pathogenic bacterium which specifically colonizes the gastric epithelium causing chronic gastritis, peptic ulcer disease, and/or gastric malignancy.
The prevalence of *H. pylori* is high in the older population – presumably acquired in their childhood, mainly by fecal-oral, oral-oral or gastro-route (Kumar & Clark, 2004; Gonzalez *et al*, 2014).
The statement of purpose for this study:
1- People living in developing countries, particularly in crowded, unsanitary conditions are at increased risk of infection with the communicable disease, *H. pylori* (Stanley & swierzewski, 2008).
2- Similar to the early risk factors related to *H. pylori* infection, the risk factors of diabetes mellitus (as a non-communicable disease) often begin early in life and continue throughout adulthood (Who, 2011).
3- Considering previous studies, it can be said that there is a considerable lack of sufficient work to connect the risk factors of non-communicable and communicable disease. The main aim of this study is to determine the prevalence of *H. pylori* infection within T2DM patients and to evaluate the *H. pylori* infection and its relations to T2DM as an important disease. This study was conducted at Misurata Diabetic and Endocrine Center to help understand the *H. pylori* infection among T2DM patients. Moreover, it aims at demonstrating the diagnostic methods and to determine the relationship between this infection and the serum levels of urea, ammonia, FBS and ALT.

4-1- The prevalence of *H. pylori* infection:

This study was conducted from July to October in 2016, involving 212 subjects. 195 of them were T2DM patients while the other 17 participants

were non-diabetic. To achieve this objective, two diagnostic tools have been used to detect the *H. pylori* infection (rapid test IgG antibody & stool test).

In this study, according to the obtained results, the prevalence of *H. pylori* in DMT2 patients was 71.28% (95% Cl 64.38% to 77.52%) which is higher than that of the control group 58.82% (95% cl 32.92% to 81. 56%), p-value=0.0295.

There are several lines of evidence to implicate the increasing susceptibility to the *H. pylori* infection among diabetic patients including:

1- Diabetes-induced impairment of cellular and humoral immunity may enhance individuals' sensitivity to *H. pylori* infection.

2- Diabetes-induced reduction gastrointestinal motility and acidic secretion may promote the pathogen colonization and the infection rate in guts.

3- Altered glucose metabolism may produce chemical changes in the gastric mucosa which promote the *H. pylori* colonization.

4- Individuals with diabetes are more frequently exposed to pathogens than their healthy counterparts as they regularly attend hospital settings (He *et al,* 2014).

The above lines could explain the high prevalence rate of *H. pylori* infection among the T2DM group as opposed to the control group.

Similar results were also detected in a study conducted in Gaza Strip. More than 70.5% of the samples were found to have a positive *H. pylori* test in diabetic patients (Mazen *et al*, 2013).

In another study in Benghazi, Libya, where 200 T2DM patients had been screened, the prevalence rate was 50.5% (Lemziany, 2012). However, other study in Turkey did not reveal any significant differences between the DM group and control group with regard to *H. pylori* infection (Demir *et al*, 2008).

4-2-Detection of *H. pylori* IgG among Diabetic and Control Subjects:

In this study, the positive cases of IgG among T2DM patients were 60.5% whereas 39.48% of the cases were negative with significant statistical differences p-value= 0.003. This obviously is a high percentage where compared to the control group which showed IgG positive of 41.18% and IgG negative of 58.82%. Similar results were obtained in United Arab Emirates (Bener *et al*, 2007). Almost the same results were found in Benghazi- Libya where IgG positive was in 59.5% in T2DM group (Lemziany, 2012).

Currently, testing the *H. pylori* infection by detecting the IgG antibody remains the most commonly selected test by most providers. That can be attributed to the ease of using specimen collection, single time-point testing, and its lower cost when compared to other noninvasive tests. Serologic profiles are not affected by the prior use of proton pump inhibitors (PPIs), bismuth-containing compounds, or antibiotics (Gonzalaz *et al*, 2014).

Serological testing for IgG antibody to *H. pylori* should be ordered and results should be considered with significant caution.

The greatest concern regarding this testing method is its poor positive predictive value (PPV), particularly in regions of low *H. pylori* endemicity and antibody which often remains for years following resolution of infection. Serologic testing cannot be used to distinguish active from past infection or to document eradication of the organism following successful treatment (Glupczynski, 1998). Based on these limitations, the serologic method should be avoided when establishing a diagnosis of active *H. pylori* infection; it must be used with another method to minimize improper diagnosis and unnecessary treatment regimens.

4-3-Detection of *H. pylori* Antigen among Diabetic and Control Subjects:

In this study, the positive cases of *H. pylori* antigen reached 49.23% of T2DM patients whereas 50.77% of them were negative. Showing no significant differences, the percentage of positive cases of *H. pylori* antigen among the control group was 52.94% and the rest 47.06% were negative. Another study in Pakistan determined the relationship between T2DM and *H. pylori* infection. The study used many laboratory tests including *H. pylori* stool antigen test. Examining the diabetic group, *H. pylori* stool antigen test was positive by 54/74 (73%) whereas non-diabetic cases were positive by 38/74 (51%) (Devrajani *et al*, 2010).

In this study the percentage of *H. pylori* antibody IgG positive test on T2DM patients is higher than that of *H. pylori* antigen among T2DM patients, because the IgG titer to *H. pylori* often remains for years following the resolution of infection (Kumar & Clark, 2004). Reversing *H. pylori* antigen can be positive only when the bacteria present an active disease.

The stool antigen test (SAT) is an enzyme immunoassay designed to detect *H. pylori* in fecal specimens, stool antigen test only arises positive if *H. pylori* is present, the SAT can be used as an accurate tool to diagnose active *H. pylori* infection and to establish eradication of the organism following treatment. Additionally, the specimen collection is easy, and the test is available. Therefore, the stool test can be used as a reliable marker for the screening of *H. pylori* infection.

Similar to the IgG method, the sensitivity of the *H. pylori* antigen test is decreased if the patient has taken PPI, bismuth-containing compound, or an antibiotic (Gonzalaz *et al*, 2014). Another disadvantage of this tool is that patients may be uncomfortable with the process.

4-4- The Reliability of the Two Methods Used for the T2DM Group:

The reliability of IgG method in this study has a sensitivity rate of 78.12% (95%Cl 68.53% to 85.92%), and a specificity rate of 56.57% (95% Cl 46.23% to 66.50%), PPV 63.56%, NPV 72.73%, and an accuracy of 67.17% to diagnose the *H. pylori* infection. The testing method of this assay has low PPV and low specificity. In addition, a positive result cannot be used to predict the presence of the active disease (Glupczynski, 1998).

However, testing the *H. pylori* infection, by detecting IgG antibody remains the most commonly selected test by most providers because it is relatively cheap and readily available (Gonzalez *et al*, 2014).

H. pylori infection provokes both local and systemic antibody responses. The systemic response typically comprises a transient rise in IgM, followed by a rise in a specific IgA and IgG is maintained throughout infection (Glupczynski, 1998). Nonetheless, in this method, the IgG has unknown titers and the new infection cannot be determined using the old one (Kumar & Clark, 2004). In addition, the antibody may not detectable early in an infection, and a rise in antibody titer over a seven to ten days period does not distinguish between a present or a prior infection (Havery *et al*, 2001). Consequently, IgG detection method cannot be used alone, but rather, it must be used with another method to confirm the *H. pylori* infection in avoiding unnecessary treatment of diabetic patients.

The reliability of SAT method showed a sensitivity rate of 36.56% (95%Cl 54.20% to 70.22%), and a specificity rate of 72.73% (95% Cl 61.38% to 82.26%), PPV 78.12%, NPV 56.57%, and an accuracy of 67.17% to diagnose the *H. pylori* infection.

During this test, a high specificity rate and high PPV to detect the *H. pylori* infection is necessary to avoid giving unnecessary eradication therapy to the non-infected patients (Glupczynski, 1998).

Regarding the diagnostic tool for the detection of *H. pylori* infection, using the *H. pylori* stool antigen test (SAT), is recommended since it has proved to be rapid, noninvasive and flexible. It is also a reliable method to diagnose an active infection and confirm an effective treatment of the infection, to avoid giving unnecessary eradication therapy to non-infected subjects. In addition, the stool antigen test can be used as a reliable marker for initial screening of *H. pylori* infection.

4-5- The Correlation Between *H. pylori* and Chemical Variables:

4-5-1- Fasting Blood Sugar (FBS): The current study revealed a significant association of the blood sugar level between the T2DM patients when compared to the control group (p-value=0.000).

A decrease in insulin secretion is one of the major pathophysiological defects in T2DM. The progression from normal glucose tolerance to pre-diabetes and T2DM is characterized by continuing defects in β-cell function (Weyer *et al*, 1999).

H. pylori-induced gastritis can potentially affect the secretion of gastric related hormones such as leptin and ghrelin, as well as gastrin and somatostatin, which perhaps influence a predisposition to diabetes (He *et al*, 2014).

Gastrin increases food-related and glucose-stimulated insulin release, while somatostatin regulates pancreatic insulin secretion and inhibits insulin release. Patients with *H. pylori* infections could, therefore, have altered insulin release, as they noticeably have elevated basal and stimulated serum concentrations of gastrin and decreased somatostatin. The regulation of leptin and ghrelin produced in the stomach and involved in energy homeostasis, affects obesity, insulin sensitivity, and glucose homeostasis. This often increases evidence demonstrating that *H. pylori*

can influence the production of leptin and ghrelin, and thus could promote obesity and the development of diabetes (He *et al*, 2014).

A study by So revealed that *H. pylori* titer could independently predict abnormal pancreatic β-cell function in Chinese men (So *et al*, 2009).

Additionally, Rahman and his group showed a positive association between *H. pylori* infection and impaired insulin secretion (Rahman *et al*, 2009).

Moreover, the insulin-producing pancreatic β-cells are especially susceptible to damage by inflammation and oxidative stress (Fosslien, 2001). Therefore, it is possible that inflammation caused by *H. pylori* infection results in deficits in insulin secretion. Furthermore, it was reported in a study by Hsieh that patients with *H. pylori* infection were more likely to have had impaired insulin secretion at a young age, which may increase the risk for T2DM (Hsieh *et al*, 2013).

Many additional factors are likely involved in the relationship between *H. pylori* infection and T2DM. The T2DM affected both the cellular and humoral component of immune system since the risk of T2DM patients to the complication of the *H. pylori* infection is more comment. However, it could be said that *H. pylori* infection plays a role in type-2 diabetes mellitus, chronic inflammation and the secretion of gastric related hormones. In addition, insulin secretion deficiency implicates *H. pylori* in a predisposition to diabetes.

4-5-2- Urea: The current study revealed a non-significant difference in the level of serum urea between T2DM groups compared to the control group.

It is a prospect of no elevation in urea level between the two groups because the *H. pylori* needs the urea in order to survive and be protected by converting it to ammonia.

In the same time, with the T2DM group, there were 27 patients; the subgroup, with *H. pylori* positive and a high urea level reaching (79.41%) whereas 7 T2DM patients with *H. pylori* negative had a high urea level of (20.59%). There was a relationship of correlation between positive *H. pylori* test in T2DM group and urea elevation in blood with the significant statistical relation between the two results p-value=0.000.

As result, the high urea level may cause kidney failure in diabetic patients.

Some researchers, Mendez & Hazell and Vargha, suggested that *H. pylori* urease may be essential in maintaining intracellular nitrogen balance in the micro-organism. In addition, excessive nitrogen inside the cell produced by fast catabolism of amino acid could be disposed of excretion via the urea cycle. The association of arginase with the bacteria's membrane would place the enzyme in a good position to carry out this function. Hydrolysis of urea by urease outside the cell would avoid the formation of a concentration gradient which may drive this metabolic product back into the cytosol. However, this role of urease is compatible with acid protection and high expression of this enzyme to the central metabolism (Mendz & Hazell, 1996) and (Vargha *et al*, 1983).

Although the role of the urea cycle in prokaryotes is unclear, one of its possible functions is arginine biosynthesis, since the generation of amino acid from ornithine utilizes three metabolic steps of the cycle (Mobley *et al*, 2001).

At the same time, it does not appear that arginine is a nitrogen source for the microorganism because ammonium could be obtained by fast deamination of asparagine, aspartate, and glutamine (Mendz & Hazell, 1995).

The high level of urea in serum may be attributed to a renal disease since renal excretion rate of urea becomes slower in some of the diabetics. Urea

is produced from the oxidation deamination of amino acid in which ammonia generated is transported to the liver for the formation of urea through the urea cycle (Amartey *et al*, 2015).

In T2DM patients, the renal hypertrophy is associated with a raised glomerular filtration rate. This appears after diagnosis and it is related to the poor glycaemic control. Other causes of the high serum urea are corticosteroid treatment, tetracycline treatment, and gastrointestinal bleeding (Clark & Kumar, 2004).

Neithercut reported that the urea ratio proved that it is almost as effective in identifying the presence of *H. pylori* infection, in subjects with chronic renal failure as in subjects with chronic renal function and *H. pylori* negative (Neithercut *et al*, 1993).

Finally, the simultaneous presence of urea cycle and high active urease appears paradoxical and raise the question of; how *H. pylori* avoids an energetically expensive futile cycle? (Mobley *et al*, 2001).

4-5-3-Ammonia: The current study revealed a significant difference in the ammonia level between the T2DM group and the control group (p-value=0.005). In the main time, the T2DM group was sub-divided into two groups: 1- *H. pylori* positive with a high ammonia level 18 cases (66.67%), 2- *H. pylori* negative with a high ammonia level 9 cases (33.33%). There was a correlation between *H. pylori* positive in T2DM and the ammonia elevation in blood with a significant statistical relation p-value=0.001.

The *H. pylori* needs ammonia to survive in the harsh conditions inside human stomach, to be protected from the direct contact with the hydrochloric acid and to be secreted into the stomach. It can subsequently create a microenvironment where balance of acidity and alkalinity (pH) is near neutral. This is accomplished by urease, a powerful enzyme made by

bacteria which converts urea to carbon dioxide and ammonia (Marshall, 2002).

Based on above results, the high ammonia level in T2DM patients with positive *H. pylori* infection, the bacteria could be more active and overgrowth of the gastric mucosa. This can be caused by the diabetic disease having impairment immune system or the liver's disability to convert ammonia to urea.

The reason why plasma ammonia concentration is higher in subjects with T2DM combined with *H. pylori* positive may be the ammonia load presented to the liver that would also increase the result of *H. pylori* urease activity. Another reason may be the hepatic synthesis of urea from ammonium and bicarbonate reduced by excessive production of H ions or impaired excretion of H ions to conserve bicarbonate (Haussinger *et al*, 1985).

It is auxotrophic for several amino acids which supports the idea that the growth in vivo is strictly dependent on the gastric environment. The minimal amino acid requirements of this bacterium are arginine, histidine, isoleucine, methionine, phenylalanine, and valine, with some strains also requiring alanine or serine (Reynolds & Penn, 1994).

Amino acid utilization by *H. pylori* grown in a defined medium has been investigated by nuclear magnetic resonance (NMR) spectroscopy and amino acid analysis. It has been shown that *H. pylori* can survive using amino acid as a basic nutrient during microaerophilic growth in the absence of glucose (Mendz & Hazell, 1995).

All *H. pylori* strains produce a highly abundant urease, which accounts for up to 6% of total bacterial protein (Hu & Mobley, 1990). The *H. pylori* urease has a strong affinity for its substrate and catalyzes the hydrolysis of urea to give ammonia/ammonium and carbonic acid/bicarbonate. These

compounds have buffering properties and are essential to the protection of *H. pylori* against gastric acidity, neutralizing the bacterial microenvironment (Ferrero & Lee, 1991).

H. pylori requires a large amount of nitrogen; two major sources of nitrogen are available in the gastric environment: amino acid and urea (Nedenskov, 1994). Nitrogen metabolism in *H. pylori* generates considerable amounts of free ammonia, physiological intracellular pH; most of the ammonia generated is present in protonated form as NH_4^+, which does not diffuse freely through membranes. *H. pylori* cell, therefore, must have developed strategies for eliminating excess ammonium, which is toxic. These strategies probably involve two main mechanisms: The extrusion of ammonium by a specific transport system. No ammonium exporter has yet been described in prokaryotes, but it remains a possibility to be explored (Mobley *et al*, 2001). And the transformation of ammonium into a more diffusible and nontoxic compound, such as urea, which is the principal nitrogenous waste product in mammals (Mobley *et al*, 2001).

Several studies showed that ammonia levels in both gastric juice and blood were significantly higher in *H. pylori* infection among cirrhotic patients than those without *H. pylori* infection (Jiang *et al*, 2013, Abdel-Hady *et al*, 2007, Chakrabarti *et al*, 2002).

In addition, a study by Neithercut showed that chronic renal failure with *H. pylori* positive had a high ammonia ratio in gastric juice and plasma ammonia concentration compared with patients that have a chronic renal failure with *H. pylori* negative in gastric juice and plasma ammonia concentration (Neithercut *et al*, 1993).

Another study has shown that patients with chronic liver diseases and *H. pylori* infection have higher blood ammonia levels than those with chronic liver diseases but have no *H. pylori* infection (Nandakumar *et al*, 2003).

In addition, Gunanithi compared between hyperammonia in the controlled T2DM subjects and in uncontrolled T2DM subjects. The study showed that serum ammonia levels were significantly higher among patients with uncontrolled T2DM (Gunanithi *et al*, 2016). Consequently, diabetes is associated with the occurrence of well-described microvascular complications that affect different organs; infection disease is more prevalent in individuals with DM. The pathogenic mechanisms of hyperglycemic environment increase the virulence of some pathogens. DM is known to have disordered energy homeostasis that might also well explain the reason behind hyper ammonia in uncontrolled T2DM. Synthesis of glutamine also reduces the total free ammonia level circulating in the blood; therefore, a significant increase in blood glutamine concentration can signal hyper ammonia (Gunanithi *et al*, 2016).

4-5-4-Alanine aminotransferase (ALT): The current study revealed a significant difference in the ALT level between T2DM and the control group (p-value=0.0248).

Elevation of ALT enzyme in a T2DM patient may possibly correlates with irregular intake of the anti-diabetic drug, lipid modifying therapy, or sometimes that patient may be diagnosed with a liver disease (Elizabeth & Harris, 2005).

The most common Liver function tests (LFTs) include ALT, which measures the concentration of intracellular haptic enzyme that has leaked into the circulation and served as a marker of hepatocyte injury. Individuals with type-2 diabetes have a higher incidence of liver function test abnormalities than non-diabetic ones (Elizabeth & Harris, 2005).

Ohlson, found that elevated ALT in non-diabetic Swedish men to be a risk factor for type-2 diabetes (Ohlson *et al*, 1988). Vozaroza reported a similar observation (Vozaroza *et al,* 2002).

The most common cause of elevated LFTs in type-2-diabetic patients is a nonalcoholic fatty liver disease (NAFLD). NAFLD is a clinic-pathological condition representing a spectrum of histological findings from hepatic steatosis or fat accumulation in hepatocytes without inflammation, to hepatic steatosis with a necroinflammatory component that may or may not have fibrosis, or nonalcoholic steatohepatitis (NASH) (Elizabeth & Harris, 2005).

Polyzos showed that the contribution of *H. pylori* to NAFLD might be achieved indirectly through increasing insulin resistance (IR), or directly, illustrating that it can predict NAFLD independently from IR. Inspired by this hypothesis and studies, investigating the relationship between *H. pylori* infection and IR would be important to infer the mechanism of how *H. pylori* induce NAFLD (Polyzos *et al*, 2013).

CHAPTER V

CONCLUTION & RECOMMENDATION

Conclusion and Recommendations

5-1- Conclusion:

1- The study demonstrated that the *H. pylori* infection could be an important risk factor for the T2DM disorder.

2- (IgG) serology test to detect *H. pylori* infection should not be used alone but in combination with another diagnostic method, such as stool antigen test.

3- Stool antigen test is a reliable method to diagnose an active infection and to confirm an effective treatment.

4- The high serum sugar level in T2DM patients could increase *H. pylori* complication by the impairment of the body cellular and humoral immunity.

5- *H. pylori* infection may promote a T2DM disorder by inducing chronic inflammation and affecting the insulin regulation of gastrointestinal hormones.

6- High serum ammonia level is related to *H. pylori* infection.

5-2- Recommendations

1- *H. pylori* infection should be integrated into the routinely Para-clinical investigation of all patients with T2DM.

2- Stool antigen test has to be used to detect the active *H. pylori* infection.

3- Monitoring blood glucose level and screening the *H. pylori* infection are effective preventive measures for this life-threatening infection.

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