

Original Research

CHANGES IN THE ADRENAL CORTEX SECRETION AND SOME BLOOD INDICATORS OF INFECTION WITH *HELICOBACTER PYLORI* ISOLATED AND GENETICALLY DIAGNOSED IN OUTPATIENT CLINICS IN AL-DIWANIYAH GOVERNORATE.Nael Mohammed Sarheed^{1*}, Osamah Faisal Kokas²^{1,2}Medicine College / Al- Muthanna University, Iraq. Email: Nael.serheed@mu.edu.iq**Abstract**

Infection with *H. pylori* bacteria is considered one of the most common health problems in recent years, affecting half of the world's population. Many studies have been conducted on the effects of this infection. Therefore, our study aimed to study the change in the secretion of the adrenal cortex and its relationship to blood sugar levels, in addition to studying some physiological parameters in affected women visiting outpatient clinics in Al- Diwaniyah. 34 blood samples were collected, then the level of cortisol in the blood was measured, in addition to the blood sugar level and physiological blood parameters, as well as a bacterial study that included the isolation and diagnosis of these bacteria. The results showed a decrease in the level of cortisol in the patients' blood, while blood sugar did not show a significant increase. There was also a significant increase in the number of white blood cells and a decrease in hemoglobin and red cell indicators.

The advancements made this year in the diagnosis of *Helicobacter pylori* mostly focused on endoscopy and molecular approaches. All the specimens were obtained and subjected to standard cultural and biochemical assays. Only 29 specimens successfully proliferated and were sustained on a specified culture medium. There are 16 females, accounting for 47.05% of the total, and 13 males, accounting for 38.23%. The presence of Hp1 and Hp2 specific sectors of 16s rRNA clearly observed using genetic detection techniques on a UV transilluminator at a length of 109 bp.

Keywords: *Helicobacter pylori*, Gastritis, 16s rRNA, Cortisol, Sugar, and Blood parameters.

Introduction

Helicobacter pylori (*H. pylori*) is a type of microaerophilic. These organisms have the aptitude to flourish in the extremely acidic setting of the stomach. They are well-known for causing chronic gastric inflammation and cancer ⁽¹⁾. The organism is capable of surviving under conditions of hypoxia. Remarkably, this bacterium has the ability to switch between two distinct forms based on the specific physiological demands, such as ensuring its survival in challenging environmental circumstances (such as changes in temperature or pH, extended periods without food, and exposure to antibiotics) ⁽²⁾.

Infection with the stomach bacteria *Helicobacter pylori* is considered one of the most important topics that have been dealt with by researchers in previous periods because of its wide spread, which reaches half of the population, with the presence of variation in infections between countries according to their customs and the nature of their food ⁽³⁾⁽⁴⁾⁽⁵⁾. Studies have shown that many vital processes are affected by this, the most important of which are metabolic, respiratory, and cardiac, in addition to their main pathology in the occurrence of stomach and duodenal ulcers ⁽⁶⁾⁽⁷⁾, in addition to the hormonal status being affected by the infection, including Gastrin and Motilin. Ghrelin and Histamine ⁽⁸⁾⁽⁹⁾. It has also been indicated that adrenergic hormones are affected by these bacteria ⁽⁸⁾, as well as disturbances in blood sugar levels ⁽¹⁰⁾⁽¹¹⁾. There are also some changes in blood parameters, represented by blood physiology parameters ⁽¹²⁾.

The acquisition of infection by *H. Pylori* is primarily observed during the period of childhood, with prevalence rates ranging from 25% to 50% In children without symptoms aged between ≤ 5 years and 39% to 80% in children aged above of 5 years⁽¹³⁾. *H. pylori* produces abundant virulence-factors, counting adhesins, toxins, and pro-inflammatory proteins⁽¹⁴⁾⁽¹⁵⁾.

An optimistic connection has been recognized between *H. pylori* and both duodenal-ulcer and stomach ulcer⁽¹⁶⁾, gastritis⁽¹⁷⁾, and oesophageal cancer⁽¹⁸⁾. Two procedures for identifying *H. pylori* 1-Invasive techniques including endoscopic valuation, histologic investigation, RUT (rapid urease test), and culture media. 2- Non-invasive techniques encompass UBT (urea breath test), SAT (stool antigen test), molecular diagnostic and serology procedures⁽¹⁹⁾. The PCR primer by Hp1-Hp2 is commonly utilized to amplify a 109-bp segment of the 16S rRNA gene of *H. pylori* for the purpose of detection in clinical samples⁽²⁰⁾.

The aim of study

The aim of this study is to isolate and molecular identify *H. pylori* bacteria in patients after visiting outpatient clinics in Al-Diwaniyah Governorate, in addition to studying some changes in the secretion of the adrenal gland and its relation with diabetes, and some physiological blood parameters accompanying the infection in the women.

Materials and Methods

1- Experiment Design

A- Sample collecting

A total of 34 blood samples of gastritis patients, collected from female individuals of various ages, were obtained from the outpatient clinics at Al-Diwaniyah governorate between June and August 2018.

B- Physiological study

The samples were divided into two groups: First group, include 17 samples for patients, and the second group, include 17 samples for healthy people as a control group. Each sample was isolated into two parts in different tubes, one for containing EDTA for physiological blood tests, and the other was isolated by centrifuge for serum to conduct hormone and sugar tests.

The effect of infection with *H. pylori* bacteria on the adrenal gland was measured through the level of cortisol in the blood according to⁽²¹⁾, in addition to measuring the level of blood sugar in the way indicated by⁽²²⁾, while the amount of hemoglobin in the blood and packed cell volume were measured using the method⁽²³⁾, as well as the method⁽²⁴⁾ to calculation of red blood cells, white blood cells, MCH, MCV, and MCMC in all the samples.

C- Bacterial study

For the bacterial study, 34 biopsy samples were cultured on different media, including blood agar (Columbia agar enhanced with sheep blood 5%, Bio-Mérieux) and Helicobacter-selective agar (Pylori agar, Bio-Mérieux). Collected samples are located into aseptic glass-tubes and stored at of 4°C. Once coming at the lab, if the culturing could not be continuous within a 3-hour, the samples were laid in a deep freezer at -80°C till they could be handled further. in a micro-aerobic the specimens were cultured for 15 days at 37°C consisting of O₂ 5%, 10% carbon-dioxide, and 85% N₂. They can also be sensed via positive of oxidase, catalase, and urease tests⁽²⁵⁾.

Genomic DNA and *H. pylori* 16s rRNA for PCR-amplification. chloroform/Phenol DNA-extraction technique was utilized to gain total DNA from the biopsies⁽²⁶⁾. The occurrence of *H. pylori* was strongminded by examining the DNA isolated from biopsies using the PCR technique. Specific primers were amplified a 109 bp product equivalent to the 16S rRNA region of *H. pylori*. The PCR primers are Hp1 (5'-CTG GAG AGA CTA AGC CCT CC-3') and Hp2 (5'-ATT ACT GAC GCT GAT TGT GC-3')⁽²⁶⁾. The PCR parameters for procedure as follow: 35-cycles of 30 seconds at 95°C are denatured, for 30 seconds, at 55°C annealing and for 1 minute at 72°C extension, with 72°C for 5 minutes the final extension step. The products of PCR were amplified and then separated by

electrophoresis on a 1.5% agarose gel. A DNA 100 bp ladder was used as a size marker. Ethidium bromide (500 ng/ml) was utilized for Gel staining in order to enable bands visualization. The DNA from a patient who was confirmed to be positive for *H. pylori* was utilized as a positive control. The negative control consisted of nuclease-free water obtained from Invitrogen.

Statistical analysis

Statistical analysis was performed by SPSS program according to ⁽²⁷⁾ on all the mentioned measurements, and the probability value was tested as 0.05%.

Results & Dissection

1- Physiological study

According to the statistical results of the test data, a significant decrease appeared at the probability level ($P < 0.05$) for the level of the blood cortisol hormone in patients infected with the stomach bacteria *H. pylori* when compared to the control group (Table 1), which indicates a disturbance in the functioning of the adrenal gland in patients. On the other hand, the results showed a slight, non-significant increase ($P < 0.05$) in the blood sugar level in patients infected with this bacteria when compared to the control group (Table 1).

Table 1: Cortisol and blood sugar levels for the group of patients infected with *H. pylori* and the control group. * There is a significant difference ($P < 0.05$) when comparing the two groups.

Group Test	Patient G. n(17)	Control G. n(17)
Cortisol $\mu\text{g}/\text{dl}$	3.12 \pm 1.89 *	6.68 \pm 2.95
Blood Sugar mg/dl	114.20 \pm 40.23	91.80 \pm 9.09

Some studies have indicated that there is a change in the effectiveness of the adrenal gland in patients infected with gastric bacteria, which leads to a weakness in the effectiveness of the gland in secreting its hormones. The study agreed with ⁽²⁸⁾ in the decrease in the level of the cortisol hormone in patients infected with *H. pylori* when compared with those who were not infected, while it did not agree with what was indicated by ⁽²⁹⁾ about the increase in the cortisol hormone in patients who were exposed to indigestion Dyspepsia resulting from stomach imbalances and inflammation after infection with *H. pylori* bacteria. While there was reference previously ⁽³⁰⁾⁽³¹⁾ to the dysfunction of the adrenal cortex due to mood and nervous changes, disturbance of inflammatory parameters, and constant pain due to stomach injury in patients, as well as cases of depression, imbalance in the psychological and nervous state, and lack of sleep in patients with stomach pain ⁽³²⁾ It is expected that it is the main reason for the decrease in the hormone cortisol, which is greatly affected by neural pathways, including the vagus nerve, via the HPA axis, which may cause a change in the microbiota composition and maintain the balance of the immune system in the mucous membrane, which makes the decrease in cortisol accompany a decrease in the patient's immune parameters, such as IL-6 and IL-8, which has been indicated in some studies in patients infected with *H. pylori* bacteria ⁽²⁹⁾. Here, the study indicates that low cortisol levels may be the main reason for feeling tired, weight loss, and muscle weakness.

As for blood sugar in patients, the results of our study did not agree with some researchers who indicated a significant increase in blood sugar in patients with *H. pylori* bacteria ⁽³³⁾⁽³⁴⁾⁽³⁵⁾, as it was noted that the indication in diabetic patients was an imbalance in the gastrointestinal tract. Which makes them susceptible to infection with the bacteria ⁽³⁶⁾. Therefore, this study came to prove that infection with these bacteria does not completely lead to high blood sugar and diabetes. Rather, some studies support lack of a relationship between diabetes and infection with these bacteria ⁽³⁷⁾⁽³⁸⁾⁽³⁹⁾. On the contrary, diabetic patients may be more susceptible to infection with this bacteria ⁽⁶⁾, and the relationship between diabetes and infection with gastric bacteria is unknown ⁽⁴⁰⁾. It can also be noted in this study that the low level of cortisol in patients may be a reason that prevents high blood sugar levels, contrary to what researchers indicate, which may lead in the future to Gastric carcinoma as

indicated⁽⁴¹⁾. The study also did not agree with what was stated⁽⁴²⁾ about insulin resistance in patients infected with this bacteria, which showed the opposite through the regularity of blood sugar levels in this study.

There was also a significant increase ($P < 0.05$) in our study of the number of white blood cells in patients infected with *H. pylori* bacteria, which was noticeable when compared to those who were not infected. There was also an decrease in red blood cell indicators, which included MCV, MCH and MCHC, which were significantly lower than these values in patients infected with gastric bacteria. While a non-significant decrease ($P < 0.05$) appeared in the number of red blood cells, the amount of hemoglobin in the blood, and the packed cells volume when compared statistically to the control group representing non-infected people (Table 2).

Table 2: Physiological blood parameters for the group of patients infected with *H. pylori* and the control group. * There is a significant difference ($P < 0.05$) when comparing the two groups.

Group Test	Patients n(17)	Control n(17)
WBCs	8.25 ± 1.24 *	6.52 ± 2.13
RBCs	4.04 ± 0.20	4.16 ± 0.56
Hb g/dl	10.44 ± 1.82	11.88 ± 1.25
PCV %	33.68 ± 5.67	35.18 ± 4.47
MCV mm ³	76.38 ± 11.30 *	84.44 ± 3.93
MCH g/dl	23.62 ± 3.65 *	28.68 ± 1.67
MCHC g/dl	30.92 ± 0.97 *	34.04 ± 1.65

These bacteria have the ability to secrete substances that help them adhere to the epithelial cells of the mucosa layer in the stomach by distinguishing certain carbohydrate structures, most of which are present in blood type B, which helps them do this, also, the increase in white blood cells may attributed to the ulcers that occur in the stomach and intestine as a result of infection with this bacteria, which has been frequently referred to in previous studies⁽⁴³⁾, with reference to the presence of peptic ulcer disease that accompanied the increase. IL-8 as a result of injury. While the results did not support what was previously mentioned⁽⁴⁴⁾ that there was no significant difference in the number of white blood cells for those infected with this bacterium when compared to those who were not infected. On the other hand, the deficiency in the level of iron and vitamin B12 in the blood and the deficiency of some nutrients due to the disorder of the stomach layers, in addition to the metabolic and pulmonary disturbance that occur may be the main reason for the decrease in the quantity and volume of red cells and blood hemoglobin⁽⁴⁵⁾ Here, the results of our study support what was reported by⁽⁴⁶⁾ of a significant decrease in the amount of hemoglobin in cells due to MCH and MCV for those infected, while it differed from him in the presence of a significant difference in the percentage of MCHC in patients, which indicated the opposite, and that some of these differences were in blood parameters. Physiological impairment is attributed to a defect in the immune capacity of patients as a result of an number of neutrophils and monocytes, with a decrease in some cytokines, according to what was indicated⁽⁴⁷⁾⁽⁴⁸⁾.

2- Bacterial study

A- Sampling and species identification

A total of 34 biopsies were taken from patients with gastritis who met the specified criteria. Out of the endoscopic specimens, 6 showed no growth. Among the remaining specimens, 16 (47.05%) were from females and 13 (38.23%) were from males (Table 3). The results bare a significant ($p < 0.05$) *H. pylori* infection incidence 85.29%. The age group 36 - 45 had the highest occurrence among all seven groups (23.3%). There are no statistical differences ($p < 0.05$) conducted when correlated with age and sex. The samples were sent to the laboratory and cultivated on specific enrichment and selective media

that were previously described in order to promote the growth of *H. pylori* isolates. Colonies are characterized by their Gram-negative helical structure and can be identified by their tiny, translucent to yellowish appearance.

Table 3: *H. pylori* incidence according to age groups

Age group	No. of Specimens	Female	Male
≤ 15	1	16	13
16 – 25	3	47.05%	38.23%
26 – 35	5		
36 – 45	7		
46 – 55	6		
56 – 65	3		
66 ≥	5		

B- PCR amplification of *H. pylori* 16s rRNA.

Utilizing molecular amplification of specific sections of the 16S rRNA can serve as a reliable method for confirming the presence of *H. pylori*. This is particularly useful given the range of serological and non-specific testing available. The gel electrophoresis of 29 samples in figure (1) revealed that Hp1 and Hp2 were both positive at 109 bp, indicating the presence of *H. pylori* in cases with gastritis with a sensitivity of 95%.

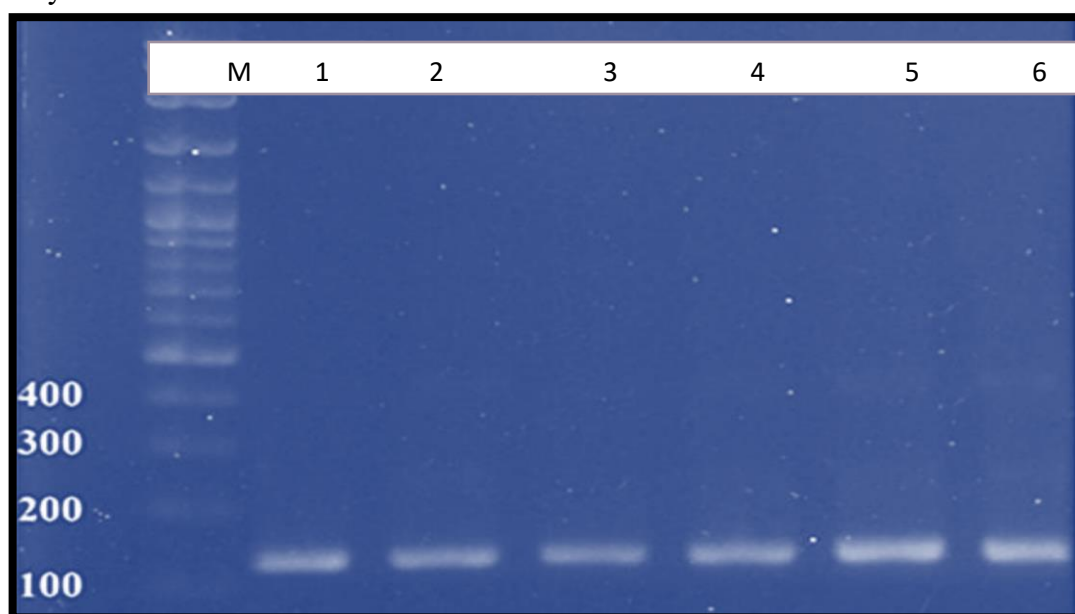


Figure 1: Amplification of Hp1 & Hp2 specific regions and visualized products at 109 bp. Lane M: 100 bp ladder. 1,2,3,4,5,6 *H. pylori* isolates.

The findings of ⁽⁴⁹⁾ indicated that the prevalence of *H. pylori* tends to rise with age. This aligns with the observed increase in *H. pylori* infection among those aged 30 and above in the present investigation. Our results clearly demonstrate that *H. pylori* prevalence is considerable, as indicated by research conducted in several Asian nations, including Bangladesh where it is reported to be 59.5% ⁽⁵⁰⁾, India 79% through using ELISA, 84% via PCR in Pakistan, and 41% in Japan by anti-*H. pylori* antibody levels measurement in urinary samples was also reported high ⁽⁵¹⁾⁽⁵²⁾. The disparity in *H. pylori* infection rates between developing countries and the industrialized world may be attributed to unfavorable socioeconomic situations and unclean lifestyles ⁽⁵³⁾.

DNA-based approaches are capable of detecting both active *H. pylori* and coccoid forms that may arise due to stress circumstances, such as those generated by antibiotics. This makes urease-based

detection methods worthless⁽⁵⁴⁾. Further research is being conducted on molecular methods for detecting *H. pylori* infection in cases of gastritis. This ongoing investigation has revealed new aspects of the infection, which has stimulated further research in this field. Like conducted in the use of 16S rRNA sequencing by NGS (Next-Generation Sequencing) for identifying *H. pylori* infections. The presence of *H. pylori* in gastric biopsy samples was determined using a 16S rRNA PCR assay. The expected PCR result of this investigation, which has been previously confirmed by other studies, was also discovered at 109 bp⁽⁵⁵⁾⁽⁵⁶⁾.

Conclusion

It can be concluded from this study that infection with *H. pylori* causes a disturbance in the adrenal cortex secretion through a decrease in cortisol, which prevents high blood sugar in patients. It was also concluded that physiological blood parameters were not significantly affected by the infection. Also, the molecular detection is cornerstone in *H. pylori* infections because there are many serological and cultural methods with low specificity so in current study, we argue the genomic detection.

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