International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Investigating the Distribution of Actinobacteria spp in Gastric Adenocarcinoma Patients with Helicobacter Pylori

Ihab Mohamed Taher[1], Mohammed Hachim Nasser[2]

[1]Al-Muthanna Health Department
Email: *ehabmuhamed8554[at]gmail.com*

[2]Al-Muthanna Governorate
Email: *aabbass12212[at]gmil.com*

Abstract: ***Background: Adenocarcinoma is the most common type of stomach cancer that arises from the epithelial glands of the stomach. Most gastric adenocarcinomas are diagnosed in advanced stages and therefore the mortality rate in them is high. Stomach cancer is the second most common cause of cancer death worldwide, accounting for more than one million deaths each year. Infection with H.pylori is a major predisposing factor for gastric adenocarcinoma. Infection with this bacterium causes chronic and persistent stomach inflammation by inducing the expression of inflammatory cytokines that play an important role in stomach cancer. In addition to host factors, H. pylori pathogenic factors increase the risk of gastric cancer, especially cagA, which is encoded by the cagA gene. The purpose of this research is to investigate the role of Actinobacteria infection in gastric adenocarcinoma in patients with Helicobacter pylori. Research method: In this study, in the patient group, there were 24 (75.0%) men and 8 (25.0%) women, and in the control group, there were 10 (50.0%) men and 10 (0.0 50 percent) of women were examined. In order to investigate Actinobacteria and Helicobacter pylori infections, RT-PCR method was used. Results: The obtained results showed that 81.3% of patients were positive for H.Pylori (Cag-A+) and 18.3% were negative for H.Pylori (Cag-A+). Therefore, most of the patients were positive for H. Pylori. Also, sick people were examined for the level of Actinobacteria. The obtained results showed that 37.5% of sick people are positive for Actinobacteria. Conclusion: Since most patients (81.3%) are positive for H. Pylori (Cag-A+), this result shows that H. Pylori plays an important role in the occurrence of gastric adenocarcinoma. Also, 37.5% of the studied patients are also positive for Actinobacteria, which shows that this bacterium can also play an important role in the progression of the disease.***

Keywords: gastric adenocarcinoma, Helicobacter pylori, Actinobacteria

1. Introduction

Gastric malignancy is one of the most important types of cancer and the most common fatal malignancy of the gastrointestinal tract, and despite a major decrease in the incidence of deaths caused by it in the last few decades, this disease is still the fourth most common cancer and the second most common cause of cancer death. remained in the world. Although stomach cancer is a global problem, nearly two-thirds of the cases of this cancer occur in developing countries (Gotoda, 2005). Despite recent advances in the diagnosis and treatment of gastric cancer, the long-term survival rate for patients with advanced gastric cancer is low because these patients often receive medical attention when they are in advanced stages of the disease (Gotoda et al., 2009) Therefore, there are no effective treatments for those suffering from this disease. Therefore, clarifying the molecular mechanisms of gastric carcinogenesis will be useful to increase the prevention, diagnosis and treatment of this disease. In many studies conducted on gastric cancer, it was shown that this cancer is a multi-gene disease and its occurrence has a complex multi-step process that includes disruption of the genetic order of proto-oncogenes and tumor suppressor genes, and recently has entered the field of miRNAs. miRNAs can act as a new type of oncogenes and tumor suppressor genes, and their positive or negative aberrant regulation and their targets are involved in various stages of cancers, including tumor growth, angiogenesis, apoptosis, and metastasis (Yasui et al., 2011). Stomach cancer is the second most common cancer in men and the fourth in women in developing countries, but the death rate is second only to lung cancer, which can be argued because of the late diagnosis of this disease. Stomach cancer is diagnosed in less than 10% of cases in the early stages, and this late diagnosis may lead to the progression of the disease. Metastasis of lymphatic, abdominal or even distant organs, as well as reducing the survival time of patients with this disease (Tanizawa and Terashima, 2010). In order to determine the optimal treatment strategy, it is necessary to perform detailed work such as chemical endoscopy or virtual staining and echo-endoscopy (Gotoda, 2005). Stomach surgery and tumor removal is one of the common treatment methods of this disease. Although surgery is the standard treatment for stomach cancer, it has been seen that it leads to poor results, hence a clear understanding of gastric carcinogenesis and the identification of new molecular markers in order to Improving the management of gastric cancer is essential (Carneiro, 2012). Adenocarcinoma is the most common type of stomach cancer that arises from the epithelial glands of the stomach. According to Iauren's histological classification, adenocarcinoma is divided into two types, diffuse and intestinal, each of which has different epidemiological and diagnostic characteristics. Its intestinal type forms a pseudo-glandular structure and its diffuse form has a sieve-like nature. Intestinal form usually develops in older people from primary damage and spreads through blood vessels to the liver. Sporadic form of gastric adenocarcinoma occurs in any age group (Lerner, 2020). Most gastric adenocarcinomas are diagnosed in advanced stages and therefore the mortality rate in them is high. Its

peripheral form is more closely related to environmental factors, and cancers of this type acquire a distinct pattern of genetic disorders. The diffuse form is more related to hereditary factors. In general, about 10% of stomach cancers occur in family groups and 1-3% are hereditary in nature. The most well-known familial syndrome of gastric cancer is hereditary diffuse gastric cancer (HDGC) (Oliveir et al., 2004). According to the estimate of the National Health Organization, the incidence of stomach cancer in Iran was 26.1 and 11.1 per 100, 000 people in men and women, respectively. It is also stated in the annual reports that in Iran, 22% of every 5, 000 deaths caused by cancer are related to stomach cancer, which is considered the second most common cancer (14%) and the fourth in women (7%) (Rugge et al., 2015). *Helicobacter pylori* is one of the successful pathogens that infects the epithelial cells of the stomach and has been classified as a first class carcinogen by the World Health Organization. In developed countries, *H. Pylori* infection is less than 50% of the adult population, and in developing countries, the rate of this infection is higher and covers nearly 80% of the adult population. This gram-negative bacillus can be colonized in the lower layers of the human stomach mucosa and survives in the stomach for a long time due to its high ability to adapt to the environment. Helicobacter pylori have several pathogenic factors such as urease, flagellum, BabA, VacA, CagA, HpaA, OipA, LPS, DupA, IceA, AlpA/AlpB, SabA, etc. It binds to the receptors of gastric epithelial cells and exerts its pathogenicity with other factors. Table No. 1-1 clearly shows the pathogenic factors of Helicobacter pylori from OipA, including BabA, VacA, CagA, and their relationship between diseases and cancer in different sources. Helicobacter pylori can cause diseases such as: duodenal ulcer, stomach ulcer, stomach cancer and lymphoma of lymphoid tissue along with gastric mucosa. Among the diseases caused by this bacterium, cancer is very important (Sugano , 2019).

2. Materials andMethods

Sample collection

In this case-control study, samples were collected along with a questionnaire on patients with gastric cancer, including (adenocarcinoma, lymphoid and non-lymphoid), following ethical rules. In this study, samples were taken from healthy and tumor cells of the stomach tissue of patients referred to 22 Bahman Hospital and Razavi Hospital in Mashhad and transferred to the laboratory of Shahin Far Medical School.

Inclusion criteria for patients included

- Patients with stomach cancer, including (adenocarcinoma, lymphoid and non-lymphoid)
- Appropriate concentration of DNA and RNA
- cDNA with suitable concentration to perform PCR

Study exclusion criteria

- Insufficient tissue volume in the paraffin block
- Defects in patients' files
- Presence of any other unreported malignancies in patients

Patients with immune system disorders

RT-PCR method

DNA extraction was performed using Biogen company kit. Then, DNA concentration and its purity were evaluated by reading DNA absorption with a nanodrop spectrophotometer. After confirming the concentration and quality of DNA, the severity of viral and bacterial infections and Helicobacter pylori was checked by Real-time PCR method. At the same time, the pathological examination was performed by a paletologist and the studied variables were determined in the patient group. In this method, RT-PCR was performed on all samples. The chemicals used in conventional PCR are shown in Table 1

Table 1: Chemicals used in PCR method

Applied volume	Chemicals
5 µl	Master mix 2x
0.5 µl	Primer 10pmol
10 nmol	DNA
maximum 10 µl	H_2O

The typical PCR plan is shown in table 2

Table 2: The typical PCR plan

45 cycles	15 min	95 °
	30 sec	95 °
	30 sec	58 °
	20 sec	72 °
	10 min	72 °

Primer

The desired primer sequence is also shown in table 3

Table 3: Primer sequence in RT-PCR method

Gene	Forward	Rivers
GAPDH	5'-ATGGGGAAGGTGAAGGTCG-3'	5'-GGGGTCATTGATGGCAACAATA-3'
H.Pylori (CAG)	5'-GTTGATAACGCTGTCGCTTC-3'	5'-GGGTTGTATGTAATTTTCCTAAA-3'
H.Pylori (VAG) s1/s2	5'-ATGGAAATACAACAAACACAC-3'	5'-CTGCTTGAATGCGCCAAAC-3'
H.Pylori (VAG) m1/m2	5'-CAATCTGTCCAATCAAGCGAG-3'	5'-GCGTCAAAATAATTCCAAGG-3'

Statistical analysis of data

In the description of the data, appropriate statistical tables and indices such as the mean, etc. have been used, and in the data analysis, the normality of the data has been investigated using the Shapiro-Wilk test. The software used in this

research is SPSS v.25 and the significance level of the tests is considered to be less than 5%.

3. Results and Discussion

Distribution of H.pylori (Cag-A+)

The results of the distribution of *H. pylori* (Cag-A+) in Figure 1 show that 81.3% of patients were positive for *H.pylori* (Cag-A+) and 18.8% were negative for *H.Pylori* (Cag-A+) as showed in figure 1 and table 4, this finding agreed with (Hanafiah et al., 2020) that found A total of 96.6% (n = 85) of the isolates were *cag*PAI-positive. Five genes in the *cag*PAI region (*cag1*, *cag5*, *cag6*, *cag8* and *cag21*) were detected in all isolates whereas *cag*2 was detected in 34.1% (n = 29) and *cag14* in 51.7% (n = 44) of the isolates. Detection of other genes ranged from 69.4 to 98.8%. also agreed with (Khodadadi et al., 2020) that collected Thirty-four *H. pylori* gastric biopsies taken from Western Iranian patients that were diagnosed as gastritis, gastric ulcers, and adenocarcinoma were used that showed showed that 86.8% of the samples were H. pylori-positive. Moreover, the cagA gene prevalence was 51.50% in the samples. In addition, the adenocarcinoma outcome was significantly related to all selected genes. Likewise, some gastric diseases such as gastric ulcers, duodenal ulcer (DU), gastritis, lymphoid, and gastroesophageal reflux disease (GERD) were observed in adenocarcinoma cases. The comparison of cagA in East Asian countries and western countries showed that gastric cancer is more strongly associated with H. pylori strains carrying East Asian cagA in geographical regions where two different strains coexist (Wen et al., 2009). In this regard, the frequency of the cagA gene in Malaysia was reported to be higher than 94% (Souod et al., 2013). In addition, cagA prevalence in China and neighboring countries such as India was reported to be 93.2% and 96.2%, respectively (Aziz et al., 2014). Such a variety in the cagA gene frequency in different countries is due to differences in the infected population, geographic condition, and strains' genetic diversity. Kamogawa-Schifter et al.'s study (Kamogawa-Schifte et al., 2018) indicated that the prevalence of the cagA gene in people suffering from duodenal ulcers and gastric cancer was 78% and 86%, respectively. Similarly, the 65.50% frequency of cagA was found in peptic ulcer isolates in a study carried out in Turkey (Bahadori et al., 2017) Furthermore, cagA pervasiveness in Mexican patients was reported to be 57% in chronic gastritis, 58.3% in gastric cancer, and 61.4% in gastric ulcer (Roman-Roman et al., 2017).

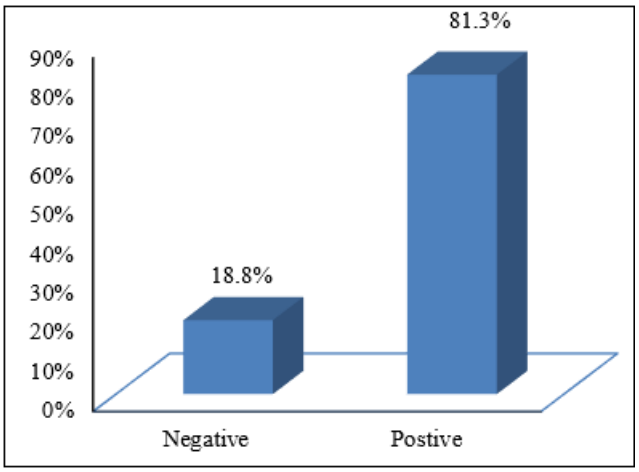

Figure1: Frequency distribution of H.pylori (Cag-A+)

Table 4: Distribution of H.pylori (Cag.A +)

H.pylori (Cag-A+)	n	%
negative	6	18.8%
positive	26	81.3%
total	32	100.0%

Distribution of Actinobacteria

The results of Actinobacteria distribution in figure 2 show that 37.5% of people were positive for Actinobacteria and 62.5% were negative for Actinobacteria. Recent evidence linking non-*Helicobacter* organisms with chronic gastritis includes studies in which the pH increases in response to treatment with histamine 2 (H2) receptor antagonists or proton pump inhibitors (PPIs)(Stockbruegger et al., 1985) A bacterial mixture consisting of *Acinetobacter* spp., *Pseudomonas* spp. and *Corynebacterium* spp. has been shown to stimulate gastrin and IL-8 (Ofori-Darko, et al., 2000) *A. lwoffii* not only colonizes the mouse stomach, but also induces hypergastrinemia, gastritis and increases gastric epithelial cell numbers, as observed with *H. pylori*(Zavros et al., 2002).

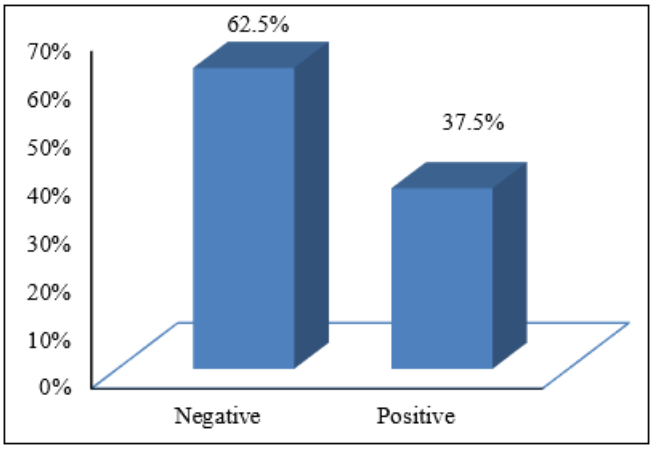

Figure2: Frequency distribution of Actinobacteria

Relationship between age of patients and Actinobacteria

The results of the relationship between patient age and Actinobacteria in figure 3 and table 5 showed that there is no significant difference (Student's test) between the average Actinobacteria of the two groups (P-Value=0.451). these findings were agreed with (Li et al., 2022) that studied potential association of the gut microbiota

composition, especially the abundance of *Actinobacteria*, as well as the differentiation of functional and resistance genes with age that found total of 11 phylum abundances in each group, of which only *Synergistetes* was significantly enriched in elderly subjects ($P = 0.013$), but interestingly were not detected in young adults. The top 5 phyla were *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Verrucomicrobia*. Compared with elderly subjects, only the relative abundance of *Firmicutes* and *Actinobacteria* in young adults seemed to be higher and the relative abundance of *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia* appeared to be lower, but there statistical significance Jeffery et al. reported that the abundances of core microbiota such as *Bacteroides*, *Alistipes* and *Parabacteroides* were greater in elderly subjects, with the most diverse microbiota being *Coprococcus*, *Prevotella* and *Catenibacterium*, which were easily affected by diet [Perez-Cobas et al., 2003]

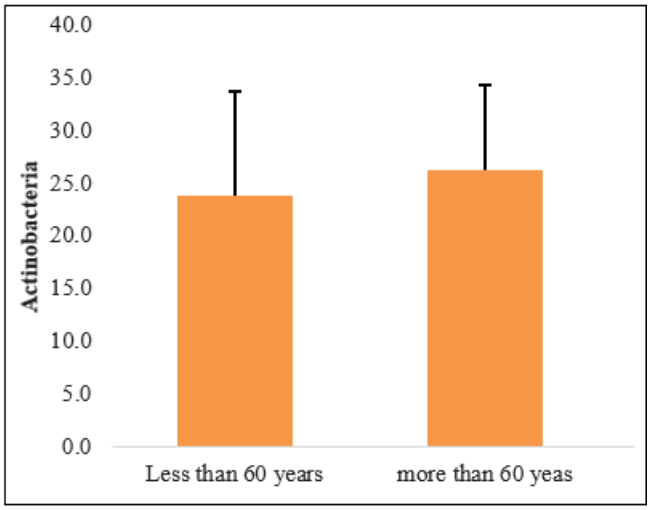

Figure3 Relationship of age with Actinobacteria

The relationship between gender of patients and Actinobacteria

As can be seen in table 5, there is no significant difference (Student's test) between the average Actinobacteria of the two groups (P-Value=0.678). Shin et al. (2019) looked at the connection between gut bacteria and women's and men's serum levels of estradiol and testosterone. Using 16s rRNA gene sequencing, the fecal microbiota of 57 men (n = 31) and women (n = 26) was evaluated. Participants were divided into three groups: Low, Medium, and High, according to the men's and women's serum testosterone and estradiol levels, respectively. There was a strong correlation between testosterone levels and the abundance of Acinetobacter, Dorea, Ruminococcus, and Megamonas in men. These findings give basic information useful for creating communication networks between human and microbial populations and show a correlation between sex steroid hormone levels and gut microbial diversity and composition.

Table 5: Relationship between gender and Actinobacteria

Gender	Mean	SD	min	max	Test statistics P-Value
Female	24.06	9.77	11.08	36.78	t=0.42
Male	25.59	8.68	0.205	11.29	P-Value=0.678

Tumor stage Relationship with Actinobacteria

As can be seen in figure 4, there is a significant difference between the Actinobacteria average of the three groups (P-Value=0.001**). Also, the result of pairwise comparisons using Tukey's method shows that there is a statistically significant difference between II-IV and IV-III (P<0.05*).

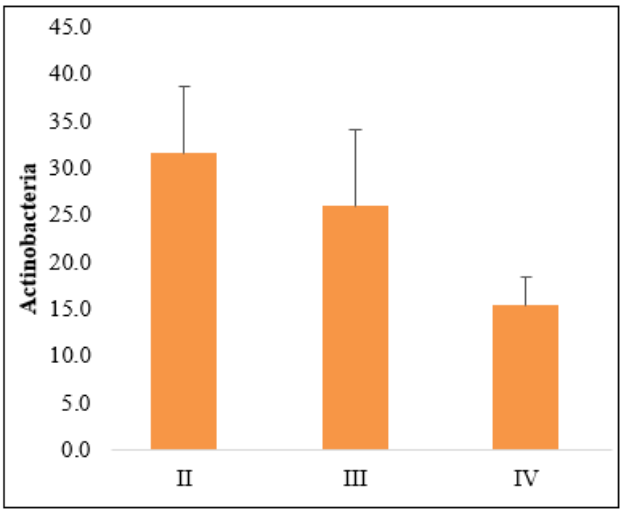

Figure4: Stage relationship with Actinobacteria

Reduced levels of Corynebacterium, Lachnoanaerobaculum, and Halomonas were recently found in lung tumor samples from patients with lung cancer (Najafi et al., 2021). On the other hand, a study conducted by Apopa and associates found that lung cancers had higher concentrations of Actinobacteria, Firmicutes, Cyanobacteria, Acidobacteria, and Chloroflexi. Furthermore, with relative abundances of 57% and 24%, respectively, the phyla Bacteriodetes and Proteobacteria predominated in lung cancer samples (Apopa et al., 2018). 89 patients with non-small cell lung cancer (NSCLC) had lung tumor samples sequenced using 16S rRNA, which revealed that Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes predominated in the tumors and surrounding tissue samples. However, at the phylum level, no notable variations were found. Pseudomonas, Burkholderia, and Aquabacterium were less prevalent in late-stage cancers than in early-stage tumors, whereas Corynebacterium, Sphingomonas, Streptococcus, Neisseria, Halomonas, Kocuria, Parvimonas, and Rothia were more prevalent (Kovaleva et al., 2020). According to Yu et al., non-malignant lung samples had a larger alpha diversity than malignant ones. Eighty percent of lung tissues that were not malignant had Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria. The findings revealed that non-malignant lung tissue samples contained Acinetobacter, Pseudomonas, Ralstonia, and two unidentified genus-level groupings. Adenocarcinoma samples showed lower Ralstonia and higher Thermus than squamous cell carcinoma samples. Thermus levels were shown to be greater in stages IIIB and IV of lung cancer (Yu et al., 2016).

Relationship between H. pylori andActinobacteria with Tumor grade

Figure 7 shows the relationship between Actinobacteria and tumor grade in patients with positive and negative *H. pylori*. The average of Actinobacteria in grade one patients with positive and negative *H. pylori* is 25.96 and 29.89 respectively, the average of Actinobacteria in grade two

patients with positive and negative *H. Pylori* is 22.23 and 34.17 respectively, and the average Actinobacteria in grade three patients with positive and negative *H. pylori* is equal to 18.81 and 0, respectively.and negative *H. pylori* is 22.23 and 34.17 respectively, and the average Actinobacteria in grade three patients with positive and negativeH. Pylori is .equal to 18.81 and 0, respectively

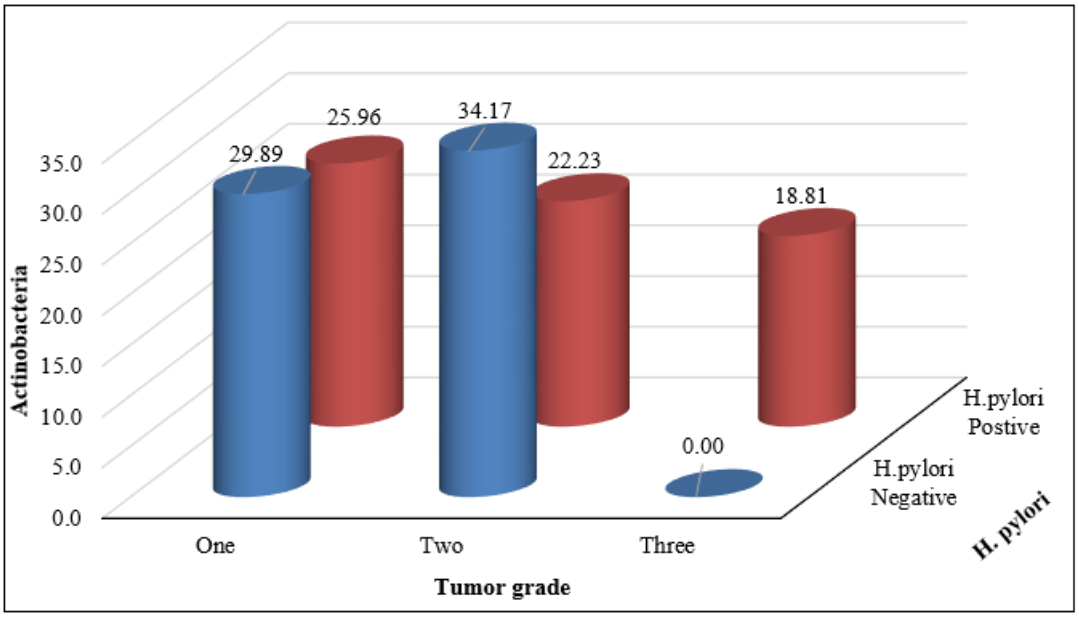

Figure 7: Relationship between H. pylori and Actinobacteria with Tumor grade

Infection with *H.pylori* is a major predisposing factor for gastric adenocarcinoma. Infection with this bacterium causes chronic and persistent stomach inflammation by inducing the expression of inflammatory cytokines that play an important role in stomach cancer. In addition to host factors, *H. pylori* pathogenic factors increase the risk of gastric cancer, especially cagA, which is encoded by the cagA gene. Different studies have shown that people who are infected with Helicobacter strains with cagA protein containing the EPIYA-D motif or a large number of EPIYA-C motifs, compared to people who are infected with Helicobacter strains lacking this protein, have a high risk of contracting They have stomach cancer(Keikha & Karbalaei, 2021). The pathogenic complexity of Helicobacter pylori is due to the fact that, on the one hand, by having a series of factors, it induces apoptosis, and on the other hand, it has factors that induce cell proliferation. In order to cause disease, the most important event is the initial binding to the receptors on the surface of the host cell(Olivares et al., 2005)

In terms of the gastric microbiota, the human stomach was formerly thought to be a sterile organ because of its high acidity (Espinoza et al., 2018). Nonetheless, H. pylori can live in the stomach's extremely acidic environment and colonize the human gastric mucosa (Schulz et al., 2015). Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, and Proteobacteria are the main microbes in the healthy human stomach environment, according to the unique features of the gastric microbiota that have been discovered with the development of new methods for analyzing the microbial community (Guo et al., 2020; Guo et al., 2021). Because H. pylori dominates the stomach in people with the infection, non-H. pylori microbial abundances significantly decline (Das et al., 2017). Other symptoms of stomach microbiota dysbiosis have been discovered in addition to the alterations in microbial composition. In terms of microbial alpha diversity, Gantuya et al. found that H. pylori-infected patients had significantly lower microbial diversity than those who were H. pylori-negative (Gantuya et al., 2019). Helicobacter abundance and the diversity of the stomach microbiome were found to be negatively correlated in another investigation (Das et al., 2017). H. pylori infection causes changes in the organization of the microbial community (beta diversity) in addition to microbial alpha diversity. A population-based investigation found that the beta diversity clearly distinguished between the H. pylori positive and negative groups (Llorca et al., 2017). Additionally, research on the ecological interactions of bacteria in the stomach environment revealed changes in the relationships between H. pylori and other microbes. In particular, network analyses revealed that Helicobacter had adverse interactions with other gastric microbiome microorganisms, according an Indian study employing 16S rRNA gene sequencing (Das et al., 2017); Similar results were observed by another Chinese investigation (Guo et al., 2020). In terms of the quantity of interactions, Coker et al. discovered that an H. pylori infection lowers the quantity of interactions with the gastric microbiota (Coker et al., 2017).

4. Conclusion

Since most patients (81.3%) are positive for H. pylori (Cag-A +), this result indicates that H. pylori has an important role in the development of gastric adenocarcinoma. Also, 37.5% of the studied patients are positive for Actinobacteria, which showed that this bacterium can also play an important role in disease progression.

References

[1] Gotoda, T. Endoscopic resection of early gastric cancer. Gastric Cancer 10, 1–11 (2007).

[2] Gotoda, T., Yamamoto, H. & Soetikno , R. Endoscopic submucosal dissection of early gastric cancer. J Gastroenterol 41, 929–942 (2006).
[3] Yasui, W., Oue, N., Aung, P. et al. Molecular-pathological prognostic factors of gastric cancer: a review. Gastric Cancer 8, 86–94 (2005).
[4] Park, YM., Cho, E., Kang, HY. et al. The effectiveness and safety of endoscopic submucosal dissection compared with endoscopic mucosal resection for early gastric cancer: a systematic review and metaanalysis . Surg Endosc 25, 2666–2677 (2011).
[5] Tanizawa, Y., Terashima, M. Lymph node dissection in the resection of gastric cancer: Review of existing evidence. Gastric Cancer 13, 137–148 (2010).
[6] Carneiro, F. Hereditary gastric cancer. Pathology 33, 231–234 (2012).
[7] Lerner, BA, Llor, X. Genetic Gastric Cancer Risk Syndromes. Curr Treat Options Gastro 18, 604–615 (2020).
[8] Oliveira, C., Suriano, G., Ferreira, P. et al. Genetic Screening for Familial Gastric Cancer. Hereditary Cancer Clin Pract 2, 51 (2004).
[9] Rugge M., Fassan M., Graham D. (2015) Epidemiology of Gastric Cancer. In: Strong V. (eds) Gastric Cancer. Springer, Cham.
[10] Sugano, K. Effect of Helicobacter pylori eradication on the incidence of gastric cancer: a systematic review and meta-analysis. Gastric Cancer 22, 435–445 (2019).
[11] Hanafiah, A., Razak, S. A., Neoh, H. M., Zin, N. M., & Lopes, B. S. (2020). The heterogeneic distribution of Helicobacter pylori cag pathogenicity island reflects different pathologies in multiracial Malaysian population. Brazilian Journal of Infectious Diseases, 24(6), 545-551.
[12] Khodadadi, P., Kargar, M., Bijanzadeh, M., Doosti, A., & Aghaei, S. (2020). Association of cagA, cagC, virB2, and vacA Subtypes of Helicobacter pylori with Adenocarcinoma Development in Iranian Patients. Jundishapur Journal of Microbiology, 13(7).
[13] Wen S, Moss SF. Helicobacter pylori virulence factors in gastric carcinogenesis. Cancer Lett. 2009;282(1):1-8. [PubMed ID: 19111390]. [PubMed Central ID: PMC2746929].https://doi.org/10.1016/j.canlet.2008.11.016.
[14] Souod N, Kargar M, Doosti A, Ranjbar R, Sarshar M. Genetic Analysis of Helicobacter PyloriIsolates and Their Relationship with Gastroduodenal Diseases in the West of Iran. Iran Red Crescent Med J. 2013;15(5):371-5. [PubMed ID: 24349721]. [PubMed Central ID: PMC3838643].https://doi.org/10.5812/ircmj.3732.
[15] Aziz F, Chen X, Yang X, Yan Q. Prevalence and correlation with clinical diseases of vacAgenotype among gastric patients from Northeast China. Biomed Res Int. 2014;2014:142980. [PubMed ID: 24949419]. [PubMed Central ID: PMC4052682].https://doi.org/10.1155/2014/142980.
[16] Kamogawa-Schifter Y, Yamaoka Y, Uchida T, Beer A, Tribl B, Schoniger-Hekele M, et al. Prevalence of Helicobacter pylori and its CagA subtypes in gastric cancer and duodenal ulcer at an Austrian tertiary referral center over 25 years. PLoS One. 2018;13(5). e0197695. [PubMed ID: 29813089]. [PubMed Central ID: PMC5973618].https://doi.org/10.1371/journal.pone.0197695.
[17] Bahadori A, Somi MH, Doran F, Eftekharsadat AT. Determination of correlation between principal genotypes of vacA genotypes and clinical out come in patients suffering from active chronic gastritis and gastric adenocarcinoma from Iran and Turkey. Biomed Res Int. 2017;28(4):1743-8.
[18] Roman-Roman A, Martinez-Carrillo DN, Atrisco-Morales J, Azucar-Heziquio JC, Cuevas-Caballero AS, Castanon-Sanchez CA, et al. babA2 increase the risk of ulcer and gastric cancer in patients from Southern Mexico. Gut Pathog. 2017;9:18. [PubMed ID: 28413454]. [PubMed Central ID: PMC5390388]. https://doi.org/10.1186/s13099-017-0167-z.
[19] Stockbruegger, R. W. (1985). Bacterial overgrowth as a consequence of reduced gastric acidity. Scandinavian Journal of Gastroenterology, 20(sup111), 7-15.
[20] Ofori-Darko, E., Zavros, Y., Rieder, G., Tarlé, S. A., Van Antwerp, M., & Merchant, J. L. (2000). An OmpA-like protein from Acinetobacter spp. stimulates gastrin and interleukin-8 promoters. Infection and immunity, 68(6), 3657-3666.
[21] Zavros, Y., Rieder, G., Ferguson, A., & Merchant, J. L. (2002). Gastritis and hypergastrinemia due to Acinetobacter lwoffii in mice. Infection and immunity, 70(5), 2630-2639.
[22] Li, J., Si, H., Du, H., Guo, H., Dai, H., Xu, S., & Wan, J. (2021). Comparison of gut microbiota structure and Actinobacteria abundances in healthy young adults and elderly subjects: a pilot study. BMC microbiology, 21, 1-10.
[23] Perez-Cobas AE, Gosalbes MJ, Friedrichs A, Knecht H, Artacho A, Eismann K, et al. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut. 2013;62(11):1591–601
[24] Shin, J. H., Park, Y. H., Sim, M., Kim, S. A., Joung, H., & Shin, D. M. (2019). Serum level of sex steroid hormone is associated with diversity and profiles of human gut microbiome. Research in microbiology, 170(4-5), 192-201.
[25] Najafi S, Abedini F, Azimzadeh Jamalkandi S, Shariati P, Ahmadi A, Gholami Fesharaki M. The composition of lung microbiome in lung cancer: A systematic review and meta-analysis. BMC Microbiol (2021) 21(1):315. doi: 10.1186/s12866-021-02375-z [DOI] [PMC free article] [PubMed] [Google Scholar]
[26] Apopa PL, Alley L, Penney RB, Arnaoutakis K, Steliga MA, Jeffus S, et al. Parp1 is up-regulated in non-small cell lung cancer tissues in the presence of the cyanobacterial toxin microcystin. Front Microbiol (2018) 9:1757. doi: 10.3389/fmicb.2018.01757 [DOI] [PMC free article] [PubMed] [Google Scholar]
[27] Kovaleva O, Podlesnaya P, Rashidova M, Samoilova D, Petrenko A, Zborovskaya I, et al. Lung microbiome differentially impacts survival of patients with non-small cell lung cancer depending on tumor stroma phenotype. Biomedicines (2020) 8(9)349. doi: 10.3390/biomedicines8090349
[28] Yu G, Gail MH, Consonni D, Carugno M, Humphrys M, Pesatori AC, et al. Characterizing human lung

tissue microbiota and its relationship to epidemiological and clinical features. Genome Biol (2016) 17(1):163. doi: 10.1186/s13059-016-1021-1 [DOI] [PMC free article] [PubMed] [Google

[29] Keikha, M., & Karbalaei, M. (2021). EPIYA motifs of Helicobacter pylori cagA genotypes and gastrointestinal diseases in the Iranian population: a systematic review and meta-analysis. New Microbes and New Infections, 41, 100865.

[30] Olivares, D. G. J. P. P. M. J., Gisbert, J. P., & Pajares, J. M. (2005). Helicobacter pylori infection and gastric mucosal epithelial cell apoptosis. Revista Espanola de Enfermedades Digestivas, 97(7), 505.

[31] Espinoza J. L., Matsumoto A., Tanaka H., Matsumura I. (2018). Gastric microbiota: An emerging player in helicobacter pylori-induced gastric malignancies. Cancer Lett. 414, 147–152. doi: 10.1016/j.canlet.2017.11.009.

[32] Schulz C., Koch N., Schutte K., Pieper D. H., Malfertheiner P. (2015). H. pylori and its modulation of gastrointestinal microbiota. J. Dig Dis. 16 (3), 109–117. doi: 10.1111/1751-2980.12233 [DOI] [PubMed] [Google Scholar]

[33] Guo Y., Zhang Y., Gerhard M., Gao J. J., Mejias-Luque R., Zhang L., et al. (2020). Effect of helicobacter pylori on gastrointestinal microbiota: A population-based study in linqu, a high-risk area of gastric cancer. Gut 69 (9), 1598–1607. doi: 10.1136/gutjnl-2019-319696 [DOI] [PMC free article] [PubMed]

[34] Guo Y., Li X., Wang Z., Yu B. (2021). Gut microbiota dysbiosis in human hypertension: A systematic review of observational studies. Front. Cardiovasc. Med. 8 (414). doi: 10.3389/fcvm.2021.650227 [DOI] [PMC free article] [PubMed] [Google Scholar]

[35] Das A., Pereira V., Saxena S., Ghosh T. S., Anbumani D., Bag S., et al. (2017). Gastric microbiome of Indian patients with helicobacter pylori infection, and their interaction networks. Sci. Rep. 7 (1), 15438. doi: 10.1038/s41598-017-15510-6 [DOI] [PMC free article] [PubMed] [Google Scholar]

[36] Gantuya B., El-Serag H. B., Matsumoto T., Ajami N. J., Oyuntsetseg K., Azzaya D., et al. (2019). Gastric microbiota in helicobacter pylori-negative and -positive gastritis among high incidence of gastric cancer area. Cancers (Basel) 11 (4). doi: 10.3390/cancers11040504 [DOI] [PMC free article] [PubMed] [Google Scholar]

[37] Llorca L., Perez-Perez G., Urruzuno P., Martinez M. J., Iizumi T., Gao Z., et al. (2017). Characterization of the gastric microbiota in a pediatric population according to helicobacter pylori status. Pediatr. Infect. Dis. J. 36 (2), 173–178. doi: 10.1097/inf.0000000000001383 [DOI] [PubMed] [Google Scholar]

[38] Coker O. O., Dai Z., Nie Y., Zhao G., Cao L., Nakatsu G., et al. (2017). Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 67 (6), 1024–1032. doi: 10.1136/gutjnl-2017.