Gastric Flora in Patients with Helicobacter Pylori Infection with and without Dyspepsia

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Abstract

Background: The changes in the gastric environment that occur because of *Helicobacter Pylori (H. pylori)* infection are complicated . The combination of these changes and gastric microbiota could lead to progression of many gastric disorders. **Objective**: This study aimed to assay the gastric bacterial microbiota in patients infected with *H. pylori* with dyspeptic symptoms and without. **Patients and Methods:** This study included 70 naive infected *H. pylori* patients .Cases were divided into two equal groups, Group 1: patients with chronic dyspeptic symptoms 3 months ago diagnosed by Rome IV criteria . Group 2: patients without dyspeptic symptoms. Upper esophagogastroduodenoscopy done for all participants and gastric biopsies were taken for bacterial cultures that were assayed by Vitek 2 system to identify colonies of gastric microbiota for all patients. **Results:** isolated gram-negative organisms were higher in non-dyspeptic than dyspeptic group (P = 0.015). Isolated *Staphylococci* were significantly higher in dyspeptic group compared to non-dyspeptic group (P = 0.003). *Streptococci* and *Klebsiella* were significantly decreased in dyspeptic group than non-dyspeptic group (P = 0.019 and 0.001 respectively) but other genus were insignificantly different between both groups. **Conclusion :** Gastric microbiota could affect the appearance of dyspepsia in *H. pylori* infected patients.

Keywords : Gastric microbiota, *Helicobacter Pylori*, Dyspepsia.

Introduction:

H. pylori infection results in persistent active inflammation of gastric mucosa, which can lead to atrophic gastritis and intestinal metaplasia, both of which enhance the chance of developing gastric cancer. The clinical finding of infection is determined by a complex interaction of bacterial pathogenic factors, host defense mechanisms, and environmental variables [1]. Numerous organisms other than *H. pylori* have been isolated from hypochlorhydria patients' stomachs, involving *Escherichia coli, Campylobacter jejuni, Lactobacillus, Streptococcus, Pseudomonas, Xanthomonas, Proteus, Klebsiella,* and *Neisseria species*[2]. Functional dyspepsia is described as the appearance of upper gastrointestinal distress symptoms without any identified structural abnormalities during diagnostic work-up most notably upper gastrointestinal endoscopy such as ulcer or masses [3]. Dyspeptic symptoms can be reflux-like, with primary symptoms of heartburn and regurgitation; dysmotility-like, with early satiation and nausea; or ulcer-like, with pain and vomiting [4]. Numerous mechanisms for the pathophysiology of dyspepsia have been hypothesized including acid hypersecretion, delayed stomach emptying, visceral hypersensitivity, psychosocial variables, and *H. pylori* infection[3] . *H. pylori* infection may cause dyspeptic symptoms through multiple mechanisms such as alterations in gastric acid media, active inflammation of gastric mucosa, and post-infective sequel in gastroduodenal mucosa[5]. Virulence factors of *H. pylori* have studied more and more, host factors as diet also studied but the answer still unclear[2] .This study aim was to assay the gastric bacterial microbiota in cases infected with *H. pylori* with and without dyspeptic symptoms by Vitek2 system, which is a fully automated microbiological system that utilizes growth-based technology for accurate microbial identification [6].

Patients and Methods:

This cross sectional comparative study included naive 70 patients with *H. pylori* infection diagnosing by *H. pylori* antigen in stool using On-Site *H. pylori* Ag rapid test for the qualitative detection of *H. pylori* antigen using lateral flow immunoassay , from Kafrelsheikh Liver Diseases Research Institute endoscopy room from May 2019 to April 2021. The study was approved by the research committee of Faculty of Medicine, Benha University. An informed written

consent was obtained from all patients included in the study with exclusion of patients with history of antibiotic , acid suppressive therapy one month ago, chronic non-steroidal anti-inflammatory drugs (NSAIDs) use and any organic disease on endoscopy (ulcers, masses, cancer, stricture, or Gastric antral vascular ectasia (GAVE).

Patients were classified into two equal groups : Group 1 included 35 patients with dyspeptic symptoms for the past three months (symptom started at least 6 months before diagnosis regard as Rome IV criteria by one or more of: bothersome postprandial fullness, bothersome early satiation , bothersome epigastric pain, bothersome epigastric burning) and no indication of structural disease by upper endoscopy[7]. Group 2: included 35 patients without dyspeptic symptoms came to endoscopy room for screening of esophageal varices and/or anemia for investigations.

All patients underwent the following:

1.Full history taking involving drug history including recent antibiotics, NSAIDs, steroids and acid suppressive therapy.

2.Laboratory investigations including complete blood count (CBC), liver tests and renal functions tests.

3.*H. pylori* antigen detection in stool, using On-Site *H. pylori* Ag rapid test ctk biotech, inc . Poway ,USA . The principles of this test was prescribed in its pamphlet as using lateral flow immunoassay for the qualitative detection of *H. pylori* antigen in specimen. The test strip consists of aburgundy-colored conjugate pad involving monoclonal anti-*H. pylori* antibody conjugated with colloidal gold (anti-H.p. conjugates), and a nitro-cellulose membrane strip containing a test line (T line) and a control line. the specimen migrates across the cassette by capillary activity. Antigens from *H. pylori* will link to anti- *H. pylori* conjugates if they are present in the material. The immunological complex is subsequently trapped by the pre-coated antibody on the membrane, resulting in the formation of a burgundy-colored T line, indicating a positive test result for *H. pylori*

4.Abdominopelvic ultrasound, to detect liver texture, hepatomegaly, splenomegaly, exclude biliary diseases, any abdominal masses or disorders that cause symptoms conflicting as dyspepsia.

5.Upper gastrointestinal endoscopy done using two Oesophago-Gastro-Duodenoscopies; Olympus system (GIF-XQ165) and Fuji (EG450-HR) system.

Multiple gastric biopsy were taken . With diligent sterile precautions, during light sedation of patients, and after rinsing the endoscope channel with sterile normal saline, two sets of mucosal biopsy samples were obtained from the stomach antrum and body using single-use forceps.

6. Bacterial cultures: A homogenizer was used to disseminate the selected samples. Each homogenate was injected into sterile tryptic soy broth before plating on blood, MacConkey and Mueller Hinton agar plates. They were then cultured for 5-7 days at 37 °C . All colonies that appeared were assayed by Vitek 2 system to identify non-H Pylori colonies and determine the gastric microbiota for all patients. Using VIETK 2 system [version: (08.01) bioMérieux, USA]. VITEK 2 system operates by incubating and interpreting automatically using colorimetric reagent cards. The reagent cards have 64 wells, each of which can contain a unique test substrate. Numerous metabolic processes such as acidification, alkalinization, enzyme hydrolysis, and growth in the appearance of inhibitory chemicals are quantified using substrates. Bar codes on the cards carry information on the product's kind, lot number and expiration date as well as a unique identity which can be connected to the sample pre or past the card is loaded on to the system.

Statistical analysis:

Once the data was gathered, a code sheet was created. The data were organized, tabulated, presented, and analyzed using IBM's SPSS V25 software in the United States of America. Quantitative variables were presented as mean and standard deviation SD and compared between the two groups utilizing unpaired Student's t- test. Qualitative variables were presented as frequency and percentage (%) and were analyzed utilizing the Chi-square test or Fisher's exact test when appropriate. A two tailed P value < 0.05 was considered statistically significant.

Results

The patients' demographic data and characteristics [age, gender, residency, and specific habits (smoking and addiction) were summarized in (Table 1). That were insignificantly different between both groups . Regarding past history and manifestations in both groups, only vomiting was significantly increased in dyspeptic group 15 (42.86%) than non-dyspeptic group 1 (2.86%) (P=0.001) (Table 2), **N.B:** all participants in dyspeptic group were selected according to Rome IV criteria

complaining of one or more of these symptoms (postprandial fullness, early satiation, epigastric pain, epigastric burning). As regards clinical examination, pallor and splenomegaly were insignificantly different in both groups (Table 2).

As regard laboratory investigations (platelets, TLC, total serum bilirubin, serum albumin, ALT, AST, creatinine and viral markers) were insignificantly different in both groups except hemoglobin was significantly lower in non-dyspeptic group (10.28 ± 1.48 g/dL) compared to dyspeptic groups (10.99 ± 1.34 g/dL) (P=0.039**)** as in (Table 3). As regards endoscope results, in the dyspeptic group erythematous± edematous mucosal appearances of gastric mucosa and erythematous± edematous appearance of duodenal mucosa were significantly higher (P=0.008) and (P=0.02) respectively, while esophageal varices grade I and II were insignificantly different (Table 4). From the 70 biopsy samples, 93 isolates were obtained, median range from 1 to 2 isolates per patient (Table 5). Single gram positive isolates were higher in dyspeptic group than non-dyspeptic group (p= 0.082), while mixed isolates were insignificantly different between both groups as in (table 5). Isolated gram negative organisms were significantly higher in non-dyspeptic group compared to dyspeptic group (p=0.015) and isolated gram positive and anaerobic organisms were insignificantly different between both groups (Table 6).

According to organisms' genus, *Staphylococcus* was found in 16 (45.71%) patients in dyspeptic group and in 4 (11.43%) patients in non-dyspeptic group. *Streptococcus* was found in 6 (17.14%) patients in dyspeptic group and in 15 (42.86%) patients in non-dyspeptic group. *Lactobacillus* was found in 9 (25.71%) patients in dyspeptic group and in 4 (11.43%) patients in non-dyspeptic group. *Granulicatella* was found in 2 (5.71%) patients in non-dyspeptic group, *Klebsiella* was found in 10 (28.57%) patients in non-dyspeptic group. *Pseudomonas* was found in 3 (8.57%) patients in dyspeptic group and in 3 (8.57%) patients in non-dyspeptic group. *Ochrobactrum* was found in 2 (5.71%) patients in dyspeptic group. *Sphingomonas* was found in one patient (2.86%) in non-dyspeptic group. Isolated *Staphylococci* were significantly higher in dyspeptic group compared to non-dyspeptic group, *streptococci* and *Klebsiella* were significantly decreased in dyspeptic group than non-dyspeptic group (Table 7).

Table 1: Patients' demographic data in both groups

		Dyspeptic group (N = 35)	Non-dyspeptic group (N = 35)	P value
Age (years)	Mean ± SD	46.06 ± 11.91	47.66 ± 10.42	0.552
	Range	20-65	22-65	
Gender	Male	17 (48.57%)	25 (71.43%)	0.225
	Female	18 (51.43%)	10 (28.75%)	
Residency	Rural	25 (71.43%)	21 (60%)	0.314
	Urban	10 (28.57%)	14 (40%)	
Specific Habits	Smoking	6 (17.14%)	10 (28.75%)	0.225
	Addiction	0 (0%)	1 (2.86%)	1

Table 2: Past history, manifestation and clinical examination in both groups

	Dyspeptic group (N = 35)	Non-dyspeptic group (N = 35)	P value
Diabetes mellitus	5 (14.29%)	9 (25.71%)	0.054
Hypertension	4 (11.43%)	8 (22.86%)	0.084
Surgery	9 (25.71%)	9 (25.71%)	1
Loss of weight	3 (8.57%)	1 (2.86%)	0.614
Fatigue	10 (28.5%)	16(45.7%)	0.137
Odynophagia	0 (0%)	3 (8.57%)	0.239
Vomiting	15 (42.86%)	1 (2.86%)	**0.001***
Pallor	9 (25.71%)	15 (42.86%)	0.134
splenomegaly	2 (5.71%)	6 (17.14%)	0.259

Table 3: laboratory findings in both groups

Laboratory investigations		**Dyspeptic group (N = 35)**	**Non-dyspeptic group (N = 35)**	**P value**
Hemoglobin (g/dL) (12-17.5)	**Mean ± SD**	10.99 ± 1.34	10.28 ± 1.48	**0.039***
	Range	8.5 – 13.8	8.1 – 14.2	
Platelets (cell/mm^3) 150000-400000	**Mean ± SD**	226885 ± 77508	209257 ± 77249	0.344
	Range	80000 - 410000	75000 – 353000	
WBC (cell/mm^3) (4500-1100)	**Mean ± SD**	7245 ± 1397	6505 ± 2008	0.078
	Range	5000 – 11000	3000 – 10400	
Total serum bilirubin (mg/dL)≤1	**Mean ± SD**	0.90 ± 0.17	0.95 ± 0.24	0.288
	Range	0.6 – 1.2	0.5 – 1.2	
Albumin (mg/dL) (3.5-5)	**Mean ± SD**	4.23 ± 0.58	4.03 ± 0.61	0.170
	Range	3.4 – 5.3	3 – 5.2	
ALT (U/L) Up to 40	**Mean ± SD**	33.80 ± 11.95	35.63 ± 13.31	0.547
	Range	19 – 76	20 – 72	
AST (U/L) Up to 40	**Mean ± SD**	31.09 ± 8.55	34.97 ± 12.98	0.144
	Range	16 – 59	15 – 74	
Creatinine (mg/dL) (0.7-1.2)	**Mean ± SD**	0.94 ± 0.17	1.02 ± 0.34	0.247
	Range	0.6 – 1.4	0.5 – 1.6	
Virology	**HBsAg**	0 (0%)	2 (5.71%)	1
	HCV Ab	2 (5.71%)	8 (22.86%)	0.084

Table 4: endoscopic findings in both groups

Endoscopic findings	Dyspeptic group (N = 35)	Non-dyspeptic group (N = 35)	P value
Gastric mucosa appearance			
Normal	20 (57.1%)	30 (85.7%)	
Erythematous ± edematous	15 (42.9%)	5 (14.3%)	**0.008***
Others	-	-	
Duodenal mucosal appearance			
Normal	19 (54.29%)	29 (82.86%)	
Erythematous ±edematous	16 (45.71%)	6 (17.14%)	**0.02***
Others		-	
Esophageal varices (EV)			
Grade 1	1 (2.86%)	4 (11.43%)	**0.357**
Grade 2	2 (5.71%)	6 (17.14%)	

Table 5: Bacterial isolates in each case in both groups

Bacterial isolates	Dyspeptic group (N = 35)	Non-dyspeptic group (N = 35)	P value
Mixed isolates	10 (28.57%)	14 (40%)	0.450
Single isolates			
-Gram positive	17 (48.57%)	9 (25.71%)	**0.082**
-Gram negative	4 (11.43%)	9 (25.71%)	0.218
-Anaerobes	3 (8.57%)	0	0.239
No isolates	1 (2.86%)	3 (8.57%)	0.614

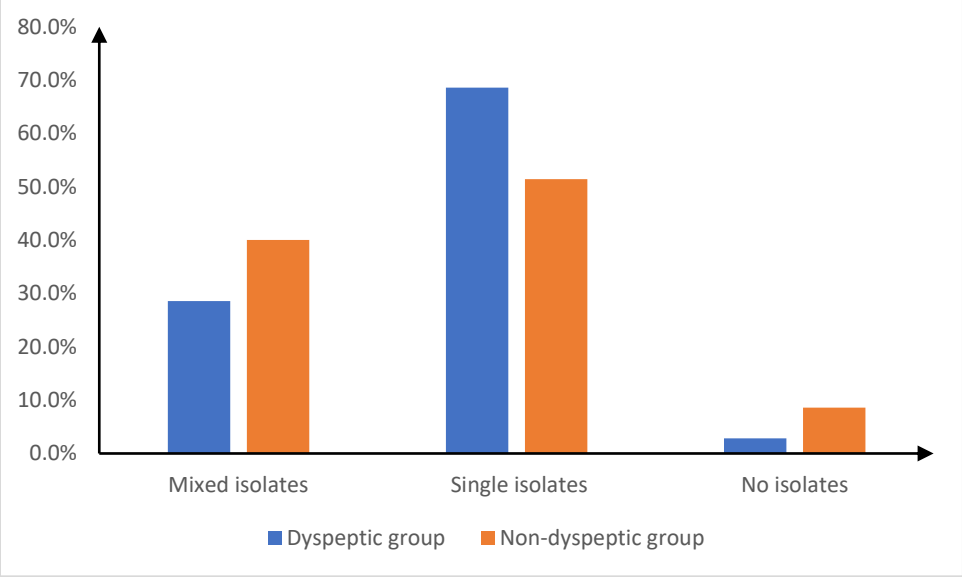

Figure 1: Bacterial isolates in both groups

Table 6: Bacterial cultures in both groups

Bacterial cultures	Dyspeptic group (N = 35)	Non-dyspeptic group (N = 35)	P value
Gram positive	31 (88.57%)	25 (71.43%)	0.133
Gram negative	10 (28.57%)	21 (60%)	**0.015***
Anaerobes	4 (11.43%)	2 (5.71%)	0.673

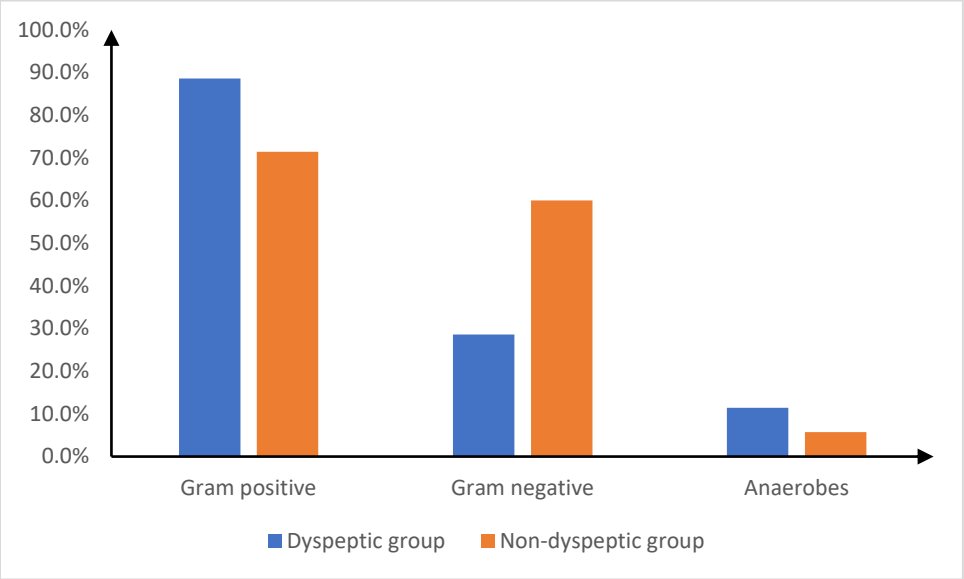

Figure 2: Bacterial cultures in both groups

Table 7: Organisms' genus in both groups

Organisms' genus		Dyspeptic group	Non-dyspeptic group	P value
Gram positive genus	**Staphylococcus**	16 (45.71%)	4 (11.43%)	**0.003***
	Streptococcus	6 (17.14%)	15 (42.86%)	**0.019***
	Lactobacillus	9 (25.71%)	4 (11.43%)	0.133
	Granulicatella	0 (0%)	2 (5.71%)	0.493
Gram negative genus	**Acinetobacter**	1 (%)	0 (0%)	1
	Citrobacter	1 (%)	0 (0%)	1
	Escherichia	2 (%)	7 (%)	0.151
	Klebsiella	0 (0%)	10 (28.57%)	**0.001***
	Pseudomonas	3 (8.57%)	3 (8.57%)	1
	Ochrobactrum	2 (5.71%)	0 (0%)	0.493
	Sphingomonas	0 (0%)	1 (2.86%)	1
Anaerobic genus	**Morganella**	3 (8.57%)	1 (2.86%)	0.614
	Aeromonas	1 (2.86%)	1 (2.86%)	1

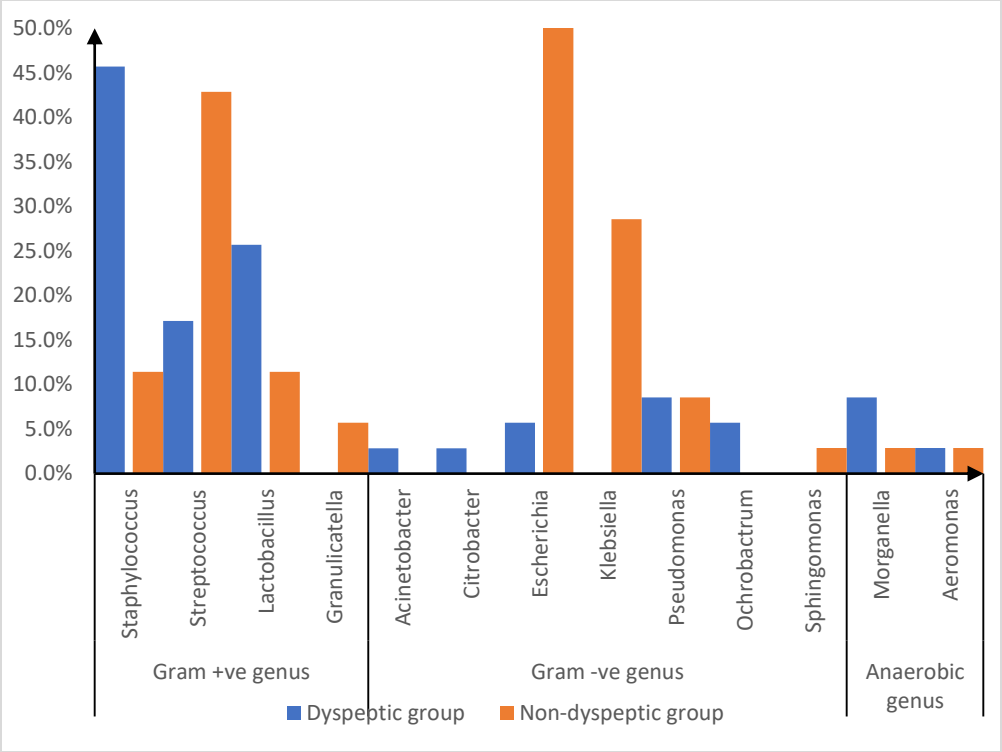

Figure 3: Organisms' genus in both groups

Discussion

Up to 50% of the population in worldwide is infected with *H. pylori*. It has been linked to a variety of gastroduodenal (duodenal ulcers, MALT lymphoma, and gastric cancer) and extra-intestinal (e.g., refractory anemia, idiopathic thrombocytopenic purpura) diseases[8]. Why just a tiny proportion of people infected by *H. pylori* develop illness and the other not? this is the topic of much examination. Numerous pathogenic factors have been identified within the organism that enable colonization and development to disease[9]. Recently, attention has shifted to host factors, with an increased emphasis on the involvement of the stomach microbiota, notably in the etiology of gastric cancer in the presence of *H. pylori* infection [10]. More frequently than the development of organic disease, people with *H. pylori* infection have symptoms of chronic dyspepsia also in absence of organic disease (so-called functional dyspepsia) [11]. *H. pylori* has been implicated in the pathogenesis of gastroduodenal disease (acid-peptic disorders, low-grade MALT lymphoma, and cancer) [12]. However, of the

question is why only some people have disease progression but the majority do not . A function for variables in the host environment (gastric microbiome) has lately attracted considerable attention[13] .However, we are unaware of any research that have sought to discriminate the stomach microbiota of persons with *Helicobacter pylori* infection between those who have chronic dyspepsia and those who do not . Finally, there is data on the stomach microbiota in the West, but there are few publications on the gastric microbiota in developing countries.[10].

In line with current study, numerous investigations discovered that both dyspeptic and non-dyspeptic groups had comparable rates of special behaviors (smoking ,alcohol, coffee) as in another study which reported that smokers was 39% of participants [14] .In this study smoking was in 17.14% of dyspeptic patients which was in line with the same study that demonstrated that 17.07% were smokers[15]. In the current study, residency in rural areas was more prevalent in patients with dyspepsia. This was in contrast to another study **,** that demonstrated no predominance in residency in its locality[15],this difference may be due to the different geographical distribution. In the present study, loss of weight was in 8.57% and vomiting was in 42.86%. This was in line with other study that showed that vomiting was in (34.1%) in dyspeptic patients with *H. pylori* [14]. In this study laboratory investigations (platelets, TLC, total serum bilirubin, serum albumin, ALT, AST, creatinine and viral markers) showed insignificant differences between both groups except hemoglobin was significantly lower in non-dyspeptic group compared to dyspeptic group (P=0.039) as quite many cases in non-dyspeptic group complaining of anemia for investigation . Endoscopic findings in the current study showed that normal gastric mucosal appearance was 57.1% in dyspeptic group and 85% in non-dyspeptics with significant difference between two groups, and edematous ± erythematous gastric mucosa was significantly higher in dyspeptic group 42.9% than non-dyspeptic 14.3% P=0.008 also normal duodenal appearance was 54.29% in dyspeptic group and was 82.86% in non-dyspeptic and edematous ± erythematous duodenal appearance was significantly higher in dyspeptic group 45.71% than non-dyspeptic 17.14% P=0.02 ,esophageal varices grade I and II were insignificantly different in both groups P= 0.357 .Other structural endoscopic findings as ulcer, masses, GAVE and erosions were

excluded [6],also no gastric varices in each group. while another one found that 82.3% of patients with dyspepsia had features of gastritis[16]. This difference may be attributed to small sample size.

From the 70 biopsy samples, 93 isolates were obtained, median range from 1 to 2 isolates per patient. The more obvious observation is that single gram positive isolates were higher in dyspeptic group than non-dyspeptic group while mixed isolates were insignificantly different between both groups. Bacterial cultures in current study showed that isolated gram negative organisms were significantly higher in non-dyspeptic group compared to dyspeptic group (P = 0.015) and isolated gram positive and anaerobic organisms were insignificantly different between both groups (P = 0.133 and 0.673). At a genus level isolated *Staphylococci* were significantly higher in dyspeptic group compared to non-dyspeptic group (P = 0.003), *Streptococci* and *Klebsiella* were significantly decreased in dyspeptic group than non-dyspeptic group (P = 0.019 and 0.001 respectively). These results fairly agreed with other study in which 74 gastric biopsy samples obtained from patients with or without chronic dyspepsia, that found *Staphylococcus spp., Streptococcus spp. and Lactobacillus spp*. as the most frequently identified organisms in the samples investigated. Intriguingly, the flora of H. pylori-positive individuals with chronic dyspepsia was dominated by *Staphylococcus* and *Lactobacillus* (in that order of predominance); in those without dyspepsia, *Streptococcus, Staphylococcus, and Klebsiella* predominated **[11]**. In agreement with current study, earlier research found that *Staphylococcus spp*. and *Lactobacillus spp*. were shown to be more prevalent in chronic dyspeptics, whereas *Streptococcus spp*., *Pseudomonas, Escherichia coli*, and *Klebsiella pneumoniae* were found to be more prevalent in non-dyspeptics**[17]**. *Staphylococcus and Klebsiella* are two of these species that produce urease. Notably, according to another study *Streptococcus* was the most prevalent genus in the duodenal mucosa of both control group and patients with dyspepsia *Streptococcus* had an inverse connection with the anaerobic genera *Prevotella, Veillonella, and Actinomyces*, which were all considerably reduced in individuals with dyspepsia**[18].** Conversely, in contrast with current study a Japanese study, in 11 chronic dyspepsia and seven healthy subjects were collected, it showed that *Streptococcus* was significantly elevated in chronic dyspepsia

and its relative abundance also positively correlated with symptoms severity[19]. The difference between these findings and this study findings could be attributed to variation in ethnicity, food habits or antibiotics abuse and selection bias.

In contrast to this study in which all patients had *H. pylori* infection another study compared the composition of the stomach microbiota between 24 patients with dyspepsia and 21 age- and gender-matched healthy controls, authors discovered that the dyspepsia group's microbiota was characterized by an elevation in *Bacteroidetes* to *Proteobacteria* ratio and a complete lack of *Actinobacteria,* whereas the control group's microbiome had a reduced *Bacteroidetes*to *Proteobacteria* ratio and *Actinobacteria* were present[20]. A variety of ways for H. pylori's interaction with non- *H. pylori* microbes in the stomach was proposed . *H. pylori*'s relationships with other bacteria found in the stomach may be impacted by human reaction, it is reported that *H. pylori* produces unique niches in the stomach that allow bacteria to survive and colonies[21]**.** In the same context in a prospective cohort research was conducted to compare the fundamental physiological features of gastric fluid and the microbial profile in 44 patients with dyspepsia with 44 healthy controls, authors reported the abundance of genus *Prevotella* and *Bifidobacterium / Clostridium* was higher in dyspeptics than in healthy controls[22].

The changes that occur in the gastrointestinal environment because of *H. pylori* infection are complicated and include several components. An association of these would assess not only the composition of the gastric microbiota but also the progression of various diseases[23]. The current study findings of gastric microbiota domination by higher prevalence of *Staphylococcus* and *Lactobacillus* in patients with H. pylori-positive chronic dyspepsia and by *Streptococcus* followed by *Klebsiella and E. coli* in individuals without dyspepsia, this adds another layer of complexity to the study of microorganisms and their interactions in the etiology of symptoms. These outcomes raise the potential that one or more of these entities (bacteria-bacteria, bacteria-host, or bacteria-host-bacteria) may be involved in the production of symptoms associated with *H. pylori* infection. The breadth of interactions between human-associated microorganisms is unknown, as is the effect on host health or disease[24].

These results contribute to the growing body of knowledge that individual bacteria associated with specific gastrointestinal

diseases may not be the only actors but may also be impacted in their pathogenicity by the community in which they exist. While this is an exciting concept, it is too early to tell whether managing concurrent microbiota will give an alternative strategy of inhibiting or treating certain diseases or symptoms, so complete microbiome mapping is required to detect the influence of ethnicity on the gut microbiota to give attention to microbes that cause disease or that disrupt normal microbial balance and contribute to perturbations in the GI microbiota and contribute to illness.

Conclusion: There is difference in *H. pylori* infected patients regarding gastric microbiota with and without dyspeptic symptoms. Interaction between *H. pylori* and specific gastric microbiota could play a role in incidence of dyspepsia.

Drawback: The drawback of this study is the low sample size, histopathological examination of biopsy samples to assess the severity of gastritis was not done due to economic issues, also culture of the *H. pylori* organisms couldn't be done due to technical obstacles (needs selective media as Clombia agar, needs microaerophilic atmosphere and high grade of freezing for preservation of specimen. *H.pylori* antigen not analyzed by ELISA and finally the nutritional state and habits of cases not documented.

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