The relationship between *Helicobacter pylori* associated gastric pathology and bacterial load

Noha Abouseada[1]*, Manal M Baddour[1], Nahed Baddour[2], WalaaMostafa[1], Yosri Taher[3], Ahmed F Mahmoud [4].

[1]Medical Microbiology and Immunology Department, Faculty of Medicine, Alexandria University.
[2]Pathology Department, Faculty of Medicine, Alexandria University.
[3] Internal Medicine, Faculty of Medicine, Alexandria University.
[4]Paediatric gastroenterology, Faculty of Medicine, Alexandria University.
noha.abouseada@alexmed.edu.eg, manal.baddour@alexmed.edu.eg, nahedbaddour@yahoo.com, drwalaa.wassal@yahoo.com, yosritaher2000@gmail.com, fouad_ped@yahoo.com

Abstract: Helicobacter pylori (H. pylori) is the most common chronic human bacterial infection, affecting up to 50% of the world's population. An accurate and reproducible technique for the detection of Helicobacter pylori (H. pylori) DNA within the gastric mucosa is needed. In this study, we examined a quantitative real-time PCR assay to determine the levels of H. pylori, and correlated the results with the histopathological changes. The severity of the inflammatory changes were correlated with the presence of the virulence factor CagA. *H. pylori* DNA load was estimated by real-time PCR. Conventional PCR was used to identify the CagA gene. Histopathological microscopic examination was done for grading of gastritis according to the updated Sydney classification. Six histopathological parameters were studied; severity and depth of inflammatory response, presence or absence of activity, glandular atrophy, the presence or absence of lymphoid aggregates, and gastric eosinophilia. A correlation study was done between quantity of *H. pylori* DNA and various histopathological parameters of gastritis. There was an overall significant correlation between quantity of *H. pylori* DNA and the severity of histopathological parameters of gastritis. There was a significant correlation between age and bacterial load of *H. pylori* as well as a positive correlation between CagA and severity of inflammation.
[Noha Abouseada, Manal M Baddour, Nahed Baddour, WalaaMostafa, Yosri Taher, Ahmed F Mahmoud. **The relationship between *Helicobacter pylori* associated gastric pathology and bacterial load.** *Life Sci J* 2016;13(10):1-8]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 1. doi:10.7537/marslsj131016.01.

Keywords: Helicobacter pylori, gastritis, chronic inflammation, real-time PCR, *CagA*

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram negative, curved microaerophilic rod. It is a member of the family *Helicobacteracaeae*, with a narrow host range, mainly humans and some non-human primates. *H. pylori* has been implicated in the pathogenesis of gastritis, peptic ulcer and gastric cancer [1]. Prevalence of this pathogen varies greatly from 25% in developed countries to 90% in developing areas[2-5]. In developing countries *H. pylori* is highly prevalent even in children, while in developed countries it is of lower prevalence among children and adolescents than adults and elderly [6]. Infection with *H. pylori* has been inversely correlated with the socioeconomic status, and while prevalence remains high in the developing world, it is declining in developed countries, and the incidence of new cases is much attributed to immigrants from developing countries [7].

Gastric *H. pylori* infections cause chronic active gastritis in all patients, and most patients do not develop further complications. The severity of this infection and its distribution within the stomach is multi-factorial, depending on the infecting strain, host immune response, diet and acid production levels[8,9]. Cytotoxin-associated gene A (*CagA*) is a 140 kDa highly immunogenic protein that is encoded by the *CagA* gene[10]. The presence of *CagA* in *H. pylori* strains was correlated with virulence, as it induces morphological changes, vacuolation and degeneration of *in-vitro* cultured cells. The gene *CagA* was found in 50% to 70% of *H. pylori* strains and is considered a marker for the presence of a genomic pathogenicity island PAI, which is approximately 40kb, encoding about 29 to 31 proteins [10-12].

Infection with *CagA* positive strains usually results in a high inflammatory response, together with an increased risk of developing conditions such as peptic ulcer or gastric cancer. This outcome is mainly seen in Western countries, but not in Asian populations. Having said that, *CagA* PAI negative strains have also been recovered from patients with gastric ulcer disease and gastric cancer, but at lower rates[13-16].

CagA PAI proteins form a type IV secretion system, which interacts with gastric epithelial cells resulting into injection of *CagA*, peptidoglycan and other bacterial factors in host cells. This will eventually result in a series of cell signaling events ending with morphological changes of the gastric epithelial cells. *CagA* PAI proteins also modulate the immune response against *H. pylori* by inducing apoptosis of T-cells[17-19].

The aim of this study was to estimate the *H. pylori* DNA load in gastric biopsy samples from Egyptian patients undergoing endoscopy and biopsy for clinical gastritis, and to relate the bacterial load to the various pathological findings. Also, to detect the presence or absence of the *CagA* virulence gene and its relation to bacterial load.

2. Methods

Five hundred and twenty one young patients with clinical gastritis were admitted to Alexandria University Hospitals for gastroscopy and biopsy during the period between December 2012 and December 2013. Multiple biopsies were taken and histopathological examination was done. Histological grading of gastritis according to the updated Sydney classification was recorded(RRR).

Bacterial quantitation was performed on 92 randomly chosen *H. pylori*-positive formalin – fixed, paraffin- embedded gastric biopsy samples previously collected from patients suffering from clinical gastritis.

DNA was isolated from each biopsy, using a DNA extraction kit (iNtRON Biotechnology, Inc., Korea) accordingto the manufacturer's instructions and used for the molecular analysis.

Bacterial quantification was performed applying the technology of real-time PCR using Applied Biosystems Step One ™ real time PCR system. Real-time PCR was performed using final solution of 15 µl containing 2 µl of extracted DNA, 200 mMdNTPs, 1.5 mMMgCl2, 9.5 µL 2X SYBR Green Master mix, 0.2 mM primers (5′-TTATCGGTAAAGACACCAGA AA- ′ and the reverse is 5′ATCACAGCGCATGTCTTC- ′, 2) as previously reported[20]. Cycling conditions consisted of an initial denaturation at 95˚C for 30 s, followed by 40 cycles with denaturation at 95˚C for 5 s, annealing at 54˚C for 5 s and extension at 72˚C for 8 s.

The forward and reverse primers *CagA*/ConF (5'-GTGCCTGCTAGTTTGTCAGCG-3') and *CagA*/Con-R (5'-TTGGAAACCACCTTTTGTATTAGC-3') were used to amplify a 402-bp fragment of the *CagA* gene using conventional PCR [21]. The amplification cycles included an initial denaturation at 95˚C for 3 minutes, followed by 30 cycles of denaturation at 95˚C for 30 s, annealing at 50˚C for 45 s and extension at 72˚C for 45 s. Final extension was carried out at 72˚C for 5 min.

Data were collected, revised, verified, and analyzed statistically using Statistical Package for Social Sciences Program Windows (SPSS v17). The following tests were used in this study: Mann Whitney test and Kruskal Wallis test were used for comparison between different degrees of histopathological (continuous) variables regarding quantity of *H. pylori* DNA. *p*-values lessthan 0.05 were considered to indicate statistical significance.

3. Results

Out of 521 cases suffering from clinical gastritis, 217 cases (41.6%) were proven to be *H. pylori* positive by histopathological examination among young patients(up to 20 years)in a period of one year. Ninety two out of the 271 *H. pylori* positive cases were randomly chosen for further study. Out of the 92 samples examined, 39 samples (42.3%) were from male patients and 53 samples (58.2%) were from female patients. Age of the patients ranged from one to twenty years.

Histopathological examination was done for histological grading of gastritis according to the updated Sydney classification (RR). Six histopathological parameters were studied; severity and depth of the inflammatory response, activity of the lesion, glandular atrophy, lymphoid aggregation, and gastric eosinophilia (eosinophilic infiltration of blood vessels and lamina propria). Additionally, *H. pylori* DNA was quantitated from the paraffin embedded biopsy samples by real-time PCR.

Histopathological data showed that 7.6% of the samples had no significant inflammation of the gastric mucosa, 27% showed mild inflammation of the gastric mucosa, 40.1% showed moderate degree of inflammation whereas 23.9% showed severe degree of inflammation. No eosinophils were seen in 16.3% of the cases while 78.2% showed mild grade of gastric eosinophilia, 3.2% showed moderate eosinophilia and 1% showed severe gastric eosinophilia.

As regards activity 21.7% showed no activity in gastric mucosa, 63% showed a mild degree of activity, 15.2% showed a moderate degree of activity. No severe degree of activity was detected in the collected samples.

Regarding atrophy of the gastric mucosa 70% displayed non atrophic gastritis, 23.9% displayed mild degree of atrophic gastritis while 5.4% displayed moderate degree of atrophic gastritis. Regarding lymphoid aggregation, 28.2% of samples showed none in the gastric mucosa while 71.7% showed lymphoid aggregation.

The virulence gene *CagA* was detected in 50 out of the 92 *H. pylori* positive cases studied, accounting for 54%. The mean of the *H. pylori* DNA quantity was significantly higher in *CagA* positive cases, $p<0.001$ (Fig.1).

The association between the presence of *CagA* virulence gene and the various pathological parameters was also studied. Each histopathologic feature studied was classified into absent, mild, moderate or severe according to the Modified Sydney Classification.

Bacterial load was significantly higher in all cases where histopathological features of inflammation, neutrophil activity, eosinophilic infiltration and gastric atrophy were moderate or severe ($p<0.01$) (Table 1). However, no significant differences were detected between moderate and severe degrees of histpathological abnormalities detected. Lymphoid aggregation was only assessed as present or absent, and significant difference in the quantity of *H. pylori* DNA was detected among cases with aggregation than those without.

It was noted that *CagA* positive isolates were found where pathological changes were moderate or severe. In biopsy samples where inflammation was severe or moderate 100% and 62.2% were *CagA* positive, respectively. All biopsies with severe and moderate eosinophilic infiltration harbored *CagA* positive *H. pylori* DNA. All cases with moderate activity were *CagA* positive and those with no neutrophil activity showed 43.1% *CagA* positivity. *CagA* was positive in all cases with moderate atrophy and 66.2% where there was lymphoid aggregation. (Table 2).

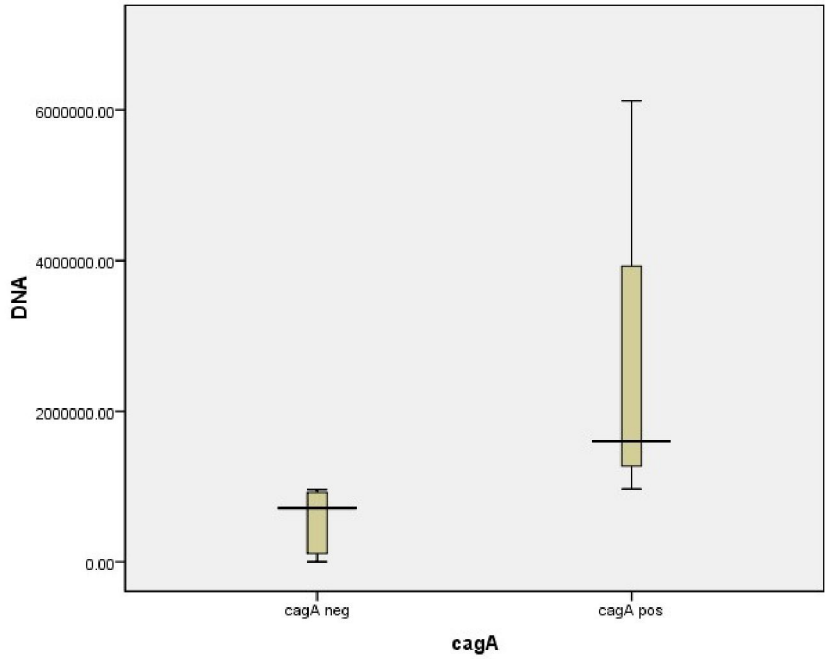

Figure 1. *H. pylori* DNA quantities measured from biopsy specimens in *CagA* negative and positive strains.

Table 1: Distribution of the studied samples according to the variable degrees of inflammation

	No	**Mild**	**Moderate**	**Severe**
Inflammation	7.6% **(no= 7)**	27.1% **(no= 26)**	40.2% **(no=37)**	23.9% **(no=22)**
Gastric eosinophilia	16.3% **(no=15)**	78.2% **(no=73)**	3.2% **(no=3)**	1% **(no=1)**
Neutrophil activity	21.7% **(no=20)**	63% **(no=58)**	15.2% **(no=14)**	0% **(no=0)**
Atrophy	70.6% **(no=65)**	23.9% **(no=22)**	5.4% **(no=5)**	0% **(no=0)**

Table 2: Relationship between *H. pylori* DNA load and severity of different histopathological changes

	Absent	**Mild**	**Moderate**	**Severe**
Inflammation	7.6% (N=7)	27.1% (N=26)	40.2% (N= 37)	23.9% (N= 22)
Quantity of *H. pylori*DNA ($\times 10^4$) Mean ± SD Median	**0.0 ± 0.0 0.0**	**90.53 ± 29.0 87.08**	**104.79 ± 64.48 108.75**	**375.67 ± 180.16 412.38**
p value for Mann Whitney test		$p_1<0.001^*$	$p_2<0.001^*$	$p_3<0.001^*$
Gastric eosinophilia	16.3% (N= 15)	78.2% (N= 73)	3.2% (N= 3)	1% (N= 1)
Quantity of *H. pylori*DNA ($\times 10^4$) Mean ± SD Median	**57.20 ± 64.81 60.98**	**170.78 ± 157.37 112.01**	**301.91 ± 269.06 162.60**	**550.38 550.38**
p value for Mann Whitney test		$p_1<0.001^*$	$p_2= 0.017^*$	
Neutrophil activity	21.7% (N= 20)	63% (N= 58)	15.2% (N= 14)	0% (N= 0)
Quantity of *H. pylori* DNA ($\times 10^4$) Mean ± SD Median	**58.93 ± 5166.05 68.59**	**126.16 ± 92.10 110.38**	**448.96 ± 173.77 524.40**	**-**
p value for Mann Whitney test		p1<0.001*	p2<0.001*	
Atrophy	70.6% (N= 65)	23.9% (N= 22)	5.4% (N= 5)	0% (N= 0)
Quantity of *H. pylori*DNA ($\times 10^4$) Mean ± SD Median	**114.88 ± 115.59 92.92**	**228.15 ± 182.70 140.43**	**458.88 ± 179.79 518.43**	**-**
p value for Mann Whitney test		p1= 0.001*	p2<0.001*	
Lymphoid aggregation	70.6% (N= 24)	23.9% (N= 68)		
Quantity of *H. pylori*DNA ($\times 10^4$) Mean ± SD Median	**79.64 ± 120.75 68.71**	**189.26 ± 164.72 126.50**		
p value for Mann Whitney test		$<0.001^*$		

p_1: p value for Mann Whitney test for comparing between absent and Mild
p_2: p value for Mann Whitney test for comparing between absent and Moderate
p_3: p value for Mann Whitney test for comparing between absent and severe

Table 3: Distribution of *CagA* gene in tested biopsy samples in relation to various histopathological grades.

		CagA +		*CagA*-	
		N	**%**	**N**	**%**
Inflammation	Absent (N=7)	0	0	7	100
	Mild (N=26)	5	19.2	21	80.8
	Moderate (N=37)	23	62.2	14	37.8
	Severe (N=22)	22	100.0	0	0.0
Total		50		42	
Gastric eosinophilia	Absent (N=15)	0	0.0	15	100.0
	Mild (N=73)	46	63.0	27	37.0
	Moderate (N=3)	3	100.0	0	0.0
	Severe (N=1)	1	100.0	0	0.0
Total		50		42	
Neutrophil activity	Absent (N=20)	3	13.6	17	77.3
	Mild (N=58)	33	56.9	25	43.1
	Moderate (N=14)	14	100.0	0	0.0
	Severe (N=0)	-		-	
Total		50		42.0	
Atrophy	Absent (N=65)	28	43.1	37.0	56.9
	Mild (N=22)	17	77.3	5.0	22.7
	Moderate (N=5)	5	100.0	0.0	0.0
	Severe (N=0)	-		-	
Total		50		42.0	
Lymphoid aggregation	Absent (N=24)	5	20.8	19.0	79.2
	Present (N=68)	45	66.2	23.0	33.8
Total		50		42.0	

4. Discussion

H. pylori is recognized as the most common chronic human bacterial infection, affecting up to 50% of the world's population and it plays a crucial role in the pathogenesis of upper GI disease including gastritis, peptic ulcer disease and gastric cancer. [9]

The present study was an attempt to find if there is any relationship between *H. pylori* DNA load (and hence bacterial count) in the gastric mucosa of the stomach and the severity of the pathological changes. Also, to determine if there is any association between *CagA* and *H. pylori* DNA bacterial load.

Real time PCR proved to be a useful tool for detecting *H. pylori* DNA from paraffin embedded gastric biopsy samples. This study confirmed a significant correlation between quantity of *H. pylori* DNA and gastric inflammation as described before, where a positive correlation between microorganism density and the presence of a marked inflammatory infiltration of the gastric mucosa suggests that high bacterial loads are associated with increased acute mucosal damage and long-term changes in the gastric mucosa. The influence of *H. pylori* density reduction on the improvement of gastric mucosal changes was observed in studies using 'clearance' therapies[22]. Other studies revealed a significant correlation between bacterial load and markers of inflammatory cells suggesting IFN-γ activity was associated with the *H. pylori* density and correlated significantly with inflammation (active and chronic). These results were in agreement with **Marshall and Warren (1984)** who reported a positive correlation between microorganisms and the presence of a marked inflammatory infiltration of the gastric mucosa, in fact, this was what lead them to consider *H. pylori* as a cause of chronic active gastritis[23,24].

The present study elucidated a weak positive correlation between quantity of *H. pylori* DNA and atrophic gastritis (not atrophic gastritis, this is the end stage it is diagmnosed chronic gastritis with atrophy not total atrophy remember these are young patients. Atrophic gastritis is usually found in old age). Shukla

et al. suggest that the chances of finding *H. pylori* in biopsy specimens become less when the changes of chronic atrophic gastritis are settled in the stomach. These changes lead to absence or decrease in the *H. pylori*load in the stomach probably due to lack of nutrients for this organism[25,26]. They suggest this may be the probable reason for finding no association between chronic atrophic gastritis with *H. pylori* DNA level[26].

Weck*et al.* suggested that the association of *H. pylori* infection with chronic atrophic gastritis is much stronger than reported in epidemiologic studies that did not take disease-related loss of the infection/infective agent into account[27].

As glandular atrophy progresses, *H. pylori* positivity declines, which can be explained by the fact that *H. pylori* colonizes the epithelium, hence it disappears from areas of intestinal metaplasia. Another reason is that the hypochlorhydric stomach is not optimum for the organism which only favours the narrow pH of the partially acidic environment.[28,29]. Thus, absence of H. pylori from the atrophic stomach does not eliminate its role in the pathogenesis of gastriris.[28].

This study displayed a significant correlation between quantity of *H. pylori* DNA and density of lymphoid aggregation which agreed with the study done by Chen XY *et al.* who concluded that the prevalence and density of lymphoid follicles and aggregates in gastric antral mucosal biopsies correlated closely with *H. pylori* infection and eradication of *H. pylori* infection resulted in a decrease of lymphoid tissue hyperplasia in the gastric antral mucosa[30]. Pereira and Medeiros state that chronic infection with *H. pylori* is significantly associated with the induction of gastric lymphoid follicles, representing the proposed first step in MALT lymphomagenesis of lymphoid expansion[31,32]. In addition, the bacterium can be histologically identified in the gastric mucosa of the majority of gastric MALT lymphomas, with some series describing incidences as high as 92%, although the density and detectability of *H. pylori* decrease as the histology progresses from chronic gastritis to gastric MALT lymphoma. These data suggest that bacterial colonization is important for early lymphomagenesis, but becomes less relevant as the disease progresses[31].

There is also a significant correlation between quantity of *H. pylori* DNA and gastric eosinophilia concordant with Nagy *et al.* who concluded that *H. pylori* enhance eosinophil recruitment to colonized gastric mucosa. This may play a significant role in pathogenesis and disease. Indicating that *H. pylori* increases production of the chemokines CCL2, CCL5, and granulocyte-macrophage colony-stimulating factor by gastric epithelial cells and that these molecules induce eosinophil migration. These events are mediated by the Cag pathogenicity island and by mitogen-activated protein kinases, suggesting that eosinophil migration orchestrated by *H. pylori* is regulated by a virulence-related locus[33]. Other studies suggest that eosinophil infiltration and degranulation may be associated with *H. pylori* gastritis. They postulated that the release of toxic cationic proteins from eosinophils contributes to the inflammatory changes present in *H. pylori* gastritis[34].

CagA was present in a high number of biopsy samples, particularly where all types of inflammatory parameters were severe. This correlates well with the fact that Cag A protein plays a dual role as a pro and anti-inflammatory protein, where it is implicated in the release of IL-8 and NFκB[35,36].

In conclusion, our work shows that the Q-PCR technique is easy to perform and it permits a concomitant rapid, reliable determination and quantification of *H. pylori*. There is a correlation between bacterial load of *H. pylori* and various degrees of inflammation of the gastric mucosa and high bacterial loads are associated with increased acute mucosal damage and long-term changes in the gastric mucosa. Severe inflammatory changes are associated with the presence of the *CagA* gene.

Corresponding author:
Institutional Mailing address:
Faculty of Medicine, Alkhartoum square, Azarita. Alexandria, Egypt.
noha.abouseada@alexmed.edu.eg

Acknowledgements
Authors have no conflict of interests.

References

1. Kargar M, Baghernejad, M., Doosti, A. and Ghorbani-, Dalini S: Clarithromycin resistance and 23S rRNA mutations in Helicobacter pylori isolates in Iran. *African Journal of Microbiology Research* 2011, 5:853-856.
2. Godoy APO, Ribeiro, M.L., Benvengo, Y.H.B., Vitiello, L., Miranda, M.C.B., Mendonça, S. and Pedrazzoli, J., Jr.: Analysis of antimicrobial susceptibility and virulence factors in Helicobacter pylori clinical isolates. *BMC Gastroenterology* 2003, 3:20.
3. Kargar M, Souod, N., Doosti, A. and Ghorbani-Dalini, S.: Prevalence of Helicobacter pylori vacuolating cytotoxin A gene as a predictive marker for different gastrodoudenal diseases. *Iranian Journal of Clinical Infectious Diseases* 2011, 6:85-89.

4. Kusters JG, Van Vliet, A.H.M. and Kuipers, E.J.: Pathogenesis of Helicobacter pylori Infection.*Clinical Microbiology reviews* 2006, 19.
5. Wu CC, Chou, P.Y., Hu, C.T., Liu, Z.C., Lin, C.Y., Tseng, Y.H. and Lin, N.T: Clinical relevance of the vacA, iceA, cagA, and flaA genes of Helicobacter pylori strains in eastern Taiwan. *Journal of Clinical Microbiology* 2005, 43:2913-2915.
6. Pounder RE, Ng D: The prevalence of Helicobacter pylori infection in different countries. *Aliment Pharmacol Ther* 1995, 9 Suppl 2:33-39.
7. Perez-Perez GI, Olivares AZ, Foo FY, Foo S, Neusy AJ, Ng C, Holzman RS, Marmor M, Blaser MJ: Seroprevalence of Helicobacter pylori in New York City populations originating in East Asia. *J Urban Health* 2005, 82:510-516.
8. Blaser MJ, Atherton JC: Helicobacter pylori persistence: biology and disease. *J Clin Invest* 2004, 113:321-333.
9. Kusters JG, van Vliet AH, Kuipers EJ: Pathogenesis of Helicobacter pylori infection. *Clin Microbiol Rev* 2006, 19:449-490.
10. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N, et al.: Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci U S A* 1993, 90:5791-5795.
11. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, Bukanov NO, Drazek ES, Roe BA, Berg DE: Analyses of the cag pathogenicity island of Helicobacter pylori. *Mol Microbiol* 1998, 28:37-53.
12. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A: cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A* 1996, 93:14648-14653.
13. Beales IL, Crabtree JE, Scunes D, Covacci A, Calam J: Antibodies to CagA protein are associated with gastric atrophy in Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 1996, 8:645-649.
14. Go MF, Graham DY: Presence of the cagA gene in the majority of Helicobacter pylori strains is independent of whether the individual has duodenal ulcer or asymptomatic gastritis. *Helicobacter* 1996, 1:107-111.
15. Hamlet A, Thoreson AC, Nilsson O, Svennerholm AM, Olbe L: Duodenal Helicobacter pylori infection differs in cagA genotype between asymptomatic subjects and patients with duodenal ulcers. *Gastroenterology* 1999, 116:259-268.
16. Peek RM, Jr., Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC, Blaser MJ: Heightened inflammatory response and cytokine expression in vivo to cagA+ Helicobacter pylori strains. *Lab Invest* 1995, 73:760-770.
17. Moese S, Selbach M, Kwok T, Brinkmann V, Konig W, Meyer TF, Backert S: Helicobacter pylori induces AGS cell motility and elongation via independent signaling pathways. *Infect Immun* 2004, 72:3646-3649.
18. Naumann M, Wessler S, Bartsch C, Wieland B, Covacci A, Haas R, Meyer TF: Activation of activator protein 1 and stress response kinases in epithelial cells colonized by Helicobacter pylori encoding the cag pathogenicity island. *J Biol Chem* 1999, 274:31655-31662.
19. Selbach M, Moese S, Backert S, Jungblut PR, Meyer TF: The Helicobacter pylori CagA protein induces tyrosine dephosphorylation of ezrin. *Proteomics* 2004, 4:2961-2968.
20. He Q, Wang JP, Osato M, Lachman LB: Real-time quantitative PCR for detection of Helicobacter pylori. *J Clin Microbiol* 2002, 40:3720-3728.
21. Meine GC, Rota C, Dietz J, Sekine S, Prolla JC: Relationship between cagA-positive Helicobacter pylori infection and risk of gastric cancer: a case control study in Porto Alegre, RS, Brazil. *Arq Gastroenterol* 2011, 48:41-45.
22. Varbanova M, Malfertheiner P: Bacterial load and degree of gastric mucosal inflammation in Helicobacter pylori infection. *Dig Dis* 2011, 29:592-599.
23. Paz Bouzal JI GI, Abadl MM, Muñoz E, Rincón MR, Bullón A: Chronic gastritis associated with H. pylori. Correlation between histological and bacteriological findings. *Histol Histopath* 1991, 6:485-489.
24. Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984, 1:1311-1315.
25. Konturek PC, Kania J, Konturek JW, Nikiforuk A, Konturek SJ, Hahn EG: H. pylori infection, atrophic gastritis, cytokines, gastrin, COX-2, PPAR gamma and impaired apoptosis in gastric carcinogenesis. *Med Sci Monit* 2003, 9: SR53-66.
26. Shukla SK, Prasad KN, Tripathi A, Ghoshal UC, Krishnani N, Nuzhat H: Quantitation of Helicobacter pylori ureC gene and its comparison with different diagnostic techniques and gastric histopathology. *J Microbiol Methods* 2011, 86:231-237.

27. Weck MN, Gao L, Brenner H: Helicobacter pylori infection and chronic atrophic gastritis: associations according to severity of disease. *Epidemiology* 2009, 20:569-574.
28. Clyne M, Labigne A, Drumm B: Helicobacter pylori requires an acidic environment to survive in the presence of urea. *Infect Immun* 1995, 63:1669-1673.
29. Dixon M: Pathology of Gastritis and Peptic Ulceration. In *Helicobacter pylori: Physiology and Genetics*. Edited by Mobley HLT MG, Hazell SL: ASM Press; 2001.
30. Chen XY, Liu WZ, Shi Y, Zhang DZ, Xiao SD, Tytgat GN: Helicobacter pylori associated gastric diseases and lymphoid tissue hyperplasia in gastric antral mucosa. *J Clin Pathol* 2002, 55:133-137.
31. Pereira MI, Medeiros JA: Role of Helicobacter pylori in gastric mucosa-associated lymphoid tissue lymphomas. *World J Gastroenterol* 2014, 20:684-698.
32. Siddiqui ST, Naz E, Danish F, Mirza T, Aziz S, Ali A: Frequency of Helicobacter pylori in biopsy proven gastritis and its association with lymphoid follicle formation. *J Pak Med Assoc* 2011, 61:138-141.
33. Nagy TA, Allen SS, Wroblewski LE, Flaherty DK, Slaughter JC, Perez-Perez G, Israel DA, Peek RM, Jr.: Helicobacter pylori induction of eosinophil migration is mediated by the cag pathogenicity island via microbial-epithelial interactions. *Am J Pathol* 2011, 178:1448-1452.
34. McGovern TW, Talley NJ, Kephart GM, Carpenter HA, Gleich GJ: Eosinophil infiltration and degranulation in Helicobacter pylori-associated chronic gastritis. *Dig Dis Sci* 1991, 36:435-440.
35. Lin WC, Tsai HF, Kuo SH, Wu MS, Lin CW, Hsu PI, Cheng AL, Hsu PN: Translocation of Helicobacter pylori CagA into Human B lymphocytes, the origin of mucosa-associated lymphoid tissue lymphoma. *Cancer Res* 70:5740-5748.
36. Tanaka H, Yoshida M, Nishiumi S, Ohnishi N, Kobayashi K, Yamamoto K, Fujita T, Hatakeyama M, Azuma T: The CagA protein of Helicobacter pylori suppresses the functions of dendritic cell in mice. *Arch Biochem Biophys* 2010, 498:35-42.

10/10/2016