Research Article

Increased Production of Matrix Metalloproteinases in *Helicobacter pylori* Infection that Stimulates Gastric Cancer Stem Cells

Gelaan SZ Saleh[1], Mona IH El-Enbaawy[2], Sahar S Abd El-Rahman[3] and Sherif Marouf[2]

[1]Department of Microbiology, The central Armed Forces laboratories for Medical research and Blood Bank, Cairo, Egypt
[2]Department of Microbiology, Faculty of Veterinary Medicine, Cairo, Egypt
[3]Department of Pathology, Faculty of Veterinary Medicine, Cairo, Egypt
***Corresponding author:** drsherif_marouf@yahoo.com

ABSTRACT

Background and aim: *Helicobacter pylori* (*H. pylori*) is an incriminated pathogen causing diseases in both animals and humans and considered a zoonotic pathogen. *H. pylori* infection is considered a cause of gastric cancer, which rests a significant health care challenge. This study analyzes the expression pattern of matrix metalloprotein 2 (MMP-2) in patients with *Helicobacter pylori*-associated gastritis and the effect of *H. pylori* on gastric cancer stem cells, as well as study the role of helicon bacteriosis in dog in transmission of *H. pylori* infection to human. **Materials and methods:** Fifty-five of each sample (gastric biopsy, blood and stool) were collected from patients suffering from dyspepsia, chronic vomiting and perforated peptic ulcers and also from apparent healthy dogs. The investigation detected *H. pylori* by serological and histopathological examination. Biopsies were stored in physiological saline for identification of *H. pylori* by conventional time PCR. MMP-2 and Gastric cancer stem cells were then identified by immunohistochemistry. **Results:** Serological identification for *H. pylori* Antigen and Antibodies revealed (63% human, 50% dogs) and (87% human, 90% dogs) respectively were positive. Genotyping of *H. pylori* based on 16S rRNA gene showed 54.5% of human and 35% of dogs were positive. Immunohistochemistry revealed strong expression of CD44 in *H. pylori*- associated gastric cancer cases, MMP-2 expression was observed in all neoplastic lesions associated with *H. pylori* infection. **Conclusion:** *H. pylori* infection affects gastric mucosa and induces changes in gastric stem cells altering their differentiation and increased expression of MMP's and CD44 with a resultant potentiation of oncogenic alteration. In addition, the up-regulation of both markers could be an instrumental to interpret the origination of gastric cancer.

Key words: *Helicobacter pylori*, MMP-2, Gastric cancer, Gastric cancer stem cell, Dogs.

INTRODUCTION

Helicobacter pylori is a microaerophilic gram-negative bacterium which infects the stomach epithelial liner. *H. pylori* play a role as a reason of peptic ulcer disease (Marshall and Warren, 1983). *H.pylori*-associated diseases result in a considerable public health burden, and it has been suggested that all *H. pylori*-infected persons should receive treatment unless there are competing considerations (Sugano *et al.,* 2015). The World Health Organization guesses that *Helicobacter* spp. occur in 70% of individuals in developing countries and 30% of people in developed nations (Chung *et al.,* 2013). The persistent infection with *H. pylori* and the expansion of *H. pylori*–associated gastritis still not clearly understood, it is supposed that the communication between immune response and gastric epithelium made by *H. pylori* is a causative issue. The first line of host defence it not the only role of the gastric epithelial cells but likewise can produce factors that call immune cells that increased inflammatory response. Matrix metalloproteinases are among the numerous molecules made by gastric epithelial cells in reaction to infection (Lv*et al.,* 2019). To regulate the incidence of gastric cancer, and to deliver effective treatment, there is a critical need to identify effective biomarkers with therapeutic rate that are related with cancer stem cells (Goel *et al.,* 2015). Stem cells seem to be the ideal cellular goals for the accumulation of genetic changes, given their fundamental properties of durability and self-renewal (Rossi *et al.,* 2008). CD44 is identified as a downstream goal of Wnt/β-catenin pathway and is stated in different types of tissues, and solid tumors such as gastric cancer (Rocco *et al.,* 2012). The expressing cells CD44 have cancer stem cell structures as there are able

to, regenerate, tumorigenic and generate phenotypically varied non tumorigenic cells. In detail, relatively little information is obtainable about the effects of *H. pylori* infection effect on stem cells at present, while *H. pylori* has been shown to attack epithelial cell and their progenitors (Necchi *et al.,* 2007). A related occurrence of *Helicobacter* spp. has been stated in companion animals, the particular broadcast mode of *H. pylori* it's not totally clarified. But, it is recognized that *H. pylori* colonizes the gastric mucosa, suggesting that the infection take place through the gastro-oral, oral-oral, fecal-oral routes or by zoonotic transmission (Junqueira *et al.,* 2017). The present investigation was to assist diagnosis of gastric cancer caused by *H. pylori* and to confirm relation between human and companion animals in transmission of *H. pylori.*

MATERIALS AND METHODS

Animal ethics

This study was carried out according to the principles of the Declaration of Egypt and approved from Veterinary Medicine Cairo University Institutional Animal Care and Use Committee Ref; VETCU1022109065.

Human ethics

The study had full ethical approval from Kobry-Elkobah Military Ethics Committee according to the principles of the Declaration of Egypt.

Samples
Stool and blood samples

Seventy-five of each stool and blood (55 humans, 20 dogs) samples were collected. Stool was examined for evaluation of *H. pylori* with stool antigen test; On Site *H. pylori* Ag Rapid test-Cassette (CTK Biotech, USA). Blood samples were centrifuged at 1,000xg for 10 min. The serum was kept at -20°C until analyzed by Enzyme Linked Immunosorbent Assay (ELISA). Measurements of *H. pylori* Ab (IgA) were performed using QUANTA Lite *H. pylori* IgA ELISA (Inova Diagnostics, USA) according to manufacturer directions. *H. pylori* Ab titers of ≥25 U/mL considered positive.

Human and dogs' biopsies samples

Underwent routine protocols for endoscopy, fifty-five gastric biopsy were collected from Kobry-Elkobah Military Hospital, Cairo, Egypt. The patients were suffering from dyspepsia, chronic vomiting, and perforated peptic ulcer. All the patients were asked to fill the inclusion questioner. Twenty pet dogs biopsies from antrum and corpus underwent routine anesthetic protocols for endoscopy at faculty of veterinary medicine Cairo University were sampled through the working channel for PCR and histopathological study.

Molecular techniques

Extraction of genomic DNA from Gastric biopsy was done by using QIAamp DNA Mini kit (Qiagen, Germany) according to the manufacture protocol. The amplification reactions were performed in a total volume of 25µl containing12.5µlof Emerald Amp GT PCR master mix (2x premix), 1µl of each primer (20pmol), 4.5µl of nuclease free water and 6µl of DNA template. Primer

sequences and conditions are clarified in Table 1. The mixture of PCR reactions was subjected to 35cycles and analyzed by 1% agarose gel electrophoresis. The gel was stained with ethidium bromide and examined under UV transilluminator for the presence of the amplified DNA (Image Quant 400, GE Healtcare).

Histopathological examination

Gastric biopsies of the suspected cases were fixed in 10% buffered neutral formalin. A routine processing of the fixed specimens was carried out by dehydration in graded series of alcohol followed by xylol clearance and finally embedded in Paraffin blocks. The later blocks were serially sectioned to obtain sections at 4-5 µm thickness which stained with hematoxylin and eosin. Giemsa stain was used for staining of *H pylori* in the examined gastric biopsies (Bancroft and Gamble, 2008).

Immunohistochemistry

The expression of CD44 and MMP-2 was identified immunohistochemically on paraffin sections of biopsy gastric specimens with various neoplastic reactions using avidin-biotin peroxidase technique (DAB, Sigma) according to previously mentioned method (Hsu *et al.,* 1981). The sections were mounted on sterile slides (Dako, Glostrup, Denmark), routinely dewaxed then rehydrated. The activity of the endogenous peroxidases was overcome with hydrogen peroxide then washed in double-distilled water. Antigen retrieval was carried out in microwave in 0.01 mol/L citric acid. Then the slides were incubated at 4°C overnight with monoclonal antibodies for CD44 and MMP-2 (Dako Corp, Carpenteria, CA) at dilutions of (1:100 and 1:200) respectively. Immunoreactive complexes were detected with the avidin-biotin peroxidase (ABC peroxidase kit, Vectastain®, Vector Laboratories, Burlingame, CA, USA) method and were visualized by chromogen 3,3 -diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co.). Mayer's hematoxylin was used as a counterstain and finally examined under a light microscope.

RESULTS

Recovered rate of *H. pylori* from serotyping.

The recovery rate of *H. pylori* based on serological technique (Table 1) were 63.6% (35/55) for human stool and 50% (10/20) for dogs faeces using *H. pylori* Ag rapid test. The Antibody detection rate was higher in dogs' serum with a percentage of 90% (18/20) but human serum was 87.2% (48/55) based on ELISA technique.

PCR amplification

Amplification of *H. pylori* genomic DNA revealed the expected 109 bp fragment (Fig. 1). The PCR assay was positive in 30 of 55 human specimens with percentage 54.5% and 7 of 20 dog's specimen with percentage 35% (Table 1).

Histopathologic lineaments of *H. pylori*-associated various gastric neoplastic reactions

Various biopsy specimens taken from cases with different neoplastic reaction were shown to be associated with H. pylori infection with a particular adenocarcinoma cases (Table 1).

Table 1: Oligonucleotide primers and PCR conditions

Target gene	Primer sequence (5' → 3')		PCR fragment (bp)	PCR conditions	Reference
16S rRNA	HP1-F HP2-R	CTGGAGAGACTAAGCCCTCC ATTACTGACGCTGATTGTGC	109	95°C for 5 min, 35 cycles of 95°C for 1 min, 56°C for 2 min, 72°C for 4 min, and 72°C for 5 min	Shahi *et al.*, 2015

Table 2: Presenting different types of gastric neoplastic biopsy specimens associated with *H. pylori* infection.
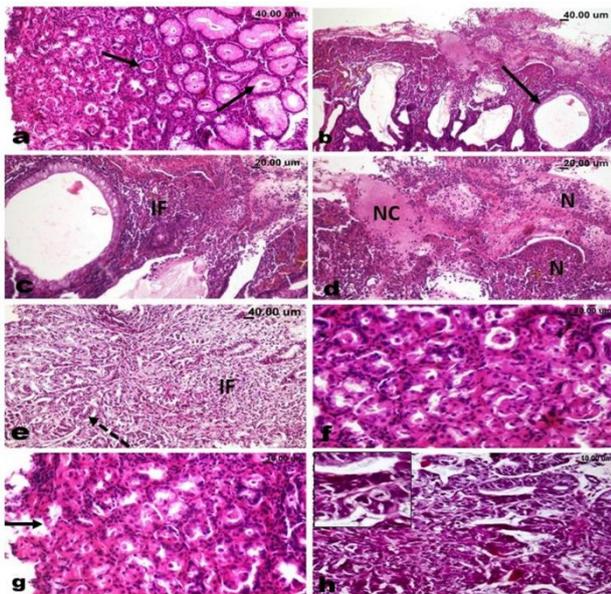
			No lesion	Gastric polyps: Hyperplastic polyps Fundic gland polyps	Dysplastic reaction	Gastric adenoma	Adenocarcinoma: Tubular, Papillary, Solid	Total positive
Positive *H.pylori* Ab (IgA) in serum	Dog	%	90	-	-	-	-	90
				-			-	
							-	
		N	18	-	-	-	-	18
				-			-	
							-	
	Human	%	7.2		10.9	18.1	9	87.2
				12.7			3.6	
				9			16.3	
		N	4	7	6	10	5	48
				5			2	
							9	
Positive *H.pylori* Ag in stool samples	Dog	%	50	-	-	-	-	50
				-			-	
							-	
		N	10		-	-	-	10
				-			-	
				-			-	
	Human	%	9	9	10.9	7.2	7.2	63.6
				7.2			3.6	
							9	
		N	5		6	4	4	35
				5			2	
				4			5	
Positive *H.pylori* by PCR	Dog	%	35	-	-	-	-	35
				-			-	
							-	
		N	7	-	-	-	-	7
				-			-	
							-	
	Human	%	2		9	5.4	5.4	54.5
				7.2			3.6	
				9			12.7	
		N	1	4	5	3	3	30
				5			2	
							7	
Total Number and Percent of the examined cases	Dog	%	100		-	-	-	
				-			-	
				-			-	
		N	20		-	-	-	
				-			-	
				-			-	
	Human	%	10.9		12.7	20	9	
				14.5			3.6	
				10.9			18.1	
		N	6		7	11	5	
				8			2	
				6			10	
Type of lesion			No lesion	Gastric polyps: Hyperplastic polyps Fundic gland polyps	Dysplastic reaction	Gastric adenoma	Adenocarcinoma: Tubular, Papillary, Solid	Total positive

Gastric polyps which showed *H. pylori* infection were of two types; hyperplastic polyps and fundic gland polyps. The former characterized by abundant glandular structures and heavy inflammatory cells infiltration including lymphocytes, plasma cells, and few eosinophils (Fig. 2a) with interepithelial lymphocytic infiltration. One case with fundic gland polyp showed marked ulceration and characterized by irregularly dilated fundic glands with severe inflammatory reaction, hemorrhage and mucosal ulceration (Fig. 2b and c). The ulcerated areas revealed marked necrosis, inflammatory exudate and heavy neutrophilic cells infiltration admixed with few lymphocytes and plasma cells (Fig. 2d). A case with dysplastic reaction was positive for *H. pylori* infection, it was characterized by haphazard proliferation of the gastric gland with a typic cells and pattern as well as irregularly formed glandular structure, the stroma is heavily infiltrated by mononuclear inflammatory cells (Fig. 2e). Gastric adenoma was diagnosed in two cases and revealed small compact glandular growths with hyperchromatic
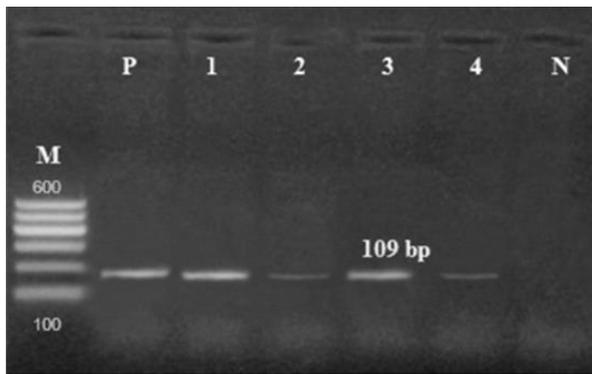
Fig 1: 1% Agarose gel electrophoresis of 16S rRNA gene after PCR. Lane M: 100- 600bp DNA ladder; Lane 1,2,3,4 were positive at 109bp. Lanes P: Control positive (*H. pylori*). Lane N: control negative (*E.coli*).


Fig. 2: H&E stained sections of gastric biopsy specimens associated with *H. pylori* infection; (a) Hyperplastic polyp showing abundant glandular structures (arrow) and heavy inflammatory cells infiltration. (b-c) Fundic gland polyp showing; (b)irregularly dilated fundic glands (arrow) with (b) severe inflammatory reaction (IF), hemorrhage and (c) mucosal ulceration, necrosis (NC), inflammatory exudate and heavy neutrophilic cells infiltration (N). (e) Dysplastic reaction presenting haphazard proliferation of the gastric gland with atypic cells and pattern (dashed arrow) with heavy stromal mononuclear inflammatory cells infiltration (IF). (f)Gastric adenoma showing small compact glandular growths with hyperchromatic nuclei, mild stromal reaction and sometimes (g) superficial erosions (arrow) covered by pink hemorrhagic debris. (h) Gastric adenocarcinoma showing irregular proliferation of atypic cells with hyperchromatic nuclei, pleomorphism, few mitotic figures (upper corner).

nuclei and few mitotic figures (Fig. 2f) with mild stromal reaction. In one case it appeared with superficial erosions (Fig. 2g and h) that were covered by pink hemorrhagic debris.

In regards to cases with gastric adenocarcinoma, variable pictures were observed; the most common of which, was characterized by irregular glandular proliferation with atypic cells that presented hyperchromatic nuclei, pleomorphism, few mitotic figures,
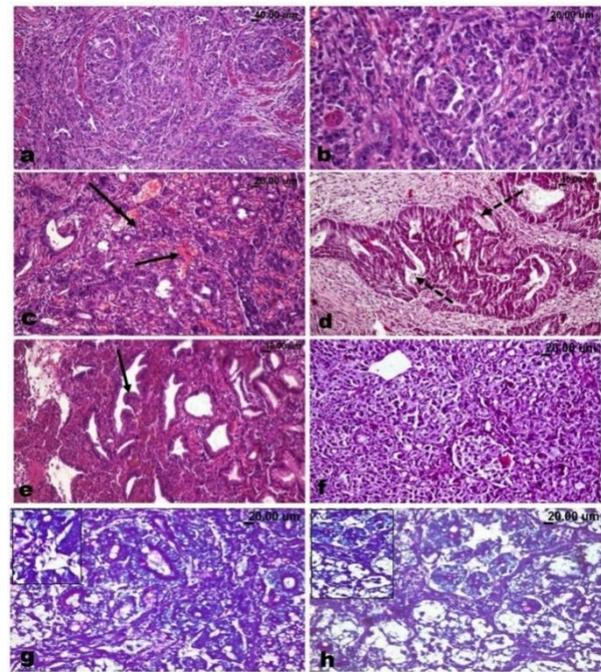
Fig. 3: H&E stained sections of biopsy specimens of gastric adenocarcinoma showing; (a and b) irregular glandular proliferation with atypic cells with hyperchromatic nuclei, pleomorphism with (c) marked angiogenesis. (d) Tubular adenocarcinoma demonstrating irregularly distended, branched or fused tubules, with intraluminal mucus, nuclear and inflammatory debris (dashed arrow). (e) Papillary adenocarcinoma showing epithelial projections with central fibrovascular core. (f) Solid pattern adenocarcinoma showing solid masses of atypical irregular glandular hyperplasia with intense pleomorphism and scares irregular atypic glandular. (g and h) Giemsa staining demonstrated positive *H. pylori* in cases with adenocarcinoma.
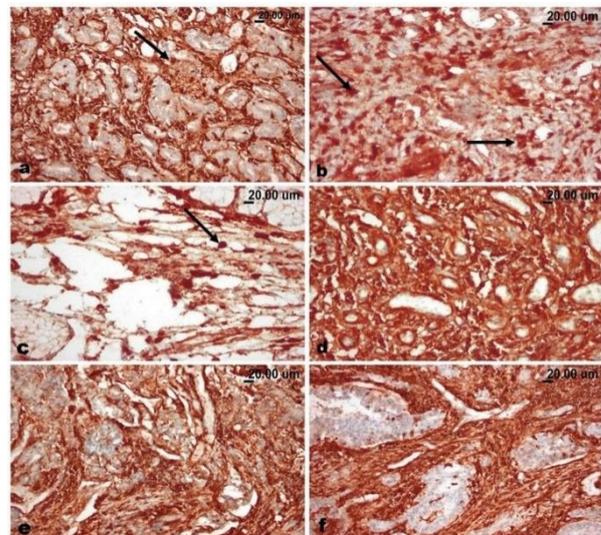
Fig. 4: Immunohistochemistry micrograph showing the expression of CD44 (a-c) and MMP-2 (d-f) in adenocarcinoma cases.

scares apoptosis and some vacuolated and necrotic cells (Fig. 3a and b) with marked angiogenesis (Fig. 3c). Tubular adenocarcinoma demonstrated various sizes, irregularly distended, branched or fused tubules, with intraluminal mucus, nuclear and inflammatory debris (Fig. 3d). Papillary form (Fig. 2e) was characterized by

epithelial projections scaffolded by a central fibrovascular core. Solid pattern adenocarcinoma was another picture that characterized by marked atypical irregular glandular hyperplasia into a solid mass of a typic tumor cells with intense pleomorphism with scares irregular a typic glandular pattern (Fig. 3f). Giemsa staining demonstrated positive appearance of *H. pylori* in several cases (Fig. 3g and h).

Intensive expression of CD44 and MMP-2 in gastric cancer cases

Immunohistochemistry revealed strong expression of CD44 in *H. pylori*-associated gastric cancer cases (Figs. 4a and b) primarily in the stromal cells and extended peripherally (Fig. 4c). The expression was limited to a small number of epithelial cells in gastric adenomas. However, MMP-2 expression was observed in all neoplastic lesions associated with *H. pylori* infection. The overall intensity of MMP-2 was higher in all types of adenocarcinoma (Figs. 4d-f).

DISCUSSION

Infections with *H. pylori* become a significant public health issue in many countries. Several studies indicate that diseases as stomach cancer, chronic gastritis and peptic ulcer are caused by *H. pylori* (Cardenas *et al.*, 2008). The present study examined the expression pattern of matrix metalloprotein 2 (MMP-2) in patients with *Helicobacter pylori*-associated gastritis and to investigate the effect of *H. pylori* on gastric cancer stem cells and to confirm relation between human and companion animals in transmission of *H. pylori*. In this study, blood serum and stool samples of total 75 patients (55 human, 20 dogs) were analyzed concerning the distribution of *H. pylori* antibody level in serum and antigen existence in stool, And in the cooperative estimation of the two tests, an elevated positivity rate in serum Ab was found and there was a significant difference among results. The reasons for *H. pylori* IgA being higher than antigen in the present study is the IgA antibody positivity ongoing in the serum. IgM class antibodies develop first. And then, IgG and IgA antibodies progress systematically and locally in gastric mucosa and the level of these two antibodies can be reserved from months to years in *H. pylori*-infected patients (Fox *et al.*, 2009). The present results of detection of *H. pylori* antigen from human stool indicated that 35 out of 55 (63.6 %) were positive for *H. pylori* antigen and 50% (10/20) for dogs faeces were positive for *H. pylori* antigen. Similar results were reported by (Mishra *et al.*, 2008, Jafar *et al.,* 2013) Overall, a total of 75 sera samples from dogs (n = 20) and human (n = 55) were tested. A relatively high percentage of dogs were found to have antibodies to *H. pylori* by ELISA kit (90%; 18/20) as previous studies (Elhariri *et al.*, 2017). A total of 87.2% (48/55) of human sera were found to have antibodies to *H. pylori*. Similar results reported by (Shi *et al.*, 2008) Serology is commonly used but cannot distinguish the difference between an active infection and a past infection (Meijer *et al.*, 1997). PCR is more precise in identification when compared to other methods; the product of PCR can be managed with restriction enzymes to confirm *H. pylori* strains. Also, starting with the PCR, DNA sequencing can be complete to confirm mutations, which no other

technique is proficient of doing. The selected targets for these PCR methods included the 16S rRNA, which has been broadly used and has been established to have a increased sensitivity (Shahi *et al.*, 2015). Hp1-Hp2 PCR was applied to gastric biopsy specimens from *H. pylori*-infected patients, a 109-bp PCR product was generated from all 75 biopsies specimens (55 human, 20dog) tested (Fig. 1), the given results was 30 positive out of 55 human gastric biopsies with a percentage 54.5% and 7 positive from 20 dog gastric biopsies with percentage 35% this finding consistent with results of previous studies (Ogiwara *et al.*, 2009, Basiri *et al.*, 2014). The PCR method detects *Helicobacter* spp. DNA, but cannot differentiate the infection is active or not (Sjö¨din *et al.*, 2011). It is supposed that dogs and cats are risk factors of a *Helicobacter* spp. infection in humans, whereby the bacteria are spread through licking animals. This concept is reported by Ekman *et al.* (2013), who found *Helicobacter* spp. in many saliva samples. The present study revealed that incidence of *H. pylori* among dyspeptic patients (Table 2) with normal Pathological findings was 10.9% among human samples and 100% among Dog samples. The negative results of pathological findings in dogs samples despite the positive detection of *H. pylori* by PCR and serological test may be because of the patchy distribution of organisms within the stomach, or may be indicate an inactive *H. pylori* infection or the animals were infected with other species of *Helicobacter* that gave a false positive results with the used primers in PCR. Studies evaluating the prevalence of numerous species of *Helicobacter* in the canine stomach appear to confirm this finding. *H. pylori* infrequently occur in dogs. Taking this into consideration, Studies including the histological evaluation of dogs' stomach exposed the presence of the bacterium as a predominant incidence in the body and gastric fundus. However, the grade of colonization by *H. pylori* do not relate directly with the diagnosis of insignificant to moderate gastritis in dogs (Eaton *et al.*, 1996).On the other hand we establish a large association between *H. pylori* infection and different type of gastric neoplastic lesion among human cases as show on Table 2, similar observations were reported by (Gisbert and Calvet, 2011) However, to verify conclusively that *H pylori* is responsible for the augmented gastric cancer risk, future interventional studies were necessary. New studies have demonstrated the existence of cancer stem cells (CSCs) in solid tumors, which share many features with tissue stem cells, such as self-regeneration and differentiation, and are mainly responsible for supporting the growth of tumors (Vermeulen *et al.*, 2008). *H. pylori* is capable to motivate the migration characterization of mesenchymal stem cells (MSCs) directly in in-vivo and in vitro studies (Zhang *et al.*, 2016). So, it provides an unusual microenvironment with exceptional situation in stomach. This study indicates that *H. pylori* as incorporator part of this microenvironments can transform the regulation of carcinogenesis- and metastasis-relevant genes expression on migrated cells like MSCs. In this study we try to clarify the role of *H. pylori* infection in inducing gastric cancer We have established that *H. pylori* infection causes upregulation of MMP-2 in epithelial cells, according to previous studies there is an association between the expression of MMP-2 and gastric cancer, more than 50% of reported studies showed that irregular

changes in MMP-2 expression show a critical role in tumor metastasis in gastric cancer (Fereshte*et al*., 2019). In this study we show that human *H. pylori* induced gastritis is associated with a substantial increase in MMP-2, activity in the gastric mucosa Fig 4 (d-f). Previous studies have shown same results (Mori *et al*., 2003).Virulence genes of *H. pylori* especially cag A adhere to the cellular matrix and up-regulate MMP as we demonstrates this change certainly corresponds to an epithelial–mesenchymal change, with adequate decrease in epithelial cell markers and an increase in mesenchymal cell markers. Moreover, high level of CD44 was expressed by these cells, which is a marker of gastric CSC and which present CSC-like properties (Bessede *et al*., 2014). In this study Immunohistochemistry revealed strong expression of CD44 in *H. pylori*-associated gastric cancer cases (Figs. 4a and b) primarily in the stromal cells and extended peripherally (Fig. 4c). The expression was restricted to a minor number of epithelial cells in gastric adenomas. This result agrees with (Bessède *et al*., 2014).

Conclusion

Several studies reported that the cause gastric cancercan be *H. pylori* infection. *H. pylori* affect gastric mucosa with changes in gastric stem cells and their differentiation, through upregulation of MMP's and expression of CD44 so this may potentiate oncogenic alteration. Companion animals play a silent role in zoonotic transmission of *Helicobacter* spp.

Author contributions

All authors contributed to the reagents/materials/ analysis tools, collected the material, analyzed the data and wrote and revised the manuscript.

REFERENCES

Bancroft JD and Gamble M, 2008. Theory and Practice of Histological Techniques. 6th Ed, Churchill Livingstone, Elsevier, China.

Basiri Z, Safaralizadeh R, Bonyadi MJ, *et al*., 2014. Helicobacter pylori vacA d1 genotype predicts risk of gastric adenocarcinoma and peptic ulcers in northwestern Iran. Asian Pac J Cancer Prev, 15: 1575.

Bessede E, Staedel C, Acuna Amador LA, *et al.,* 2014. Helicobacter pylori generates cells with cancer stem cell properties via epithelial–mesenchymal transition–like changes. Oncogene, 33: 4123-31.

Cardenas VM, Dominguez DC, Puentes FA, *et al*., 2008. Evaluation of a novel stool native catalase antigen test for Helicobacter pylori infection in asymptomatic North American children. J Pediatr Gastroenterol Nutr, 46: 399-402.

Chung JW, Ha M, Yun SC, *et al*., 2013. Meta-analysis: sequential therapy is superior to conventional therapy for Helicobacter pylori infection in Korea. Korean J Gastroentero, 62: 267-271.

Eaton KA, Dewhirst FE, Paster BJ, *et al*., 1996. Prevalence and varieties of Helicobacter species in dogs from random sources and pet dogs: animal and public health implications. J Clin Microbiol, 34: 3165-3170.

Ekman E, Fredriksson M and Trowald-Wigh G, 2013. Helicobacter spp. in the saliva, stomach, duodenum and faeces of colony dogs. Vet J, 195: 127-129.

Elhariri M, Elhelw R, Hamza D, *et al*., 2017. Serologic evidence and risk factors for Helicobacter pylori infection in animals and humans. J Infect Dev Ctries, 11: 414-419.

Bibak F, Ahmadi S, Khateri Z, *et al*., 2019. The role of matrix metalloproteinase-2 expression in gastric cancer susceptibility: A Systematic Review. Int J Cancer Manag, September; 12: e94185.

Fox JG and Megraud F, 2009. Helicobacter, In: Murray PR (editor), Manual of Clinical Microbiology. 9th ed. Volume 1, Ankara, pp: 947-62.

Gisbert JP and Calvet X, 2011. Review article: common misconceptions in the management of Helicobacter pylori-associated gastric MALT-lymphoma. Aliment. Pharmacol Ther, 34: 1047-1062.

Goel G and Sun W, 2015. Advances in the management of gastrointestinal cancers-an upcoming role of immune checkpoint blockade. J Hematol Oncol, 8: 86.

Hsu SM, Raine L and Fanger H, 1981. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. Am J Clin Pathol, 75: 816-821.

Jafar S, Jalil A, Soheila N, *et al*., 2013. Prevalence of Helicobacter pylori infection in children, population-based cross sectional study in west Iran. Iran J Pediatr, 23: 13-18.

Junqueira ACM, Ratan A, Acerbi E, *et al*., 2017. The microbiomes of blowflies and houseflies as bacterial transmission reservoirs. Sci Rep, 7: 1-15.

Marshall BJ and Warren JR, 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet, 1(8390): 1311–1315.

Meijer BC, Thijs JC, Kleibeuker JH, *et al.,* 1997. Evaluation of eight enzyme immunoassays for detection of immunoglobulin G against Helicobacter pylori. J Clin Microbiol, 35: 292 4.

Mori N, Sato H, Hayashibara T, *et al.,* 2003. Helicobacter pylori induces matrix metalloproteinase-9 through activation of nuclear factor kappa B. Gastroenterology, 124: 983–92.

Necchi V, Candusso ME, Tava F, *et al*., 2007. Intracellular, intercellular and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by Helicobacter pylori. Gastroenterology, 132: 1009–1023.

Ogiwara H, Sugimoto M, Ohno T, *et al.,* 2009. Role of deletion located between the intermediate and middle regions of the Helicobacter pylori vacA gene in cases of gastroduodenal diseases. J Clin Microbiol, 47: 3493-500.

Rocco A, Liguori E, Pirozzi G, *et al*., 2012. CD133 and CD44 cell surface markers do not identify cancer stem cells in primary human gastric tumors. J Cell Physiol, 227: 2686–2693.

Rossi DJ, Jamieson CH and Weissman IL, 2008. Stems cells and the pathways to aging and cancer. Cell, 132: 681–696.

Shahi H, Reiisi S, Bahreini R, *et al*., 2015. Association between Helicobacter pylori cagA, babA2 Virulence Factors and Gastric Mucosal Interleukin-33 mRNA Expression and Clinical Outcomes in Dyspeptic Patients. IJMCM, 4: 227-34.

Shi R, Xu S, Zhang H, *et al*., 2008. Prevalence and risk factors for Helicobacter pylori infection in Chinese populations. Helicobacter, 13: 157-165.

Sjo¨din S, Trowald-Wigh G and Fredriksson M, 2011. Identification of Helicobacter DNA in feline pancreas, liver, stomach and duodenum: comparison between findings in fresh and formalin-fixed paraffin-embedded tissue samples. Res Vet Sci, 91: e28-30.

Sugano K, Tack J, Kuipers EJ, *et al,* 2015.Kyoto global consensus report on Helicobacter pylori gastritis. Gut, 64: 1353-1367.

Vermeulen L, Sprick MR, Kemper K, *et al,* 2008. Cancer stem cells-Old concepts, new insights. Cell Death Differ, 15: 947-958.

Yi-pin Lv, Cheng P, Zhang JY, Mao FY, *et al*., 2019. Helicobacter pylori–induced matrix metallopeptidase-10 promotes gastric bacterial colonization and gastritis. Sci. Adv, 5: eaau6547.

Zhang X, Hua R, Wang X, FY 2016. Identification of stem-like cells and clinical significance of candidate stem cell markers in gastric cancer. Oncotarget, 7: 9815-9831.