International Journal of Applied and Natural Sciences (IJANS)
ISSN 2319-4014
Vol. 2, Issue 4, Sep 2013, 109-116

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

STUDY THE EFFECT OF OXIDATIVE STRESS AND SEVERAL BIOCHEMICAL FEATURES IN PATIENTS WITH *HELICOBACTER PYLORI* (*H PYLORI*) BACTERIA

ATHEER A. MEHDE & TAKWA A. CHECHAN

Department of Acceptable Analysis, Health and Medical Technical College, Baghdad, Iraq

ABSTRACT

Objective: Gastric ulcer, also known as peptic ulcer, is a localized area of erosion in the stomach lining, resultin abdominal pain, possible bleeding, and other gastrointestinal symptoms. The most common cause of gastric ulcer is a stomach infection associated with the *Helicobacter pylori* (*H pylori*) bacteria. The spread of *H pylori* among humans is not completely understood; it may spread through contaminated food and water. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. The aim of the current study is to investigate the oxidative stress in patients with *H. pylori* and compare to control group.

Methods: A total of 60 males were studied in Digestive Centerand Central Health Laboratory, Baghdad, Iraq. The samples were collected within 3 months, starting from July till September 2011. The pepsinogenI(PGI), pepsinogen II(PGII), Anti-*Helicobacter pylori*IgG and gastrin 17 in sera of patients and control are measurement after subjected all patients for clinical diagnosis and examination of (gastroscopic) under the supervision of a committee of doctors jurisdiction. Laboratory investigations including gastric panel tests, serum total antioxidant capacity (TAA), malondialdehyde (MDA), Glutathione (GSH), Vitamin C, Vtamin E, uric acid and TAA / MDA ratio . The control group consisted of 40 healthy individuals who were not complaining of any gastro intestinal problem.

Results & Conclusions: The present study showed increase in the mean level of Anti-*H.pylori*Ab-IgG more over observed that the level of circles accounting PGI and PGII and PGII / PGI ratio which increased in patient group when compared to control group, while the mean level of gastrin17 was decreased in patient group compared to control. On the other hand the mean level of MDA in the sera of patients with *H.pylori*showed a significant increase [P <0.001] compared to control group, while significant decrease in TAA, vitamin C, glutathione , vitamin E and TAA/MDA ratio in patient groups compared to control. The current study concludethatoxidative stress may play important role in development of Gastric ulcer disease, also TAA/MDA ratio may be used as marker to diagnosis the development the infection in patient with *H.pylori*

KEYWORDS: Folic Acid, Total Antioxidant Capacity, Malondialdehyde, Pepsinogen**,** Gastrin17, and *Helicobacter pylori*

INTRODUCTION

Gastric ulcer resulted from persistent erosions and damage of the stomach wall that might become perforated and developed into peritonitis and massive haemorrhage as a result of inhibition in the synthesis of mucus, bicarbonate and prostaglandins [(1)]. The acid out- put in gastric ulcer patients had been found to be within or below normal levels, but slow gastric motility and slow gastric emptying may predispose a person to gastric ulcer [(2)]. Various factors can contribute to the formation of gastric ulcer such as the infection of stomach by *Helicobacter pylori*, the frequent use of nonsteroidal anti-inflammatory drugs (NSAIDs) aspirin, and alcohol [(3)].

*Helicobacter.Pylori(H.pylori)*It lives in the human stomach exclusively and it is the only known organism that can thrive in that highly acidic environment [(4)]. *Helicobacter pylori* causes three different types of immune reactions acute, chronic active, and the atrophic stages of gastritis. These are all stages of inflammation caused by the immune system [(5)].When *H. pylori* infect the stomach or duodenum, it does a number of things to become established in the tissue. It must penetrate the mucus layer, attach to the surface of the epithelial cell, and acquire nutrients in order to survive [(6)].

The biological oxidative effects of free radicals on macromolecules are controlled by a spectrum of enzymatic and nonenzymaticantioxidants An impaired antioxidant defense system is widely regarded as a cause of oxidative damage accumulation in cells. Various markers of oxidative damage have been identified. Malondialdehyde (MDA), which is one of the most popular markers, was designed to indicate lipid peroxidation. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells [(7)]

MATRIAL AND METHODS

The sampling procedure was done in 60male patients with *H.pylori* in (26-61 year) .None of these patients and control were on a special diet, or taking any antioxidant drug except (voltarin, paracetetol). Patients were chosen from the patients referred to the Digestive Centerand Central Health Laboratory, Iraq. The samples were collected within 3 months, starting from July till September 2011.Patients were compared with 40 healthy control subjects were included (men age 28-61 year). Blood samples were obtained from the patients the diagnosis based on the clinical and endoscopy examination under supervision of physciains or surgery specialists. Five milliliters (5ml) venous blood samples were collected , the samples were transferred into clear plain tubes and centrifuged within 30 minutes of collection, then serum from all blood samples were separated for measuring *H.pylori*, PGI, PG II, gastrin 17, TAA, MDA, glutathione, vitamin. E, vitamin C and uric acid .The sera were stored in the freezer at about – 20 C° for maximum of fortnight before analyses. The serum Anti-*H.pylori*IgGAb, PGI, PGII and gastrin 17 were measured by double sandwich enzyme – linked linkedimmunosorbent assay (ELISA).Total antioxidant activity (TAA) in serum samples was carried out according to Rice–Evans and Miller [(8)]. Serum MDA was determined according to the modified method of Benge[(9)]. The serum glutathione concentration was measured according to Ellman's method[(10)]. Vitamin C levels were estimated by the method Washington and Toronto[(11)].Vitamin E levels were determined according to a modified of Hashim and Schuttringer[(12)]. The serum uric acid levels was measured by spectrofotomateric methods.

All statistical analyses in studies were performed using SPSS version (statistical package for social sciences) Ver.17 [SPSS Inc. Chicago IL] .Descriptive analysis was used to show the mean and standard deviation of variables.

The significance of difference between mean values was estimated by Student T-Test. The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

RESULTS

The mean level of Anti- *H.pylori*- IgGAb was 79.12± 11.07 EIU in sera of *H.pylori* patients group in comparison with the mean serum concentration of control group 13.39±3.19EIU which showed significant increase ($p<0.001$), as shown in table 1.

The mean concentration of PGI, PGII and PGI/PGII were 181.88± 23.15 , 15.47± 1.49 and 11.71± 2.10, respectively in sera of patients group while the mean concentration of the gastrin 17 (1.37± 0.28) was showed significantly decrease ($p<0.001$) ,as shown in table 1.

Table 1: The Mean Distribution of Anti-*H.pylori*-IgGAb, PGI, PGII, PGI/ PGII Ratio and Gastrin 17 in Sera *H.pylori* Patients Group and Control Group

Characteristic	Patients Group	Control Group	P Value
Anti-*H.pylori*-IgGAb(EIU)	79.12± 11.07	13.39±3.19	<0.001
PGI(µg/l)	181.88± 23.15	72.32±15.51	<0.001
PGII(µg/l)	11.71± 2.10	8.80±1.41	<0.001
PGI/PGII	15.47± 1.49	8.21±1.00	<0.001
Gastrin 17(Pmol/l)	1.37± 0.28	8.60±1.24	<0.001

The mean levels of sera TAA, glutathione, vitamin C, vitamin E and TAA/MDA ratio showed. A significant decrease in patients group when compared to control group(P<0.001), while MDA showed a significant increase in *H.pylori*patientsgroup in comparison to control group(P<0.001) .It was no significant difference in serum uric acid when compared to control group (P> 0.05) as shown in table 2 .

Table 2: The Mean Distribution of TAA, MDA, Glutathione, Vitamin C, Vitamin E, Uric Acid and TAA/MDA Ratio in Sera *H.pylori* Patients Group and Control Group

Characteristic	Patients Group	Control Group	P Value
TAA(µmol/l)	374.83± 10.35	413.55±5.55	<0.001
MDA(µmol/l)	4.50± 0.67	1.42±0.12	<0.001
Glutathione(µmol/l)	304.45± 8.40	344.83±7.27	<0.001
VitaminE(mg/dl)	0.93± 0.15	1.36±0.18	<0.001
VitaminC(mg/dl)	1.46± 0.09	1.66±0.02	<0.001
Uricacid(mg/dl)	6.62± 0.34	6.04±0.37	<0.05
TAA/MDAratio	85.56± 15.75	292.45±21.03	<0.001

There were a different correlations between Anti-*H.pylori*-IgGAb ,PGI ,PGII, PGI/ PGII ratio and gastrin 17 in sera *H.pylori* patients groupwith antioxidant parameters while there were no correlationin control group as shown in table 3. There were a negative significant correlated TAA/MDA ratio with Anti–H. PyloriAb-IgG [r=-0.91,p<o.o1],PGI(µg/l) [r=-o.72,p<0.01,n=60] and PGII(µg/l) [r=-0.41 ,p<0.01],While positivecorrelation between TAA/MDA ratio and gastrin 17 (Pmol/l)[r=0.84, p<0.01] table 3.

Table 3: Correlations between Anti-*H.pylori*-IgGAb, PGI, PGII, PGI/ PGII Ratio and Gastrin 17 in Sera *H.pylori* Patients Group with Antioxidant Parameters

Characteristic	Anti-*H.pylori*-IgGAb(EIU)		PGI(µg/l)		PGII(µg/l)		Gastrin 17 (Pmol/l)	
	r	p	r	p	r	p	r	p
TAA [µmol/L]	-0.90	0.01	-0.72	0.01	-0.45	0.01	0.83	0.01
MDA [µ mol/L]	0.93	0.01	0.79	0.01	0.44	0.01	-0.79	- 0.01
Glutathione(µmol/l)	-0.82	0.01	-0.81	0.01	-0.39	0.01	0.68	0.01
VitaminE(mg/dl)	-0.83	0.01	-0.64	0.01	-0.36	0.01	0.71	0.01
VitaminC(mg/dl)	-0.93	0.01	-0.78	0.01	-0.46	0.01	0.76	0.01
TAA/MDA ratio	-0.91	0.01	-0.72	0.01	-0.41	0.01	0.84	0.01

DISCUSSIONS

The *H. pylori* infection is one of the most common chronic infections in the majority of the global population [(13)]. The results in the present study agreed with another research which reported the presence of B-lymphocytes and plasma cells in the gastric mucosa is evidence of an active humoral response in chronic infection.[(14)]

In *H.pylori* infection, the significant elevation of IgG class antibodies to *H.pylori* in human serum or plasma are found.[(15)] . Most research groups find an IgG assay to give the best discrimination between *H.pylori* positive and negative patients. However, the activity or severity of the gastric inflammation failed to correlate with the titer of the systemic response. Several studies showed that serum PGI increase due to inflammation. [(15)] The PGI is a precursor enzyme to

pepsin and is synthesized by the chief cells of the gastric corpus (from so called oxyntic glands of the gastric mucosa), the major part of the PGI is secreted into the gastric lumen but a small amount can be found in serum.[16] PGI and PGII, the two main precursors of pepsin, are both produced by the chief and mucous neck cells of the stomach, and were measured as markers of gastric atrophy. PGII is also produced by pyloric glands. In gastritis, serum PGI and PGII levels increase due to inflammation. The *H.pylori* infection ongoing in relation with gastritis activity and inflammation and stimulating the PGs to release. [17] Serum levels of the PGI/PGII ratio were used in several studies as markers of gastric mucosal changes [17,18]. These findings suggest that serum pepsinogen levels, especially PGI/PGII ratio, are a new tool that may be found to be clinically useful in evaluation of treatment outcome in patients with H.pylori-associated gastritis [19]. The results about Gastrin levels agreed with other studies. [20,21] As revealed by these studies, Gastrin is a polypeptide hormone produced by G cells in the lateral walls of the glands in the antral portion of the gastric mucosa in response to a meal. [21]Gastrin secretion is particularly stimulated by protein digestion in the stomach, particularly small peptides and amino acids in the lumen of the stomach, which act directly on the G cells. The most potent stimuli for gastrin secretion are phenylalanine and tryptophan and stimulated by gastric distension and by vagal stimulation [21].

H.pylori induces infiltration and activation of neutrophils and macrophages. [22] One characteristic event in inflammation is the infiltration of affected tissue by neutrophils, which produce large amounts of ROS in host defence reactions .Enhanced ROS levels due to neutrophil infiltration and increased oxidative DNA damage have been reported in *H.pylori* infected patients[22,23].The increased level of pro-oxidative factors and decreased level of TAA in sever oxidative stress can modulate may processes in gastric epithelium [24] .

The present result in table 3 showed that *H.pylori* may be associated with increased oxidative stress and decreased serum TAA. Lipid peroxidation is one of the reactions set into motion as a consequence of the formation of these radicals in cells and tissue. The initiation of peroxidation has been considered the proximal cause of cell membrane destruction and cell damage[25]. Increased amounts of MDA have been found in patients infection with H.pylori[26]. The significant correlation between MDA and gastric panel test may be demonstrated that increased levels of MDA are generated in *H.pylori* –infected gastric epithelial cells and that this may be one mechanism leading to apoptosis associated with infected.

The current result agreed with other studies, who have showed that serum glutathione (GSH) an important endogenous antioxidant, are significantly reduced in the patients infected with *H.pylori* as compared to non-infected ones. Similar findings have been reported by another study regarding the effects of *H.pylori* on the glutathione content of the gastric mucosa, they have shown that GSH levels are significantly lower in the patients infected with *H.pylori* than those without infection[27].A deficiency of GSH puts the cell at risk for oxidative damage [28].*H.pylori* directly decrease cellular glutathione.[27] these may be demonstrate the correlation between glutathione and gastric panel test.

Vitamin E may play a part in gastro duodenal disease was suggested by studies performed in rats showing a protective effect for α-tocopherolaginast gastric mucosal injury induced by a variety of agents.[28,29] As our knowledge, no previous study refers to the significant correlation between vitamin E PGI , PGII and gastrin 17 in *H.pylori* patients while no significant association was found between them in control , these correlation may reflect immobilization of antioxidant defenses to the sites of maximal infection in the stomach.

Most reports have not found an association between serum vitamin C concentration and H.pylori[30,31]. The current study showd an association between serum vitamin C and *H.pylori* while to knowledge the significant correlations between serum vitamin C and level of PGI ,PGII and gastrin 17 in *H.pylori* patients group are the first population –based study of

Iraqi people to report such associations. If these findings are confirmed by other investigators and are linked causally, higher intakes of vitamin C may be a protective factor in the preventation of *H.pylori* infection

There were a negative significant correlated TAA/MDA ratio with Anti–H.PyloriAb-IgG [r=-0.91, p<o.o1], PGI(µg/l) [r=-o.72, p<0.01,n=60] and PGII(µg/l) [r=-0.41, p<0.01], While positive correlation between TAA/MDA ratio and gastrin 17 (Pmol/l)[r=0.84, p<0.01] table 3. There has been no other study has showed to these differences, oxidative stress is as state in which toxic reactive oxygen species (ROS) overcomes the endogenous antioxidant defense the host.(19) The state results in an excess of free radical , which can react with cellular lipids, proteins ,and nucleic acids, leading to cellular and eventual organ dysfunction .Gastric inflammation is a highly complex biochemical protective response to cellular /tissue injury. A large amount of evidence suggests that *H.pylori* infection is major causative factors in the pathogenesis of gastric mucosal oxidatative injury in humans.(19) The significant correlation between TAA/MDA ratio and gastric panel test in *H.pylori* patients table 3 may be explain these fact .

This observation is detected for the first time to the best of our knowledge .The present study suggests that a more detailed research should be used when the role of TAA/MDA is investigated in patients with *H.pylori*. In conclusion, oxidative stress TAA/MDA ratio may be used as marker to diagnosis the development the infection in patient with H.pylori

REFERENCES

1. Cryer, B. and Spechler J.; (2006); Peptic Ulcer Disease; Sleisenger and Fordtran's Gastrointestinal and liver disease; 2: 1091.
2. Lingappa , V., Ganong W. and Lange J.;(2005);. Pathophysiology of Disease. 4th ed., Appelton and Lange.
3. Valle,D.;(2005); Peptic ulcer diseases and related disorders; Harrison's Principles of Internal Medicine; 16thed.; Mc.Graw. Hill, 1746.
4. Makola, D., Peura ,D., Crowe, S.;(2007); *Helicobacter pylori* infection and related gastrointestinal diseases; J.Clin.Gastroenterol. ; 41:6: 548-558.
5. Weck M.; Gao L.; Brenner H.; (2009); Helicobacter pylori infection and chronic atrophic gastritis associations according to severity of disease; Epidemiology; 20: 569–574.
6. Crabtree J. and Naumann M.; (2006); Epithelial cell signaling in Helicobacter infection; Curr. Signal Transduction Therapy; 1:53-65.
7. Shanmugam, N.; Figarola, J.L.; Li, Y.; Swiderski, P.M.; Rahbar, S.; Natarajan, R.;(2008);Proinflammatory effects of advanced lipoxidation end products in monocytes.; Diabetes;57:879–888.
8. Rice-Evans ,C.,RNJ.Mille;(1994);Total antioxidant status in sera and body fluids .In:Methods in Enzymology .New York: Academic Press, 279-293.
9. Benge J.A. and Aust S.D.; (1978); Estimation of serum Malondialdehyde level in hoffee P.A .and Jones M.E. (eds), Methods in Enzymology Hoffee Jones. Academic Press, New York, San Francisco, London, A Subsidinary of Harcoart Brace Jovanovich, Publisher, 51: 302.
10. Ellman G.; Dtetermination of thiol concentration ; (1959); Biochem .Biophys ; 2:82:70-77.

11. Pesce A. and Kaplan A.;(1978);; Methods in clinical chemistry, the C.V. Mosby Company- Washington D.C Toronto; 590.

12. Hashim S. and Schuttringer G.; (1966);Rapid determination of tocopherol in marco-and microquantities of plasma, results obtained in various nutrition and metabolic studies; Am. J. Clin. Nutr.; 19:2: 137- 145.

13. Noah D., OkomoM., EloumouBagnakaS., Ngaba G, AlongeI., PaloheimoL, Njoya O.; (2012); Assessing GastroPanel serum markers as a non-invasive method for the diagnosis of atrophic gastritis and *Helicobacter pylori* infection, Open Journal of Gastroenterology; 2: 113-118

14. OkomoM., Noah D., Ngaba G, EloumouBagnakaS., Alonge, L. Paloheimo L. and NjoyaO.; (2012); Association between the immune responsesagainst Helicobacter pylori infections andthe concentration of atrophic gastricbiomarkers in Cameroon Journal of Applied Medical Sciences; 1:1 15-26

15. Irvani S, Hashemi MR, Moghadam KG, Saeidee S, Khavaran K, Najari O, Ranavardi M, Nasiri S, Salmasian H, Rohanizadegan M.;(2010); Accuracy of serum pepsinogens I and II, gastrin-17 and anti-helicobacter pylori antibodies in histological diagnoses of atrophic gastritis; 56:1:13-7.

16. Cox, Michael; Nelson, David R.; Lehninger, Albert L ;(2008);Lehninger principles of biochemistry. San Francisco: W.H. Freeman.

17. Bölükbas C.; Bölükbas F.; Övünc O.; Kilic G.; DalayR.;et.al.; (2006); Relationship between *Helicobacter.pylori* status and serum pepsinogens as serologic markers in atrophic gastritis; Turk.J. Gastroenterol; 17: 172-176.

18. A.Alverez , S.Ibiza , C.Hernandez, A.Alvarez- Barrientos , VJ.Esplugues , S.sara, (2006); Gastrin induces leukocytete-endothelial cell interactions in vivo and contribute to the inflammation caused by *H.pylori*. The FASEB journal 20, 2396-2398

19. Song HJ, Jang SJ, Yun SC, Park YS, Kim MJ, et al.; (2010); Low Levels ofPepsinogen I and Pepsinogen I/II Ratio are Valuable Serologic Markers forPredicting Extensive Gastric Corpus Atrophy in Patients Undergoing EndoscopicMucosectomy. Gut Liver; 4: 475–480.

20. Kang JM, Kim N, Yoo JY, Park YS, Lee DH, et al.; (2008); The role of serumpepsinogen and gastrin test for the detection of gastric cancer in Korea. Helicobacter 13: 146–156.22.

21. Haj-Sheykholeslami A, Rakhshani N, Amirzargar A, Rafiee R, ShahidiSM,et al.; (2008); Serum pepsinogen I, pepsinogen II, and gastrin 17 in relatives ofgastric cancer patients: comparative study with type and severity of gastritis. ClinGastroenterolHepatol; 6: 174–179.

22. Arend A.; Loime L.;Roosar P.; Soom M.; LoivukeneK.;et.al.; (2005); *Helicobacter pylori* substantially increases oxidative stress in indomrthacin-exposed rat gastric mucosa; Medicina (Kaunas); 41:4:343-346.

23. SZ.Ding , Y.Minohara ,Xj.Fan , J.wang ,VE.Reyes , J.patel et al ; (2007); Helicobacter pyloriinfection induces oxidative stress and programmedcell death in human gastric epithelial cells . Infect Immun; 75:4030-4039.

24. SoundravallyRajendiran, Bobby Zachariah, and AbdoulHamide;(2012); Increased Protein Carbonylation and Decreased Antioxidant Status in Anemic *H. Pylori* Infected Patients: Effect of Treatment,Saudi J Gastroenterol; 18:4: 252–256.

25. A.Valado , TC.Paula , P.Leonal , C.FontesAnaerobic exercise , oxidative stress-Effect of theintensive exercise on NO and Malondialdehyde ,proceedings of the 2007 WSEAS Int Conferenceon Cell and molecular Biology (Bio'07) Biophysics and Bioengineering Athens , Greecp566

26. Vijayan G, Sundaram RC, Bobby Z, Hamide A, Selvaraj N, Dasse NR.; (2007); Increased plasma malondialdehyde and fructosamine in anemic *H pylori* infected patients: Effect of treatment. World J Gastroenterol.;13:796–800

27. Hanna C,DorataS,MichatS,MalgorzataP,anaZofia P;(2005);Glutathione level and activity of GSH dependent enzymes in gastric carcinoma patients –a preliminary report.,Gastroenterologia polska;12:2:107-111.

28. Rui-Li Z., Wen-Da Luo, Tie-Nan Bi and Shen-K.;(2012); Evaluation of Antioxidant and Immunity-Enhancing Activitiesof*Sargassumpallidum* Aqueous Extract in Gastric Cancer Rats,Molecules, 17, 8419-8429

29. Hando O, Naito Y, Yoshikawa T. (2010); *Helicobacter pylori*; a ROS-inducing bacterial species in the stomach, Inflamm Res.;59:997–1003

30. Sun YQ, Girgensone I, Leanderson P, Petersson F, Borch K.;(2005); Effects of antioxidant vitamin supplements on *Helicobacter pylori*-induced gastritis in Mongolian gerbils. Helicobacter.;10:33–42

31. Kamiji MM, Oliveira RB.;(2005); Effect of vitamin C administration on gastric colonization by *Helicobacter pylori*. Arq Gastroenterol.;42:167–72