OPEN ACCESS

GAVIN PUBLISHERS

Family Medicine and Primary Care: Open Access

Research Article

Yordanova M. J Family Med Prim Care Open Acc 3: 139.

DOI: 10.29011/2688-7460.100039

Oxidative Stress and the Antioxidant Capacity of Plasma in Patients with *Helicobacter pylori* -Positive Gastroduodenitis and the Effect on Serum Iron Levels

Mariana Yordanova[1,2*]

[1]Military Medical Academy, Multiprofile Hospital for Active Treatment, Varna, Bulgaria

[2]Department of General Medicine and Clinical Laboratory, Medical University of Varna "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria

[*]**Corresponding author:** Mariana Yordanova, Department of General medicine and Clinical laboratory, Medical University of Varna "Prof. Dr. Paraskev Stoyano", Varna, Bulgaria, Military Medical Academy, Multiprofile Hospital for Active Treatment,

Citation: Yordanova M (2019) Oxidative Stress and the Antioxidant Capacity of Plasma in Patients with *Helicobacter pylori* -Positive Gastroduodenitis and the Effect on Serum Iron Levels. J Family Med Prim Care Open Acc 3: 139. DOI: 10.29011/2688-7460.100039

Received Date: 30 November, 2019; **Accepted Date:** 17 December, 2019; **Published Date:** 23 December, 2019

Abstract

Aim: To evaluate the oxidative stress and antioxidant protection in patients with chronic Gastroduodenitis and the relationship between their levels with the etiologic agent *H. pylori* and how this affects iron homeostasis.

Introduction: Several records indicate that oxidative stress plays a significant role in the pathogenesis and progression of chronic inflammatory diseases of the gastrointestinal tract. *H. pylori* infection generates free oxygen and nitrogen species (ROS/RNS) cause damage to the gastric mucosa.

Materials and Methods: 55 patients with gastritis and 80 healthy volunteers were studied, with a mean age of 59.69±11.52 and 53.8 ±0.8 years, respectively. Both groups define routine laboratory parameters (including CRP and Iron). Serum levels of antibodies against *H. pylori* (ELISA DiaMetra Italy) and antigenic presence in faecal matter were examined. Patients undergo endoscopic examination. Oxidative stress (dROMs) and serum antioxidant capacity (BAP) were determined spectrophotometrically on a semi-automatic Carpe diam analyzer. (Diacron Labs Italy). Statistical methods used: T-test for comparison of mean values, descriptive and correlation analysis.

Results: The patient group showed significantly elevated mean dROMs values compared to the control group. Levels in patients with *H. pylori* infection differ significantly from HP negative ones. The BAP test showed a significant decrease compared to the control group. A similar relationship exists between the two patient groups for the BAP test. A moderate positive correlation between CRP and dROMs was found, and a moderate negative correlation with the BAP test. Increasing dROMs and decreasing BAP results in a decrease in serum iron levels.

Conclusion: Determination of oxidative stress levels, antioxidant protection, and iron homeostasis may be used in the monitoring of patients with chronic Gastroduodenitis.

Keywords: Oxidative stress; Antioxidant capacity; CRP; *H. pylori*; Iron

Introduction

Chronic gastritis is accepted as a disease of civilization. Globally, they are a common disease, with about 1/2 of people over 50 years old. In clinical practice, there is some underestimation of chronic gastritis as a severe disease, despite its prominent role in the pathogenesis of peptic ulcer and gastric cancer.

Chronic gastritis is divided into several types. Type A develops by autoimmune mechanisms, and Type B gastritis affects the antrum of the stomach and in more than 85% of cases is due to *H. pylori* (HP) infection. There is also gastritis type C, which is caused by chemical effects - NSAIDs and aggressive action of bile in reflux to the stomach. The Gastrointestinal (GI) tract is a key source of Reactive Oxygen (ROS) and Nitrogen (RNS) compounds. Gastritis is characterised by a chronic non-specific inflammatory process of the gastric mucosa of diffuse and focal character, gradually leading to atrophy. Infiltration of inflamed gastric mucosa with neutrophils/or macrophages producing inflammatory cytokines and other mediators contributes to oxidative stress. Multiple endogenous and exogenous factors are the cause of gastritis and peptic ulcer, the formation of free radicals

being closely linked to them (smoking, alcohol, stress, medicines, foods, ionising radiation, infections, ischemic conditions, etc.).

Reactive Oxygen Species (ROS) are by-products of normal cellular metabolism. Low and moderate amounts of ROS have a beneficial effect on several physiological processes, including the killing of invading pathogens, wound healing and tissue regeneration processes [1]. ROS include radical compounds such as superoxide (O2·), hydroxyl radicals (HO·), lipid hydroperoxides, and reactive nonradical compounds including singlet oxygen ($^1O2$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), chloramines (RNHCl), and ozone (O_3) [2]. Oxidative stress is viewed as a state of imbalance between ROS generated and host endogenous antioxidant protection [3]. Excess free radicals can react with cellular lipids, proteins and nucleic acids, leading to cellular damage and eventual organ dysfunction. Although free radicals are generated continuously, the body is equipped to protect itself against the harmful effects of ROS with the help of antioxidants. The antioxidants are compounds that discard, clear and suppress the formation of free radicals or the opposition to their actions. Enzyme antioxidants include (superoxide dismutase (SOD), catalase and thioredoxin- and glutathione-dependent peroxidase (s) and reductase. Non-enzymatic antioxidants include vitamins A, C and E; glutathione; α-lipoic acid; carotenoids; trace elements such as copper, zinc and selenium; Coenzyme Q10 (CoQ10); and cofactors such as folic acid, uric acid, albumin and vitamins B_1, B_2, B_6 and B_{12} [1].

Helicobacter pylori is a spiral gram-negative, microaerophilic bacterium, highly adapted, that selectively colonizes the human stomach [4]. It is transmitted by faecal-oral, gastro-oral and oral-oral routes, assuming that the infection is mainly in childhood. According to statistics, it affects around 3-4 billion people worldwide, with the rate of infection in developing countries very high, reaching up to 80% [5]. During the host colonization process, *H. pylori* induces a strong inflammatory response, characterized histologically by superficial epithelial degeneration and infiltration of the gastric mucosa by inflammatory cells [3]. Polymorphonuclear cells, macrophages and lymphocytes infiltrating lamina propria and intraepithelial space generate accumulation of ROS and oxidative damage to DNA in the gastric mucosa. Approximately 50% of *H. pylori* strains produce thermally labile, protease-sensitive vacuole cytotoxin [6], and express different enzymes that play a role in mucosal damage [7] mucosal permeability is altered and this contributes to the generation of specific immune responses. The most important enzyme of *H. pylori* is urease, which produces ammonia. This enzyme protects HP from gastric acid of the stomach, breaks down the mucus layer and adheres to the gastric epithelium with a toxic effect on it [8] [8]. *H. pylori* isolated from antrum and stomach body in more than 80% of patients [5].

Determination of oxidative stress in gastric inflammation may be necessary for a better understanding of its pathophysiology. The existing relationship between *H. pylori*, the specific and nonspecific immune response of the host, the generation of oxidative stress and impaired mucosal antioxidant balance are leading in the strategy for the treatment of GI diseases.

The purpose of this study is to evaluate the oxidative stress and antioxidant protection in patients with chronic Gastroduodenitis and the relationship between their levels with the etiologic agent *H. pylori* and how this affects iron homeostasis.

Materials and Methods

The patient group includes 55 patients with gastritis at an average age of 57.93±12.08 (range 30-78) years admitted to the Gastroenterology Department of Military hospital -Varna with pain and dyspeptic syndrome. The control group consisted of 80 healthy volunteers, with an average age of 53.86±8.67 (range 30-72) years, undergoing routine annual prophylactic examinations. All participants enrolled in the study have signed informed consent. The study was approved by the local Ethical Committee (Protocol No 64/13.07.2017). Patients were selected according to the following inclusion criteria: patients with disease activation, including subjective complaints, endoscopically confirmed chronic gastritis, and HP infection. Exclusion criteria are the presence of a malignant complication and/or recent surgery. Both groups define routine laboratory parameters, including CRP as a marker of inflammation. Venous blood is taken for biochemical testing. The blood serum was removed by centrifugation for 10 minutes, 3500 rpm. Serum levels of antibodies against *H. pylori* (ELISA DiaMetra Italy) and antigen present in the faecal mass (qualitative test) were examined. Oxidative stress (dROMs) and serum antioxidant capacity (BAP) were determined spectrophotometrically on a semi-automatic Carpe diam analyzer. (Diacron Labs Italy). d-ROMs test is based on the ability of a biological sample to oxidize an aromatic amine (DPPD). Hydroperoxides, chloramines and their derivates (a class of ROMs), after reacted with an opportunely buffered chromogen, develop a coloured derivative, which is photometrically detected. The concentration of this class of ROMs, that directly parallels with colour intensity is expressed as Carratelli Units (1 CARR U = 0.08 mg% hydrogen peroxide).The range in healthy peoples is 250-300 U CARR. Increased values directly correlate to increased levels of oxidative stress.

The BAP test is a spectrophotometric test measuring the antioxidant capacity(AOC) of a sample placed in a solution of ferric chloride and its ability to reduce iron from ferric Fe^{3+} to Ferro Fe^{2+} ion.For both tests, quality control is carried out in two levels.

CRP and serum iron are determined by routine methods of a biochemical analyzer Olympus AU 640 with Beckman Coulter reagents. Patients undergo endoscopic examination to evaluate inflammatory changes in the gastric/duodenal mucosa. According to the *H. pylori* infection test, the patient group was divided into two subgroups: *HP* - positive and negative.

Statistical methods

Data analysis was done using GraphPad Prism v. 6.0 software by standard statistical methods (descriptive statistics, non-parametric T-test (Mann- Whitney) for mean comparison, and Spearman correlation analysis. Biochemical parametric data were presented as mean ± standard deviation. Statistical significance was indicated at $p<0.05$ (Tables 1-5) (Figures 1-5).

Results

Studied persons	Man (N)	Woman (N)	Total (N)
***HP* Positive** **Age(mean±SD)**	23 55.22±11.86	16 58.0±12.77	39 56.35±12.14
***HP* Negative** **Age(mean±SD)**	11 63.55±10.88	5 58.0±12.23	16 63.77±10.05
Total patients group **Age(mean±SD)**	34 57.91±12.13	21 57.95±12.31	55 57.93±12.08
Control group **Age(mean±SD)**	40 55.0±8.52	40 52.76±8.77	80 53.86±8.67

Table 1: Demographic character of participants.

Endoscopic Dx	Regional (n=26)	Diffuse (n=29)
Erythematous gastritis	15	10
Erosive gastritis	11	16
Atrophic gastritis	0	3

Table 2: Endoscopic characteristics of change in the gastrointener/duodenal mucosae of the total study patient group.

Disease and Causes	Drug (n=28)
Hypertension	b-blockers, Ca antagonists (n=6)
Infection	Antibiotic + Fe drug (n=5)
Heart failure	Anti-arrhythmic n(n=3)+ Aspirin
Joint diseases and gout	NSAID (n=10)
Alcohol	N=2
Smoking	N=3
Combination of several drug	N=10

Table 3: Concomitant chronic diseases and used drug that lead to the induction of gastritis.

Parameters	Control group	*HP* Positive	*HP* Negative
dROMs UCARR*	337.7± 35.24	402.5± 95.36	350.3±71.23
BAP umol/l**	2624±279.9	2258± 295.5	2440±212.2

*d-ROMs is a patented test by Diacron International sas, Grosseto (Italy; 1 UCarr equals 0.08 mg/dL of H_2O_2. The range in healthy peoples is 250-300 U CARR. Increased values directly correlate to increased levels of oxidative stress.

**The reference values of normal level of antioxidant defense is > 2200 µmol/L (reference iron -reducing agent: Vitamin C).

Table 4: dROMs and BAP test levels in the study groups.

d-ROMs Test main reactions (Reactive Oxygen Metabolites Test)

– R-OOH is a generic hydroperoxide.

– R-O• is the alkoxyl radical of a generic hydroperoxide.

– R-OO• is the hydroperoxyl radical of a generic hydroperoxide.

– A-NH2 is N, N-diethyl-paraphenylendiamine (chromogenic substrate).

– [A-NH2•]+ is the coloured radical cation of the chromogenic substrate.

BAP test allows to measure the chemically active antioxidant capacity (scavengers) of the plasma barrier. In particular it includes antioxidants both of exogenous nature (ascorbic acid and tocopherols) and of endogenous nature (uric acid, bilirubin and albumin).

Parameters	Control group	*HP* Positive	*HP* Negative
CRP** mg/l	2.38±1.59	14.75±14.95	5.34± 5.56
Iron *serum µmol/L	16.82±7.32	10.90± 3.57	14.58±6.24
* The reference serum serum iron by TPTZ method is 10.6-32.0 µmol/L and the range is valid for both sexes. ** the CRP reference limit is up to 5 mg/L			

Table 5: Values of the inflammatory marker CRP and serum iron in the study groups and the role of *H. pylori*.

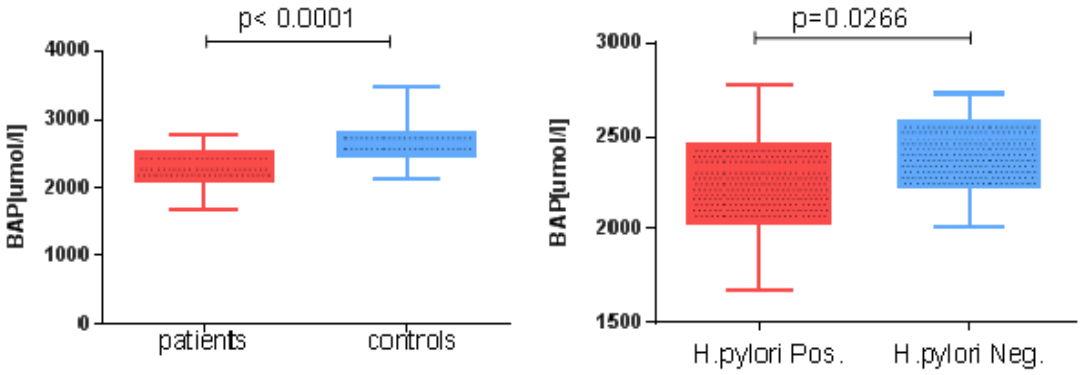

Figure 1: BAP test values of two groups (controls and patient group), and two patient group (HP positive and HP Negative).

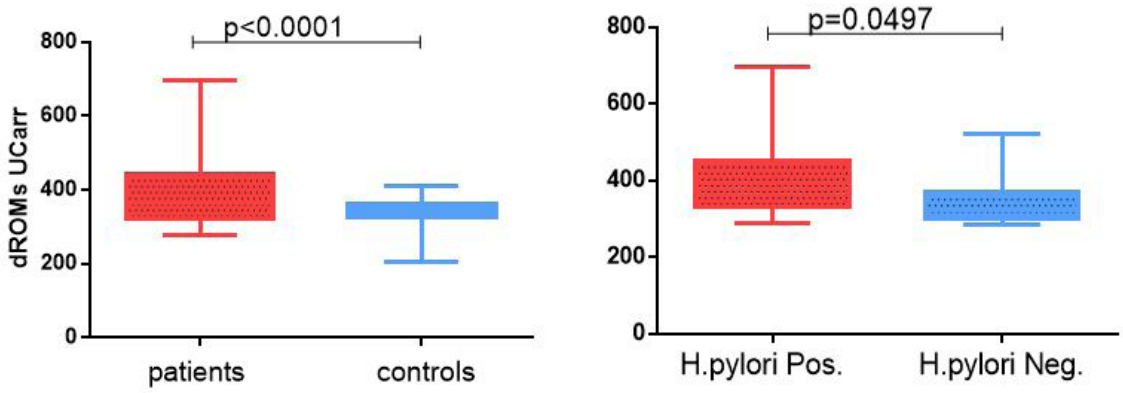

Figure 2: dROMs test values of two groups (controls and patient group), and two patient group (HP positive and HP Negative).

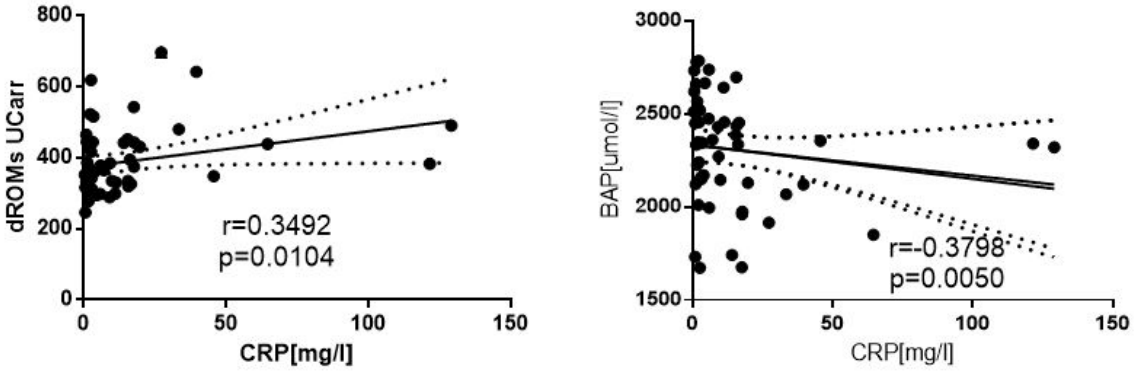

Figure 3: Correlation between CRP, dROMs, and BAP test serum levels in gastritis patients.

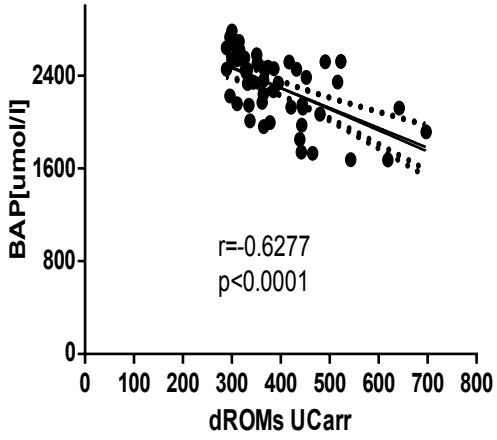

Figure 4: Correlation between dROMs levels and BAP test in patients with chronic gastritis.

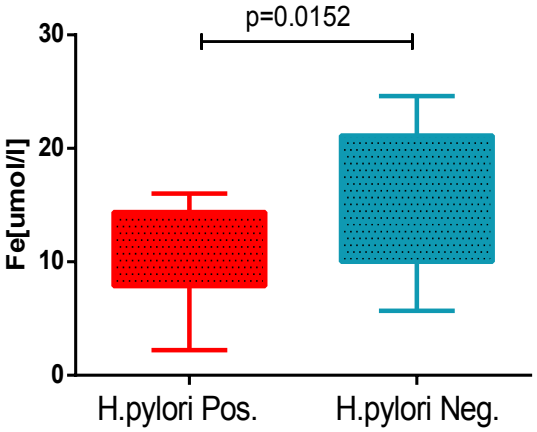

Figure 5: Iron values two patient group HP positive and HP Negative.

Discussion

Laboratory markers for the diagnosis of chronic gastritis are few and not specific enough. Some of the indicators change as a result of the inflammatory response, clinical manifestations and complications of their course. In the pathogenesis of the development of Gastro-Intestinal tract (GI) diseases including gastroduodenal ulcers [9] inflammatory bowel disease [10,11] and malignant diseases of GI such as gastric [12] and colorectal cancer [13], the primary role is played by oxidative stress. The gastrointestinal tract is prone to ROS attack as it directly contacts the external environment. The digestive process itself, the mucosal immune system and the commensal microflora, are all potential sources of ROS. Two major enzymatic reactions generate ROS in the GI tract - hypoxanthine/xanthine oxidase (HX/XO) and the NADPH oxidase system. In the body, the highest concentration of XO is found in the GI tract. Reactive Oxidative Species (ROS) are exclusively increased in the gastric mucosa of individuals with *H. pylori* and are directly related to bacterial load [14]. Together with phagocytic cells (monocytes, neutrophils), microorganisms co-operate in the generation of large amounts of O2 and damage to the gastric mucosa [15]. The hyperoxide radical has a short half-life and is eventually reduced to H_2O_2 by the Fenton reaction. *H. pylori* infection likely impairs the ability of the GI tract to protect against ROS-mediated damage [16]. HP maintains a delicate balance between activating inflammatory processes and preventing the negative effects of inflammation by limiting the degree of immune effector activity [17].

Our study showed higher levels of plasma oxidative stress in patients with HP gastritis compared to controls and negative for HP. HP negative gastritis is predominantly of group C, drug-induced, dietary disorders and possibly ischemic changes from comorbidities or bad habits. Higher CRP values were found in the HP positive group as an inflammatory marker. Colonization of the stomach by *H. pylori* induces chronically active gastritis in almost all infected individuals, but most patients clearly do not develop any apparent clinical signs of infection [18]. More severe disorders and inflammation of the gastro-duodenal mucosa such as peptic ulcers, erosive pangastritis are accompanied by higher levels of CRP. Histological studies in patients with chronic gastritis have found that the degree of *H. pylori* infection and the severity of mucosal damage are directly related to the increased neutrophilic infiltration of the mucosa [3,19]. The pronounced changes in the mucosa and the inflammatory process are associated with the presence in the HP of the cytotoxin-related gene (CagA), the vacuolating cytotoxin VacA, which determine its greater virulence [18]. CagA strains show enhanced oxidative breakdown in Polymorphonuclear cells (PMNs) [14]. Increased levels of d-ROMs correlate well with increased CRP levels and inflammatory response. We observe a moderate correlation between the two laboratory indicators (r = 0.3494) (Figure 3). Increasing oxidative stress leads to a decrease in plasma antioxidant protection, with a significant negative correlation between the two tests (r = -0.6277) (Figure 4). Reduction in BAP test levels is most pronounced in patients with HP positive gastritis (Figure 1). It has been established that infected individuals have a significant decrease in levels of Vitamin E and C. Infection with *H. pylori* causes increased consumption of vitamin C and impairs vitamin D secretion in the gastric [4]. This is likely to further contribute to the oxidative stress during HP infection and the lower antioxidant capacity in patients with chronic gastritis and peptic ulcer disease [20]. Dietary intake is an important source of antioxidants. Potential factors such as malnutrition or malabsorption lead to the lack of absorption of valuable vitamins, trace elements and antioxidants. The imbalance between ROS and AOC leads to increased oxidative stress and mucosal damage. Naito Y. et al. found a decrease in the levels of a very important antioxidant enzyme SOD, which plays a major role in neutralizing hyperoxide radical in neutrophilic inflammatory infiltration. HP, on the other hand, tries to avoid the attack of phagocytic cells and the generated high levels of ROS [21]. The bacterium also produces several of ROS scavenging enzymes (catalase and SOD) and promote the production of hydroxyl radicals through the Fenton reaction.

Chronic gastritis and peptic ulcer disease are known etiological causes leading to iron deficiency anemia. Iron is a major vital trace element for almost all organisms, including HP, but free iron can be potentially toxic due to its catalytic effect on the formation of hydroxyl radicals [18]. Inflammation is an important factor in the release of free iron in the upper digestive tract. Our study found significantly lower levels of iron in the HP positive patient group. Although they remain within the reference range, mean serum iron values differ significantly from the control group and HP negative patients. These results can be explained by the active inflammatory response and competition of the microorganism with the host for iron. The trace elements play the role of cofactors of several *H. pylori* enzymes for its adaptation to the acidic environment of the stomach or catalyse the formation of toxic hydroxyl radicals [4]. The absorbed food in the stomach provides iron in the form of hemin and non-hemin iron. Non-heme iron represents> 80% of dietary imported iron. Reduction from Ferric ions (Fe^{+++}) to Ferro ions (Fe^{++}) is essential for its absorption in the body. Due to the low pH and digestive enzymes in the gastric lumen, iron is separated from the binding ligands and reduced. *H. pylori* and the host compete for free iron [18]. There is evidence that, through this mechanism of competition, and with the presence of a regulator of iron uptake (Fur) in *H. pylori*, the microorganism may cause iron deficiency in the host. This process is exacerbated by poor iron intake of food. Several authors suggest a link between iron deficiency anaemia and *H. pylori* infection [22-24] reduction of ascorbic acid and hypoacidity in the stomach. A recent report reveals the important role of bacterial CagA in maintaining iron homeostasis [18]. H. pylori and PMN work together to damage gastric mucosa. Oxidation-induced changes in iron balance increase the sensitivity to oxidative damage to the gastric mucosa. The reduction of Fe^{2+} is a signal of oxidative stress in the gastric mucosa and the control of its homeostasis during oxidative stress should be investigated [25].

Conclusion

In conclusion, the determination of oxidative stress levels, antioxidant protection and iron homeostasis can be used in the monitoring of patients with chronic gastroduodenitis. Understanding of cellular and molecular mechanisms, approaches to the radical eradication of HP infection, can prevent the development and progression of mucosal atrophy, metaplasia, and possibly gastric cancer. The assessment of the need for the addition of antioxidants to conventional therapy is also an important part of the therapeutic management of the inflammatory process and oxidative stress in chronic gastrointestinal tract diseases.

References

1. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE (2014) Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 94: 329-354.
2. Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87: 245-313.
3. Bodger K, Crabtree JE (1998) Helicobacter pylori and gastric inflammation. Br Med Bull 54: 139-150.
4. Dovhanj J, Kljaić K, Dodig-Curković K, Curković M, Volarević M, et al. (2009) Helicobacter pylori, zinc and iron in oxidative stress-induced injury of gastric mucosa. Mini Rev Med Chem 9: 26-30.
5. Katsarov K, Takov D, Dunkov Z (2010) Gastritis. Medinfo No.11.
6. Cover TL (1996) The vacuolating cytotoxin of Helicobacter pylori. Mol Microbiol 20: 241-246.
7. Graham DY, Malaty R, Goodgame R, Ou CN (1996) Effect of cure of *H. pylori* infection on the gastric mucosal permeability. Gastroenterology 110: 122.
8. Labigne A, de Reuse H (1996) Determinants of Helicobacter pylori pathogenicity. Infect Agents Dis 5: 191-202.
9. Peng YC, Hsu CL, Tung CF, Chou WK, Huang LR, et al. (2008) Chemiluminescence assay of mucosal reactive oxygen species in gastric cancer, ulcer and antral mucosa. Hepatogastroenterology 55: 770-773.
10. Grisham MB (1994) Oxidants and free radicals in inflammatory bowel disease. Lancet 344: 859-861.
11. Pavlick KP, Laroux FS, Fuseler J, Wolf RE, Gray L, et al. (2002) Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. Free Radic Biol Med 33: 311-322.
12. Kekec Y, Paydas S, Tuli A, Zorludemir S, Sakman G, et al. (2009) Antioxidant enzyme levels in cases with gastrointesinal cancer. Eur J Intern Med 20: 403-406.
13. Inokuma T, Haraguchi M, Fujita F, Tajima Y, Kanematsu T (2009) Oxidative stress and tumor progression in colorectal cancer. Hepatogastroenterology 56: 343-347.
14. Zhang QB, Nakashabendi IM, Mokhashi MS, Dawodu JB, Gemmell CG, et al. (1996) Association of cytotoxin production and neutrophil activation by strains of Helicobacter pylori isolated from patients with peptic ulceration and chronic gastritis. Gut 38: 841-845.
15. Martin HM, Hancock JT, Salisbury V, Harrison R (2004) Role of xanthine oxidoreductase as an antimicrobial agent. Infect Immun 72: 4933-4939.
16. Oh TY, Yeo M, Han SU, Cho YK, Kim YB, et al. (2005) Synergism of Helicobacter pylori infection and stress on the augmentation of gastric mucosal damage and its prevention with alpha-tocopherol. Free Radic Biol Med 38: 1447-1457.
17. Han YM, Park JM, Jeong M, Yoo JH, Kim WH, et al. (2015) Dietary, non-microbial intervention to prevent Helicobacter pylori-associated gastric diseases. Ann Transl Med 3: 122.
18. Pich OQ, Merrell DS (2013) The ferric uptake regulator of Helicobacter pylori: a critical player in the battle for iron and colonization of the stomach. Future Microbiol 8: 725-738.
19. Danese S, Cremonini F, Armuzzi A, Candelli M, Papa A, et al. (2001) Helicobacter pylori CagA-positive strains affect oxygen free radicals generation by gastric mucosa. Scand J Gastroenterol 36: 247-250.
20. O'Connor HJ, Schorah CJ, Habibzedah N, Axon AT, Cockel R (1989) Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease, and possible sources. Gut 30: 436-442.
21. Naito Y, Yoshikawa T, Ando T, Kishi A, Ueda S, et al. (1992) Changes in superoxide dismutase activity in the gastric mucosa of peptic ulcer patients. J Clin Gastroenterol 14: S131-S134.
22. Cardenas VM, Mulla ZD, Ortiz M, Graham DY (2006) Iron deficiency and Helicobacter pylori infection in the United States. Am J Epidemiol 163: 127-134.
23. Qu XH, Huang XL, Xiong P, Zhu CY, Huang YL, et al. (2010) Does Helicobacter pylori infection play a role in iron deficiency anemia? A meta-analysis. World J Gastroenterol 16: 886-896.
24. Annibale B, Capurso G, Delle Fave G (2003) The stomach and iron deficiency anaemia: a forgotten link. Dig Liver Dis 35: 288-295.
25. Kato S, Osaki T, Kamiya S, Zhang XS, Blaser MJ (2017) Helicobacter pylori sabA gene is associated with iron deficiency anemia in childhood and adolescence. PLoS One 12: e0184046.