PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 October 21; 12(39): 6361-6365
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Inducible nitric oxide synthase polymorphism is associated with the increased risk of differentiated gastric cancer in a Japanese population

Yasuyuki Goto, Takafumi Ando, Mariko Naito, Hidemi Goto, Nobuyuki Hamajima

Yasuyuki Goto, Mariko Naito, Nobuyuki Hamajima, Department of Preventive Medicine / Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
Takafumi Ando, Hidemi Goto, Department of Gastroenterology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
Supported by a Grant-in-Aid for Scientific Research on Special Priority Areas of Cancer from the Japanese Ministry of Education, Culture, Sports, Science and Technology
Correspondence to: Yasuyuki Goto MD, PhD, Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. ey-goto@med.nagoya-u.ac.jp
Telephone: +81-52-7442133 Fax: +81-52-7442971
Received: 2006-08-10 Accepted: 2006-08-22

Abstract

AIM: To examine the association of inducible nitric oxide synthase (iNOS) C150T polymorphism with gastric cancer, as well as with gastric atrophy and *H pylori* seropositivity.

METHODS: A single nucleotide polymorphism of iNOS C150T was examined for 454 Japanese health checkup examinees (126 males and 328 females) aged 35 to 85 years without a history of cancer and 202 gastric cancer patients (134 males and 68 females) aged 33 to 94 years with pathologically confirmed diagnosis of gastric adenocarcinoma.

RESULTS: The iNOS C150T polymorphism was not associated with gastric atrophy or with *H pylori* seropositivity. The odds ratio (OR) of the *C/T* + *T/T* for gastric cancer was increased without statistical significance (OR=1.19, 95% confidence interval (CI): 0.68-2.08). In the differentiated subgroup (n = 113), however, the OR of the *C/T* genotype for gastric cancer was significant (OR = 2.02, 95% CI: 1.04-3.92) relative to the *C/C* genotype. In addition, considering the location of gastric cancer (n = 105), there were significant differences between the controls and non-cardia group with the OR of 2.13 (95% CI: 1.08-4.18) for *C/T* and 1.94 (95% CI: 1.00-3.78) for *C/T* + *T/T*.

CONCLUSION: The iNOS C150T polymorphism is associated with the risk of *H pylori*-related gastric cancer in a Japanese population. This polymorphism may play an important role in increasing the risk of gastric cancer in Asian countires with the highest rates of gastric cancer.

Key words: Gastric cancer; Inducible nitric oxide synthase; Polymorphism; *H pylori*

Goto Y, Ando T, Naito M, Goto H, Hamajima N. Inducible nitric oxide synthase polymorphism is associated with the increased risk of differentiated gastric cancer in a Japanese population. *World J Gastroenterol* 2006; 12(39): 6361-6365

http://www.wjgnet.com/1007-9327/12/6361.asp

INTRODUCTION

Gastric cancer is the second most frequent cancer in the world, accounting for a large proportion of cancer cases in Asia, Latin America, and some countries in Europe[1]. *H pylori* strains carrying the cytotoxin-associated gene A (*cagA*) are strongly associated with increased risk of gastric adenocarcinoma[2]. However, only some of those infected with *H pylori* developed *H pylori*-related gastric cancer, even in Asian countries including Japan with high prevalence of *cagA*-positive *H pylori* infection. Therefore, it is important to examine any host genetic predisposition to *H pylori*-related gastric cancers.

Nitric oxide (NO) produced by activated phagocytes has been reported to play a role in the processing of carcinogenesis[3-5]. NO is synthesized enzymatically from L-arginine by a family of three distinct nitric oxide synthase (NOS) isoforms[5,6]. Two NOS isoforms, eNOS (expressed in vascular endothelial cells) and nNOS (expressed in neurons of the central and peripheral nervous system), are constitutively expressed and are calcium dependent. The third isoform, inducible NOS (iNOS), is calcium independent, and expressed in response to bacterial endotoxins and cytokines to cause sustained NO release. iNOS is one of the most important enzymes involved in the pathway of reactive oxygen and nitrogen species metabolism in the presence of *H pylori* infection. iNOS can produce larger amounts of NO than either eNOS or nNOS. iNOS expression in the gastric mucosa is higher in *H pylori* positive gastric cancer patients than in

H pylori negative patients[7].

Several types of polymorphisms have been identified in the promoter region of the iNOS gene: G to C at -954, C to T at -1173, and tandem repeat number polymorphism of (TAAA)n and (CCTTT)n[8-11]. iNOS production is mainly regulated at the transcriptional level[12].The human iNOS gene comprises 27 exons, with the transcription start site in exon 2 (E2) and the stop codon in E27[13]. Johannesen *et al*[14] have detected 10 polymorphisms in 8 exons of the iNOS gene, of which one polymorphism (C150T) in exon 16 resulting in an amino acid substitute, Ser608Leu, showed the only distorted transmission in the transmission disequilibrium test. This polymorphism is associated with cigarette- and alcohol- induced gastric cancer in Chinese population[15], indicating that iNOS Ser608Leu allele may have a dramatic effect on the enzyme activity.

Accordingly, we have investigated the associations of iNOS C150T polymorphism with *H pylori* seropositivity, gastric atrophy and gastric cancer in Japanese population.

MATERIALS AND METHODS

Study subjects

Detailed information of the characteristics of healthy controls and gastric cancer patients in this study has been published in our previous paper[16]. Briefly, the control group included 454 health checkup examinees (126 males and 328 females) aged 35 to 85 years with no history of cancer who attended a health checkup program supported by the Nagoya Municipal Government in August and September, 2000. The case group consisted of 202 patients (134 males and 68 females) aged 33 to 94 years with a pathologically confirmed diagnosis of gastric adenocarcinoma undergone tumor resection in different hospitals affiliated to Nagoya University. Informed consent was obtained from all subjects. Approval for the study was given by the relevant ethical committees.

Tests for H pylori antibody and pepsinogens

Anti-*H pylori* IgG antibody tests, high molecular weight campylobacter-associated protein (HM-CAP) ELISA (Enteric Products Inc., Westbury, NY) and HM-CAP with antigens extracted from clinically isolated Japanese *H pylori* strain (J-HM-CAP) ELISA (Kyowa Medex, Tokyo, Japan), were used for the identification of *H pylori*-infected participants. An ELISA value of 2.3 or over was regarded as positive for both tests. The infection was confirmed in all gastric cancer cases by culture and bacteriological tests (Gram-negative, oxidase, catalase, and urease test–positive spiral, curved rods) using biopsy specimens before gastric resection. Pepsinogens I and Ⅱ (PG I and PG Ⅱ) in serum were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). Gastric atrophy was defined as PG I < 70 ng/mL and PG I/PG Ⅱ ratio < 3. These parameters for atrophy are widely used in Japan and have been validated against histological confirmatory studies.

Genotyping

DNA was extracted from the buffy coat fraction by Qiagen QIAamp DNA blood mini kit (QIAGEN Inc.,Valencia,CA). We carried out PCR-RFLP to identify iNOS C150T gene genotype as previously described by Shen *et al*[15]. However, the way to do PCR was somewhat different. Genomic DNA was used per 25 μL of reaction with 0.12 mmol/L dNTPs, 25 pmol of each primer, 0.5 units of "AmpliTaq Gold", and 2.5 μL GeneAmp 10 × PCR buffer including 15 mmol/L $MgCl_2$ (Perkin-Elmer Corp., Foster City, CA). Amplification conditions were 10 min of initial denaturation at 95℃, followed by 30 cycles of 1 min at 95℃, 1 min at 58℃ and 1 min at 72℃, then a final extension at 72℃ for 5 min. The PCR product was digested with a restriction enzyme (*Tsp* 509 I) by the same way as previously described by Shen *et al*[15].

Table 1 The sex-age-adjusted ORs and 95% CIs of the *iNOS* C150T genotypes for gastric atrophy (GA) among *H pylori* seropositive controls

iNOS polymorphism	*n*	GA *n* (%)	OR	95% CI
C/C	217	121 (55.8)	1.00	Reference
C/T	30	16 (53.3)	0.90	0.42-1.94
T/T	3	0 (0)	0	-
C/T+T/T	33	16 (48.5)	0.75	0.36-1.56

Table 2 The sex-age-adjusted ORs and 95% CIs of the *iNOS* C150T genotypes for gastric cancer

iNOS polymorphism	Cases[1] *n* (%) (*n* = 201)	Controls *n* (%) (*n* = 454)	OR	95% CI
C/C	175 (87.1)	403 (88.8)	1.00	Reference
C/T	25 (12.4)	48 (10.6)	1.22	0.69-2.17
T/T	1 (0.50)	3 (0.66)	0.71	0.07-7.54
C/T + T/T	26 (12.9)	51 (11.2)	1.19	0.68-2.08

[1]One case subject could not be genotyped.

Statistical analysis

The strength of associations of *H pylori* seropositivity, gastric atrophy and gastric cancer with the iNOS C150T polymorphisms was measured as odds ratios (ORs). ORs adjusted for sex and age with 95% confidence intervals (CIs) were calculated using logistic regression analysis. Hardy-Weinberg equilibrium was tested for iNOS C150T polymorphism. We used two-sided *P* values. $P < 0.05$ was considered statistically significant. These calculations were performed by computer program STATA Version 8 (STATA Corp., College Station, TX).

RESULTS

The characteristics of study subjects have been described elsewhere[16]. Only one case could not be genotyped. The distributions of the iNOS C150T gene was in the Hardy-Weinberg equilibrium ($\chi^2 = 1.37$ and $P = 0.24$). The iNOS C150T polymorphism had no significant effect on *H pylori* seropositivity. Table 1 shows the sex-age-adjusted ORs and 95% CIs of the iNOS C150T genotypes for gastric atrophy among *H pylori* seropositive controls. The iNOS C150T polymorphism was not associated with gastric

Table 3 The distribution of the *iNOS* C150T genotype in case, considering the location of gastric cancer and the phenotype *iNOS*

Polymorphism	Phenotype[1]	Total cases[2] *n* (%) (*n* = 185)	OR	95% CI	Non-cardia *n* (%) (*n* = 177)	OR	95% CI	Cardia *n* (%) (*n* = 8)	OR	95%CI
C/C	Differentiated	93 (82.3)	1.00		86 (81.9)	1.00		7 (87.5)	1.00	
C/T	Differentiated	20 (17.7)	2.02	1.04-3.92[a]	19 (18.1)	2.13	1.08-4.18[a]	1 (12.5)	1.28	0.15-11.0
T/T	Differentiated	0 (0)	0		0 (0)	0		0 (0)	0	
C/T + T/T	Differentiated	20 (17.7)	1.84	0.96-3.54	19 (18.1)	1.94	1.00-3.78[a]	1 (12.5)	1.16	0.14-9.95
C/C	Undifferentiated	69 (95.8)	1.00		69 (95.8)	1.00		0	-	
C/T	Undifferentiated	2 (2.78)	0.25	0.06-1.06	2 (2.78)	0.25	0.06-1.06	0	-	
T/T	Undifferentiated	1 (1.39)	1.74	0.17-18.1	1 (1.39)	1.74	0.17-18.1	0	-	
C/T + T/T	Undifferentiated	3 (4.17)	0.35	0.10-1.17	3 (4.17)	0.35	0.10-1.17	0	-	

[a]$P < 0.05$ *vs* control group. [1]Information on gastric cancer phenotype was not available for 16 cases; [2]One case subject with undifferentiated type could not be genotyped.

atrophy. Table 2 shows the sex-age-adjusted ORs and 95% CIs of the iNOS genotypes for gastric cancer. The OR of the *C/T+T/T* genotype for gastric cancer was increased without significance.

In the same way as our previous report[17], we divided case subjects into differentiated and undifferentiated type according to their tumor phenotypes, referring to Nakamura *et al*[18] (Table 3). We could not get information on the phenotypes for 16 cases. In the differentiated type subgroup, the OR of the *C/T* genotype for gastric cancer was significant (OR = 2.02, 95% CI: 1.04-3.92). Considering the location of gastric cancer, there were significant differences between the controls and non-cardia group with the OR of 2.13 (95% CI: 1.08-4.18) for *C/T* genotype and 1.94 (95% CI: 1.00-3.78) for *C/T+T/T* genotype. The prevalence of *H pylori* seropositivity in the cases and healthy controls was 100% and 55.1%, respectively. All non-cardia subgroups with differentiated phenotype had gastric atrophy. On the other hand, atrophy was present in 34.8% of 454 controls, 21 of which were seronegative.

Especially compared with *H pylori* seropositive controls or gastric atrophy positive controls, the corresponding ORs were 2.20 (95% CI: 1.08-4.49) for *C/T* genotype and 1.94 (95% CI: 0.97-3.89) for *C/T+T/T* genotype.

DISCUSSION

This study showed that the iNOS C150T polymorphism was associated with gastric cancer where the cell type was differentiated type and located in non-cardia, namely *H pylori*-related gastric cancer. Our control iNOS gene frequency is coincident with the results presented by Shen *et al*[15], which represents the genotype frequency in China.

We could not evaluate the interaction between iNOS C150T polymorphism and smoking-induced risk of gastric cancer as the study of Shen *et al*[15], because we have no information on smoking habit in case group. iNOS is expressed in response to bacterial endotoxins and cytokines to cause sustained NO release. This excess NO would contribute to the development of gastric atrophy, reacting with superoxicde produced by *H pylori* infection[19] to form peroxynitrite which has strong oxidizing properties. We chose the seropositive controls as the comparison group. The iNOS C150T polymorphism, however, was not associated with gastric atrophy as the precursor lesion of gastric cancer in this study.

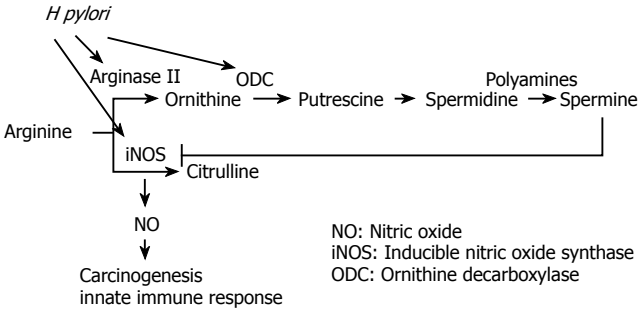

Figure 1 Regulation of Nitric oxide (NO) and polyamine pathways. *H pylori* induces inducible nitric oxide synthase (iNOS) expression, arginase II and ornithine decarboxylase (ODC). iNOS competes with arginase for arginine. Gastric cancer relates to the high expression of iNOS. *H pylori*-induced iNOS is inhibited by spermine produced by the arginase-ODC pathway.

It was reported that immune dysregulation induced by *H pylori* in which stimulated spermine synthesis by the arginase-ornithine decarboxylase (ODC) pathway inhibits iNOS translation and NO production, can lead to persistence of the bacterium[20] (Figure 1). We hypothesized that the iNOS C150T polymorphism might affect *H pylori* infection. But, this gene polymorphism was not associated with *H pylori* seropositivity in this study, confirming the finding that there is no correlation between *H pylori* infection and iNOS expression[21]. On the contrary, it has been reported that iNOS detection is significantly associated with *H pylori* infection[22,23].

There are many reports concerning the high expression of iNOS in gastric cancer, which increases with the stage of cancer and lymph node metastasis[24-26]. It was reported that the long forms of iNOS promoter region are associated with intestinal gastric cancer in Japanese women[27]. Nam *et al*[28] reported that iNOS contributes to *H pylori*-associated gastric carcinogenesis, and that *H pylori* is associated with non-cardiac tumor but not with cardiac tumor[29]. The

high incidence of *H pylori* infection in patients with gastric cancer including both intestinal type and diffuse type, particularly in those with intestinal type has been confirmed[30]. Our study has confirmed that iNOS C150T polymorphism is related to the risk of *H pylori*-related gastric cancer, by stratification of iNOS genotype frequency among cases according to the location of gastric cancer and histologic phenotype. It may take decades for superficial gastritis to progress to atrophic gastritis. *H pylori* infection is highly associated with each stage of this progression[31]. Loss of serological markers of *H pylori* infection following onset of severe atrophy and intestinal metaplasia is a well described phenomenon, including in Japan[32]. Considering these facts, the comparison with the controls with *H pylori* seropositivity and/or gastric atrophy might be adequate to evaluate the effect of the genotype on *H pylori*-related gastric cancer risk. In fact, the corresponding OR was significantly stable (OR = 2.20 for *C/T*, 95% CI: 1.08-4.49) in our study.

iNOS differs independently of Ca^{2+} from the constitutive forms of NOS (eNOS and nNOS). One major divergence in the close sequence similarity is a 40-50-amino acid insert in the middle of the FMN-binding domain of eNOS and nNOS. When this insert is removed from nNOS, the deleted mutant retains maximal NO synthesis activity at a lower concentration of free Ca^{2+} than the wild type enzyme[33]. The iNOS C150T polymorphism is located at the position near to this deletion. The amino acid change in E16 may be of functional interest. As in the study of Shen *et al*[15], our study indicated that those with *T* allele might increase iNOS expression and the level of NO in gastric mucosa. But specific functional tests of this polymorphism remain to be elucidated.

It is important to discuss our finding that iNOS C150T polymorphism is associated with *H pylori*-related gastric cancer but not with *H pylori* infection or with gastric atrophy (especially the association with the latter two is controversial). iNOS-derived NO is a central effector molecule in the innate immune response to pathogens, with essential antimicrobial functions in host defense. *H pylori* induces both arginase Ⅱ and ODC in macrophages[34] (Figure 1). Arginase converts L-arginine to L-ornithine, which is metabolized by ODC to produce putrescine that is converted to polyamines (spermidine and spermine). Spermine inhibits *H pylori*-stimulated NO production in macrophages by a post-transcriptional effect on *iNOS* translation[20]. The temporal switch of arginine as a substrate for the cytostatic iNOS/NO axis to the pro-growth argininase/ornithine/polyamine and proline axis is regulated by inflammatory cytokines as well as interregulated by the arginine metabolites themselves. Satriano[35] has proposed that agmatine, converted from arginine by arginine decarboxylase, coordinates the early and repair phase pathways of arginine in the inflammatory response as a gating mechanism at the transition from the iNOS/NO axis to the arginase/ODC/polyamine axis. Therefore the stage of *H pylori* infection and gastric atrophy might follow the arginase/ODC/polyamine axis. On the other hand, the stage of gastric cancer might follow the iNOS/NO axis. In addition, there is no significant association between cagA positive *H pylori* strains and iNOS expression[20]. CagA might affect the mechanisms that regulate this temporal switch, resulting in the rginase/ODC/polyamine axis.

In conclusion, iNOS C150T polymorphism is associated with the risk of *H pylori*-related gastric cancer in Japanese and Chinese population. This polymorphism may play an important role in increasing the risk of gastric cancer in Asian countries with the highest rates of gastric cancer. The confirmation of this finding requires much larger studies of different ethnic groups to stimulate the interest in the molecular mechanisms of this polymorphism function.

ACKNOWLEDGMENTS

The authors are grateful to Ms Yoko Mitsuda for her technical assistance and Dr Nobuyuki Katsuda for his cooperation concerning the participant enrollment.

REFERENCES

1 **Parkin DM**, Pisani P, Ferlay J. Global cancer statistics. *CA Cancer J Clin* 1999; **49**: 33-64, 1
2 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115
3 **Grisham MB**, Ware K, Gilleland HE Jr, Gilleland LB, Abell CL, Yamada T. Neutrophil-mediated nitrosamine formation: role of nitric oxide in rats. *Gastroenterology* 1992; **103**: 1260-1266
4 **Esumi H**, Tannenbaum SR. U.S.-Japan Cooperative Cancer Research Program: seminar on nitric oxide synthase and carcinogenesis. *Cancer Res* 1994; **54**: 297-301
5 **Ohshima H**, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat Res* 1994; **305**: 253-264
6 **Nathan C**, Xie QW. Nitric oxide synthases: roles, tolls, and controls. *Cell* 1994; **78**: 915-918
7 **Goto T**, Haruma K, Kitadai Y, Ito M, Yoshihara M, Sumii K, Hayakawa N, Kajiyama G. Enhanced expression of inducible nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients. *Clin Cancer Res* 1999; **5**: 1411-1415
8 **Kun JF**, Mordmüller B, Lell B, Lehman LG, Luckner D, Kremsner PG. Polymorphism in promoter region of inducible nitric oxide synthase gene and protection against malaria. *Lancet* 1998; **351**: 265-266
9 **Hobbs MR**, Udhayakumar V, Levesque MC, Booth J, Roberts JM, Tkachuk AN, Pole A, Coon H, Kariuki S, Nahlen BL, Mwaikambo ED, Lal AL, Granger DL, Anstey NM, Weinberg JB. A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. *Lancet* 2002; **360**: 1468-1475
10 **Bellamy R**, Hill AV. A bi-allelic tetranucleotide repeat in the promoter of the human inducible nitric oxide synthase gene. *Clin Genet* 1997; **52**: 192-193
11 **Xu W**, Liu L, Emson PC, Harrington CR, Charles IG. Evolution of a homopurine-homopyrimidine pentanucleotide repeat sequence upstream of the human inducible nitric oxide synthase gene. *Gene* 1997; **204**: 165-170
12 **Cho HJ**, Xie QW, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Nathan C. Calmodulin is a subunit of nitric oxide synthase from macrophages. *J Exp Med* 1992; **176**: 599-604
13 **Xu W**, Charles IG, Liu L, Moncada S, Emson P. Molecular cloning and structural organization of the human inducible nitric oxide synthase gene (NOS2). *Biochem Biophys Res*

Commun 1996; **219**: 784-788
14 **Johannesen J**, Pie A, Pociot F, Kristiansen OP, Karlsen AE, Nerup J. Linkage of the human inducible nitric oxide synthase gene to type 1 diabetes. *J Clin Endocrinol Metab* 2001; **86**: 2792-2796
15 **Shen J**, Wang RT, Wang LW, Xu YC, Wang XR. A novel genetic polymorphism of inducible nitric oxide synthase is associated with an increased risk of gastric cancer. *World J Gastroenterol* 2004; **10**: 3278-3283
16 **Goto Y**, Ando T, Yamamoto K, Tamakoshi A, El-Omar E, Goto H, Hamajima N. Association between serum pepsinogens and polymorphismof PTPN11 encoding SHP-2 among *Helicobacter pylori* seropositive Japanese. *Int J Cancer* 2006; **118**: 203-208
17 **Goto Y**, Ando T, Goto H, Hamajima N. No association between EGF gene polymorphism and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 2454-2456
18 **Nakamura K**, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann* 1968; **59**: 251-258
19 **Bagchi D**, Bhattacharya G, Stohs SJ. Production of reactive oxygen species by gastric cells in association with *Helicobacter pylori*. *Free Radic Res* 1996; **24**: 439-450
20 **Bussière FI**, Chaturvedi R, Cheng Y, Gobert AP, Asim M, Blumberg DR, Xu H, Kim PY, Hacker A, Casero RA Jr, Wilson KT. Spermine causes loss of innate immune response to *Helicobacter pylori* by inhibition of inducible nitric-oxide synthase translation. *J Biol Chem* 2005; **280**: 2409-2412
21 **Son HJ**, Rhee JC, Park DI, Kim YH, Rhee PL, Koh KC, Paik SW, Choi KW, Kim JJ. Inducible nitric oxide synthase expression in gastroduodenal diseases infected with *Helicobacter pylori*. *Helicobacter* 2001; **6**: 37-43
22 **Pignatelli B**, Bancel B, Estève J, Malaveille C, Calmels S, Correa P, Patricot LM, Laval M, Lyandrat N, Ohshima H. Inducible nitric oxide synthase, anti-oxidant enzymes and *Helicobacter pylori* infection in gastritis and gastric precancerous lesions in humans. *Eur J Cancer Prev* 1998; **7**: 439-447
23 **Mannick EE**, Bravo LE, Zarama G, Realpe JL, Zhang XJ, Ruiz B, Fontham ET, Mera R, Miller MJ, Correa P. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. *Cancer Res* 1996; **56**: 3238-3243
24 **Rajnakova A**, Moochhala S, Goh PM, Ngoi S. Expression of nitric oxide synthase, cyclooxygenase, and p53 in different stages of human gastric cancer. *Cancer Lett* 2001; **172**: 177-185
25 **Feng CW**, Wang LD, Jiao LH, Liu B, Zheng S, Xie XJ. Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: correlation with clinical features. *BMC Cancer* 2002; **2**: 8
26 **Song ZJ**, Gong P, Wu YE. Relationship between the expression of iNOS,VEGF,tumor angiogenesis and gastric cancer. *World J Gastroenterol* 2002; **8**: 591-595
27 **Tatemichi M**, Sawa T, Gilibert I, Tazawa H, Katoh T, Ohshima H. Increased risk of intestinal type of gastric adenocarcinoma in Japanese women associated with long forms of CCTTT pentanucleotide repeat in the inducible nitric oxide synthase promoter. *Cancer Lett* 2005; **217**: 197-202
28 **Nam KT**, Oh SY, Ahn B, Kim YB, Jang DD, Yang KH, Hahm KB, Kim DY. Decreased *Helicobacter pylori* associated gastric carcinogenesis in mice lacking inducible nitric oxide synthase. *Gut* 2004; **53**: 1250-1255
29 **Whiting JL**, Hallissey MT, Fielding JW, Dunn J. Screening for gastric cancer by *Helicobacter pylori* serology: a retrospective study. *Br J Surg* 1998; **85**: 408-411
30 **Caputo S**, Mosca F, Persi A, Ettaro G, Scaringi S, Russo G, Piazza D. [*Helicobacter pylori* and gastric cancer. The incidence of infection in personal experience]. *Minerva Chir* 2002; **57**: 649-655
31 **Faisal MA**, Russell RM, Samloff IM, Holt PR. *Helicobacter pylori* infection and atrophic gastritis in the elderly. *Gastroenterology* 1990; **99**: 1543-1544
32 **Asaka M**, Sugiyama T, Nobuta A, Kato M, Takeda H, Graham DY. Atrophic gastritis and intestinal metaplasia in Japan: results of a large multicenter study. *Helicobacter* 2001; **6**: 294-299
33 **Daff S**, Sagami I, Shimizu T. The 42-amino acid insert in the FMN domain of neuronal nitric-oxide synthase exerts control over Ca(2+)/calmodulin-dependent electron transfer. *J Biol Chem* 1999; **274**: 30589-30595
34 **Gobert AP**, Cheng Y, Wang JY, Boucher JL, Iyer RK, Cederbaum SD, Casero RA Jr, Newton JC, Wilson KT. *Helicobacter pylori* induces macrophage apoptosis by activation of arginase II. *J Immunol* 2002; **168**: 4692-4700
35 **Satriano J**. Arginine pathways and the inflammatory response: interregulation of nitric oxide and polyamines: review article. *Amino Acids* 2004; **26**: 321-329

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Ma WH