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DETECTION OF *P53* and *Bax* genes ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION

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ABSTRACT

H. pylori is considered one of the most common human pathogen worldwide, with infection rates much higher in developing countries than in developed nations. The aim of this study is the investigation of some candidate genes include *P53* and *Bax* genes mutation associated with *H. pylori* infection and study the role of *H. pylori* virulence genes on the induction of *P53* and *Bax* genes mutation. The results show a total of 92 gastric biopsies gave positive results for the presence of *H. pylori* diagnosed by molecular technique. Only (49) cag a positive *H. pylori* strains obtain from gastric biopsy of patient suffering from *H. pylori* infection which also harboring *cag E* and *vacA* genes. Single Strand Conformation Polymorphism (PCR-SSCP) technique used to identify mutations in the *p53* gene in several exons. Where 16 alterations in exon E5A, 19 alterations in exon E5B6A, 17 alterations in exon E7 and only two in exon E6B; but no alteration was appeared in exon E8 of *P53* gene. In the same way, PCR-SSCP analysis detected alterations in *Bax* gene where only 14 positive samples gave alteration in the sequence of *Bax* gene in exons E1, E4 and E6. Regarding to exon 1 of *Bax* gene, it was appeared that only 10 samples gave positive gene locus mutation. This may attribute to *H. pylori* which stimulate *Bax* mutation through its ability to produce cag A protein which has the ability to cause changing in gene sequence and function. It is concluded that *p53* and *Bax* genes mutation is important tool in the screening of genetic alteration associated with *H. pylori* infections.

Key words: *H. pylori*, Gastric biopsy, PCR- SSCP technique, *P53*gene mutation, *Bax* gene mutation.

INTRODUCTION

Helicobacter pylori was definitively identified by Robin Warren and Barry Marshall through cultivation of bacteria from gastric biopsy samples which usually seen for nearly a century by pathologists. The WHO and the IRAC recognized this pathogen as a group one carcinogen for stomach cancer during 1994, also currently it is counted the most common etiologic agent of infection associated with cancer diseases, which account about 5% of the worldwide cancer problem. Moreover, it influences the prognosis of gastric cancer (Wroblewski and Peek, 2013; Yong *et al*., 2015).

Mostly, *H. pylori* colonization does not produce any symptoms. Conversely, long-term *H. pylori* infection significantly enhances the risk of developing gastro duodenal illness include gastritis, peptic ulcer disease, gastric cancer and mucosa associated lymphoid tissue lymphoma (Bauer and Meyer, 2011). Besides, some researches supplied clear evidence that *H. pylori* infection significantly enhances the risk of gastric cancer (Oda *et* al,. 2002; Farzam *et al*., 2014; Graham, 2014). However, development of gastric cancer was reported in about 3 % of *H. pylori* infected patients (Malnick *et al*., 2014)

The most important host factor associated with severe illness caused by *H. pylori* infection are P53 (tumor-suppressor gene), and *Bax* gene dysfunction. p53 which is responsible for regulation of cell proliferation and apoptosis and Bax is also involved in the regulation of apoptosis. However, *p53*and *Bax* alteration is correlated with particular consequences of gastric carcinogenesis (Shadifar *et al*., 2015).

MATERIALS AND METHODS

Patients: A total of 92 biopsy specimens were taken from patients with *H. pylori* infection, who admitted to specialized center of digestive system and endoscopy unit of Medical Marjan City at Babylon governorate/ Iraq during a period from February to September 2016, Samples were collected according to ethical approval of Ministry of Iraqi health. Evaluation of *H. pylori* infection was tested by cultivation and PCR amplification of *ureC and ureA* using gastric biopsy specimens. *H. pylori* infection was considered when any one of these assays were positive. Besides, molecular investigation of *H. pylori* virulence markers include *cagA*, *cag E* and *vacA* was also done. The PCR primers design and conditions was done with some modification according to that reported in He *et al*., (2002) and Bessa *et al*., (2014).

Investigation of *P53* and *Bax* Gene Mutations Related to *H. Pylori* Infection: Genomic DNA extraction from gastric biopsy is achieved according to the method recommended by the manufacturing company (Geneaid/ Korea) using (gSYNC^{TM} DNA Mini Kit). The PCR-primers and their corresponding genes are shown in (table-1) PCR amplification of exons (5 – 8) of the *p53* gene and *Bax* gene (Deguchi *et al*., 2001 and Oda *et al*., 2002). The PCR conditions were involved: after an initial denaturation, (35 – 40) cycles at 94°C for 1–1.5 min, (58° C - 62°C) for 1–2 min and (72°C) for 1–2 min was carried out in a thermal cycler (Clever, USA). The molecular markers used to amplify exons (1, 4, 5, 6) of the *Bax* gene amplification conditions were an initial denaturation, (35–40) cycles of the reaction mixture at

(94°C) for 1 min, (52° - 62°C) for 1 min and (72°C) for 1 min were included. PCR amplicon were investigated by 1.6% agarose gel electrophoresis.

These genes were screened for detection novel mutations. And a group of samples was analyzed by SSCP technique. The amplicons were diluted 1/10 V\V with sterile distal water. Equal amounts of diluted sample and SSCP loading buffer were heated to (95°C) for 3 minutes to denature the samples, and directly put on ice to avoid re-annealing of DNA strands. Then electrophoresis of 3 µl of each sample was done. The SSCP gels consisted of 1× TBE buffer (Tris–borate–EDTA), (8-10)% polyacrylamide and 5% glycerol. Setting agents were 15µl (25%w/v)ammonium persulphate and 15µl *N, N, N′,N*-tetramethylethylenediamine (TEMED) for every 10ml of non-denaturing gel and SYBR green. Electrophoresis was done at room temperature (20–24°C), using 0.5× TBE buffer. The gels were electrophoresed for 2–3 hours at 170 Volt. Single strands of DNA were seen and the pattern of each mutant band was compared with that of normal pattern of healthy individual biopsy.

Table 1: Primers sequences and their corresponding genes.

Genes	Primers 5'→3'		Product size	References
P53-E5A	**Forward**	TTCCTCTTCCTGCAGTACTC	**152 bp**	**Saxena *et al.*, 2012**
	Reverse	TCCGTCATGTGCTGTGACTG		
P53-E5B6A	**Forward**	GCCATCTACAAGCAGTCACA	**167 bp**	
	Reverse	GCCAGACCTAAGAGCAATCA		
P53-E6B	**Forward**	TTAGGTCTGGCCCCTCCTCA	**132 bp**	
	Reverse	AGTTGCAAACCAGACCTCAG		
P53-E7	**Forward**	TTGTCTCCTAGGTTGGCTCT	**136 bp**	
	Reverse	CAAGTGGCTCCTGACCTGGA		
P53-E8	**Forward**	TGGTAATCTACTGGGACGGA	**149 bp**	
	Reverse	CTGCTTGCTTACCTCGCTTA		
Bax-E1	**Forward**	CGTTCAGCGGGGCTCTCA	**207 bp**	**Deguchi *et al.*, 2001**
	Reverse	CAGGCCGGTAGGAAGGAT		
Bax-E4	**Forward**	TCTCCTGCAGGATGATTGC	**209 bp**	
	Reverse	TCCCCAGGTCCTCACAGAT		
Bax-E5	**Forward**	CACGCAGTGGGGACAAGGTT	**192 bp**	
	Reverse	GCGGTGGTGGGGGTGAGGAG		
Bax-E6	**Forward**	CCCCTGGCCGAGTCACTGAA	**237 bp**	
	Reverse	AATGCCCATGTCCCCCAATC		

RESULTS

Detection of *P53* Gene Mutation: Among 92 *H. pylori* infected patients diagnosed by cultivation and PCR detection of *ureC* and *ureA* genes as a diagnosis biomarker, only patients were infected with *H. pylori* strain harboring full signal of virulence genes include *cag A, cagE* and *vacA* indicted that these strains were more virulent.

Beside mutations of the *p53* gene in exons (5 to 8) were identified by PCR-SSCP technique. However, PCR was used to amplify exons 5,6,7 and 8 of *p53* gene which are identified to be mutational hot spots. PCR-SSCP technique has detected mutations in the *p53* gene in several exons. Where sixteen alterations in exon E5A, nineteen alterations in exon E5B6A, seventeen alterations in exon E7 and only two in exon E6B but no alteration was seen in exon E8 of *P53* gene.

All samples were positive PCR amplification of *P53* genes after detection by agarose gel electrophoresis. The second step in the mutational screening analysis were the denaturation prior to loading, which is the essential step for SSCP techneque. The altered patterns of DNA bands observed on the SSCP gels indicated of the confirmational change occur in the single stand DNA whene seperated in a polyacrylamide gel with electrophorasis(1&2).

Detection of *Bax* Gene Mutation: PCR-SSCP analysis detected alterations in *Bax* gene were only positive samples gave alteration in the pattern of *Bax* gene in exons E1, E4 and E6. As shown in the figures (figures numbers pleas). As for the exon 1

of *Bax* gene, it was seen that only 10 samples had positive gene locus mutation. This may attribute to *H. pylori* which stimulate *Bax* mutation through its ability to produce cag A protein which have ability to cause changing in gene sequence and function.

According to the results which obtained in this study, different DNA bands size were detected in *Bax* mutant (more than three bands) whereas the original gene gave only three bands. In case of exon 4 mutation in *Bax* gene, it was noticed in only two sample, and the other after digestion gave the same that bands which indicated of absence of alteration in *Bax* gene at this locus. At the same time, it was found that only two mutations were detected in exon 6, where the mutant gene gave four bands compared to the three bands presented in the normal genes. Whereas no alteration in exon 5 of *Bax* gene was observed in the current study.

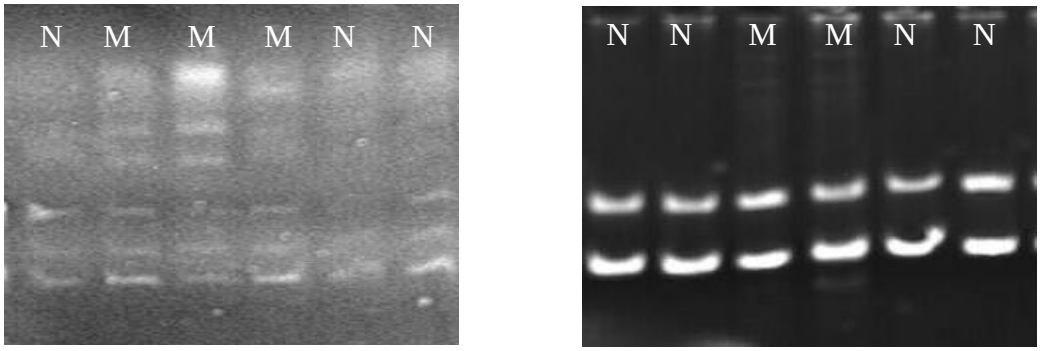

Figure 1**: A** Polyacrylamide gel electrophoresis for variant detection of ***P53*-E5B6A** using SSCP technique, visualized under U.V. Light after staining with SYBR green**. B** for ***P53*-E5B6A**

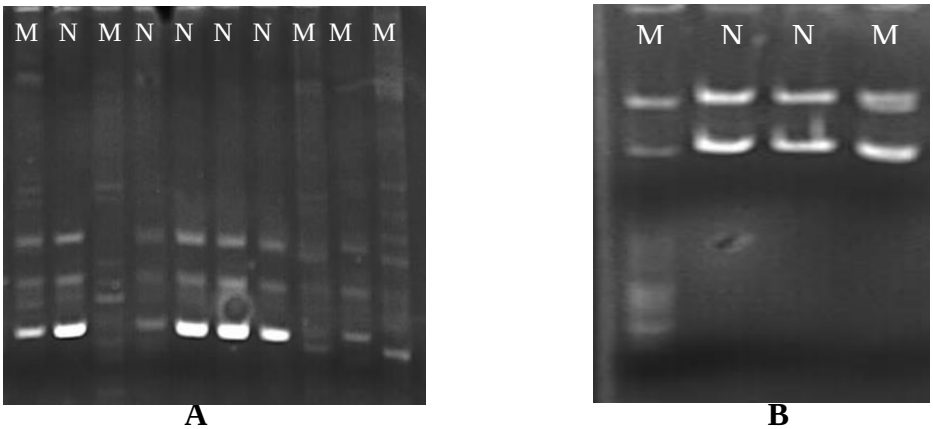

Figure 2: **A** Polyacrylamide gel electrophoresis for variant detection of ***Bax*-E1** using SSCP technique, visualized under U.V. light after staining with SYBR green**. B** Polyacrylamide gel electrophoresis for variant detection of ***Bax*-E4** using SSCP technique, visualized under U.V. light after staining with SYBR green**.**

DISCUSSION

In this study, PCR- PCR-SSCP method is used for P53 mutation detection and analysis which is based on the phenomenon that a change of base sequence (mutations) has an effect on the folding conformation of single-stranded DNA and the observation of the effect of SSCP on the migration of the DNA during electrophoresis gel. Detection of mutations by this method has an approximately 90% sensitivity and specificity. There are five regions located within tumor suppresser gene (*p53*) that have a high degree of homology in vertebrates and that are also greatly conserved in evolution. Any alteration in the sequences of these regions have been correlated with cellular transformation. The most common of p53 mutations in human tumors appear within four exons originate in these conserved regions represented exons (5,6,7,8).

In the current study, all *p53* mutations were detected in cagA positive *H. pylori* patients. Suggested that the oncogenic function of *H. pylori* cagA protein which was injected to host cell by type four secretion system may induced *P53* mutation. This result was correlated with Szkaradkiewicz *et al*., (2015) who indicated that the development of *H. pylori* (cagA+) positive gastric adenocarcinomas is associated with abnormalities in function of p53 protein and suggested that *H. pylori* (*cagA*+) may induce *p53* mutations already at the early phase of carcinogenesis. These findings seems to be suppor-

ted by studies of Kountouras *et al*., (2008) who detected *p53* mutations in 30% cases of intestinal metaplasia and in 58% patients with gastric dysplasia.

Many researchers study the relation between p53 gene mutation and *H. pylori* infection associated with more severe illness. Some studies indicated that *H. pylori* infection induces p53 gene alteration in gastric carcinogenesis are correlated with the results of this study (Masaaki *et al*., 2007; Rafiei and Hosseini, 2012; Shadifar *et al*., 2015).

Furthermore, CagA is the most important pathogenic element in the gastric tumorgenisis of *H. pylori* infection. Conspicuously, the p53 gene is highly expected to carry p53 mutations in gastric tumors with CagA-positive *H. pylori* strains. Many searches proposed that epithelial cells infected with CagA-positive *H. pylori* encourage the expression of cytidine deaminase, which may be a mechanism of p53 mutation accumulation during *H. pylori*-associated gastric carcinogenesis (Matsumoto *et al*., 2007; Shimizu *et al*., 2014). Deguchi *et al*., (2001) who proposed that CagA positive *H. pylori* strains may have a critical role in the prognosis of stomach cancer in an individual with *p53* alterations. Also, they recommended that investigation of CagA positive *H. pylori* may be valuable in finding patients with *p53* mutation.

Molecular markers that can find patients with a past of *H. pylori* infection and associate with significant risk of progress of gastric cancer remain an important aim of research, as are predictive markers for gastric cancer development and targeted treatments. Investigation of powerful combination biomarkers depending on the bacterial genotypes, inflammation, and host genetic and phenotypic profiles should supply much essential tools for screening, prevention, and treatment of severe illness associated with *H. pylori* infection.

One of the most important biomarkers linked to carcinogenesis caused by *H. pylori* infection are *P53* (tumor-suppressor gene), and *Bax* gene mutation. P53 which is responsible of regulation of cell proliferation and program cell death and *Bax* is also implicated in the regulation of apoptosis. Moreover, *p53* and *Bax* alteration is correlated with the particular events in the gastric tumorgenesis (Rath *et al*., 2015). The experimental study done by Ohnishi *et al*., (2008) was directly demonstrated the oncogenic potential of CagA through the detection that transgenic mice systemically expressing *cagA* spontaneously progressed gastrointestinal carcinomas and hematopoietic malignancies. Also, transgenic expression of CagA enhanced intestinal carcinogenesis caused by a mutation in the tumor-suppressor gene *P53* (Neal *et al*., 2013), giving more in vivo evidence for the oncogenic ability of CagA. Even though the contribution of other *H. pylori* proteins to disease development, for example vacuolating cytotoxin A (VacA) has also been appeared, collecting evidence clearly points to a central role of CagA in gastric carcinogenesis (Yamaoka, 2010; Antonia and Sepulveda, 2013; Ibraheem and Al-Ardhi, 2017; Abbas et.al, 2017)

However, Bartchewsky *et al*. (2010) demonstrated that in *H. pylori* infection, manifested by chronic gastritis, an increased Bax expression initiates cell apoptosis, but in patients with gastric cancer the pathogen induces the anti-apoptotic gene *bcl-2*. In addition, as presented in vitro investigation demonstrate that the *H. pylori*-secreted vacuolating cytotoxin – VacA exerts a pro-apoptotic effect on epithelial cells, acting in an opposite manner to anti-apoptotic action of CagA (Oldani *et al*., 2009; Matsumoto *et al*., 2011).

Moreover, only few studies have illustrated a relationship of cag E with gastric cancer (Chomvarin, *et al*., 2008; Bessa, *et al*., 2014). Whereas, other researches indicated the existence of such gene in early stage of tumor and a correlation with other pathogenic markers, representing that there is a role for *cag*E in gastric carcinogenesis (Lime, *et al*., 2010; Lime, *et al*., 2011).

CONCLUSION

It is concluded that recognition of genetic mutation for *P53* and Bax genes may accrue due to effect of pathogenic strains of *H. pylori*.

REFERENCES

Abbas, M. H., A. K. Al-Yasseen, et al., Prevalence of Staphylococcus Aureus among gingivitis in patient with orthodontic wires in Kufa City /Iraq. Pak. J. Biotechnol.14: 91-96 (2017).

Antonia R., Sepulveda M.D., *Helicobacter*, Inflammation, and Gastric Cancer. Curr Pathobiol Rep. 1(1): 9–18 (2013).

Bartchewsky W. Jr, Martini M.R., Squassoni A.C., Alvarez M.C., Ladeira M.S., Salvatore D.M,, Trevisan M.A., Pedrazzoli J. Jr and Ribeiro M.L., Effects of *Helicobacter pylori* infection on the expressions of Bax and Bcl-2 in patients with chronic gastritis and gastric cancer. Dig. Dis. Sci. 55(1): 111–116. (2010).

Bauer B. and Meyer T.F., The Human Gastric Pathogen *Helicobacter pylori* and Its Association with Gastric Cancer and Ulcer Disease. Hindawi Publishing Corporation Ulcers. ID 340157: 23 pages (2011).

Bessa PPde-N., Barbosa F.C., Carmo A.P.S., Furtado G.B., Barroso F.C. and Rabenhosrt

S.H.B., Presence of the Genes *cag*A, *cag*E, *vir*B11 and Allelic Variation of *vac*A of *Helicobacter pylori* Are Associated with the Activity of Gastritis. Open Journal of Gastroenterology 4: 347-355 (2014).

Choi I.J.. Current evidence of effects of *Helicobacter pylori* eradication on prevention of gastric cancer. Korean J. Intern. Med. 28: 525-37 (2013).

Chomvarin C., Namwat W., Chaicumpar K., Mairiang P., Sangchan A., Sripa B., Tor-Udom S. and Vilaichone R.K., Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. Int J Infect Dis. 12:30–36 (2008).

Deguchi R., Takagi A., Kawata H., Inoko H. and Miwa T., Association between cagA1 *helicobacter pylori* infection and *p53*, *bax* and *transforming growth factor*-B-*RII* gene mutations in gastric cancer patients. *Int. J. Cancer* 91: 481–485 (2001).

Farzam A., Najafipour R., Johari P., Farivar T.N., Prevalence of *Helicobacter pylori* in Formaldehyde Fixed Paraffin Embedded Gastric Tissues of Gastric Cancer Patients by Scorpion Real-Time PCR Assay. Biotech. Health Sci. 1(3): e25836 (2014).

Graham D.Y., History of *Helicobacter pylori*, duodenal ulcer, gastric ulcer and gastric cancer. World J. Gastroenterol. 20(18): 5191-5204. (2014).

He O, Wang J-P, Osato M and Lachman LB. Real-Time Quantitative PCR for Detection of *Helicobacter pylori*. Journal of Clinical Microbiology Pp. 3720–3728 (2002).

Ibraheem, N. J. and G. H. Al-Ardhi, Evaluation of serotonin hormone levels associated with obesity in some adult men. *Pak. J. Biotechnol.* 14: 215-220 (2017).

Kim Y-I., Cho S-J., Lee J.Y., Kim C.G., Kook M-C., Ryu K.W., Kim Y-W and Choi I.J., Effect of *Helicobacter pylori* Eradication on Long-Term Survival after Distal Gastrectomy for Gastric Cancer. Cancer Res Treat. 48(3):1020-1029. (2016).

Kountouras J., Zavos C., Chatzopoulos D. and Katsinelos P., New aspects of *Helicobacter pylori* infection involvement in gastric oncogenesis. *J. Surg. Res.*146(1): 149–158. (2008).

Liao J., Wen S., Cao L., Zhou Y. and Feng Z., Effect of Eradication of *Helicobacter pylori* on Expression Levels of FHIT, IL-8 and P73 in Gastric Mucosa of First-Degree Relatives of Gastric Cancer Patients. PLoS ONE 10(4): e0124576 (2015).

Lima V.P., de Lima M.A., Ferreira M.V., Barros M.A. and Rabenhorst S.H., The relationship between Helicobacter pylori genes cagE and virB11 and gastric cancer. Int. J. Infect. Dis. 14: e613–e617 (2010).

Lima V.P., Silva-Fernandes I.J.L., Alves M.K.S. and Rabenhorst S.H.B.. Prevalence of *Helicobacter pylori* genotypes (*vac*A, *cag*A, *cag*E and *vir*B11) in gastric cancer in Brazilian's patients: An association with histopathological parameters. Cancer Epidemiology 35 (5): e32-e37 (2011).

Malnick S.D.H., Melzer E., Attali M., Duek G., Yahav J., *Helicobacter pylori*: Friend or foe? World J. Gastroenterol. 21; 20(27): 8979-8985 (2014).

Masaaki K., Kazunari M. and Tadayoshi O., Expression of mutant *type-p53* products in *H. pylori*-associated chronic gastritis. World J. Gastroenterol. 13: 1541-46 (2007).

Matsumoto A., Isomoto H., Nakayama M., Hisatsune J., Nishi Y., Nakashima Y., Matsushima K., Kurazono H., Nakao K., Hirayama T. and Kohno S., *Helicobacter pylori* VacA reduces the cellular expression of STAT3 and pro-survival Bcl-2 family proteins, Bcl-2 and Bcl-XL, leading to apoptosis in gastric epithelial cells. Dig. Dis. Sci. 56(4): 999–1006 (2011).

Matsumoto Y., Marusawa H., Kinoshita K., Endo Y., Kou T., Morisawa T., Azuma T., Okazaki I.M., Honjo T. and Chiba T., *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nature medicine. 13: 470-476 (2007).

Neal J.T., Peterson T.S., Kent M.L. and Guillemin K., *H. pylori* virulence factor CagA increases intestinal cell proliferation by Wnt pathway activation in a transgenic zebrafish model. Dis. Model. Mech. 6: 802–810 (2013).

Oda T., Murakami K., Nishizono A., Kodama M., Nasu M., Fujioka T., Long-term *Helicobacter pylori* infection in Japanese monkeys induces atrophic gastritis and accumulation of mutations in the p53 tumor suppressor gene. Helicobacter 7:143-151 (2002).

Ohnishi N., Yuasa H., Tanaka S., Sawa H., Miura M., Matsui A., Higashi H., Musashi M., Iwabuchi K. and Suzuki M., Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc. Natl. Acad. Sci. USA 105: 1003-1008 (2008).

Oldani A., Cormont M., Hofman V., Chiozzi V., Oregioni O., Canonici A., Sciullo A., Sommi

P., Fabbri A., Ricci V. and Boquet P., *Helicobacter pylori* counteracts the apoptotic action of its, VacA toxin by injecting the CagA protein into gastric epithelial cells. *PLoS Pathogens* 5(10): e1000603 (2009).

Rafiei A. and Hosseini V., Inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection affect gastric cancer risk. World J. Gastroenterol. 18: 4917-24 (2012).

Rath S., Das L., Kokate S.B., Pratheek B.M., Chattopadhyay S., Goswami C., Chattopadhyay R., Crowe E.S. and Bhattacharyya A. Regulation of Noxa-mediated apoptosis in *Helicobacter pylori*–infected gastric epithelial cells. FASEB J. 29(3): 796–806 (2015).

Saxena A., Shukla S.K., Prasad K.N. and Ghoshal U.G., Analysis of p53, K-ras gene mutation & *Helicobacter pylori* infection in patients with gastric cancer & peptic ulcer disease at a tertiary care hospital in north India. Indian J. Med. Res. 136: 664-670 (2012).

Shadifar M., Ataee R., Ataie A., Gorgi A.M.H., Nasrabadi N.N. and Nouri S., Genetic and molecular aspects of *Helicobacter pylori* in gastritis, pre- cancerous conditions and gastric adenocarcinoma. Gastroenterol. Hepatol. Bed. Bench. 8(1): S15-S22 (2015).

Shimizu T., Marusawa H., Matsumoto Y., Inuzuka T., Ikeda A., Fujii Y., Minamiguchi S., Miyamoto S., Kou T., Sakai Y., Crabtree J.E. and Chiba T., Accumulation of somatic mutations in TP53 in gastric epithelium with *Helicobacter pylori* infection. Gastroenterology 147: 407-417 e403 (2014).

Shin S.H., Jung D.H., Kim J-H., Chung H.S., Park J.C., Shin S.K., Lee S.K. and Lee Y.C., *Helicobacter pylori* Eradication Prevents Metachronous Gastric Neoplasms after Endoscopic Resection of Gastric Dysplasia. PLoS ONE 10(11): e0143257 (2015).

Suzuki M., Mimuro H., Kiga K., Fukumatsu M., Ishijima N., Morikawa H., Nagai S., Koyasu S., Gilman R.H., Kersulyte D., Berg D.E. and Sasakawa C., *Helicobacter pylori* CagA Phosphorylation-Independent Function in Epithelial Proliferation and Inflammation. Cell Host & Microbe Elsevier Inc. 5(1): 23–34 (2009).

Szkaradkiewicz A., Karpiński1 T.M., Majewski J., Malinowska K., Gośliński-Kuźniarek O. and Linke K., The Participation of p53 and bcl-2 Proteins in Gastric Carcinomas Associated with *Helicobacter pylori* and/or Epstein-Barr Virus (EBV). Polish Journal of Microbiology 64(3): 211–216 (2015).

Wroblewski L.E. and Peek Jr R.M., *H. pylori* in gastric carcinogenesis-mechanisms. Gastroenterol. Clin. North Am. 42(2): 285-298 (2013).

Yamaoka Y., Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat. Rev. Gastroenterol Hepatol. 7: 629–641 (2010).

Yong X., Tang B., Li B-S., Xie R., Hu C-J., Luo G., Qin Y., Dong H., Yang S-M., *Helicobacter pylori* virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. BioMed Central (PMC) 4702319. Cell Common Signal 13: 30 (2015).