Helicobacter pylori: The association between *CagA* positivity and p53 expression

Ahmed Sattar Abood[1] *, Samir Sabaa Raheem[2], Ali Ibrahim Ali Al-Ezzy[3] and Nidhal Abdulmohaimen Mohammed[4]

Abstract— *H. pylori* had been recognized as the causative agent of several gastric diseases and possessed wide variety of virulence factors, one of most important factors is *CagA*. *p53* have central role in many cellular activities, one of them is the apoptosis. This study investigated the expression of p53 in the gastric epithelial cells in patients with *Helicobacter pylori* gastric illness in association with *CagA* positivity. Paraffin embedded tissue had been made form biopsies taken from 30 patients undergo O.G.D. and selected according to exclusion criteria. In addition, 10 apparently healthy volunteers were included as a control group. Procedures of ISH and IHC were used to detect *CagA* and p53 respectively. The *CagA* cytotoxin was detected in 21(70.0%) patients out of 30 and 9 (30.0%) gave negative results, p53 immunostaining shows 26/30 cases strong staining and 4/30 cases gave a moderate staining. The *CagA* positive *H. pylori* strains causes a higher p53 expression than the *CagA* negative strains.

Keywords: - Apoptosis, CagA, Gastritis, *Helicobacter pylori*, p53.

1 INTRODUCTION

H. *pylori* has been recognized as the etiological agent of different gastric diseases such as gastritis ,chronic atrophic gastritis ,peptic ulcers and gastric cancer (Vorobjova 2008) .The *H. pylori* genome (1.65 million bp) codes for about 1500 proteins. The genome of *H. pylori* changes continuously during chronic colonization of an individual host by importing small pieces of foreign DNA from other *H. pylori* strains during persistent or transient mixed infections (Sebastian and Pierre 2002)

One of the best-acknowledged virulence factors in *H. pylori* is the cytotoxin-associated pathogenicity island, *Cag-PAI*. The *Cag-PAI* is 40 kb in size and composed of 27 open reading frames (Olfat 2003). *CagA* expression has been considered as a main marker for the presence of *Cag-PAI* and is one of the most immunogenic proteins of *H. pylori* (AL-Ezzy 2014). *CagA* status is highly associated with the pathogenicity of *H. pylori* strains (Brito 2003). The *Cag-PAI* encodes a type IV secretion system (T4SS) for the delivery of *CagA*. For a long time it was believed that *CagA* can be randomly injected into epithelial cells(Torres 2008). Apoptosis possessed a central mechanism for regulating the number of cells in adult tissue and is present as a physiological phenomenon in the normal gastrointestinal tract (Al-Ezzy 2014). Although apoptosis may be a natural physiological occurrence, excessive apoptosis results in tissue damage (Yamasaki 2006). Apoptosis and proliferation are tightly regulated processes within cells. Among apoptosis related genes, p53, is of particular importance (Ashktorab 2008)

The tumor suppressor protein p53 plays a central role in cell cycle regulation, DNA replication, DNA repair, Anti-inflammatory, senescence and apoptosis (Herrmann 2003). Although the precise mechanisms by which p53 acts as a tumor suppressor gene are not known, accumulating evidence suggests that normal wild - type p53 acts as a "molecular policeman", preventing propagation of genetically damaged cells,(Kati 1999).p53 execute its effects on intrinsic apoptotic pathway via Bax, apaf-1, and casp-9. among these factors, Bax which is member of pro-apoptotic class of Bcl-2 family directly activated by p53(Olivares 2005)

This study tried to investigate the impact of *CagA* positivity on the p53 expression.

2 MATERIALS AND METHODS

A total of thirty patients had been included in the present study according to the exclusion criteria which are:-(a) Receiving H. pylori eradication therapy. (b) Receiving proton pump inhibitors. (c) Receiving H2-blockers. (d) In the past six weeks had received bismuth compounds or Antibiotics. (e) Receiving any of the non-steroidal anti-inflammatory drugs. (f) Rapid urease test result was negative.

According on these criteria 19 males and 11 females with a mean of age 39.47 years (range between 16 and 70 years) were chosen. Twenty-one patients were presented with Antral Gastropathy and/or Gastritis, while the rest patients were suffering from chronic atrophic gastritis. Ten apparently healthy volunteers (7 males and 3 females) with the mean age 42.3 years and age range (17-63) years were enrolled as control.

Paraffin embedded sections of gastric tissue were cut into 4-5 µm thickness, mounted onto positively charged slides (Fisher brand, superfrost /plus; U.S. Pat. 4481246) and drained the slides by fluff less blotting papers and left overnight to dry at room temperature (Divjak 2002).

Procedure of In Situ Hybridization was carried out according to (Mohammed and Abood 2010) to detect the *CagA* cytotoxin. Immunohistochemistry (IHC) had been used to evaluate the expression of p53. The procedure of IHC was preformed according to manufacturer's instruction, using Monoclonal Mouse Anti p53 (DakoCytomation: Clone/REF: -PAb240, Class /subclass:- IgG1-Kappa, Code No.:- M3566.) and Im-

1. Department of Biology - College of Education Al-Iraqia University
2. Department of Biology - College of science – Al-Muthana University.
3. Department of Pathology - College of Veterinary Medicine – Diyala University.
4. Department of Microbiology – College of Medicine – Al-Nahrain University.
* Corresponding author: alwazeer81@yahoo.com

munohistochemistry detection kit DakoCytomation LSAB2 System- HRP (Code KO673 DakoCytomation, USA).

The primary antibody diluted by the common antibody diluent 1:50 optimal antibodies concentration may vary depending on specimen and preparation method, thus optimization had been done. Both positive and negative controls were included for each run of Immunohistochemistry. The negative control was obtained by replacing the primary antibody with PBS buffer. Positive control was obtained by using tonsil tissue (Backus 2002)

The expression of p53 protein was measured by counting the number of positive cells with brown (DAB) nuclear staining under light microscopy X40. For the evaluation of p53 expression, immunostaining was assessed semi quantitatively using a scoring system for both intensity and extent of staining as shown in table 1 (Teh 2002).

The data processing was done by using Statistical Package of Social Science (SPSS) version 16.

Table (1) The semi quantitatively us-ing a scoring system for p53 immunostaining.

p53	*Score*	*Intensity*	*Stained cells (%)*
Negative	*0*	*No staining*	*<10*
Positive	*1*	*Weak*	*10-30*
	2	*Moderate*	*31-50*
	3	*Strong*	*>50*

3 Results

The *CagA* cytotoxin was detected by I.S.H. in 21 (70.0%) patients out of 30 and 9 (30.0%) gave negative results. In p53 immunostaining, the positive gastric epithelial cells showed brown nuclear staining, see figure (1). In patients group 26/30 cases gave a strong staining and 4/30 cases gave a moderate staining. The percentages of stained cells of p53 by immunohistochemistry in terms of mean, Std. Deviation, and standard error are shown in table (2).

An independent sample t-test was conducted to find out the differences in ex-pression percentage of p53 among patients group depending upon *CagA* positivity in gastric tissue sections. We found out that there is a high significant differences ($p \leq 0.001$) in p53 expression between *CagA* pos-itive and *CagA* negative patients, see table (3).

In order to investigate the correlation among the expression pattern Pearson corre-lation statistical tests was used. We found out that there is strong significant positive linear correlation (Pearson correlation Coef-ficient= 0.794, p=0.000) between *CagA* pos-itivity and p53 expression percentage.

Finally, we investigated the correla-tion between *CagA* positivity and expression scoring of p53. We found out that there is strong significant positive linear correlation (Pearson correlation Coefficient= .599, p=0.000) between *CagA* positivity and p53 scores.

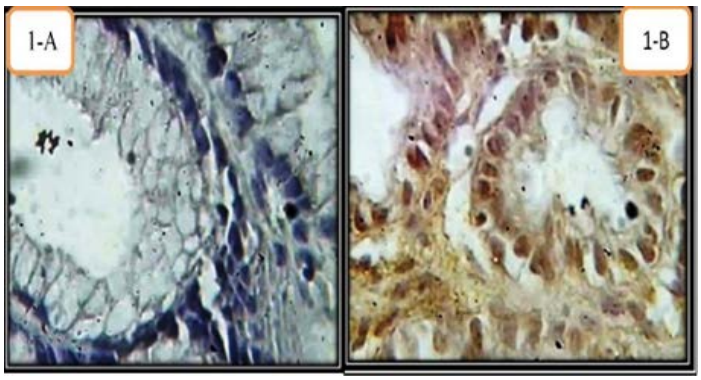

Figure (1): Immunohistochemical staining of p53 in gastric tissue sections (X40). DAB nuclear and peri-nuclear staining (brown). Counter stained by Hematoxylin. (A) No staining. (B) Strong staining.

Table (2): Descriptive data for p53 expres-sion in patients group.

Marker	Cag-A positivity	N	Mean	Std. Deviation	Std. Error Mean	T-Test p value
P53	Negative	9	52.378	11.915	3.972	≤0.001
	Positive	21	74.137	5.512	1.203	

Table (3): independent sample t-test com-parison between *CagA* Positive and *CagA* negative patients for p53 Expression.

4 Discussion

Current study revealed that 70 .0% of patients had positive I.S.H. results for *CagA*, which considered high percentage if we assumed that the clinical presentation of patients are not severe (severe gastric illness include ulcers, intestinal metaplasia and can-cer) and can be described as non-aggressive lesions. Obtainment of such percentage may be due to that the majority of patients group were suffering from antral gastritis. There is a model proposing that infection with H. pylori begins in the antrum and then spreads throughout the fundus (Hofman 2007; Al-Ezzy 2015).

Regarding the *CagA* positivity, there was a high significant differences ($p \leq 0.001$) in p53 expression between *CagA* positive and negative patients with strong positive linear relationship between *CagA* positivity and p53 expression. This results suggesting that the H. pylori strains that pos-sessed the genes to produce *CagA* had the ability to induce high levels of p53 expres-sion more than those which are *CagA* negative. Eventually these strains, *CagA* positive, cause much more severe lesions at the site of infection by increasing the apoptotic process. This result come in line with (Teh 2002), They observed that cases with extremely strong staining were all from the *CagA*-positive cases. Besides that, there was stronger association between p53 positive staining and *CagA*-positive than *CagA*-negative strains with statistical significance.

tumor suppressor p53 is a direct transcription activator of the human Bax gene and under that of c-myc (Cartron 2003). p53 might have a separate cytoplasmic role in directly regulating the Bax-dependent mitochondrial pathway to cell death (Jerry 2002). Current result come in line with (Yang 2003; Liu 2005) , found that H. pylori leads to apoptosis in the gastric epithelium through up-regulation of Bax. Other found significant association between H. pylori in-fection and the expression of Bax (Twaij 2006).

Current immunohistochemical study for expression of p53 does not provide ade-quate information about the dysfunction of the protein and gene mutation, may be due to the antibody used, staining methods or the criteria used. In addition, immunohisto-chemistry has been shown to have a discord-ancy rate of 30~35% when compared with techniques that determine p53 gene status, including single strand conformation poly-morphism polymerase chain reaction analysis and direct DNA sequencing (Kahlenberg 2000).

5 Conclusion

The strains of H. pylori with *CagA* positive induce high expression levels of the p53, in the epithelial cells of the gastric mucosa, than the *CagA* negative strains.

References

[1] AL-Ezzy, A. I. A. (2014). "Immunopathological Role of FAS-FASL Apoptotic Pathway in H. pylori CagA Positive Associated Chronic Atrophic Gastritis in Iraqi Patients." Journal of Biology, Agri-culture and Healthcare 4(23): 67-74.

[2] Al-Ezzy, A. I. A. (2014). "Molecular and Immunopathological role of nuclear factor k b detected by insitu hybridization in pathogenesis of chronic atrophic gastritis in Iraqi patients." International Jour-nal of Advanced Research 2(1): 67-78.

[3] Al-Ezzy, A. I. A. (2015). "Molecular and Immunopathological role of Gastric versus lymphocytes Inter-leukin 8 Gene Expression in H.pylori induced Fas-Fasl apoptotic pathway in Gastroduodenal Ulcer in Iraqi patients." Journal of Biology, Agriculture and Healthcare 5(5): 141-153.

[4] Ashktorab , H. D. R. H., Mohaiza M,Dashwood;Zaidi, Syed I;Hewitt, Stephen M;Green, William R;Lee, Edward L;Daremipouran, Mohammadreza;Nouraie, Mehdi;Malekzadeh, Reza; (2008). "H. pylo-ri Induced Apoptosis in Human Gastric Cancer Cells Mediated via the Release of Apoptosis In-ducing Factor from Mitochondria." Helicobacter 13(6): 506-517.

[5] Backus, H. V. G., CJ;Vos, W;Dukers, DF;Bloemena, E;Wouters, D;Pinedo, HM;Peters, GJ; (2002). "Dif-ferential expression of cell cycle and apoptosis related proteins in colorectal mucosa, primary colon tumours, and liver metastases." Journal of clinical pathology 55(3): 206-211.

[6] Brito, C. A. S., Len ra,Juc Norma,Leal, Nilma C,Souza, Wayner de,Queiroz, Dulciene,Cordeiro, Francis-co,Silva, Norma Lucena, (2003). "Prevalence of cagA and vacA genes in isolates from patients with Helicobacter pylori-associated gastroduodenal diseases in Recife, Pernambuco, Brazil." Mem3|rias do Instituto Oswaldo Cruz 98(6): 817-821.

[7] Cartron, P.-F. J., Philippe;Oliver, Lisa;Meflah, Khaled;Vallette, Franois M; (2003). "Impact of proapop-totic proteins Bax and Bak in tumor progression and response to treatment."

[8] Divjak, M. G., Eric M;Walters, E Haydn; (2002). "Improvement of non-radioactive in situ hybridization in human airway tissues: use of PCR-generated templates for synthesis of probes and an anti-body sandwich technique for detection of hybridization." Journal of Histochemistry & Cyto-chemistry 50(4): 541-548.

[9] Herrmann , M. K., J R; (2003). Apoptosis and Autoimmunity, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 3-527-30442-8.

[10] Hofman, V. J. M., Chimene;Brest, Patrick D;Lassalle, Sandra;Le Brigand, Kevin;Sicard, Dominique;Raymond, Josette;Lamarque, Dominique;H buterne, Xavier A;Mari, Bernard; (2007). "Gene expression profiling in human gastric mucosa infected with Helicobacter pylori." Modern Pathology 20(9): 974-989.

[11] Jerry, D. J. M., Lisa M;Becker, Klaus A;Blackburn, Anneke C; (2002). "Hormonal control of p53 and chemoprevention." Breast Cancer Research 4(3): 91.

[12] Kahlenberg, M. S. S., Daniel L;Rodriguez Bigas, Miguel A;Weber, Thomas K;Driscoll, Deborah L;Anderson, Garth R;Petrelli, Nicholas J; (2000). "p53 tumor suppressor gene mutations predict decreased survival of patients with sporadic colorectal carcinoma." Cancer 88(8): 1814-1819.

[13] Kati, V. S., Ivanka;Moritani, Suzuko;Nagorni, Aleksandar;Veli kovi , Ljubinka; (1999). "Over-expression of p53 protein in gastric adenomas." pathology 3(5): 6.

[14] Liu, H.-F. L., Wei-Wen;Wang, Guo-An;Teng, Xiao-Chun; (2005). "Effect of Helicobacter pylori infection on Bax protein expression in patients with gastric precancerous lesions." World journal of gastroenterology 11(37): 5899.

[15] Mohammed, N. A. and A. S. Abood (2010). "Detection of CagA in H. Pylori Gastric Illness Using in Situ Hybridization." Diyala Journal for Pure Science 6(3): 85-90.

[16] Olfat, O. (2003). Helicobacter pylori Bacterial Adherence and Host Response. UME University medical dissertations,(Sweden). New Series No, ISBN 91-7305-537-9.

[17] Olivares , D. G. J. P. P. M. J. (2005). "Helicobacter pylori infection and gastric mucosal epithelial cell apoptosis " Rev Esp ENFERM Dig Madrid 97 (7): 505-520.

[18] Sebastian, S. and M. Pierre (2002). "Helicobacter pylori infection " N Engl J Med 347(15): 1175-1186.

[19] Teh, M. B. T., Kong;Leng Seet, Bee;Guan Yeoh, Khay; (2002). "Study of p53 immunostaining in the gas-tric epithelium of cagA positive and cagA negative Helicobacter pylori gastritis." Cancer 95(3): 499-505.

[20] Torres , J. B., Steffen (2008). "Pathogenesis of Helicobacter pylori infection." Helicobacter 13(s1): 13-17.

[21] Twaij, A. D. (2006). Invasive and Non Invasive Methods for the Detection of H. pylori With Some Mo-lecular Aspects of its Pathogenesis (Bax mRNA by I.S. H. and Bcl-2 by I.H.C.).

[22] Vorobjova, T. M., Heid IngridUibo, Raivo , (2008). "Immune response to Helicobacter pylori and its as-sociation with the dynamics of chronic gastritis in the antrum and corpus." APMiS 116(6): 465-476.

[23] Yamasaki, E. W., Akihiro;Kumatori, Atsushi;Nakagawa, Ichiro;Funao, Junko;Nakayama, Masaaki;Hisatsune, Junzo;Kimura, Miyuki;Moss, Joel;Hirayama, Toshiya (2006). "Helicobacter pylori vacuolating cytotoxin induces activation of the proapoptotic proteins Bax and Bak, lead-ing to cytochrome c release and cell death, independent of vacuolation." Journal of Biological Chemistry 281(16): 11250-11259.

[24] Yang, Y. D., CS;Peng, JZ;Wong, B CY;Lam, SK;Xia, H HX; (2003). "Effect of Helicobacter pylori on apopto-sis and apoptosis related genes in gastric cancer cells." Molecular Pathology 56(1): 19