Helicobacter pylori Infection Mediates Transcriptional Alteration of Genes Involved in DNA Damage Response

Amiratabak Rajaei
Islamic Azad University
Armin Ghameshlou
Islamic Azad University
Reza Shirkoohi
Imam Khomeini Hospital Complex, Tehran University of Medical Sciences
Abbas Shakoori Farahani
Imam Khomeini Hospital Complex, Tehran University of Medical Sciences
Seyedeh Zohre Mirbagheri
Tehran University of Medical Sciences
Ronak Bakhtiari
Tehran University of Medical Sciences
Melikasadat Haeri
Islamic Azad University
Ali Rashidi-nezhad
Imam Khomeini Hospital Complex, Tehran University of Medical Sciences
Masoud Alebouyeh (masoud.alebouyeh@gmail.com)
Shahid Beheshti University of Medical Sciences

Research Article

Keywords: Double-strand DNA breaks, Error-prone DNA repair pathways, Gastritis, Helicobacter pylori, Non-homologous end joining DNA repair system

Posted Date: February 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1339574/v1

Abstract

Background: Gastritis is among the most common human diseases worldwide. Although the involvement of *Helicobacter pylori* infection as a class I human carcinogen for gastric cancer progression is accepted, it is not well known how gastritis progression to atrophy and stomach cancer occurs. In this case-control study, the potential link of *H. pylori* infection with alteration in the transcription of genes involved in DNA Damage Response pathways was investigated among the patients with gastritis.

Methods: To measure the difference in relative mRNA expression level of *ATM*, *CHEK2*, *TP53, DCLRE1C, POLM, XRCC4* genes between *H. pylori* infected and non-infected patients, gastric biopsies of 30 *H. pylori* infected patients with moderate chronic gastritis and 30 non-infected patients with mild chronic gastritis were analyzed.

Results: Up-regulation of genes linked to non-homologous end joining (NHEJ) pathway (*DCLRE1C, POLM,* and *XRCC*) was shown in 40% (8.44 fold ±13.91), 63.33% (15.72 fold ±33.08) and 50% (9.99 fold ±21.55), respectively, and also to DDR pathway (*ATM*, *CHEK2* and *TP53*) in 33% (2.42 fold ±3.17), 40% (2.86 fold ±3.61) and 50% (5.00 fold ±6.52), respectively. No correlation was detected among alteration in the transcription level of the studied genes and age or gender.

Conclusion: Our results provide new data that may support the potential involvement of *H. pylori* infection in the activation of genes involved in DNA damage response, mainly through non-homologous end joining DNA repair system that might be linked to mutagenesis in the pre-cancerous gastric tissue.

1. Introduction

Gastric cancer (GC) is the third leading cause of cancer mortality globally (1). Although there are supporting data about the involvement of host genetic factors, infections, and environment in the histopathological changes and gastric carcinogenesis (2), it is not well known how these factors might be involved in carcinogenesis and its progression. Chronic gastritis is a multistep, progressive and life-long inflammation (3). Most gastric adenocarcinomas, particularly those of the intestinal type, are related to a sequence of phenotypic changes of the native mucosa triggered by long standing inflammation. *Helicobacter pylori* infection is, by far, the foremost common etiological agent of chronic active gastritis and therefore, the most specific etiological factor of gastric non-syndromic oncogenesis (4). In 1994, infection with *Helicobacter pylori* was regarded as a class I human carcinogen for gastric cancer by the International Agency for Research on Cancer (IARC) (5). Following the epidemiological and cellular microbiology studies, various virulence factors in this bacterium, including CagA oncoprotein and VacA cytotoxin, as well as immune system stimulation resulting from the interaction of these factors with the host, are introduced as a reason for cancer in patients. These interactions can trigger a cytokine cascade in the gastric tissue and subsequently attract and differentiate systemic and localized immune cells, resulting in irreversible secondary genetic events in this organ (6).

DNA within our cells is continually being exposed to DNA-damaging agents. These include actinic radiation, natural and man-made mutagenic chemicals and reactive oxygen species generated by ionizing radiation (IR). Of the varied types of damage that are inflicted by these mutagens, probably the most dangerous type is the DNA double-strand breaks (DSB). There are two main pathways for DNA DSB repair: homologous recombination (HR) and non-homologous end- joining (NHEJ) (7). During HR, the damaged chromosome enters into synapsis with an undamaged DNA molecule and retrieves genetic information from, which shares extensive sequence homology. In contrast, NHEJ, which brings about the ligation of two DNA DSBs without the necessity for extensive sequence homology between the DNA ends, does not need synapsis of the broken DNA with an undamaged partner DNA molecule (8). Consequently, NHEJ is barely error-free and sequence insertions and deletions of varying lengths are usually introduced (7). Although cells may survive from lethal genomic damages through repair pathways, accumulation of deletions and insertions contribute to tumorigenesis (9).

Since little is understood about the role of *H. pylori* infection on double-strand breaks and also the induction of the NHEJ repair mechanism in patients, this study investigated the expression of genes that play a role in DNA damage response (DDR) as well as activating the NHEJ pathway to analyze the possible involvement of *H. pylori* in DNA damage in early gastritis.

2. Methods

2.1 Sampling and data collection:

A total of 180 adult patients with various gastric disorders were referred to the endoscopy ward of Firoozgar hospital and considered for endoscopic examinations from February to August 2019. Patients with acute gastritis, intestinal metaplasia, gastric neoplasia, dysplasia, also those experiencing gastrointestinal surgery or consuming antibiotics in the past three weeks were excluded from this study. Finally, gastric biopsies of all patients with mild to moderate chronic gastritis were included for further investigations after obtaining informed consent. This study was approved by the Tehran University of Medical Sciences (Code 43392-27-02-98).

2.2 Histopathological analysis and culture for *H. pylori*

Three biopsy samples were obtained from each patient. A pathologist analyzed all the samples in order to evaluate the gastritis grade based on the Updated Sydney classification of Gastritis. Biopsies were cultured in Brucella agar medium supplemented with 10% sheep blood and antibiotic supplement (Amphotericin B, trimethoprim, vancomycin, and polymyxin). Incubation was done under microaerophilic conditions up to three days. Grown colonies were screened based on their morphology, urease, catalase and oxidase tests. PCR was done on DNA extracted from biochemically confirmed colonies using specific primers targeting *glmM* gene as previously described (10).

2.3 RNA extraction, cDNA synthesis, and real-time PCR

Total RNA was extracted from gastric tissues using RNA extraction kit (Pars Tous Biotechnology, Cat NO. A101231, Mashhad, Iran) and the purity of extracted RNA was quantified with NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). Extracted RNA was used as the template for cDNA synthesis by using cDNA synthesis kit (Pars Tous Biotechnology, Cat NO. A101161, Mashhad, Iran) according to the manufacturer's instructions. The suitability and integrity of the synthesized cDNA was confirmed by PCR with B2M primers (provided in the cDNA synthesis kit) and electrophoresis, respectively. To investigate expressional changes, specific primers were designed for *CHEK2*, *DCLRE1C*, *XRCC4, TP53, POLM, ATM* and *B2M* genes using Primer3 software (version 4.1.0). During this study, the *B2M* gene was selected as Housekeeping gene. Primers were synthesized by the Metabion Company (Germany) (Table 1). SYBR Green Quantitative RT-PCR Kit (Biofact – South Korea) was used for making real-time PCR mixes. The Rotor-Gene Q instrument (QIAGEN, USA) was used for doing real-time PCRs at following conditions: 1 cycle of denaturation at 95 ^{o}C for 15 min, 40 cycles of denaturation at 95 ^{o}C for 10 seconds, amplification at 62 ^{o}C, 58 ^{o}C and 55 ^{o}C. , each for 5sec, and extension at 72 ^{o}C for 15 sec, plus one cycle of melting curve analysis. All the assays were done in duplicate. The efficiencies of the primers were calculated based on 10-fold dilution series. Relative Expression Software Tool (REST) 2009 software (developed by QIAGEN – Germany) was used for Real-Time PCR data analysis.

2.4 Statistical analysis

All statistical analyses were performed by Statistical package for social science software (SPSS, version 23). Chi-square test was applied to evaluate the correlation of categorical variables among different defined groups. Independent-Samples T-test was used to compare quantitative and qualitative variables. Levene's Test was performed to assess the possible correlation between NHEJ and DDR group genes. Differences were considered statistically significant if p values were ≤ 0.05.

3. Results

3.1 Clinicopathological characteristics of patients:

After a review of demographic, microbiological and pathological data, 60 patients were recruited in the study. . Thirty patients with *H. pylori* infection and moderate chronic gastritis were regarded as case group (Mean age of 50.10 years including 14 (46.66%) female, 16 (53.33%) Male) and the remaining 30 *H. pylori* negative patients who showed mild chronic gastritis patients were regarded as control group (Mean age of 47.07 years including 19 (63.33%) female (19) and 11 (36.66%) Male).

3.2 Result of gene expression analysis of genes involved in DDR and NHEJ pathways

The efficiency of the primers for *ATM*, *CHEK2*, *DCLRE1C*, *POLM, TP53* and *XRCC4* genes was 100%, 99%, 99%, 98%, 100% and 99% respectively. Transcriptional analysis showed up-regulation of genes linked to DDR (*ATM*, *CHEK2* and *TP53* genes) and NHEJ (*DCLRE1C*, *POLM* and *XRCC4* genes) pathways in the case group in comparison to the control group (Table 2).

3.3 Correlation of NHEJ and DDR genes in the *H. pylori* infected tissue:

To analyze the potential correlation between transcriptional alteration of genes linked to NHEJ and DDR pathways, Levene's test for equality of variances was performed. Statistical analysis showed significant correlation between NHEJ and DDR pathways genes expression in the *H. pylori* infected patients (*p* value= 0.002).

4. Discussion

H. pylori infects the stomach of about half of the world's population, while gastritis and its progresses to gastric cancer is occurs in low percentages of the infected patients (11). Although this infection is considered as a risk factor for gastric cancer, mechanisms mediating the disease in the gastric tissue are not well known. Chronic gastritis, deregulation of cell signaling pathways, mucosal damage and genomic instabilities seems critical in this interplay. Chronic inflammation is estimated to be the cause of approximately 25% of human cancers (12). In the case of gastric tissue, it is assumed that *H. pylori* infection could induce gastritis by two different mechanisms, either through toxins that are secreted by different bacterial secretory systems (e.g., CagA, VacA) or by invasion and induction of the epithelial cells to release pro-inflammatory mediators (13,14). The induced chemokines can promote neutrophil infiltration and also T lymphocytes to reinforce reactive oxygen and nitrogen species (RONS), which play key role in DNA damage and mutagenesis (15,16). Although there is a growing body of evidence supporting direct or indirect involvement of *H. pylori* in genomic instability and mutagenesis, molecular mechanisms that link DNA damage to erroneous DNA repair system, which will affect oncogenes and tumor suppressor genes, is not well known. To show this relationship, we addressed activation of NHEJ in the gastric tissue of *H. pylori* infected compared with non-infected patients. Our findings provide initial evidences of the activation of the error-prone repair pathway (NHEJ) in patients infected with *H. pylori*.

Activation of the NF-κB transcription factor, overexpression of IL8, and release of free oxygen radicals that are related to oxidative stress (ROS) in the gastric epithelial cells are associated with mutagenesis through DSBs and activation of DDR systems (17). DSBs are detected by the DNA damage sensors, namely the MRN complex (Mre11-Rad50-Nbs1), which successfully manipulates other components of the DDR pathway, including activation of *ATM* and *ATR* leading to H2AX histone phosphorylation that is followed by activation of the transducers Chk1 and Chk2, resulting in p53 activation. Cell cycle arrest through activation of TP53 is a trigger for the repair of DNA breaks (18). So far, not many studies have been done on the role of this bacterium in causing DSBs in human gastric cells. Findings from in vitro examinations suggest a possible role for VacA and CagA in DSBs development (19). Despite these shreds of evidence, it remains unclear how this pathogen may play a role in gastric carcinogenesis. Investigating a possible link between the activation of repairing mechanisms and the occurrence of errors that cause genetic mutations in the gastric tissue can clarify the relevance. In our study, among the studied genes, *POLM* and *XRCC4* that are associated with the NHEJ repair pathway, showed the highest expression in the *H. pylori* infected patients, which indirectly suggests the formation of chromosomal mutations. Interestingly, in a study of prostatic adenocarcinoma cells, increased expression of the *POLM* gene was reported, which is in line with

the results of the current study. It has also been suggested increased expression is because of the inefficiency of the HR repair pathway (20). Also, in studies on gastric and breast cancerous tissues, an increase in the *XRCC4* gene expression was reported (21,22). Kitagawa et al. introduced the expression of the *XRCC4* gene as a biomarker to detect the recurrence of breast cancer (22). Overexpression of the *DCLRE1C* gene, which rises through recruitment of NHEJ repair pathway, was previously reported in lung cancer (23). On similar study, Farkas et al. reported a rise in the expression of the *DCLRE1C* gene in colorectal cancer (24). In current study, overexpression of *DCLRE1C* can be a result of activation in NHEJ pathway in terms of compensating the induced damage by *H. Pylori.*

In the current study, the *TP53* gene from the DDR pathway showed the highest increase in expression levels in *H. pylori* infected patients, supporting a link between the cell cycle arrest and activation of the repair system in response to the induced damage on DNA. In a study on gastrointestinal cancer by Sun et al., an increase in the expression of this gene was reported in comparison to normal and benign tissues, which is to somehow in line with the current study (25). Paradoxically, in a study on *H. pylori* mediated gastric cancer by Calcagno et al., a decrease in expression of the *TP53* gene at mRNA levels was reported. In this study, cancerous tissue was used instead of inflammatory tissue (26). It is plausible that decreased expression of the *TP53* gene could be resulted from mutations that occur during the carcinogenesis process, which occur over time and can reduce the function of this gene. Another study by Nianshuang et al. examined the role of the TP53 protein in *H. pylori* infection and its association with gastric cancer. The study results suggested that *H. pylori* might use its pathogenic agents, such as CagA that can enter into the host cells, to promote degradation and reduction of TP53 protein. This could accelerate the occurrence of DSBs in host DNA (27). According to these findings, the increase in *TP53* transcription that observed in our study could be described through degradation of the protein in the infected cells and its compensation by the cell after transcription.

Decreased *CHEK2* gene expression was generally reported in studies on gastric, lung, colon, and breast cancers (28–31), which are in contrary to our findings. In a study performed by Bae et al. on animal models of normal gastric tissue to investigate the effect of *H. pylori* on *CHEK2* gene expression, it was demonstrated that the expression of this gene was increased, which is in line with the results of our study. Their findings showed increased expression of the *TP53* and *CHEK2* genes simultaneously, indicating that the cell is headed for cell death after the cell cycle arrest due to DSB (32). Moreover, *ATM* gene expression has decreased in most studies on cancerous tissues (33,34), which contrasts with the present study's findings. In general, the decreased expression of DDR-related genes in cancerous tissues may be due to multiple mutations occurs overtime which could give rise to the loss of function of these genes. The overexpression of *ATM* gene in current study could be explained due to the different essence of tissues and the mediation of *H. pylori* in this interplay.

We were the first to study the transcription of genes linked to NHEJ and DDR pathways in association to *H. pylori* infection in the precancerous gastric tissue, however, the mechanism of chromosomal mutations and genomic instabilities caused by this bacterium need further examinations. Absence of healthy individuals to measure the basic expression levels of target genes and comparing them with case group

and impossibility to study all NHEJ genes and regulators, failure to determining DNA breaks at the chromosomal stages and possible shuffling and translocations, and finally, the impossibility of investigating the genome of bacterial strains in the studied specimen samples, in order to understand their relationship with the NHEJ pathway activation are among the limitations of the present study. Future studies can identify subtypes of pathogenic factors of *H. pylori* involved in DSBs of host cells in gastric tissue. It can also be studied to identify activators of other DSB-related signaling pathways by *H. pylori* and their interaction with DDR. Additional tests to understand the type of mutations associated with the NHEJ pathway and the identification of NHEJ pathway mediators that play a vital role in the repairment of DSB in gastric tissue, and the design of appropriate drugs for therapeutic purposes, might be topics for future studies.

Conclusion

In overall, based on the results of this study, it can be suggested that *H.pylori* can directly or indirectly cause double-strand breaks in the DNA of gastric cells and activate the error-prone repair pathway, a process that may be accompanied by the cell cycle arrest.

Declarations

Acknowledgments

This article was taken from a master's thesis in Genetics and we would like to appreciate all staff of Cancer Biology Research Centre of Imam Khomeini hospital complex that helped us for this study.

Author Contributions

MA and ARN conceived and designed the experiments. SZM and MH collect the biopsy samples, demographic data and histopathological reports. AR and AG performed the laboratory experiments. ARN, AR, MA, AG, RS, RB, and ASF analyzed the data. ARN, AR, MA, AG wrote the paper. AR and AG contributed equally to this study.

Funding

No funding were accrued to this study.

Ethics approval and consent to participate

This study was approved by the ethical committee of Tehran University of Medical Sciences (accepted Number, IR.TUMS.SPH.REC.1398.167 1398/7/3) and an informed consent form was obtained from all the patients.

Availability of data and material

Not applicable

Consent for publication

The authors declare their consent for publication of the results. All the authors approved the final draft of manuscript.

Competing interests

The authors declare that they have no competing interests.

References

1. Ge S, Xia X, Ding C, Zhen B, Zhou Q, Feng J, et al. A proteomic landscape of diffuse-type gastric cancer. Nat Commun. 2018;

2. Yoo MW, Park J, Han HS, Yun YM, Kang JW, Choi DY, et al. Discovery of gastric cancer specific biomarkers by the application of serum proteomics. Proteomics. 2017;

3. Sipponen P, Maaroos HI. Chronic gastritis. Scand J Gastroenterol. 2015;

4. Rugge M, Meggio A, Pravadelli C, Barbareschi M, Fassan M, Gentilini M, et al. Gastritis staging in the endoscopic follow-up for the secondary prevention of gastric cancer: A 5-year prospective study of 1755 patients. Gut. 2019;

5. IARC. Schistosomes, Liver Flukes and Helicobacter pylori. Iarc Monogr Eval Carcinog Risks To Humans. 1994;

6. Gagnaire A, Nadel B, Raoult D, Neefjes J, Gorvel J-P. Collateral damage: insights into bacterial mechanisms that predispose host cells to cancer. Nat Rev Microbiol [Internet]. 2017;15(2):109–28. Available from: https://doi.org/10.1038/nrmicro.2016.171

7. Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis. 2002;23(5):687–96.

8. Johnson RD. Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. EMBO J. 2000;

9. Mao Z, Bozzella M, Seluanov A, Gorbunova V. Comparison of nonhomologous end joining and homologous recombination in human cells. DNA Repair (Amst). 2008;

10. Gharibi S, Falsafi T, Alebouyeh M, Farzi N, Vaziri F, Zali M. Relationship between histopathological status of the Helicobacter pylori infected patients and proteases of H. pylori in isolates carrying diverse virulence genotypes. Microb Pathog. 2017 Jun 1;110.

11. SY O, J L, JY K. Helicobacter pylori: beneficial for most? Expert Rev Gastroenterol Hepatol [Internet]. 2011 Dec [cited 2021 Sep 28];5(6):649–51. Available from: https://pubmed.ncbi.nlm.nih.gov/22017690/

12. Kawanishi S, Ohnishi S, Ma N, Hiraku Y, Murata M. Crosstalk between DNA Damage and Inflammation in the Multiple Steps of Carcinogenesis. Vol. 18, International Journal of Molecular Sciences. 2017.

13. Eck M, Schmausser B, Scheller K, Toksoy A, Kraus M, Menzel T, et al. CXC chemokines Groα/IL-8 and IP-10/MIG in Helicobacter pylori gastritis. Clin Exp Immunol [Internet]. 2000 [cited 2021 Sep 29];122(2):192–9. Available from: https://pubmed.ncbi.nlm.nih.gov/11091274/

14. N W, T S, Y O, H H, A T. Proinflammatory cytokines and Helicobacter pylori stimulate CC-chemokine expression in gastric epithelial cells. J Physiol Pharmacol [Internet]. 1997 Sep [cited 2021 Sep 29];48(3):405–13. Available from: https://pubmed.ncbi.nlm.nih.gov/9376623/

15. Naito Y, Yoshikawa T. Molecular and cellular mechanisms involved in Helicobacter pylori -induced inflammation and oxidative stress 1,2 1Guest Editor: Giuseppe Poli 2This article is part of a series of reviews on “Reactive Oxygen and Nitrogen in Inflammation.” The full list of . Free Radic Biol Med [Internet]. 2002 Aug;33(3):323–36. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0891584902008687

16. Nozawa Y, Nishihara K, Peek RM, Nakano M, Uji T, Ajioka H, et al. Identification of a signaling cascade for interleukin-8 production by Helicobacter pylori in human gastric epithelial cells. Biochem Pharmacol [Internet]. 2002;64(1):21–30. Available from: https://www.sciencedirect.com/science/article/pii/S0006295202010304

17. Kidane D. Molecular Mechanisms of H. pylori-Induced DNA Double-Strand Breaks. Int J Mol Sci [Internet]. 2018 Sep 23;19(10):2891. Available from: http://www.mdpi.com/1422-0067/19/10/2891

18. Kalisperati P, Spanou E, Pateras IS, Korkolopoulou P, Varvarigou A, Karavokyros I, et al. Inflammation, DNA damage, Helicobacter pylori and gastric tumorigenesis. Front Genet. 2017;8(FEB):1–8.

19. Gagnaire A, Nadel B, Raoult D, Neefjes J, Gorvel J-P. Collateral damage: insights into bacterial mechanisms that predispose host cells to cancer. Nat Rev Microbiol [Internet]. 2017 Feb 3;15(2):109–28. Available from: http://www.nature.com/articles/nrmicro.2016.171

20. Long G, Ouyang W, Zhang Y, Sun G, Gan J, Hu Z, et al. Identification of a DNA Repair Gene Signature and Establishment of a Prognostic Nomogram Predicting Biochemical-Recurrence-Free Survival of Prostate Cancer. Front Mol Biosci. 2021;8(March):1–13.

21. Cheng L, Qiu L, Wang M, Zhang R, Sun M, Zhu X, et al. Functional genetic variants of XRCC4 and ERCC1 predict survival of gastric cancer patients treated with chemotherapy by regulating the gene expression. Vol. 56, Molecular Carcinogenesis. 2017. 2706–2717 p.

22. Kitagawa M, Someya M, Hasegawa T, Mikami T, Asaishi K, Hasegawa T, et al. Influence of XRCC4 expression by breast cancer cells on ipsilateral recurrence after breast-conserving therapy. Strahlentherapie und Onkol. 2019;195(7):648–58.

23. Antonie L, Bessonov K. Classifying microarray data with association rules. Proc ACM Symp Appl Comput. 2011;94–9.

24. Farkas SA, Vymetalkova V, Vodickova L, Vodicka P, Nilsson TK. DNA methylation changes in genes frequently mutated in sporadic colorectal cancer and in the DNA repair and Wnt/β-catenin signaling pathway genes. Epigenomics. 2014;6(2):179–91.

25. Sun R, Wang X, Zhu H, Mei H, Wang W, Zhang S, et al. Prognostic value of LAMP3 and TP53 overexpression in benign and malignant gastrointestinal tissues. Oncotarget. 2014;5(23):12398–409.

26. Calcagno DQ, Freitas VM, Leal MF, de Souza CRT, Demachki S, Montenegro R, et al. MYC, FBXW7 and TP53 copy number variation and expression in Gastric Cancer. BMC Gastroenterol. 2013;13(1).

27. Li N, Xie C, Lu N. p53, a potential predictor of Helicobacter pylori infection-associated gastric carcinogenesis? Oncotarget [Internet]. 2016 Oct 4;7(40):66276–86. Available from: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.11414

28. Zhang P, Wang J, Gao W, Yuan BZ, Rogers J, Reed E. CHK2 kinase expression is down-regulated due to promoter methylation in non-small cell lung cancer. Mol Cancer. 2004;3:1–10.

29. Sullivan A, Yuille M, Repellin C, Reddy A, Reelfs O, Bell A, et al. Concomitant inactivation of p53 and Chk2 in breast cancer. Oncogene. 2002;21(9):1316–24.

30. Lee HE, Han N, Kim MA, Lee HS, Yang HK, Lee BL, et al. DNA damage response-related proteins in gastric cancer: ATM, Chk2 and p53 expression and their prognostic value. Pathobiology. 2013;81(1):25–35.

31. Stawinska M, Cygankiewicz A, Trzcinski R, Mik M, Dziki A, Krajewska WM. Alterations of Chk1 and Chk2 expression in colon cancer. Int J Colorectal Dis. 2008;23(12):1243–9.

32. Bae M, Lim JW, Kim H. Oxidative DNA Damage Response in Helicobacter pylori-Infected Mongolian Gerbils. J cancer Prev [Internet]. 2013;18(3):271–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25337555%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4189467

33. Suerbaum S, Michetti P, Cali S, Duynhoven Y Van, Jonge R, Suerbaum S, et al. *Helicobacter pylori* Infection. N Engl J Med. 2002;

34. Ye C, Cai Q, Dai Q, Shu XO, Shin A, Gao YT, et al. Expression patterns of the ATM gene in mammary tissues and their associations with breast cancer survival. Cancer. 2007;109(9):1729–35.

Tables

Table 1. Primer sequences used for real-time PCR.

Gene	Primer sequences (5′ to 3′)	Amplicon (bp)
ATM	F: TTGCTGACAATCATCACCAAGTTC	181
	R: GCACTATGGGACATTTCTCTCATTC	
B2M	F: AGATGTCTCGCTCCGTGG	85
	R: TGAATCTTTGGAGTACGCTGG	
CHEK2	F: TTTGCCAATCTTGAATGTGTGAATG	189
	R: GCCACTGTGATCTTCTATGTATGC	
DCLRE1C	F: CAACAGACCGCAACACTCAGA	198
	R: ATGAACTCTCTCCAGTCCTCACA	
POLM	F: TGTGAGGAGGTGGAGAGAGTTC	132
	R: GAGGTCATCTAAGGTTCGCAGTC	
TP53	F: TGGAGTATTTGGATGACAGAAACAC	187
	R: AGTAGATTACCACTGGAGTCTTCC	
XRCC4	F: AGGCTTCTGAGAGATTGGAATGATG	200
	R: GCAGTTTCCCCTTCTTGTTTGATG	

Table 2. Alteration of mRNA expression levels of genes linked to DNA repair system in the *H. pylori* infected compared with *H. pylori* non-infected patients. [a]

a: $2^{-\Delta\Delta Ct}$ in case group (Mean RQ ± SD)

Figures

Genes	Title	Function	Relative abundance of mRNA expression [a]
ATM	Ataxia telangiectasia mutated	DNA-binding, cellular response to DNA damage, cell cycle arrest,	2.42 ±3.17
CHEK2	checkpoint kinase 2	DNA damage response, DNA damage checkpoint, protein kinase binding	2.86 ±3.61
TP53	Tumor protein p53	DNA-binding, tumor suppressor, cell cycle arrest, apoptosis	5.00 ±6.52
DCLRE1C	DNA cross-link repair 1C	Damaged DNA binding, exonuclease activity, exodeoxyribonuclease activity, DBS repair via NHEJ	8.44 ±13.91
POLM	DNA polymerase mu	Gap-filling polymerase, DBS repair via NHEJ	15.72 ±33.08
XRCC4	X-ray repair cross complementing 4	DNA ligation involved in DNA repair, DSB repair via NHEJ	9.99 ±21.55

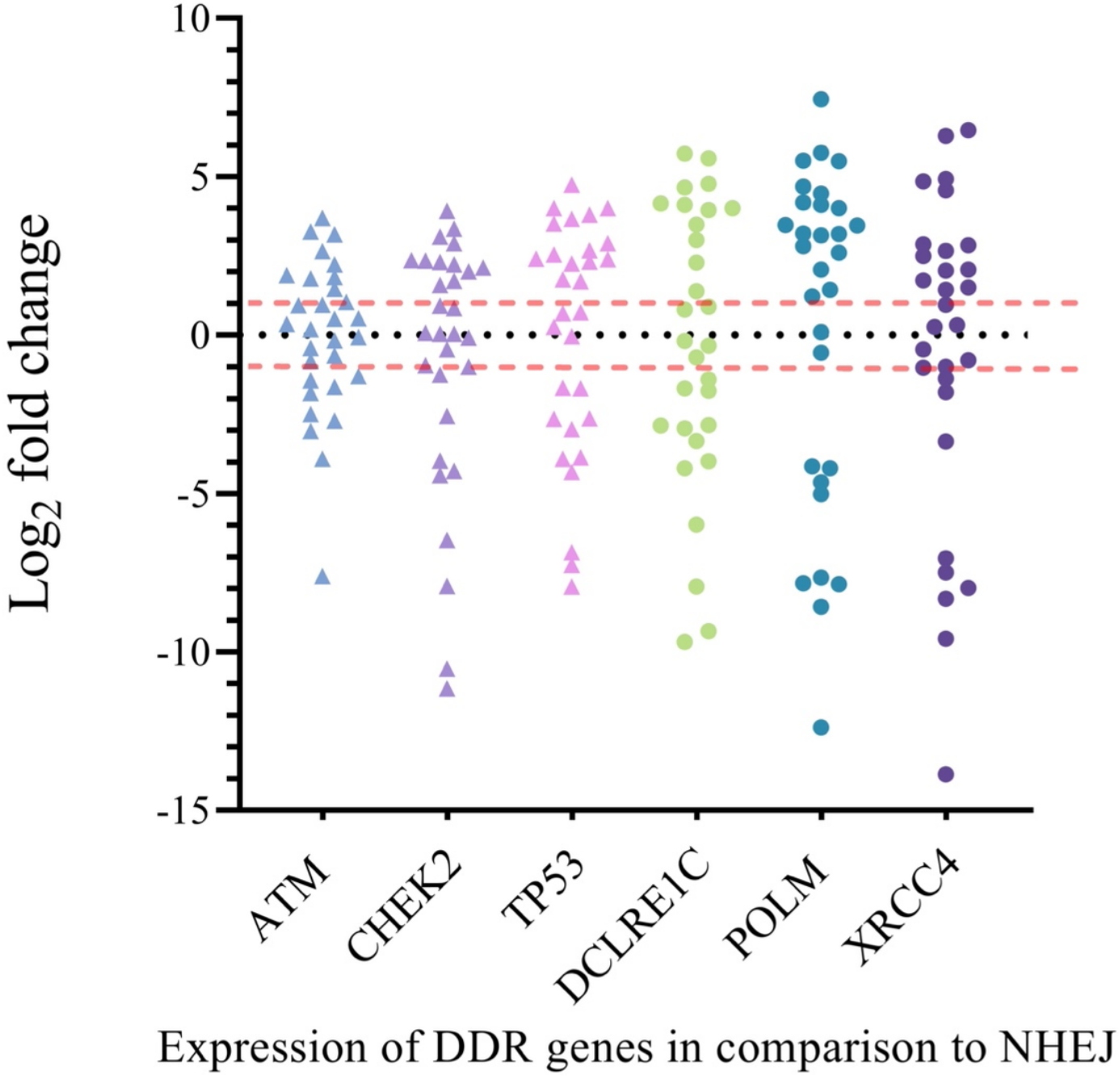

Figure 1

Relative alteration in the transcription of DNA damage response genes linked to NHEJ and DDR pathways in the H. pylori infected vs non-infected patients with gastritis. The data are presented as Log_2 fold changes in gene expression that normalized to a reference gene and relative to *H. pylori* negative samples which were considered as control group. Increased mRNA expression was defined as N-fold ≥1.0, "normal" expression as N-fold ranging from -0.9999 to 0.9999, and decreased mRNA expression as N-fold ≤ -1 (Dashed lines).