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Original Article

Activation of the DNA damage response pathway in the infected gastric tissue *with Helicobacter pylori*: a case-control study

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Abstract

Introduction: 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.

Methodology: To measure the difference in the relative mRNA expression level of *ATM*, *CHEK2*, *TP53, DCLRE1C, POLM,* and *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 between alteration in the transcription level of the studied genes and age or gender.

Conclusions: 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 a non-homologous end-joining DNA repair system that might be linked to mutagenesis in the pre-cancerous gastric tissue.

Key words: Error-prone DNA repair pathways; gastritis; *Helicobacter pylori*; NHEJ.

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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].

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 inflicted by these mutagens, 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) [5]. During HR, the damaged chromosome enters into synapsis with an undamaged DNA molecule and retrieves genetic information from it, 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 [6]. Consequently, NHEJ is barely error-free, and sequence insertions and deletions of varying lengths are usually introduced [5]. Although cells may survive lethal genomic damages through repair pathways, accumulation of deletions and insertions contributes to tumorigenesis [7].

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.

Methodology

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 examination. Gastric biopsies of all patients with mild to moderate chronic gastritis were included for further investigations after obtaining informed consent (Code of Ethics: 43392-27-02-98).

Histopathological analysis and culture for H. pylori

A pathologist analyzed all the samples 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 for 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 the *glmM* gene as previously described by Gharibi *et al.* [8].

RNA extraction, cDNA synthesis, and real-time PCR

Total RNA was extracted from gastric tissues using an RNA extraction kit, and extracted RNA's purity was quantified. Extracted RNA was used as the template for cDNA synthesis by using a cDNA synthesis kit and according to the manufacturer's instructions. The suitability and integrity of the synthesized cDNA were 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. During this study, the *B2M* gene was selected as a Housekeeping gene.

SYBR Green Quantitative RT-PCR Kit was used for making real-time PCR mixes. Real-time PCRs were done at the following conditions: 1 cycle of denaturation at 95 °C for 15 minutes, 40 cycles of denaturation at 95 °C for 10 seconds, and amplification at 62°C, 58 °C, and 55 °C, each for 5 seconds, and extension at 72 °C for 15 seconds, 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.

Statistical analysis

All statistical analyses were performed by Statistical package for social science software (SPSS, version 23). The 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.

Results

Clinicopathological characteristics of patients

60 patients were recruited in the study (30 patients with *H. pylori* infection and moderate chronic gastritis as the case group and 30 *H. pylori*-negative patients with mild chronic gastritis as a control group).

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

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 compared to the control group (Table 1) (Figure 1). Changes in gene expression were presented as Log_2 fold changes of target genes in mRNA extracts of the *case* relative to the *control*

groups. 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.

Correlation of NHEJ and DDR genes in the H. pylori-infected tissue

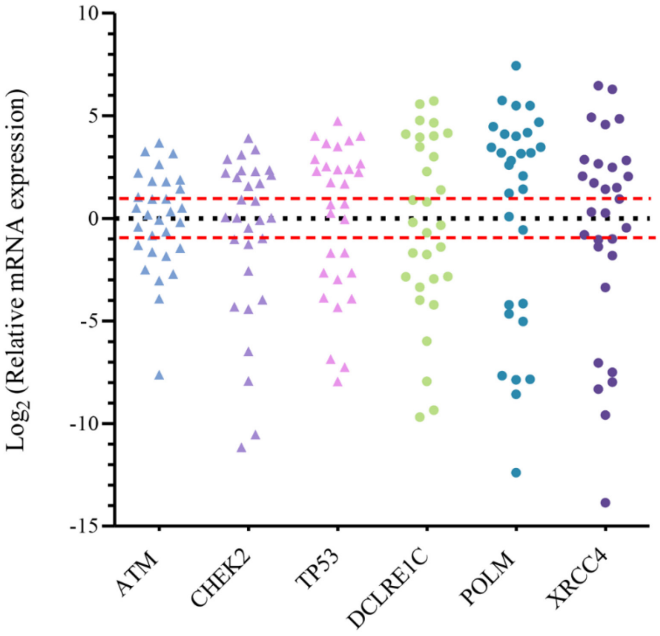
Levene's test for equality of variances was performed and statistical analysis showed a significant correlation between NHEJ and DDR pathways genes expression in the *H. pylori*-infected patients (*p-value* = 0.002).

Discussion

Chronic inflammation is estimated to be the cause of approximately 25% of human cancers [9]. 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 [10,11]. Our findings provide initial evidence 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 [12]. 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 [13]. 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.* infected *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 that increased expression is because of the inefficiency of the HR repair pathway [14]. Also, in studies on gastric and breast cancerous tissues, an increase in the *XRCC4* gene expression was reported [15,16]. Kitagawa *et al.* introduced the expression of the *XRCC4* gene as a biomarker to detect the recurrence of breast cancer [16]. Overexpression of the *DCLRE1C*

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 of fold change in the expression level of genes in the H. pylori positive relative to H. pylori negative samples (Rq). Overexpression, normal, and down-regulation were considered when Rq values were, ≥1.0, -0.9999 to 0.9999, and ≤ -1, respectively (Dashed lines).

Table 1. 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].

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

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

gene, which rises through the recruitment of the NHEJ repair pathway, was previously reported in lung cancer [17]. In a similar study, Farkas *et al.* reported a rise in the expression of the *DCLRE1C* gene in colorectal cancer [18]. In the 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, which is involved in 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. According to these findings, the increase in *TP53* transcription seems to be a compensatory mechanism, induced by the degradation of the protein in the infected cells.

Decreased *CHEK2* gene expression was generally reported in studies on gastric, lung, colon, and breast cancers [19–22], which are contrary to our findings. In general, the decreased expression of DDR-related genes in cancerous tissues may be due to multiple mutations occurring overtime which could give rise to the loss of function of these genes. The overexpression of the *ATM* gene in the current study could be explained due to the different essence of tissues and the mediation of *H. pylori* in this interplay.

According to our knowledge, this is the first study that analyzed transcription of genes linked to NHEJ and DDR pathways in association with *H. pylori* infection in the precancerous gastric tissue; however, the mechanism of chromosomal mutations and genomic instabilities caused by this bacterium need further examinations. The absence of healthy individuals to measure baseline levels of expression for the targeted genes, the impossibility of study of all NHEJ genes and regulators, the failure to determine DNA breaks at the chromosomal stages and possible shuffling and translocations, and the study of the bacterial diversity at the genomic level 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.

Conclusions

In conclusion, our results showed the involvement of *H. pylori* infection in the activation of error-prone DNA repair system in the stomach of patients with chronic gastritis. Further studies are needed to determine mediators playing a key role in this interplay.

Authors' Contributions

MA and AR-N, designed and supervised the study, do data curation, and review the manuscript. AR and AG do laboratory and molecular experiments, write a draft of the article, analyze the data, and revise the manuscript. MSH collected gastric biopsy samples, and filled out the patients questionnaire; SZM and RB cultured the gastric biopsies and characterized *H. pylori* in the patients' samples; RS, and ASF were consultants in this projects.

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