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 \textit{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 \textit{H. pylori} in DNA damage.

\section*{Methodology}

\subsection*{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).

\subsection*{Histopathological analysis and culture for \textit{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 \textit{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 \textit{glmM} gene as previously described by Gharibi et al. [8].

\section*{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 electrophoresis, respectively. To investigate expression changes, specific primers were designed for \textit{CHEK2}, \textit{DCLRE1C}, \textit{XRCC4}, \textit{TP53}, \textit{POLM}, \textit{ATM}, and \textit{B2M} genes. During this study, the \textit{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.

\section*{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 $\leq 0.05$.

\section*{Results}

\subsection*{Clinicopathological characteristics of patients}
60 patients were recruited in the study (30 patients with \textit{H. pylori} infection and moderate chronic gastritis as the case group and 30 \textit{H. pylori}-negative patients with mild chronic gastritis as a control group).

\subsection*{Result of gene expression analysis of genes involved in DDR and NHEJ pathways}
Transcriptional analysis showed up-regulation of genes linked to DDR (\textit{ATM}, \textit{CHEK2}, and \textit{TP53} genes) and NHEJ (\textit{DCLRE1C}, \textit{POLM}, and \textit{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

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.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Title</th>
<th>Function</th>
<th>Relative abundance of mRNA expression (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>Ataxia telangiectasia mutated</td>
<td>DNA-binding, cellular response to DNA damage, cell cycle arrest,</td>
<td>2.42 ±3.17</td>
</tr>
<tr>
<td>CHEK2</td>
<td>checkpoint kinase 2</td>
<td>DNA damage response, DNA damage checkpoint, protein kinase binding</td>
<td>2.86 ± 3.61</td>
</tr>
<tr>
<td>TP53</td>
<td>Tumor protein p53</td>
<td>DNA-binding, tumor suppressor, cell cycle arrest, apoptosis</td>
<td>5.00 ± 6.52</td>
</tr>
<tr>
<td>DCLRE1C</td>
<td>DNA cross-link repair 1C</td>
<td>Damaged DNA binding, exonuclease activity, exodeoxyribonuclease activity, DBS repair via NHEJ</td>
<td>8.44 ± 13.91</td>
</tr>
<tr>
<td>POLM</td>
<td>DNA polymerase mu</td>
<td>Gap-filling polymerase, DBS repair via NHEJ</td>
<td>15.72 ± 33.08</td>
</tr>
</tbody>
</table>

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

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.
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 over time 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.

References


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