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Original Article

Upregulation of XRCC1 DNA Repair Gene, Interleukin-8, and Bcl-2 Antiapoptotic Gene Levels in Kurdish Patients with Gastric Adenocarcinoma

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Abstract
Gastric cancer (GC) is one of the deadliest tumors due to its competence to invade and metastasize. The DNA repair gene (*XRCC1*), interleukin-8 (*IL-8*) gene, and B-cell lymphoma 2 (*Bcl-2*) gene play a crucial role in the development and progression of GC. This study aimed to evaluate the expression of these target genes in GC patients in the Kurdistan region of Iraq. Gastric cancer tissues were collected from 29 patients diagnosed with gastric adenocarcinoma that underwent gastric resection, and 21 tissue samples were obtained from healthy patients that underwent gastroscopy. The gastric tissues were collected in different hospitals in Erbil and Sulaymaniyah cities in the Kurdistan region of Iraq. Moreover, the data regarding *Helicobacter pylori*, age, gender, and stage of the disease were recorded and analyzed using GraphPad Prism. The gene expression levels of *XRCC1*, *IL-8*, and *Bcl-2* from gastric tissue were studied by real-time quantitative polymerase chain reaction. The results showed that *H. pylori* infection was equally distributed among males and females in the tissues of gastric patients, while most of the *H. pylori*-negative patients were females. It is also found that gastric patients aged 30-60 years old are more commonly tested for the *H. pylori* test. Accordingly, in this study, patients diagnosed with gastric inflammation more often tested positive for *H. pylori*, while patients diagnosed with gastric cancer tested negative for this infection. Additionally, it was found that the target genes (*XRCC1*, *IL-8*, and *Bcl-2*) were significantly upregulated in GC patients, compared to the healthy group. Finally, the result revealed that *XRCC1*, *IL-8*, and *Bcl-2* were upregulated in the Kurdish patients with GC, compared to the healthy control group. Targeting *XRCC1*, *IL-8*, and *Bcl-2* genes can be an interesting field and promising strategy for cancer treatment.
Keywords: Gastric cancer, *XRCC1*, *IL-8*, *Bcl-2*, Gene expression

1. Introduction

Globally, gastric cancer (GC) is the fifth foremost common cancer and the third prominent cause of death due to cancer (1). According to GLOBOCAN 2018 reports, it is the fourth cancer type in males and the seventh in females (2). Currently, inadequate data are available regarding the newest incidence of cancer, including GC in the Kurdistan region of Iraq. According to recent research conducted in Kurdistan, GC is the seventh greatest form of cancer, predominantly diagnosed amongst elderly patients (≥90 years old). Moreover, it has been reported that GC is most frequently diagnosed in the late stage, compared to the other types of cancer (3).

Chronic infection with *Helicobacter pylori* is the most prominent and evitable cause of GC, especially the

non-cardia gastric carcinoma type, which resulted in an estimated 800,000 new GC cases worldwide in 2018 (4). Following the international agency and World Health Organization study, *H. pylori* has been selected as a class I carcinogen. Other factors, such as smoking habits, diet, family history, and alcohol, are considered risk factors for GC (5).

There are various genes involved in GC pathogenesis. The alterations in genes, such as DNA repair genes (*XRCC1*), might contribute to GC progression. The importance of DNA repair systems is retaining human genome integrity via various pathways that protect against DNA damage (6). Interleukin-8 (*IL-8*) is a predominant tumorigenic cytokine that participates in chronic inflammation and GC development. The *IL-8* and its receptors play a fundamental role in the expansion and metastasis of the human stomach (7). The elevated level of *IL-8* in human stomach cancer appears to be involved in the intimate angiogenesis process (8).

The B-cell lymphoma 2 (*BCl-2*) family has long been identified for its role in apoptosis. The *BCL-2* is one of the most vital oncogenes involved in cancer by hindering apoptosis and causing the progress of the malignant disease (9). The mechanism of antiapoptotic *BCL-2* is through impeding the mitochondrial pathway and communication with other associates of the *BCL-2* lineage that leads to cell survival (10).

Although there are different treatments available, GC incidence and mortality have not decreased or stabilized yet. Therefore, researchers are interested in investigating the alteration in the level of genes that might be associated with GC development and progression. In this regard, the vital objective of this research was to find out the expression degree of cytokine *IL-8*, DNA repair gene (*XRCC1*), and antiapoptotic gene (*BCL-2*) in gastric adenocarcinoma in the Kurdish people in the Kurdistan Region of Iraq.

2. Materials and Methods

2.1. Patients and Samples

In total, 110 tissues from gastric patients were collected from November 2021 until June 2022. About 66 samples were freshly collected, including 6 resected GC tissue, biopsies from 12 patients with gastric inflammation, 4 polyps, 1 ulcer, and 43 normal gastric patients. The samples were collected and histologically confirmed at various hospitals in the Kurdistan region of Iraq, including Erbil City (Par, CMC hospital, Teaching Hospital Rezgary) and Sulaymaniyah (High-quality Anwar medical city and Smart hospital). Furthermore, 44 frozen tissue samples (23 resected gastric tissue and 21 normal gastric biopsies) were collected from Sulaymaniyah Hospital. However, the histopathological data were incomplete and, in some cases, unavailable. The tissue samples were sliced into small quantities and frozen in 10x PBS solution, then stored at -80 °C in a deep freezer at the scientific research center, Erbil Polytechnic University, until RNA extraction.

2.2. Quantitative Real-Time Polymerase Chain Reaction

Total RNA was purified from the gastric tissues of patients following the protocol of the manufacturer using a total RNA mini kit (Geneaid, Korea). The concentration and the purity of total RNA were estimated using a NanoDrop instrument (Thermo Scientific, USA). The integrity of total RNA was assessed by performing agarose gel electrophoresis. The agarose gel solution was prepared from powder agarose (Bio Tek, Canada) and dissolved in 1x Tris-borate-EDTA buffer (GeNet Bio, Korea). Eventually, the samples were loaded, and the gel electrophoresis system was initiated at 100 V, 75 mA for 60 min. The results were viewed using a gel documentation system (Biotech-Fischer, Germany).

For better RT-PCR reaction, the total RNA was treated with DNase I to remove possible DNA contamination using a DNase kit (Yekta Tejhiz, Iran) following the instructions of the manufacturer. After that, quantitative RT-PCR was carried out in a two-step process. The RNA was transcribed reversely into cDNA using the High-Capacity cDNA Reverse Transcription (RT) kit (Add Bio, Korea), and then qRT-PCR was performed using the SYBER® Green

Master mix kit (Amplicon Real Q, Denmark) according to the procedure suggested by the manufacturer. Hereafter, the required components of the mixture were added, the prepared solution was placed into a PCR instrument, and the temperature cycling protocol was used based on the protocol provided by the manufacturer. The cycling temperature consisted of priming at 25 °C for 10 min, RT at 50 °C for 60 min, RT inactivation at 80 °C for 5 min, and holding at 12 °C for 2 min. Consequently, the prepared cDNA is ready for RT-PCR.

The transcript levels of three different target genes (*XRCC1*, *IL-8*, and *BCL-2*) were assessed by operating the reactions in a BIO-RAD® MJ Mini cycler, PTC-0148 & CFD-3120 MINI OPTICON qRT-PCR™ system. A total reaction mixture of 25 µl was prepared by the addition of 5 µl of SYBER Green master mix (Denmark, Amplicon RealQ master mix green), 0.3 µl of each forward and reverse specific primers that are designed for each gene, 1,4 µl of ddH2O, and 3 µl of cDNA. A two-step qPCR method was used to amplify the target genes. All primers were designed by using GENERUNNER software (version 4) and are as follows: *XRCC1*, (F) 'CCAACCCCTGAAGAGACCAA', (R), 'TGTTCCTCACTGTCCGTGT'. *IL-8*, (F), 'CGGGAGAATATACAAATAGCAA', (R), 'TAAAGGAGAAACCAAGGCAC'. *BCL-2*, (F) 'CTGTGGATGACTGAGTACCTG', (R), 'ACAGCCAGGAGAAATCAAACA', β-actin, (F), '5-CAGCACCTTGCCCCAAAATC -3', (R), '5-TGGATGGCAAACCTCAGCTC-3'.

2.3. Statistical Analysis

The correlations among gender, age, and clinical characteristics of gastric patients were evaluated in Software Prism 9 (GraphPad Software) using chi-squared and Fisher's exact test. The results were expressed as mean values. Statistical analysis was applied in SPSS software (version 21.0) to determine differences in gene expression. The *, **, and *** indicate statistically significant *P* values of < 0.05, < 0.01, and < 0.001, respectively.

3. Results

3.1. Gender, Age, and Clinical Characteristics of Gastric Patients

Only the gender, age, and some clinical data of the 66 gastric patients were analyzed due to the available information about their characteristics. It appeared that 28 (42%) out of the total 66 patients were female with an age range of 19-89 years old (average: 36.7±15.3). Furthermore, the males constituted 38 (58%) of the gastric patients with an age range of 16-78 years old (average: 45.6±16.7).

3.2. Distribution of *Helicobacter pylori* Infection Regarding Age, Gender, and Diagnosis

In this study, the patients who tested positive for *H. pylori* were equally distributed (50%) in terms of gender, while in the *H. Pylori* negative group, the females were prominent (58%). Figure 1 demonstrates that *H. pylori-positive* cases are highest (5%) in the age groups of 30-45 and 45-60 years, while the percentage of positively tested patients drops to 3% in the younger age groups. However, patients older than 60 years old tested negative for *H. Pylori* infection (100%).

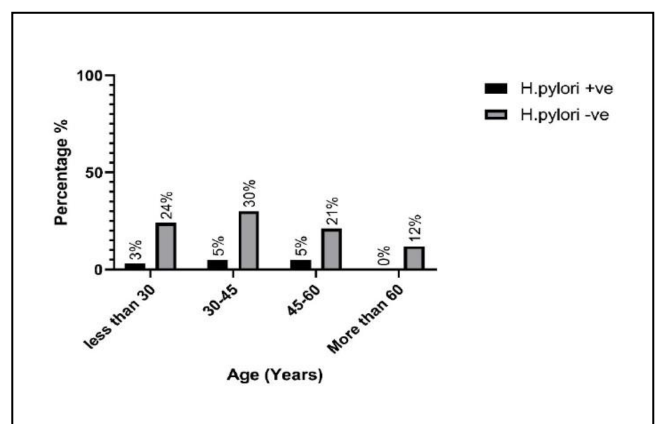

Figure 1. Distribution of *H. pylori* infection in different age groups. Most patients in the different age groups are *H. pylori*-negative, and in the age groups 30-60 years, most *H. pylori* infections are detected

Furthermore, figure 2 illustrates that all regular gastric patients and all gastric cancer patients tested negative for *H. pylori*, while most of the *H. pylori* infections were found in the gastric inflammation group (11%) followed by gastric ulcer (1%) and gastric polyp (1%).

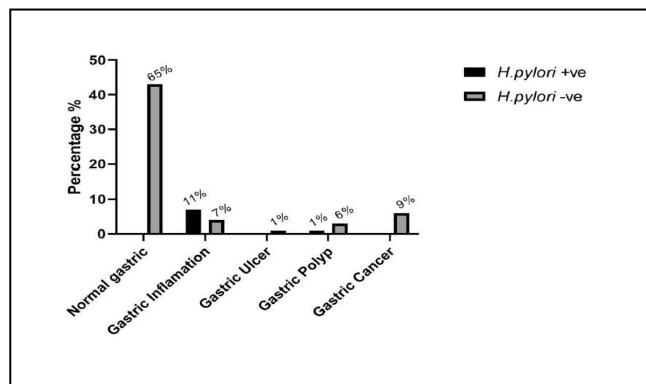

Figure 2. *H. pylori* test distribution according to patients' diagnosis. All regular diagnosed gastric patients and gastric cancer negative tested for *H. pylori*. The gastric inflammation group found the highest *number of H. pylori* positively tested patients

3.3. Assessment of Quality of Total RNA in Different Samples

To determine the quality of the extracted RNA from the gastric tissues, 1.5% gel electrophoresis was used for two samples, as shown in figure 3. It demonstrated that the 18s rRNA was visible as bright bands followed by 28s rRNA in gastric tumor tissue. This result was similar to that of the positive control samples, indicating a good RNA quality that can be used for further molecular experiments, such as cDNA synthesis from RNA.

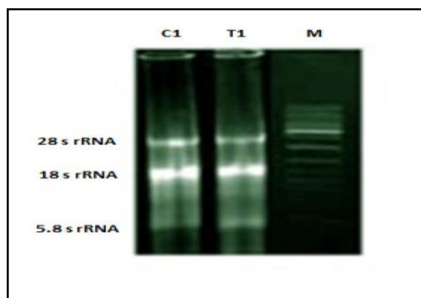

Figure 3. Agarose gel illustrates the quality of extracted total RNA from GC patient tissues. The two visible bands in lanes 1 and 2 imply a good RNA quality. Lane 1: positive control, lane 2: gastric tumor, lane 3: Marker. Total RNA is visible as bright bands in a 1,5% agarose gel, implying a proper quality of the total RNA

3.4. Quality Assessment of cDNA of the Target Genes

To measure the expression level of target genes, first, the total RNA from GC tissues was reversely transcribed into cDNA using a specific two-step kit and qRT-PCR. After cDNA synthesis, the quality of cDNA was confirmed using 1.5% gel electrophoresis. It was found that all three target genes showed a bright band with different sizes (*IL-8*: 251 bp, *XRCC1*: 230 bp, and *Bcl-2*: 124 bp), meaning that they were detected in the sample of the patients (Figure 4). Moreover, an internal control B2m with 191 bp was used to determine the quality of the cDNA reaction and all the visible bands were compared with the marker to determine the size of the bands.

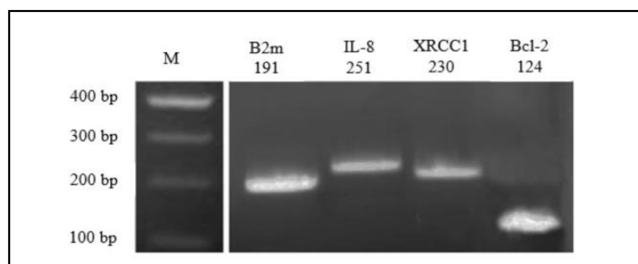

Figure 4. Quality assessment of cDNA of the target genes. The visible bright bands show different band sizes for each specific gene. Lane 1: Marker, Lane 2: internal control B2m amplicon (191bp), lane 3: *IL-8* (251bp), lane 4 *XRCC1* (230bp), and Lane 5 *Bcl-2* (124bp)

3.5. Gastric Cancer-Related Gene Expression

The relative expression of target genes in the GS patients was compared to that in the healthy control patients using the qRT-PCR assay to find out the alteration in the expression level of target genes. Figure 5 illustrates the relative expression of three different target genes. It is exhibited that the level of the DNA repair gene (*XRCC1*) is significantly ($P<0.05$) elevated in GC tissues, compared to the normal tissues (Figure 5A). Furthermore, the level of proinflammatory cytokines *IL-8* underwent a highly significant ($P<0.01$) increase in GC patients in comparison with the control group (Figure 5B). Regarding the antiapoptotic gene *BCL-2*, there was an obvious upregulation of the *BCL-2* gene in GC, while the relative expression was significantly ($P<0.001$) reduced in the control group (Figure 5C).

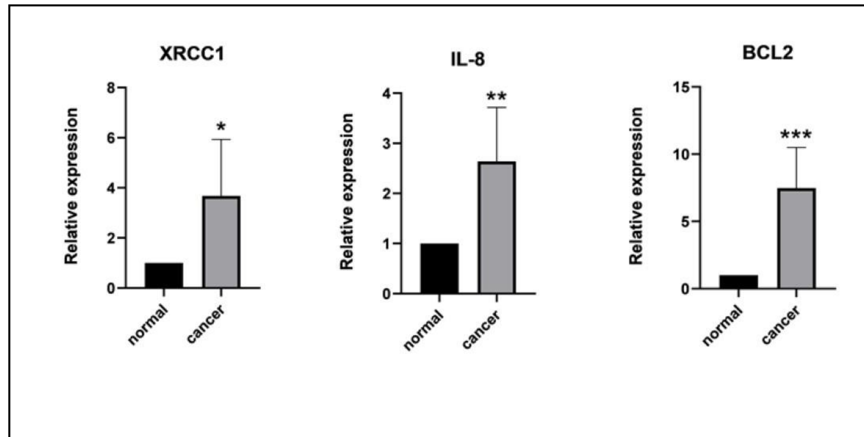

Figure 5. The relative expression of gastric cancer-related genes was measured by using the RT-PCR technique. The relative expression is significantly increased in the genes of GC patients (A) *XRCC1* gene, (B) highly significant *IL-8* gene, and (C) and highly significant *BCL-2* gene. The *, **, and *** indicates statistically significant P < 0.05, P < 0.01, P < 0.001, respectively

4. Discussion

Gastric cancer is a highly prevalent cancer that originates from the lining of the stomach and is usually identified at a progressed stage, as it manifests late phase of the disease and does not show any signs and symptoms (11). In total, 66 patients with complete available clinical data were used for correlation analysis. The *H. pylori* positive test was equally divided between both genders. This is in contrast with the findings of a study performed by Soward (12) Sowaid, Ali (12) Sowaid, Ali (12) which showed that gastric male patients were more frequently infected with *H. pylori* than gastric female patients. The small size of collected patient data due to incomplete available clinical data was probably the cause of this discrepancy. Based on the results, there was a higher percentage of *H. pylori* in the group of patients within the age range of 30-60 years old, which was in line with the findings of a previous study performed by Yang, Kartsonaki (13).

In the present study, 29 out of 110 human gastric tissue samples were confirmed as GC tissue and used to assess the relative expression level of three different target genes, including *XRCC1*, *IL-8*, and *BCL-2*, by qRT-PCR. There are several genes involved in the growth and development of GC. One of the important members of the DNA repair gene is the *XRCC1* protein which cooperates with numerous DNA repair-related proteins to complete DNA (14). The present study showed a significant elevation in the level of the *XRCC1* gene in GC patients, compared to the control group. This result contrasts with those of the study conducted by Wang, Tang (14), which found downregulation in the *XRCC1* gene expression and claimed that *XRCC1* promotes gastric carcinogenesis. Researchers have suggested that CRCC1 might be used as a biomarker to predict GC recurrence. However, limited data are available regarding the upregulation of *XRCC1* in gastric cancer (15).

Cancer is a multifactorial process that occurs in response to tissue damage or infection whereby different proinflammatory cytokine, including *IL-8*, is secreted by activated macrophages that initiate inflammation (16). It has been stated that *IL-8* is a critical regulatory component in the tumor microenvironment (17). Results of the present research showed the upregulation of the *IL-8* gene in GC patients. This result reflects those of a study carried out by Lee, Khoi (8), who also revealed elevated levels of *IL-8* in GC patients and suggested the involvement of *IL-8* in GC invasion and metastasis. Moreover, another

group of researchers has found that the high expression of *IL-8* was an independent risk factor for causing GC prognosis (15), while other investigators have astumor gastric cancer (16, 18, 19).

Alteration in the apoptosis proteins is one of the critical factors in the development of many cancers, including GC. Findings of the current study indicated significant upregulation of the *Bcl-2* gene level, compared to the control group. This finding is similar to those of the research group of Xu, Li (20), which demonstrated significantly high expression of antiapoptotic *Bcl-2* in GC and claimed that *Bcl-2* is involved in early GC development.

In conclusion, this study revealed a significant upregulation of the target genes *XRCC1*, *IL-8*, and *BCL-2* in Kurdish gastric cancer patients. These genes might play a role in the GC progression; therefore, targeting these specific genes might be an exciting and promising strategy for the treatment of GC.

Authors' Contribution

Study concept and design: Z. H.
Analysis and interpretation of data: D. A. K. and Z. H.
Drafting of the manuscript: D. A. K.
Critical revision of the manuscript for important intellectual content: Z. H.
Statistical analysis: D. A. K. and Z. H.
Study supervision: N. F. S. A. and Z. H.

Ethics

This study was approved by the Human Ethics Committee of Technical Health and Medical College, Polytechnic University, Erbil, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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