Evaluation of XRCC1, Interleukin-8, and Bcl-2 gene expression in gastric cancer patients

A Thesis

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Declaration

I declare that the master entitled (**Evaluation of XRCC1, Interleukin-8, and Bcl-2 gene expression in gastric cancer patients**) is my original work, hereby certify that unless stated, all work contained within this thesis is my independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

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We certify that we have read this thesis: (**Evaluation of XRCC1, Interleukin-8, and Bcl-2 gene expression in gastric cancer patients)** and as an examining committee examined the student(**Danya Awni Kamal**) in its content and what related to it. We approve that it meets the standard of a thesis for the degree of MSc. in MLT (Molecular biology).

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Dedication

I am dedicating this thesis to four beloved people who have meant and continue to mean so much to me.

- First and foremost, to my mom Sarwin Musa Othman whose love for me knew no bounds and, who taught me the value of hard work. Thank you somuch "Mom", I will never forget your support.
- Next, my father, Awni Kamal Ahmed, taught me how to be resilient and keep moving forward no matter what obstacles I encountered while growing up and being loved and supported financially.
- My spouse Mustafa Abdulla, who has been a continuous source of supportand encouragement throughout the difficulties of MSc and life, is also the recipient of the thesis that I would want to dedicate.
- And finally, my sister and brothers, who never stop giving of themselvesin countless ways.

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Summary

Gastric cancer (GC) is one of the deadliest tumor's due to its competence to invade and metastasize. Multiple genetic and epigenetic alterations are implicated in gastric carcinogenesis. In addition, this disease is mostly diagnosedat a late stage. The DNA repair gene x-ray repair cross-complementing protein (XRCC1), Cytokine Interleukin-8 (IL-8) gene, and anti-apoptotic B-cell lymphoma (Bcl-2) gene perform 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 (KRG). Gastric cancer tissues were taken from 29 patients that were diagnosed with gastric adenocarcinoma that underwent gastric resection and 21 tissue samples were taken from healthy patients that underwent gastroscopy.

The gastric tissues were collected in different hospitals in Erbil- and Sulaymaniyah city in the Kurdistan region of Iraq and stored at -80 ℃ for molecular purposes. Data regarding the *Helicobacter pylori (H. pylori*) infection, age, and gender of the gastric patients with their stage of the disease were recorded and analyzed using GraphPad Prism. The gene expression levels of XRCC1, IL-8, and Bcl-2 from gastric tissuewere studied by quantitative Real-Time PCR (qRT-PCR). The result 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 between 30-60 years old were more commonly positive tested for the *H. pylori* test. Furthermore, in this study patients diagnosed with gastric inflammation were more often tested positive for *H. pylori*, while patients diagnosed with gastric cancer were all negative tested 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.

Taking together, our result revealed that XRCC1, IL-8, and Bcl-2 were upregulated in gastric cancer patients compared to the healthy control group. This may indicate that these target genes could play role in gastric carcinogenesis and therefore, targeting XRCC1, IL-8, and Bcl-2 genes might be an interesting field and promising strategy for cancer treatment.

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List of abbreviations

Bcl-2	B-cell lymphoma 2
BER	Base excision repair
BID	BH3 interacting-domain death agonist
cDNA	Complementary deoxyribonucleic acid
cag PAI	Cytotoxin-associated gene pathogenicity Island
ELISA	Enzyme-Linked immunosorbent assay
GC	Gastric cancer
GE	Gastro esophagus
H.pylori	Helicobacter pylori
H.P-CSA	*Helicobacter pylori* cell surface antigens
HLO	Helicobacter Like Organism
IL-8	Interleukin-8
IgG	Immunoglobulin
IHC	Immunohistochemistry
JRSGC	Japanese Research Society for Gastric Cancer
KRG	Kurdistan Region of Iraq
MALT	Mucosa-associated Lymphoid tissue
NER	Nucleotide Excision Repair
RT-PCR	Real-time polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
RNA	Ribonucleic acid
TBE	Tris-borate-EDTA
WB	Western Blotting
XRCC1	X-ray repair cross-complementing protein

1. Introduction

Globally, gastric cancer (GC) is the fifth foremost common cancer and the third prominent cause of death due to cancer. The incidence of GC is different concerning sex and geographical variations. Males are two to three times more vulnerable than females. It has been studied that more than 50% of new cases come up in developing countries.

According to GLOBOCAN 2018 reports, GC is the fourth cancer type in males and the seventh in females. All over the world, the GC lifespan for cancer patients until 74 years old is about 1 in 54 males and about 1 in 126 females (Thrift and El-Serag 2020). Numerous researchers have been investigating the worldwide patterns in the incidence and death of gastric cancer and they have found a continued global decline. However, there are huge global variations in the incidence and mortality rates for gastric cancer and secular trends.

In recent research conducted in Kurdistan, GC is the seventh greatest form of cancer, and its predominantly diagnosed amongst elderly patients (≥45 years). Between 2016 and 2019 gastric cancer case rates were 3.6 per 100,000 males and 2.6 per 100,000 females.

Moreover, it has been reported that GC is most frequently diagnosed in the late stage of gastric cancer(M-amen, Abdullah et al. 2022). Various factors are involved in the development of gastric cancer such as embracing diet, smoking, family history, alcohol environmental factors, and bacteria. *Helicobacter pylori* contamination is one of the most predominant recognized causes for causing gastric cancer. *Helicobacter pylori* is a gram-negative bacterium that has spiral-shaped and micro-aerophilic, particularly inhabiting the host's gastric almost the whole lifetime (Machlowska, Baj et al. 2020). Chronic infection with *H. pylori* is the most prominent and evitable cause of GC especially non-cardia gastric

carcinoma type, which resulted in an estimated 800,000 new GC cases worldwide in 2018 (Rawla and Barsouk 2019).

There are various genes involved in GC pathogenesis. The alterations in genes such as DNA repair genes X-ray repair cross complement group 1 (XRCC1) might contribute to GC progression. During oxidative DNA damage, the base excision repair (BER) pathway is activated, which recognizes the DNA damage and XRCC1 participates in the pathway as a scaffold protein (Krokan, Nilsen et al. 2000). This protein recognizes DNA breaks, binds the DNA, and then finds the other parts of the repair machinery(Kubota, Nash et al. 1996, Taylor, Wickstead et al. 1998, El-Khamisy, Masutani et al. 2003). The importance of DNA repair systems is retaining human genome integrity via various pathways that protect against DNA damage (Asgerov, Şenol et al. 2019). There are varieties of research that support the XRCC1 gene as a significant risk for gastric cancer (Yuan, Deng et al. 2011, Pan, Xie et al. 2012, Qiao, Wang et al. 2013).

Alongside the DNA damage repair genes, chemokines are associated with cancer and may be a crucial biomarker for cancer development and prognosis (Poon, Fan et al. 2003). Interleukin (IL-8) is a pro-inflammatory chemokine that is in the CXC subfamily and has been shown to work as an important factor within the tumour microenvironment (Waugh and Wilson 2008). IL-8 is most likely to be generated by a diversity of human cancer cells, including gastric cancer cells (TAKAGI, KAMIYA et al. 1997). IL-8 is a predominant tumorigenic gene that participates in chronic inflammationand GC development.IL-8 and its receptors have a fundamental role in the expansion and metastasis of the human gastric (Bie, Ge et al. 2019). The elevated level of IL-8 in human gastric

cancer appears to be involved in intimately angiogenesis process (Lee, Khoi et al. 2013).

Apoptosis is a physiological programmed death of the cells which has an important role in carcinogenesis. Particular apoptosis proteins including Bcl-2 and BH3 interacting-domain death agonist (BID), are from the Bcl-2 lineage but have opposite roles (Czabotar, Lessene et al. 2014). The proteins of Bcl-2 and Bcl-xl are vital inhibitors in the apoptosis process and are usually overexpressed in many cancers (Trask, Wolf et al. 2002). Theiroverexpression is closely related to tumor initiation, aggressive, and chemoresistance (Liu, Zhang et al. 2015).

The mechanism of antiapoptotic Bcl-2 is through impeding the mitochondrial pathway and communication with other associates of the Bcl-2 lineage that led to the survival of the cell (Yin 2000, Lee, Soung et al. 2004).

Although there are different therapies available, GC incidence and mortality haven't 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. The vital objective of this research is to find out the expression degree of cytokine IL-8, DNA repair gene (XRCC1), and anti- apoptotic gene (BCL-2) in gastric adenocarcinoma patients in the Kurdistan Region of Iraq (KRG).

2. Literature Review

2.1 Gastric physiology and anatomy of stomach

The gastric is divided into five sections (figure 2.1): Cardia, Fundus, Body, Antrum, and pylorus. The cardia, located in the most proximal part of the gastric, is the section that surrounds the esophagus and gastric junction. The fundus is the upper rounded part of the gastric, followed by the corpus, which is the area between the lesser and greater curvature. Acid-secreting glands are found in the fundus, while endocrine, alkaline-secreting surface epithelium, and gastrin-secreting G-cells are found in the body. In the bottom part, the antrum is connected with the pylorus and ends with the duodenum (Persson 2009).

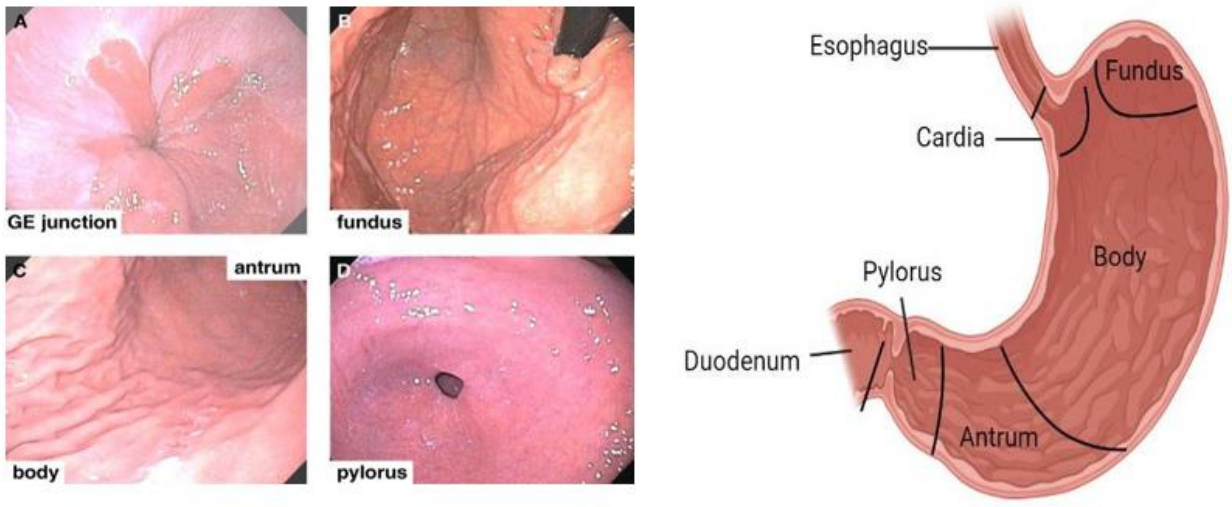

Figure 2.1 Human gastric histology and anatomy diagram. The right picture shows the visualization of histological parts is (A) GE intersection. (B) Fundus (C) Body, corpus-antrum junction, highlightingthe transition from obliquely oriented rugae to flat mucosa. (D) Pylorus (Soybel 2005). The left picture shows the upper part of the stomach that includes fundus and cardia which is connected to Esophagus. The Center of the stomach is body. The antrum comes after the body followed by pylorus which is located at the bottom of the stomach and linked to Duodenum (Soybel 2005).

2.2 Gastric cancer

2.2.1 Gastric cancer epidemiology

A multistage process is involved in the growth and progression of tumors. Typically, cancer develops after 20 to 30 years of exposure to harmful carcinogenic chemicals. Almost 990,000 individuals globally are recognized with GC, and nearly 738,000 are dying each year (Camargo, Murphy et al. 2011). Modern medical advances permit earlier detection of most tumors in their later stages, and in 50% of cases , radical resection results in recovery (Machlowska, Baj et al. 2020). Additionally, some recent epidemiological data point to a growing frequency in some young patient groups that may be brought on by autoimmunity; if this tendency is verified, it may alter the epidemiology of GC in the future (Petryszyn, Chapelle et al. 2020).

Regions with the highest likelihood of GC development includes South and Central America, East Asia, and Eastern Europe. Southern Asia, Australia, North America, North and East Africa, and New Zealand are low-risk regions. Only Japan has a moderately five-year survival rate. In Europe, the ratio varies between 10 to 30 percent (Matsuda and Saika 2013). Using the endoscopic examination method, the five-year survival rate due to the early diagnosis has improved, which causes early diagnosis of cancer. Overall, in most world parts, the prevalence of GC has declined from the past years. The incidence of a sporadic intestinal type of GC has decreased, while the incidence of diffuse-type GC has been elevated. The distal GC rate is lower than the proximal GC rate. *Helicobacter pylori* eradication, improved food conservation, improved hygiene standards, high intake of vegetables and fresh fruits could explain this trend (Sitarz, Skierucha et al. 2018).

2.2.2 Gastric cancer risk factors

Many factors impact the elevation of GC risk, like tobacco, infections, family history, age, and alcohol as shown in figure 2.2.

One of the crucial risk factors is to have a family with history of GC. The patients with a history of GC from their lineages are three times more susceptible to develop GC than individuals without such a history (Blair, McLeod et al. 2020). According to the World Cancer Research Fund/ American Institute for Cancer Research (WCRF/AICR), charbroiled animal meats, smoked foods, and salt-preserved foods promote GC progression (Fang, Wei et al. 2015). At the same time, fruits and green vegetables are protective counter the progress of GC. Food carcinogens cause changes in gene expression by interacting with gastric epithelial cells, particularly in non-cardia GC endogenous or dietary role of N-nitroso compounds have been analyzed to elevate the danger of gastric tumor (Yusefi, Lankarani et al. 2018).

It has been found that use of alcohol and cigarettes have a huge role in the development of gastric tumor. It has been shown that smokers have a eighty percent higher risk of developing gastric cancer than non-smokers (Bae 2021). Furthermore, the GC risk is measured to be eighty percent in heavy drinkers and hence they are at higher risk for GC. A cohort study showed that high consumption of alcohol is in positively related with the threat of gastric tumor and frequently drinking of alcohol is related with intestinal non-cardia carcinoma (Shin, Kim et al. 2011).

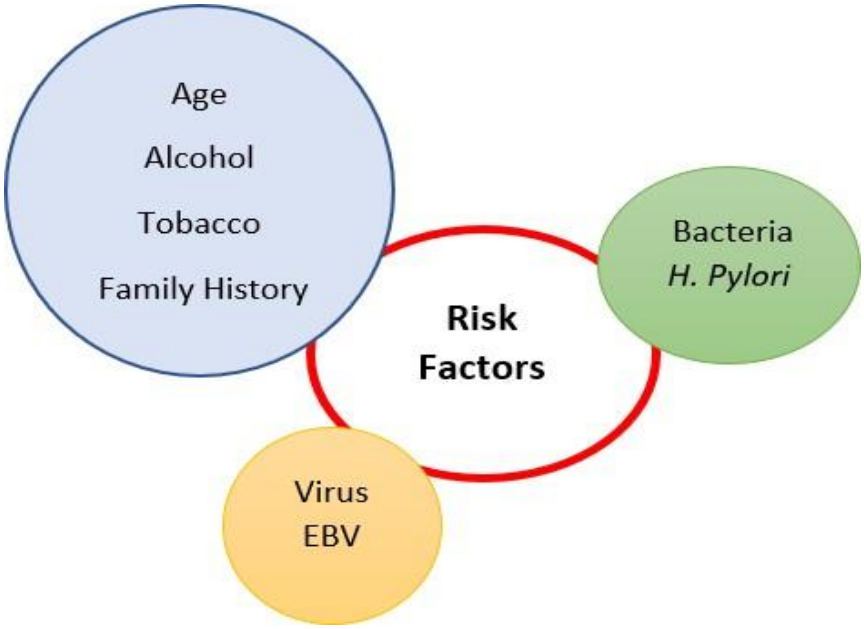

Figure 2.2 Gastric cancer risk factors. Various factors are involved in the development of gastric cancer including H. pylori bacteria, viruses such as Epstein-Bar virus (EBV) environmental factors like (age, alcohol, family history, and tobacco.

2.2.2.1 Helicobacter pylori

The Australian physician Barry J. Marshall and J. Robin Warren won the Nobel prize in medicine or physiology in 2005 for the discovery of H. pylori bacteria and its role in gastric inflammation and peptic ulcer disease (Fock, Graham et al. 2013). *Helicobacter pylori* is a gram-negative spiral-shaped and microaerophilic bacteria which is highly modified for gastric colonization in the gastric and is strongly linked to many gastroduodenal diseases such as chronic gastritis, peptic ulcer disease, atrophic gastritis, mucosa-associated lymphoid tissue (MALT) andnon-cardia gastric cancer (Wang, Simpson et al. 2015). Most other microorganisms die in the gastric because of high acidity, frequent stomach emptying, and limited nutrient availability. *Helicobacter pylori* has created many mechanisms like enzyme urease to overcome barriers of the stomach

environment Urea is converted to ammonia and carbon dioxide by the urease enzyme resulting in an increase of the pH in the environment around the bacteria, which is beneficial to the bacteria's attachment and preservation (Persson 2009). The most common manifestation of *H. pylori* is forming gastric acid, which depends on infection severity, location of the bacteria anatomically, and extension of the gastric inflammation. The bacteria cause acute gastritis and reduces blood chloride levels directly after infection, which then develops chronic gastritis affecting either the antrum that elevates acid secretion and duodenal ulcers, corpus that causes gastric atrophy, or both (Watari, Chen et al. 2014, Diaconu, Predescu et al. 2017). *Helicobacter pylori* infection are known risk factors for GC as illustrated in figure 2.3:

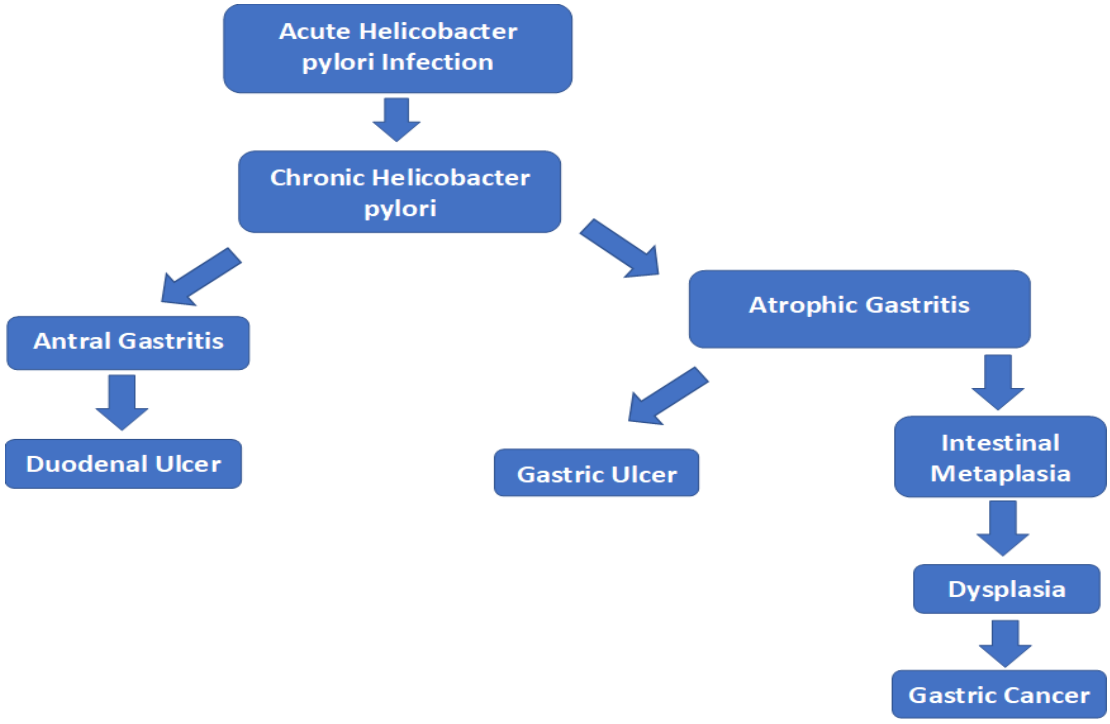

Figure 2.3 Gastric adenocarcinoma event through *H. pylori* infection. The development of gastric adenocarcinoma starts with *H. pylori* infection in the stomach. followed by gastric atrophy, gastric intestinal metaplasia, gastric dysplasia, and finally gastric adenocarcinoma (Diaconu, Predescu et al. 2017).

2.2.2.2 *Helicobacter pylori* epidemiology

The global incidence of *H. pylori* infection reaches fifty percent worldwide (Malfertheiner, Megraud et al. 2012) and in developed countries, it can increase into eighty to ninety percent (Hunt, Xiao et al. 2011). In developing nations like Saudi Arabia, Vietnam, India, or Canadian populations (Bernstein, Mckeown et al. 1999), more than eighty percent of the people are infected, while in industrialized nations, the rate of infection is as low as thirty percent (Thung, Aramin et al. 2016). Colombia has one of thehighest gastric adenocarcinoma rates in the world, with more than eighty percent of the people are infected with *H. pylori (Mannion, Dzink-Fox et al. 2021)*.

2.2.2.3 *Helicobacter pylori* detection

There are many techniques for the detection of *H. pylori* infection, which are classified as invasive and non-invasive. Rapid urease test, biopsy-based polymerase chain reaction, and microbiological culture are invasive (Sabbagh, Mohammadnia-Afrouzi et al. 2019). Urea breath tests, serological investigations, and stool antigen tests are non-invasive for *H. pylori* detection (Stefano, Rosalia et al. 2018).

Helicobacter pylori detection by serological method uses antibodies in the plasma or serum and determine the previous infection. Commercial serology test performance varies primarily due to strain heterogeneity. It embraces immunoblotting assay, conventional immunoglobulin (IgG), and ELISA (enzyme-linked immunosorbent assay). Some strains of *H. pylori* have cag PAI (cytotoxin-associated gene Pathogenicity Island) and effector protein called cag A, which is known to cause more widespread inflammation in the gastric mucosa.

Cag A antibodies are a better predictor of the previous infection and last much longer as compared to the antibodies against Helicobacter pylori cell surface antigens (HP-CSA) (Lopes, Vale et al. 2014).

The choice of the required technique strategy depends on many factors such as sensitivity, clinical status, specificity, and cost-based issues. Depending on the patient's history and clinical circumstances, each test has merits and demerits (Atkinson and Braden 2016).

2.2.3 Histological classification of gastric cancer

There are different classifications used to diagnose GC one of them is the Lauren classification. Lauren criteria is used for the histopathologic classifications ofGC over the past half-century. Lauren classification categorizes GC into three groups including diffuse, intestinal, and uncommon type which is known as intermediate type (Hwang, Lee et al. 2010). The relative prevalence is generally 54% for the intestinal type, 32% for the diffuse type, and 15% for the indeterminate type (Polkowski, van Sandick et al. 1998). Visible glands and cohesiveness between tumor cells are characteristics of intestinal carcinoma. Poorly cohesive cells that diffusely infiltrate the stomach wall and have little to no gland development make up the diffuse subtype. The cells often have a signet ring cell shape and are tiny and spherical (Hu, El Hajj et al. 2012). The tumor grade identifies whether cancer cells are regular or aberrant under a microscope. The cancer is less aggressive, spreads and grows more slowly than normal looking cells.

However, the more aberrant the cells seem, the more aggressive the cancer is likely to be and the quicker it is likely to spread. Depending on the type of cancer, there are different systems for characterizing tumor grade. But most cancers are rated as: Grade X: No grade can be given (undetermined grade); Grade 1: resemble healthy stomach cells, Undifferentiated (low grade); Grade 2: somewhat resemble a regular cell, moderately differentiated (intermediate grade); Grade 3: Unlike a typical cell, poorly differentiated (high grade).

For a long time, JRSGC classification was used for the diagnosis of the GC grade. However, in 1982 this method was converted to the Lauren classification known as Hanai's converting table as shown in table 2.1 (Hanai and Fujimoto, 1982, (Kaneko and Yoshimura 2001).

Table 2.1: Converting table from JRSGC to Lauren classification.

Lauren Classification	**JRGSC classification**
Intestinal type	Well-differentiated type
	Moderately differentiated type
Diffused type	Poorly differentiated adenocarcinoma
	Undifferentiated carcinoma

Japanese Research Society for Gastric Cancer (JRGSC)

2.3 Role of inflammatory genes in stomach cancer

Different inflammatory genes have a variety of roles in GC development and progression such as IL-1, IL-2, IL-6, IL-18, and IL-8. Particularly IL-8 has vital role in GC progression.

2.3.1 Interleukin 8 (IL-8)

Interleukin-8 (IL-8) or (CXCL8), is a neutrophil-activating peptide, that is a chemokine mainly secreted by macrophages. This chemokine participates in many cellular processes like tissue remodeling, cell proliferation, and angiogenesis. IL-8 is located on chromosome 4q12-q21, a small protein encoded by the IL-8 gene (White and Cooke 2000).

IL-8 enhances the propagation, movement, and existence of the endothelium cells, intensifying the epithelial-mesenchymal movement and survival of cancer cells and triggering macrophage and the immune system reacts at the site of the tumor. Moreover, the invasion and angiogenesis are all engaged in metastasis.

IL-8 level in the GC also affects the competence of metastasis. The overexpression of IL-8 in the GC of humans is intimately related to angiogenesis (Lee, Khoi et al. 2013). A study found that the degree of IL-8 is forth with related to the vascularity of human gastric tumor and the generated IL-8-infected cells are increasing promptly, in highly vascular neoplasms, in comparison to the control cells. In contrast, suppression of the IL-8 reduces angiogenesis in gastric tumors. (Kitadai, Haruma et al. 1998).

As illustrated in figure 2.4, IL-8 promotes endothelial cell proliferation, survival, and migration, as well as cancer cell epithelial-mesenchymal transition and survival, stimulating immune responses and macrophages at the tumor site (Yuan, Chen et al. 2005).

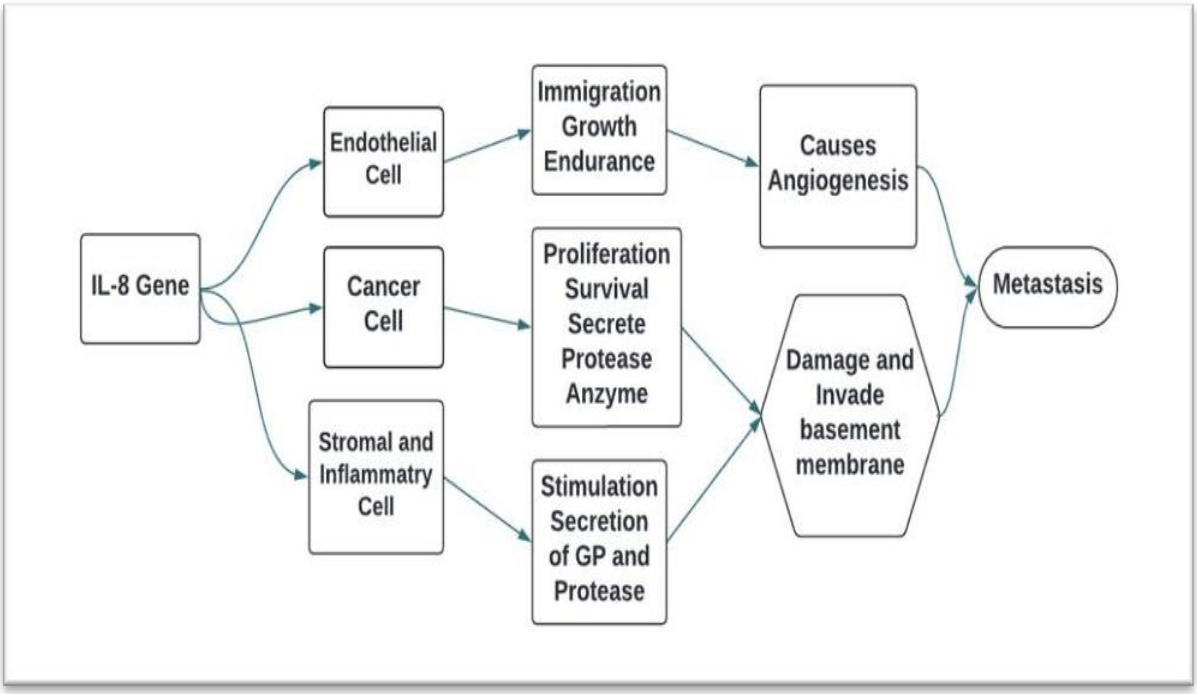

Figure 2.4 Role of IL-8 in the gastric cancer. IL-8 has effects on different cells; In endothelial cells, IL-8 causes angiogenesis and metastasis by stimulating the immigration and growth of the endothelial cells. In cancer, the cells grow out of control which stimulates the release of proteases enzyme that damage the basement membrane which stimulates invasiveness and leads to metastasis. Inflammatory cells damage the basement membrane by secreting protease enzyme that leads to metastasis (Grivennikov, Greten et al. 2010).

The role of IL-8 in the development and progression of cancer is widely studied (Table 2.2). There is an important relationship between elevated IL-8 levels in gastric mucosa and the risk of developing GC (Yamada, Kato et al. 2013). It has been analyzed that serum levels of IL-8 act as a gastric tumor marker (Macri, Versaci et al. 2006). The Clinopathological aspect of the disease, including poor diagnosis, is associated with a higher level of IL-8 mRNA in tissue extracts of the persons suffering from GC. Human regulatory elements control IL-8 expression; IL-8 presentation elevates tumorigenesis, indicating IL-8 has a significant function in gastric tumors. It is suggested that increased IL-8 levels are related to poor diagnosis as determined by histology and stage and that for more GC (Asfaha, Dubeykovskiy

et al. 2013). IL-8 has intrigued the interest of researchers with its role in GC angiogenesis and invasion. Angiogenesis is closely related to the upregulation of IL-8 in GC (Lee, Khoi et al. 2013). IL-8-infected cells produce highly vascular neoplasms which grow swiftly, and the presentation of IL-8 was in directly related to the vascularity of GC in human beings. IL-8 inhibition, on the other hand, reduces angiogenesis in gastric tumors (Kitadai, Haruma et al. 1998). *In vitro* study has shown that IL-8 activates the growth of the endothelial cell,and the creation of capillary tubes and IL-8 monoclonal antibodies can inhibit these effects. IL-8 has also been related to cell adherence and relocation in gastric tumor (Lin, He et al. 2010). The involvement of IL-8 in gastric carcinogenesis is summarized in table 2.2.

Table 2.2: IL-8 gene involved in gastric carcinogenesis.

Experimental model	**Samplesize**	**The main Technic used**	**GC association**	**IL-8 involved in gastric-cancer-related events**	**Reference**
Human	1843	IHC	Upregulated	Angiogenesis	(Wang, Hou et al. 2021)
Human	14	RT-PCR, WB, IHC	Upregulated	Angiogenesis	(Chang, Wu et al. 2022)
Cell line (SGC7901)	--	WB	Upregulated	Angiogenesis	(Shi, Li et al. 2015)
Human	111	IHC	Upregulated	Angiogenesis	(Zhai, Shen et al. 2019)
Human	75	RT-PCR	Upregulated	Angiogenesis	(Lin, He et al. 2019)

IHC (immunohistochemistry), WB (western blotting), RT-PCR (Real-time polymerase chain reaction)

2.4 DNA repair gene in gastric cancer

There are different genes involved in the DNA repair process such as XPE, XPF, XPG, XPGC, XPV, and XRCC1. One of the important DNA repair genes that have a vital role in gastric carcinogenesis is XRCC1. In this thesis, only the role of DNA repair gene XRCC1 is explained in detail.

2.4.1 XRCC1 gene

The DNA repair system plays a vital role in maintaining genetic integrity and the stability of cellular functions, through the damaged DNA caused by various endogenous and exogenous factors involving therapeutic agents, Eukaryotic and prokaryotic organisms have different repair mechanisms to protect their DNA. In mammalian cells, various DNA repair pathways repair DNA damage.

Throughout excision, chemically changed, improperly matched, unpropitiousbases (like Uracil in DNA) from the genomes and placed on the array of bases inthe direction of their locations (Asgerov, Şenol et al. 2019). Nucleotide excision repair (NER) and base excision repair (BER) are two types of excision repair. X-ray repair cross-complementing group 1 (XRCC1) encodes for a protein composed of 633 amino acids and is found on the long arm of the 19^{th} chromosome.

It is involved in the BER pathway and a change in this gene is widely implicated in cancer susceptibility (Chatterjee and Walker 2017). Recent research indicates that the XRCC1 gene is a significant risk factor for GC (Wang, Yang et al. 2020). The XRCC1 protein works in collaboration with many other proteins to promote BER. The involvement of **XRCC1** in gastric carcinogenesis is summarized in table 2.3.

Table 2.3: XRCC1 gene involved in gastric carcinogenesis

Experimentalmodel	Samplesize	Main Techni cused	GC association	XRCC1 Involved in gastric-cancer-related events	Reference
Human	89	RT-PCR	Upregulated	GC recurrence	(Wang, Yang et al. 2020)
Human	179	RT-PCR and IHC	Down-regulated	Promote GC	(Wang, Tang et al. 2010)
Human	303	RT-PCR	Up-regulated	Elevate GC risk	(Putthanachote, Promthet et al. 2017)
Human	100	RT-PCR and IHC	Down-regulated	GC progression	(Yousaf, Khan et al. 2019)
Human	1756	WB and IHC	Down-regulated	GS progression	(Wang, Wang et al. 2016)

IHC (immunohistochemistry), WB (western blotting), RT-PCR (Real-time polymerase chain reaction)

2.5 Role of the anti-apoptotic gene in gastric cancer

Apoptosis is a physiological programmed cell death that plays a major rolein the process of carcinogenesis. It involves several proteins, including (Bcl-2, Bcl-10, Bcl-x, Bcl-XL, Bcl-XS, and Bcl-w), each having different functions. In this thesis, we have focused on the BCL-2 protein.

The role of BCL-2 is explained in detail below, however, BID protein is a member of another group of the Bcl-2 family. It activates apoptosis and at the same time integrates two main apoptotic routes, connecting the membranous (external) and mitochondrial (internal) pathways. However, its role in the pathogenesis of cancer is still poorly elucidated.

2.5.1 BCL-2 gene

The Bcl-2 protein inhibits the mitochondrial pathway of apoptosis, interacting with other members of the Bcl-2 family. The increased expression shifts the balance between the pro- and antiapoptotic factors toward cell survival.Apoptotic disorders are associated with the development of many cancers, including that gastric (Kyokane, Ito et al. 1998, Yin 2000, Cory and Adams 2002, Lee, Soung et al. 2004, Kelly and Strasser 2011).

Two key features of apoptosis are mitochondrial outer membrane permeabilization and the consequent release of cytochrome *c*. The Bcl-2 family proteins regulate the process of mitochondrial outer membrane permeabilization (Bender and Martinou 2013). The proteins of Bcl-2 and Bcl-xl are crucial inhibitors in the procedure of apoptosis and are commonly overexpressed in many tumors (Hellemans, Van Dam et al. 1995, Trask, Wolf et al. 2002). Their overexpressionis closely related to tumor initiation, aggressive, and chemoresistance (Liu, Zhang et al. 2015). The resistance of cancer cells to apoptosis mediated by Bcl-2 is a distinguishing feature of cancer; inhibition of Bcl-2 antiapoptotic proteins will bea new way to enhance the effectiveness of chemotherapy. Figure 2.8shows the participation and overexpression of Bcl-2 protein during GC which inhibits the apoptosis that results in increased cell division. The involvement of BCL-2 in gastric carcinogenesis is summarized in table 2.4.

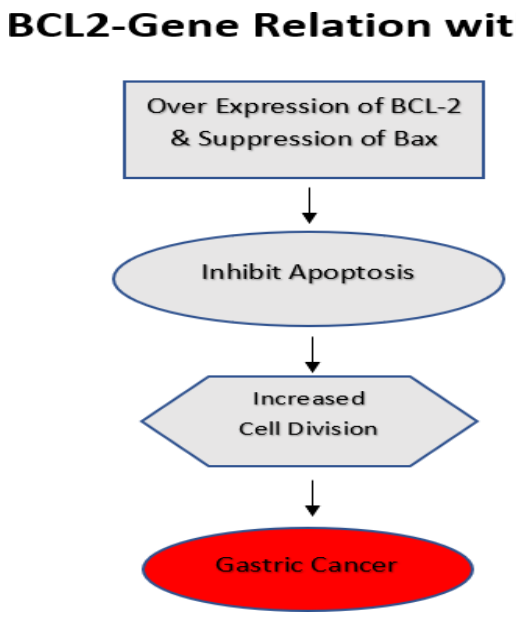

Figure 2.5 Bcl-2 gene involvement in gastric cancer development. Gastric cancer is caused by an imbalance of the two genes that participate in theapoptosis process resulting in apoptosis inhibition leading to an increase in cell division and turning into gastric cancerous cells (Adams, Clark-Garvey et al. 2019, Tao, Gu et al. 2021).

Table 2.4: BCL2- a gene involved in gastric carcinogenesis.

Exp. model	Sample size	Main Technique used	GC association	BCL-2 involved in gastric-relatedevents	Reference
Human	70	RT-PCR andWB	Up regulated	GC resistance, and progression	(Mei, Liu et al. 2021)
Human	60	RT-PCR	No change	GC progression and development	(Cui, Han et al. 2019)
Human	50	RT-PCR	Up regulated	GC metastasis	(Tao, Gu et al. 2021)
Human	88	IHC	Up regulated	GC growth and formation	(Gryko, Pryczynicz et al. 2014)
Human	95	RT-PCR	Up regulated	GC formation	(Cui, Han et al. 2019)

IHC (immunohistochemistry), WB (western blotting), RT-PCR (Real-time polymerase chain*re reaction.*

3. Material and Methods

3.1 Patients and samples

A total of 110 tissues of gastric patients were collected from November 2021 until June 2022. About 66 samples were freshly collected, including 6 resected gastric cancer tissue, biopsies from 12 gastritis patients, 4 polyps, 1 ulcer, and 43 normal gastric patients. The samples were collected and histologically confirmed at various hospitals in Erbil city, Kurdistan region of Iraq (Par, CMC hospital, Teaching Hospital Rezgary) and Sulaymaniyah (High-quality Anwar medical city and Smart hospital). Furthermore, 44 frozen cDNA samples (23 gastric cancer and 21 normal gastric) were collected and donated from Sulaymaniyah hospital, however, there were no histopathological data available for these frozen cDNA samples. This study was approved by the Human Ethics Committee of Technical Health and Medical College, Polytechnic University – Erbil. Moreover, the tissue specimens were cut into small pieces and frozen in 10x PBSsolution and then stored at -80°C in a deep freezer (Gesellschaft, Germany) at the scientific research center, Erbil polytechnic University until RNA extraction.

3.2 RNA extraction and quantification of human gastric tissues

Total RNA was purified from human gastric tissues following the manufacturer's protocol using a total RNA mini kit (Geneaid, Korea). The RNA extraction was performed in the molecular lab at the scientific research center, Polytechnic University-Erbil. The procedure is as follow:

1. A small quantity (25 mg) of frozen gastric tissues were sliced and transferred into a 1.5 microcentrifuge tube.

2. 400µl of RB buffer with 4µl β- mercaptoethanol are added to 1.5 microcentrifuge tube to lyse the cells and grounded with disposable micro pestle until a pulp is formed.
3. A filter column is placed into a clean 2 ml collection tube (supplied with the kit). The sample mixture is transferred to the filter column and centrifuged for 30 seconds at 1000×g and then the filter column is discarded.
4. The filtrate is transferred into clean 1.5 ml microcentrifuge tube and 400µl of 70% ethanol is added to the lysate for RNA binding to the filter membrane and immediately shaken vigorously for 10 seconds.
5. RB column is placed into a clean 2 ml collection tube (supplied with the kit).The sample mixture is transferred to the RB column and centrifuged at 15000×g for 1 min to separate the soluble RNA from cell debris, proteins, and other nucleic acids, the flow through was then discarded and the RB column is placed back into the 2 ml collection tube.
6. The RB column with bounded RNA was then washed three times with different wash buffers.
7. The column matrix was first washed with 400µl W1 washing solution and centrifuged at 15000×g for 30 second then the flow through was discarded.
8. Then the column matrix was washed twice with 600µl wash buffer centrifuged at 15000×g for 30 second then the flow through was discarded.
9. After washing steps, the column matrix was dried by centrifugation for 3 minutes at 16.000xg and then placed in a clean 1.5 microcentrifuge tube.
10. Eventually, 50µl of RNAase free water was added to the center of column

matrixand incubated for 2 minutes until it was absorbed by the filter matrix and the purified RNA was then eluted in a clean microcentrifuge tube by centrifuging for 1 minute at 15000×g.

The concentration and the purity of total RNA were estimated spectrophotometrically using a Nanodrop instrument (Thermo scientific, USA).

Nanodrop protocol

1. By using Kim Wipe the upper and lower pedestal surfaces were cleaned.
2. 2µL of NanoPure water was placed on the lower pedestal.
3. The sampling was lowered and pressed OK for calibration.
4. After calibration the upper and lower pedestals were wiped again with Kim Wipe to prepare for sample.

Historically, the ratio of absorbances at wavelengths has been used as a measure of purity in both nucleic acid and protein extractions. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. Similarly, absorbance at 230 nm is accepted as being the result of contamination.

3.3 Agarose gel electrophoresis

The quality of total RNA was also assessed by performing agarose gel elec trophoresis.

1. The agarose gel solution was prepared from powder agarose (Bio Tek, Canada) and dissolved in 1xTris-borate-EDTA(TBE) buffer (GeNet Bio, Korea).

2. The solution is put in a microwave until the agarose is dissolved uniformly.
3. The solution is cooled down to 60 °C, a 4µl safe gel stain (Add Bio, Korea) was added to the gel solution for visualization of the RNA bands.
4. The recommended volume of safe dye is 7 µl for 100 ml agarose solution with a gel percentage of 1.5%- 2.0 %.
5. The gel is poured into the gel casting system with the combs in place and avoiding any air bubbles and allowing the gel to set.
6. After it has set, the combs were carefully removed, and the gel was covered with 1x Tris-borate-EDTA (TBE) buffer. The RNA sample mixture was prepared by adding 5µl from the patient sample and 3µl from 6X loading dye (Add Bio, Korea).
7. The total RNA is loaded in the wells of the gel. Eventually, the gel electrophoresis system is turned on which is correctly oriented with respect to the anode and cathode, and initiated at 100V, 75mA for 60 minutes.
8. The results were viewed, using a gel documentation system (Biotech-Fischer, Germany).

3.4 DNase treatment

For better RT-PCR reaction, the total RNA was treated with DNase I for removing possible DNA contaminations by using a DNase kit (Yekta Tejhiz, Iran) following the manufacturer's instructions. Briefly, in an RNAase-free tube the following components were mixed

1. 1µg of RNA
2. 1µl of 10X reaction buffer with MgCl2,

3. 1µl of DNase I,
4. RNAase-free (1U),
5. 10µl of DEPC-treated water to RNAase free tube

the mixture was incubated at 37°C for 30 minutes. Hereafter, 1µl of 50 mM EDTA was added and incubated at 65°C for 10 minutes. The prepared RNA is used as a template for reverse transcriptase.

3.5 cDNA synthesis

Quantitative RT-PCR was carried out in a two-step process. cDNA and qRT-PCR. RNA was reverse transcribed into cDNA by using High-Capacity cDNA ReverseTranscription kit (Add Bio, Korea).

For every cDNA reaction that is prepared in a thin-walled PCR tube

1. 3µl of RNA template was used in the reaction mixture with the 8µl of Nuclease-free D.W.
2. 4µl of 5× reaction Buffer,
3. 2µl of 10mM dNTP Mixture,
4. 2µl of 10× oligo dT,

5 1µl of 20× AddScript Enzyme Solution.

After all, the required components of the mixture were added, the prepared solution was placed into a PCR Instrument, and the temperature cycling protocol was used according to the manufacturer's protocol.

The cycling temperature was as follow:

- Priming at 25°C for 10 min,
- Reverse transcription at 50°C for 60min,
- RT inactivation at 80°C for 5 min, and hold at 12°C for 2 min.

This prepared cDNA is ready for RT-PCR.

3.6 Primer Design

In the present study, expression of XRCC1, BCL-2, and IL-8 genes by Real-Time PCR was investigated. For this purpose, B2M gene, which is a housekeeping gene, was considered as an internal control. All primers were designed by using GENERUNNER software version 4.

Table 3.1: shows the characteristics of primers.

Gene	Sequence (5' → 3')	PCR product
XRCC1	F: 5- CCAACCCCTGAAGAGACCAA -3 R: 5- TGTTCCTCACTGTCCGTGT-3	230
IL-8	F: 5- CGGGAGAATATACAAATAGCAA -3 R: 5- TAAAGGAGAAACCAAGGCAC -3	251
BCL-2	F: 5- CTGTGGATGACTGAGTACCTG -3 R: 5- ACAGCCAGGAGAAATCAAACA -3	124
B2M	F: 5-CAGCACCTTGCCCCAAAATC -3 R: 5-TGGATGGCAAACCTCAGCTC-3	184

3.7 Quantitative Real-Time PCR (qRT-PCR)

qRT-PCR was performed using SYBER® Green Master mix kit (Amplicon Real Q, Denmark) According to the manufacturer's suggestion procedure. The transcript level of 3 different target genes (XRCC1, IL-8, and BCL-2) were measured by running the reactions in a BIO-RAD® MJ Mini cycler, PTC-0148 & CFD-3120 MINI OPTICON qRT-PCR™ system.

Table 3.2: Amounts of materials needed for real-time PCR reaction.

Gene name	SYBER green	Forward	Reverse	ddH_2O	cDNA
XRCC1	5 µl	0.3µl	0.3µl	1.4µl	3µl
BCL-2	5µl	0.3µl	0.3µl	1.4µl	3µl
IL-8	5µl	0.3µl	0.3µl	1.4µl	3µl
B2M	5µl	0.3µl	0.3µl	1.4µl	3µl

genes β-Actin, XRCC1, BCL-2, and IL-8.

Table 3.1: Temperature conditions and reaction time of Real-Time PCR for

	Primary denaturation	Denaturation	Annealing	Extension	Final extension
Temperature	95 °C	95 °C	60 °C	72 °C	72 °C
Time	5 minutes	30 seconds	30 seconds	30 seconds	5 minutes
Number of cycles	1	40	40	40	1

3.8 Statistical analysis

The correlations between gender, age, and clinical characteristics of gastric patients were evaluated by using software prism 9 (GraphPad software) with Chi-square, Fisher's exact test. and expressed as mean values ± SD. Statistical analysis was applied using SPSS 21.0 to determine differences in gene expression between gastric cancer patients and normal healthy patients. The *, **, and *** indicates statistically significant $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

4. Result

4.1. Gender, age, and clinical characteristics of gastric patients

In total, 110 gastric patient tissues were collected wherein 66 gastric tissues were freshly collected while the remaining 44 frozen cDNA of GC were generously donated for this study. The gender, age, and some clinical characteristics of the 66 freshly collected gastric patient's sample were analyzed due to the available information about their characteristics. It appears that women make up 28 (42%)) of the total 66 patients, and the age range is 19-89 years (average 36.7±15.3), while males compromise 38 (58%) gastric patients, and the age range varied between 16-78 years (average 45.6±16.7). The histopathological evaluation of the 6 GC patients was accomplished according to the Lauren classification which is classified into (intestinal, cohesive, diffuse, and mixed). In this classification, 2 patients were diagnosed with intestinal type, 3 patients had mixed adenocarcinoma, and 1 patient had a cohesive type.

Regarding the 44 donated frozen cDNA, it's known that 23 of these samples are cancer patients and 21 patients are considered healthy patients. Out of 23 patients 9 of these patients are 50 years old or younger while the remaining 14 GC patients are older than 50 years. It's also known that 11 out of 23 GC patients are classified as early-stage GC while 12 GC patients are classified as advanced- stage cancer.

Table 4.1: Gender, Age, and Clinical Characteristics of GC.

Characteristics of Gastric patients	**Total patients (110)**	**PatientsNo.**	**TNM staging/Laurenclassification**	**Gastrictissue**
Frozen cDNA samples	44	23 Age(year) ≤50= 9 >50=14	11 early stage	ResectedGC tissue
			12 advanced stages	
		21	N/A	Without GC (Normal)
Freshly collected gastric tissue	66	6	2 intestinal types 3 Mixed Adenocarcinomas 1 cohesive	ResectedGC tissue
		4	N/A	Polyp
		12	N/A	Gastritis
		1	N/A	Ulcer
		43	N/A	Without GS (Normal)

For the following data analysis only, freshly collected GC are used, due to the available histopathology data

H. pylori cases in 66 gastric Patients	**Male**	**Female**	**Age (years)**	**Add number of patients+ (%)**
H. pylori +ve	4 (50%)	4 (50%)	less than 30	2 (25%)
			30-45	3 (37.5%)
			45-60	3 (37.5%)
			More than 60	0 (0%)
H. pylori -ve	24 (41%)	34(58%)	less than 30	16 (27.5%)
			30-45	20 (34.4%)
			45-60	14 (24.1%)
			More than 60	8 (13.8%)
Total	28 (42%)	38(57%)		

n/a= not applicable, GC= Gastric cancer

Regarding the 44 donated frozen gastric cDNA samples, it is known that 23 of these samples are cancer patients and 21 patients are considered healthy patients. The age of 9 out of 23 patients is 50 years old or younger while the remaining 14 gastric cancer patients are older than 50 years. It's also known that 11 out of 23 of the gastric cancer patients are classified as early-stage Gastric cancer while 12 GS patients are classified as advanced-stage cancer.

In this study, figure 4.1 shows that *H.pylori* infection (*H.pylori* positivelytested patients), is equally distributed (50%) for both genders, while females are prominent in the *H.pylori* negative group (58%).

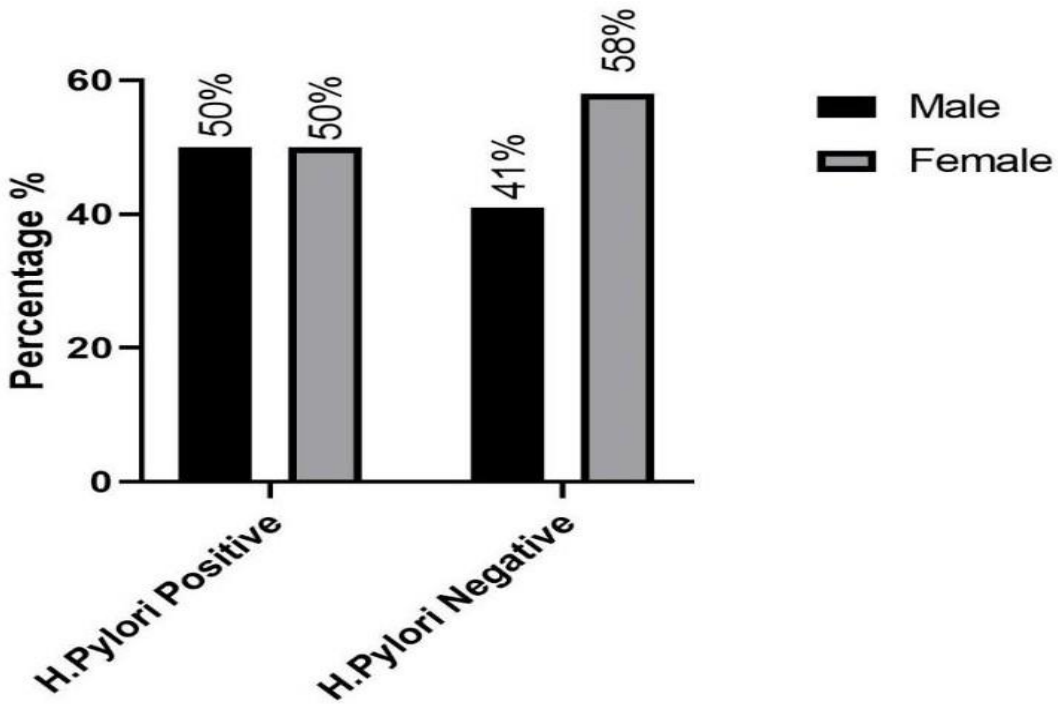

Figure 4.1 the distribution of *H.pylori* infection according to gender. The *H. pylori* infection was equally divided between males and females, whilemore females are negative tested for *H. pylori* compared to males.

4.2. Distribution of *H. pylori* infection according to age gender and diagnosis.

In this study, the *H. pylori*-positive tested patients are equally distributed (50%) for both genders, while females are prominent in the *H. pylori* negative group (58%). Figure 4.2 demonstrates that *H. pylori*-positive tests are highest (5%) in the age groups 30-45- and 45-60-years old patients and the percentage of positively tested patients is dropping to 3% in the younger age groups. However, patients older than 60 years are all negatively tested for *H. pylori* infection (100%).

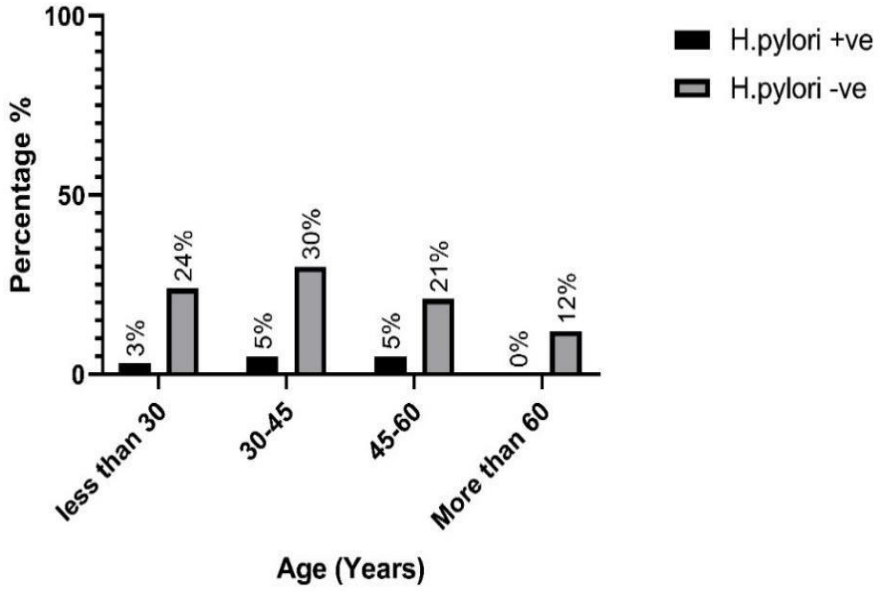

Figure 4.2 Distribution of *H. pylori* infection in different age groups. Most of the patients in the different age groups are *H. pylori*-negative and in the age groups, 30-60 years are most *H. pylori* infections detected.

Furthermore, figure 4.3 shows that all normal gastric patients and all gastric cancer patients were negative for *H. pylori* tested, while most of the *H. pylori* infections were found in the gastric inflammation group (11%) followed by gastric ulcer (1%) and gastric polyp (1%).

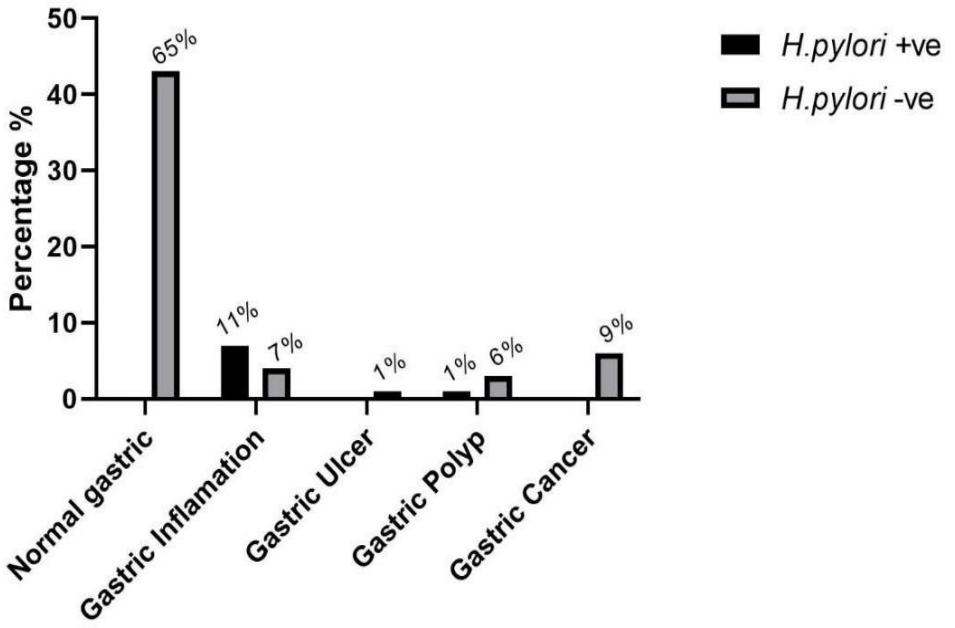

Figure 4.3 *H. pylori* test distribution according to patients' diagnosis. All normal diagnosed gastric patients and gastric cancer patients recorded as negative results for *H. pylori* infection. The highest *H. pylori* positively tested patients were found in the gastric inflammation group.

4.3. Assessment of quality of total RNA in different samples

To determine the quality of our extracted RNA from the gastric tissues, 1.5% gel electrophoresis is conducted for two samples as shown in figure 4.4. It demonstrated that the 18s rRNA is visible as bright bands followed by 28s rRNA in gastric tumor tissue. This result is similar to the positive control samples, indicating a good RNA quality that can be used for further molecular experiments such as cDNA synthesis from RNA.

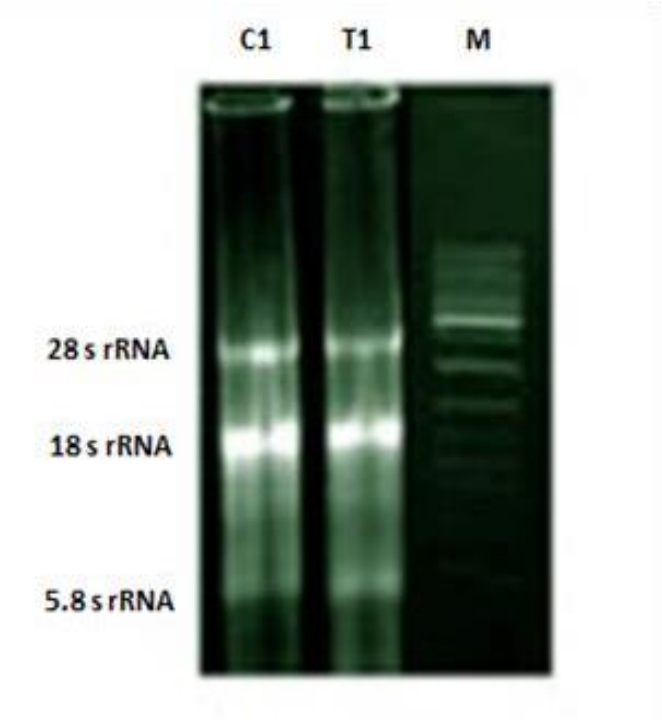

Figure 4.4 Agarose gel illustrates the quality of extracted total RNA from GC patient tissues. The two visible bands in lanes 1 and 2 imply a good RNA quality. C1: positive control, T1: gastric tumor, M: Marker. Total RNA is visible as bright bands in a 1,5% agarose gel that implies a proper quality of the total RNA.

4.4. Quality assessment of cDNA of the target genes

To measure the expression level of target genes, first, the total RNA from GC tissues is reversely transcript into cDNA using a specific two-step kit and running by qRT-PCR. After cDNA synthesis, the quality of cDNA is confirmed using 1.5% gel electrophoresis. It is demonstrated that all three target genes show a bright band with different sizes (IL-8: 251 bp, XRCC1: 230 bp, and Bcl-2: 124 bp) meaning they are detected in the sample of the patients (figure 3.5). Moreover, an internal control B2m with 191 bp is used to determine the quality of the cDNA reaction, and all the visible bands are compared with the marker to determine the size of the bands.

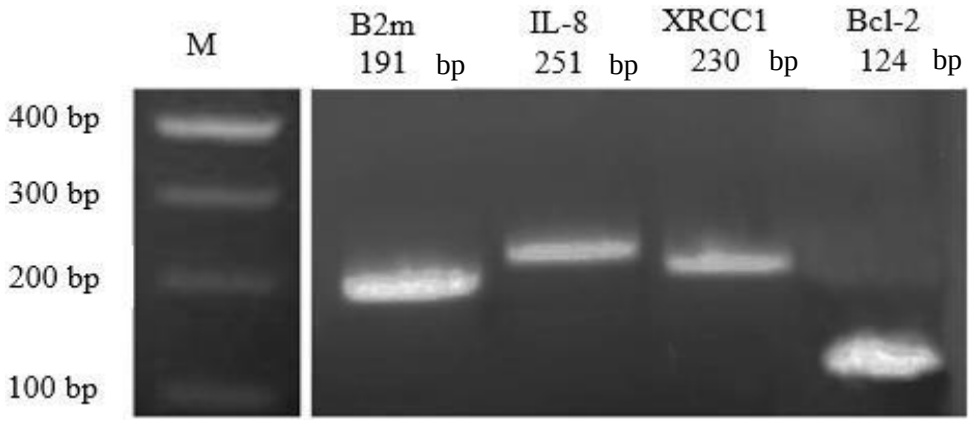

Figure 4.5 Quality assessment of cDNA of the target genes. The visible bright bands show different band sizes for each specific gene. Lane1: Marker, Lane 2: internal control B2m amplicon (191bp), lane 3: IL-8 (251bp),lane 4 XRCC1 (230bp), and Lane 5 Bcl-2 (124bp).

4.5. Gastric cancer-related gene expression

To find out the alteration in the expression level of target genes, the relative expression of target genes in the GS patients is compared to the healthy control patients using the qRT-PCR assay. Figure 6 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 in reference to normal tissues (figure 4.6A). Furthermore, there is a highly significant (p<0.01) increased level of proinflammatory cytokines IL-8 found in GC patients in comparison with the control group (figure 4.6B). Regarding the antiapoptotic gene BCL-2, there is an obvious upregulation of the BCL-2 gene in GC while the relative expression is significantly (p<0.001) dropped in the control group (figure 4.6C).

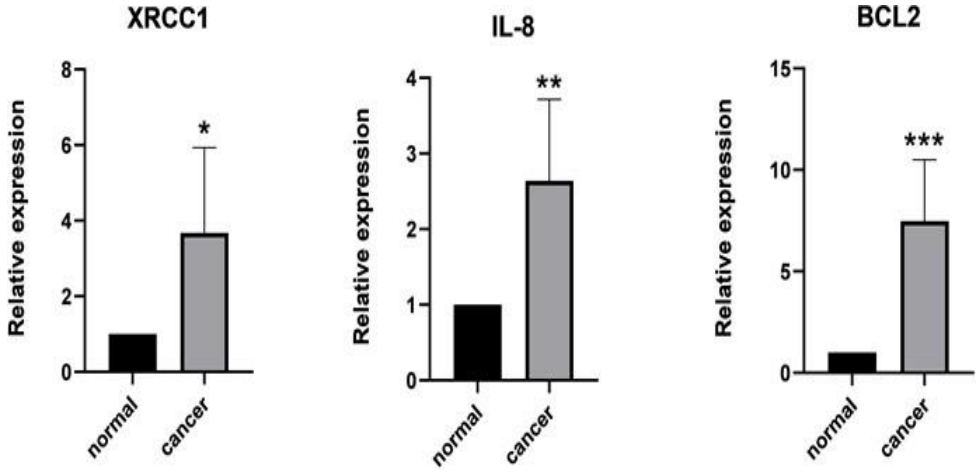

Figure 4.6 The relative expression of gastric cancer-related genes was measured by using the RT-PCR technique. The relative expression is significantly increased in the genes of GC patients (A) XRCC1 gene, (B) highly significant IL-8 gene, and (C) and highly significant BCL-2 gene. The *, **, and *** indicates statistically significant P < 0.05, P < 0.01, P < 0.001, respectively.

4.6. Collection of gastric patients' data from 2013 to 2021

A total of 1237 patients' data were received from PAR hospital between 2013 and 2021 including their age, gender, and clinical characteristics of gastric patients that underwent gastroscopy. The majority of the patients had inflammation only,

some of the patients had HLO and inflammation. Out of all collected gastric data, 101 patients were diagnosed with GC. There was a marginal difference between the male and female cases with greater cases in males. The number of gastric cancer cases was 623 (50%) in males as opposed to614 (49.6%) in females as shown in table 4.2

Table 4.2 clinical characteristics of gastric patients.

Cases +Total	**Total F(%)**	**Male F(%)**	**Femal eF (%)**	**<30 yearsF (%)**	**30-45 yearsF (%)**	**45-65 yearsF (%)**	**>65 yearsF (%)**
Normal	27(2)	13(48)	14(52)	7(26)	9(33)	7(26)	4(15)
Inflammation	829 (67)	426(51)	403(49)	136(16)	239(29)	261(31)	193(23)
HLO & Inflammation	191(15)	87(70)	104(54)	29(15)	61(32)	56(29)	45(24)
Ulcer	27(2)	19(31)	8(30)	2(7)	2(7)	6(22)	17(63)
Polyp	62(5)	19(58)	43(69)	3(5)	11(18)	24(39)	24(39)
Cancer	101(8)	59(58)	42(42)	2(2)	8(8)	36(36)	55(54)
1237	1237(100)	623(50)	614(49.6)	179(14)	312(27)	390(32)	338(27)

F=frequency HLO= Helicobacter like organisms

Table 4.2 shows the frequency of gastric cases collected in different hospitals between 2013 to 2021. In total 1237 gastric cases were collected with equal gender distribution. Furthermore, the majority of the cases (67%) were diagnosed with inflammation, while another 15% were diagnosed with HLO and inflammation. Out of 1237 patients only 101 patients were diagnosed with gastric cancer. However, 5% of the cases were identified as ulcer and only 2% of the patients had polyp and

similar result was diagnosed as normal. Moreover, more than half (54%) of the gastric patients were older than 65 years at the day of diagnosis and 36% of the patients were diagnosed at age 45-65 years. Eight percent of the gastric patients were young adult patients between 30 and 45 years old and 3% were younger than 30 years at diagnosis stage. Furthermore, inflammation appears to occur mostly in patients from 30-65 years, while ulcer and polyps are mostly diagnosed in patients at age 45-65 years.

This table 4.3 is describing the cancer grading for different genders and age groups. The majority of the cases is found in G3 with poorly differentiated adenocarcinoma and the distribution of males (51.5 %) and females 48.4% were similar in advanced grade gastric cancer of the males were diagnosed in advanced grades than females were male and was female. Furthermore, this tables indicates that the more the persons get older the more they are diagnosed with GC as compared to the other age groups older than 60 years had the higher rate to develop GC.

Table 4.3: Clinicopathological features and histological grading.

Histological Grading	**Gender**		**Age groups (Years)**			
	Frequency Male (%)	**Frequency Female (%)**	**<30**	**30-45**	**45-60**	**>60**
G1 (well differentiated)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
G2 (Moderately differentiated)	8(53)	6(47)	0(0)	0(0)	3(23)	10(77)
G2+G3 (Moderate&Poor)	8(73)	3(27)	1(9)	4(36)	5(45)	1(9)
G3 (Poorly differentiated)	34(51.5)	32(48.4)	1(2)	4(8)	19(37)	39(75)

F=frequency

According to the Laurens classification the majority of cases had diffused type GC with a higher rate for male patients as compared to female patients and the elderly had the most diffused type cases as shown in table 4.4.

Table 4.4: Clinicopathological features and Laurens's classification.

Laurens Classification	**Gender**		**Age groups (Years)**			
	Male F (%)	Female F (%)	<30	30-45	45-60	>60
Intestinal type	21(60)	14(40)	0(0)	2(6)	13(37)	20(57)
Diffused type	28(52.8)	25(47)	2(4)	6(11)	14(26)	31(58)

F=frequency

This table describes the type of cancer based on gender and age group.

5. Discussion

Gastric carcinoma is a common malignancy around the world and common malignant tumors that endanger human health (Li, Xu et al. 2018). The occurrence and development of GC is a multistage process, involving multiple gene and molecular level changes. In addition, Gastric cancer is one of the highly prevalent cancers that originate 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 (Yeni, Korkut et al. 2021).

Smoking is one of the risk factors that increase the chance of developing gastric cancer. A prospective Japanese study discovered that the risk of stomach cancer was higher when smoking and *H.pylori* infection were combined than when each factor was present alone (Ladeiras-Lopes, Pereira et al. 2008, Shikata, Doi et al. 2008, Wang, Meng et al. 2014).

Other risk factor for gastric cancer development is familiar history. One of the studies that examined the relation between metabolic variables and stomach cancer in a large group of people getting health checks. According to the study, gastric cancer was significantly higher in older patients as well as patients that were infected with *H.pylori* and in patients that having the disease in their family (Youn Nam, Park et al. 2019).

In total 66 patients with complete available clinical data were used for correlation analysis. The *H. pylori* positive test was equally divided in both genders. This is in contrast with the findings of Sowaid et al. (Izaldeen Sowaid, Ali et al. 2022) that found gastric male patients are more frequently infected with *H. pylori* than gastric female patients. The small sample size included in this study due to incomplete

available clinical data is probably the cause of the discrepancy in this result. There is a higher percentage of *H. pylori* in the patient group of 30- 60 years, this result is in line with the previous study Yang et al. (Yang, Kartsonaki et al. 2021). Chronic gastritis brought on by *H.pylori* infection can develop in to gastric atrophy, Intestinal metaplasia, dysplasia, and ultimately gastric cancer. Chronic gastritis brought on by *H.pylori* infection can develop in to previous studies have found that GC patients are significantly more likely to have *H.pylori* than non-GC patients, and they have also suggested a link between *H.pylori* status and the prognosis of GC(Carrasco and Corvalan 2013). However, our most recent study has not found any conclusive evidence of this link.

In this study, among 110 human gastric tissues, 29 samples were confirmed as GC tissue and used to assess the relative expression level of three different targetgenes including XRCC1, IL-8, and BCL-2 by qRT- PCR. Regarding 1237 gastric patient data collected from PAR hospital, 101 patients were diagnosed with GC. Regarding the subtypes, there are 35 intestinal-type GC and 53 diffused-type GC. The diffused type of GC prevalence has increased compared to the intestinal type, which is in agreement with the research group of Machlowska (Machlowska, Baj et al. 2020) study and while it is in contrast with the study of HU and his colleagues (Hu, El Hajj et al. 2012). Intestinal gastric adenocarcinoma includes 21 males and 14 females. This result is supported by the study of Waldum and his colleagues (Marques et al) (Marques-Lespier, Gonzalez-Pons et al. 2016) wherethey suggest that the intestinal type of GC is more frequent in men than in women.

Furthermore, in diffused GC the prevalence of men was higher than females 28:25 and among older patients. However, the findings of Hu and his colleagues indicates that the incidence of diffused subtype is higher among female and younger patients (Hu, El Hajj et al. 2012). On the other hand, Paulo et al conducted a study about GC and the results indicated higher in younger patients (Assumpção, Barra et al. 2020).Wang et al showed that men present with larger, higher grade GC than women (Li, Wei et al. 2020), which is in parallel with recent research.

Furthermore, our result shows the greatest incidence of GC in the late stage G3, which is in line with the study of Abdullah et al (Abdullah, Amin et al. 2022).
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 (Wang, Tang et al. 2010). The present study showed a significant elevation level of the XRCC1 gene in GC patients compared to the control group. This result is in contrast with the study of Wang and his colleagues (Wang, Tang et al. 2010) that found downregulation in the XRCC1 gene expression and claim that XRCC1 promotes gastric carcinogenesis. Researchers suggest that XRCC1 might be used as a biomarker for the prediction of GC recurrence. However, limited data is available regarding the upregulation of XRCC1 in gastric cancer (Wang, Yang et al. 2020).

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 (Li, Xu et al. 2018). It has been stated that IL-8 is a key regulatory component in the tumor microenvironment

(Campbell, Maxwell et al. 2013). The result of our research shows the upregulation of the IL-8 gene in GC patients. This result reflects those of Lee et al. (2013) who also revealed an elevated level of IL-8 in GC patients and suggest the involvement of IL-8 in GC invasion and metastasis. Moreover, another group of researchers found that high expression of IL-8 was an independent risk factor for causing GC prognosis (Wang, Yang et al. 2020), while other investigators associated high IL- 8 expression levels with tumor angiogenesis, as well as metastasis in gastric cancer (Shi, Li et al. 2015, Li, Xu et al. 2018, Sun, Xiang et al. 2019).

Alteration in the apoptosis proteins is one of the key factors in the development of many cancers including GC. In the current study, there is a significant upregulation of the Bcl-2 gene level observed compared to the controlgroup. Our finding is in a similar vein to the research group of Xu et al. (Xu, Li et al. 2001) that demonstrated significantly high expression of antiapoptotic Bcl-2 in GC, claims 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 and therefore targeting these specific genes might be an interesting and promising strategy for the treatment of GC.

Conclusion

- There was overexpression of the target genes (XRCC1, IL-8, and Bcl-2) found in gastric cancer patients compared to healthy individuals.
- The study's findings revealed that males and females were equally affected by *H. pylori* infection in the tissues of gastric patients, while the majority of the *H. pylori*-negative patients were females.
- *H. pylori* test results for gastric patients between the ages of 30 and 60 are more frequently positive.
- The result of collected data showed that elderly and patients with advanced stages of stomach cancer had a higher prevalence of the disease. The majority of the gastric cancer patients were more often diagnosed at advanced stage
- Males are predominant in the diffused type of gastric cancer.

Recommendation

The research presented in this thesis has uncovered important new informationfor the field of GC and highlighted potential research avenues.

- ❖ Conducting sanger sequencing for other genes that's related to gastric cancer development.
- ❖ Measuring the expression of target genes (XRCC1, IL-8, and Bcl-2) on protein level using western blotting.
- ❖ Immunohistochemistry of target genes (XRCC1, IL-8, and Bcl-2) to determine the distribution of genes in healthy and unhealthy patients.
- ❖ In future other researchers must put their results in National Center for Biotechnology Information (NCBI) for Kurdish population.

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Appendices

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Upregulation of XRCC1 DNA repair gene, Interleukin-8, and Bcl-2 antiapoptotic gene levels in Kurdish patients with gastric adenocarcinoma

Document Type : Original Articles

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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 Bcl-2 gene perform 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 Kurdistan region of Iraq (KRG). Gastric cancer tissues were taken from 29 patients that were diagnosed with gastric adenocarcinoma that underwent gastric resection and 21 tissue samples were taken from healthy patients that underwent gastroscopy. The gastric tissues were collected in different hospitals in Erbil- and Sulaymaniyah city in the Kurdistan region of Iraq and data regarding the Helicobacter pylori (H. 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 quantitative Real-Time PCR (qRT-PCR). The result 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 from 30-60 years old are more commonly positive tested for the H. pylori test. Furthermore, in this study patients diagnosed with gastric inflammation are more often tested positive for H. pylori, while patients diagnosed with gastric cancer were all negative tested for this infection. Additionally, it found that the target genes (XRCC1, IL-8, and Bcl-2) were significantly upregulated in GC patients compared to the healthy group.

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پوختە

هەڵسەنگاندنی دەربڕینی XRCC1، ئینتەرلوکین-8، و BCL-2 لە نەخۆشانی شێرپەنجەی گەدە

شێرپەنجەی گەدە یەکێکە لە کوشندەترین جۆرەکانی شێرپەنجە بەهۆی توانای داگیرکرن و تەشەنە کردنی. چەندین جۆر گۆڕانکاری بۆماوەیی و ئیپیجینێتیک بەشدارن لە دروستبوونی شیرپەنجەی گەدە. لەگەڵ ئەوەش ئەم نەخۆشییە زیاتر لە قۆناغێکی پێشکەوتوو دەستنیشان دەکرێت. جینی چاککەرەوەی (DNA)، پرۆتینی تەواوکەری یەکتر چاککردنەوەی تیشکی ئێکس (XRCC1) ، جینی سایتۆکاین ئینتەرلوکین (IL-8)، و جینی لیمفۆمای خانەی (B)، دژە ئەپۆپتۆتیک (BCL-2)، ڕۆلێکی گرنگ لە گەشەسەندن و پێشکەوتنی شێرپەنجەی گەدە دەگێرن. ئامانجی ئەم تۆێژینەوەیە دەرخستنی ئاستی ئەم جینانەیە لە نەخۆشەکانی هەرێمی کوردستان.

شانەکانی شێرپەنجەی گەدە لە ٢٩ نەخۆش وەرگیراون کە تووشی شێرپەنجەی گەدە بوون و نەشتەرگەری گەدەیان بۆ کراوە و ٢١ نموونەی شانە لە نەخۆشە تەندروستەکان بە پشکنینی نازوور وەرگیراون. شانەکانی گەدە لە نەخۆشخانە جیاوازەکانی شارەکانی هەولێر و سلێمانی لە هەرێمی کوردستان کۆکراونەتەوە و لە ٨٠ پلەی سیلیزی هەڵگیراون.

زانیارییەکان سەبارەت بە هەوکردنی بەکتریای گەدە ، تەمەن و ڕەگەزی ئەو ناخۆشانەی گەدە کە یەک لە قۆناغەکانی نەخۆشییەکەیان هەبووە تۆمارکران و بە بەکارهێنانی گرافپاد پریزم شیکرانەوە. ئاستی دەربڕینی جینەکان لە شانەکانی گەدە بە کوانتیتەیتڤ ریل تایم پی سی ئار (RT-PCR) لێکۆڵینەوەی لەسەر کرا. ئەنجامەکە دەریخست کە هەوکردنی بەکتریای گەدە بە یەکسانی لە نێوان نێر و مێ لە شانەکانی ناخۆشانی گەدە دابەش بووە. ئەوە لە کاتێکدا زۆرینەی ئەو ناخۆشانەی کە بەکتریای گەدەیان نێگەتیڤ بووە ئافرەت بوون. هەروەها دەرکەوتووە کە نەخۆشانی گەدە لە نێوان ٣٠-٦٠ ساڵ بوون زیاتر بەکتریای گەدەیان بۆ دەرچووە.

جگە لەوەش لەم تۆێژینەوەیەدا ئەو نەخۆشانەی کە تووشی هەوکردنی گەدە بوون زۆرترینییان بەکتریای گەدەیان هەبوو، لە کاتێکدا ئەو نەخۆشانەی کە تووشی شێرپەنجەی گەدە بوون، کەسیان بەکتریای گەدەیان نەبوو. سەرەڕای ئەوە، دەرکەوت کە جینەکانی (IL-8, BCL-2, XRCC1) لەو نەخۆشانەی کە شێرپەنجەی گەدەیان هەیە بەشێوەییکی بەرچاو بەرز بووەنەوە، بەراورد بەو کەسانەی کە تەندروستن.

ئەمە لەوانەیە ئاماژە بەوە بکات کە ئەم جینە ئامانجدارانە دەتوانن ڕۆڵ بگێڕن لە تەندروست بوونی شێرپەنجەی گەدە و هەربۆیە، ئامانجکردنی جینەکانی (XRCC1-BCL-2,IL-8) لەوانەیە بواریکی سەرنجڕاکێش و ستراتیژی بەڵێندەر بێت بۆ چارەسەرکردنی شێرپەنجە.

هەڵسەنگاندنی دەربڕینی XRCC1، ئینتەرلوکین-8، و BCL-2 لە نەخۆشانی شێرپەنجەی گەدە

نامەکە

پێشکەش بە ئەنجومەنی کۆلێژی تەکنیکی تەندروستی و پزیشکی هەولێر کراوە لە زانکۆی پۆلیتەکنیکی - هەولێر

وەک بەشێک لە پێداویستیەکانی بەدەستهێنانی پلەی ماستەر لە بەشی شیکاری نەخۆشیەکان

لە لایەن

دانیە عونی کمال

بەکالۆریۆس لە کۆلیژی تەکنیکی تەندروستی- زانکۆی پۆلیتەکنیک - هەولێر-2013

بە سەرپەرشتیاری

پ.ی. نەبەز فیصل شاکر

د. ژوان محمد اسماعیل

هەولێر – کوردستان

حوزەیران-٢٠٢٢