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# Gene Expression Analysis of *c-Jun* and *NF-kB (p65)* in Gastroduodenal Disorders

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**Abstract:** Gastroduodenal diseases, often associated with *Helicobacter pylori* (*H. pylori*) infection, represent significant global health challenges. Understanding the molecular mechanisms and gene expression patterns involved in these conditions is essential for advancing therapeutics. Two key contributors to gastroduodenal disorders are *c-Jun* and *NF-kB (p65)*, transcription factors that regulate gene expression and inflammation in the gastrointestinal tract. This study delves into the gene expression profiles of *c-Jun* and *NF-kB (p65)* in *H. pylori*-related gastroduodenal disorders. Quantitative PCR was employed to evaluate gene expression in gastric biopsies from 88 participants categorized into four groups based on *H. pylori* status and gastrointestinal conditions: *H. pylori*-positive gastritis, peptic ulcers, gastric cancer patients, and *H. pylori*-negative healthy controls. The comparison in fold change among these groups revealed upregulation of *c-Jun* and *NF-kB (p65)* across all patient categories. Notably, gastric cancer patients exhibited the highest fold change in *c-Jun* (2.49745), while peptic ulcer patients displayed the most substantial increase in *NF-kB (p65)* (4.87057). It was concluded that *c-Jun* and *NF-kB (p65)* signaling pathways play pivotal roles in *H. pylori*-associated gastroduodenal diseases, offering valuable insights into molecular mechanisms and potential therapeutic targets.

**Keywords:** Gastroduodenal diseases, Gene Expression, *c-Jun*, *NF-kB (p65)*, *H. pylori*, qPCR.

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## Introduction

Gastroduodenal disorders, including peptic ulcers and gastric cancer, pose formidable global health challenges. These conditions, characterized by mucosal damage in the gastrointestinal tract, contribute significantly to worldwide morbidity and mortality (1).

Chronic inflammation is a hallmark of these disorders, orchestrated by diverse immune responses (2, 3). They often originate from *H. pylori* infection, a bacterium colonizing the stomach and causing chronic inflammation and tissue injury (4, 5). Approximately 10% of those infected face the risk of developing peptic ulcers during their lifetime (6). Conversely, gastric cancer, responsible for a substantial share of global cancer-related mortality, that is closely linked with *H. pylori* infection (7, 8). Within the intricate immune landscape of these disorders, *c-Jun* emerges as a pivotal transcription factor within the Jun family, orchestrating immune responses by governing genes implicated in inflammation and cellular differentiation. Dysregulation of *c-Jun*

has garnered attention for its involvement in immune-related diseases and cancer progression(9). Similarly, *Nuclear Factor kappa B (NF-κB)*, a transcription factor family, holds a central role in immune processes. Notably, the *p65* subunit of *NF-κB* takes center stage in regulating immune and inflammatory responses, culminating in the transcription of pro-inflammatory genes, firmly establishing it as a key regulator in immune-related diseases (10). This article is poised to delve into the intricate roles played by *c-Jun* and *NF-κB (p65)* in immune regulation within the realm of gastroduodenal disorders. The aim of this study is to provide invaluable insights into the immune mechanisms underpinning peptic ulcers and gastric cancer, potentially paving the way for groundbreaking therapeutic strategies.

**Materials and methods**

The study adhered to ethical standards and was approved by the Research Ethics Committee No. 13179. In line with the Declaration of Helsinki, full informed consent was obtained from a total of 163 participants, ranging in age from 12 to 71, including 134 *H. pylori*- positive patients and 29 *H. pylori*-negative healthy controls. The study was conducted from April 2021 to August 2022 at the Gastroenterology and Hepatology Teaching Hospital in Baghdad Medical City. All patients exhibited gastric disease symptoms, such as abdominal pain, bloating, and nausea, and were referred for esophagogastroduodenoscopy (OGD). Those with a smoking history, medication use, or certain conditions (cardiovascular disease, hypertension, diabetes, asthma, renal failure, anemia, neoplasia, or immunological diseases) were excluded. Clinical and demographic data, as well as gastric biopsies, were collected during the study. A specialist physician diagnosed all cases and grouped them into four categories: patients with gastritis, peptic ulcers (PU), gastric cancer (GC), and healthy individuals.

**Sample collection**

Four gastric biopsies were collected from each subject using a sterile technique under the doctor's supervision. The biopsies were immediately flash-frozen in liquid nitrogen and then stored at -80 °C in an ultra-low temperature freezer.

***H. pylori* detection**

For the detection of *H. pylori*, polymerase chain reaction (PCR) was employed using a set of primers targeting the *UreA* gene. The primer sequences were as follows: forward primer 5'-TCGTTGTCTGCTTGCCTATC-3' and reverse primer 5'-TCGGCTCACACTTCCATTTC-3', with a product size of 205 bp. These primers were carefully designed, and their specificity was verified using the Primer BLAST tool on the National Center for Biotechnology Information (NCBI) website. Furthermore, to optimize the PCR process, annealing temperatures were systematically optimized. This was achieved using a gradient thermal cycler (Eppendorf, Germany) with a range of temperatures. A specific protocol was employed to amplify DNA samples through PCR reactions. To achieve a final volume of 25 µL, we added the following components: 12.5 µl GoTaq G2 Green Master Mix 2X (Promega, USA), 1 µl (0.1 µM) from each primer, 6.5 µl (40 ng) of DNA sample, and nuclease-free water. The thermal cycler (Eppendorf, Germany) was used to amplify the PCR reactions. The PCR program included one denaturing cycle at 95 °C for 5 min during pre-incubation, followed by thirty-five amplify cycles. Each

amplification cycle included denaturation at 94 °C for 1 min, annealing at 53 °C for 45 s, and extension at 72 °C for 1 min. Finally, one final extension cycle was added at 72 °C for 5 min.

The results of PCR amplification were analyzed using an electrophoresis system (Cleaver Scientific, UK) as follows: The PCR amplification products and a 100 bp step DNA marker (Promega, USA) were loaded onto a 2% pre-stained agarose gel (Promega, USA). The agarose gel was submerged in a 1X TAE buffer. Electrophoresis was conducted at 90V for 60 minutes to separate the DNA fragments based on size. The separated bands were then visualized using the Gel-documentation-system (ATTO, Japan). The results were validated by positive control DNA extracts from an Iraqi standard strain (IRQ19-106) recorded in the NCBI GenBank database with accession number JX164111.

**RNA extraction**

The RNA was extracted and purified from the biopsies using the SV total RNA isolation system kit (Promega, USA) following the manufacturer's instructions. Subsequently, the concentration and purity of RNA samples were assessed using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, USA). The assessment involved checking for an acceptable purity range, which is indicated by a 260/280 ratio of approximately 2 and a 260/230 ratio ranging from 1.8 to 2.2. This rigorous RNA extraction and purification process ensured the high quality and integrity of the obtained RNA samples, which are crucial for downstream molecular analyses in this study. The RNA was then stored at -80 °C for further analysis.

**cDNA synthesis**

The RNA was converted to cDNA using thermal cycler (Eppendorf, Germany). The reaction was prepared by adding the following components: 4 µl of All-In-One 5X RT MasterMix (abm, Canda), 1 ng – 2 µg RNA, and up to 20 µl nuclease-free water. The PCR program included one cycle at 37 °C for 15 mins, followed by one cycle at 60 °C for 10 mins, then one cycle at 95 °C for 3 mins, and finally one cycle at 4 °C for 5 mins. The cDNA was then ready for downstream analysis by qPCR.

**Quantification of *c-Jun* and *NF-kB (p65)***

For the quantification of *c-Jun* and *NF-kB (p65)*, quantitative PCR was employed using sets of primers targeting *c-Jun* and *NF-kB (p65)* genes, which were normalized to a reference gene. The primers sequences were as follows: *c-Jun* forward primer 5'-GGTGCCAACTCATGCTAACG-3' and *c-Jun* reverse primer 5'-TCTCTCCGTCGCAACTTGTC-3', with a product size of 130 bp. *NF-kB (p65)* forward primer 5'-TGCCGAGTGAACCGAAACTC-3' and *NF-kB (p65)* reverse primer 5'-AGCCTGGTCCCGTGAAATAC-3', with a product size of 109 bp. And for the reference gene, *β-actin* forward primer 5'-AAACTGGAACGGTGAAGGTGAC-3' and *β-actin* reverse primer 5'-CTCGGCCACATTGTGAACTTTG-3', with a product size of 70 bp. These primers were carefully designed, and their specificity was verified using the Primer BLAST tool on the National Center for Biotechnology Information (NCBI) website. Furthermore, to optimize the PCR process, annealing temperatures were systematically optimized. This was achieved using a gradient thermal cycler (Eppendorf, Germany) with a range of temperatures.

A specific protocol was employed to amplify cDNA samples through qPCR reactions. To achieve a final volume of 20 µL, the following components were added: 10 µl GoTaq qPCR Master Mix (Promega, USA), 1 µl (0.1 µM) from each primer, 4 µl of cDNA sample, and nuclease-free water. The RT PCR (Qiagen, Germany) was used to amplify the qPCR reactions. The qPCR program included one denaturing cycle at 95 °C for 2 mins during pre-incubation, followed by forty amplify cycles. Each amplification cycle included denaturation at 95 °C for 15 sec, annealing at 58 °C for 45 s, and extension at 72 °C for 15 sec. Finally, one final cycle was added for the melt ranged 55-99 °C. The results of qPCR amplification were analyzed using a Livak method and the fold change was calculated.

**Statistical analysis**

The statistical analysis was conducted using IBM SPSS version 27 software (IBM, Armonk, NY). Descriptive statistics, including the mean and standard deviation, were calculated for each experimental group. The normality of the data distribution was assessed using the Shapiro-Wilk test, revealing that the data did not follow a normal distribution. An independent-Samples Kruskal-Wallis Test was employed to explore potential differences between the groups. A p-value less than 0.05 is considered significant.

**Results and discussion**

The Ct values and melting curves obtained from the qPCR analysis provide crucial information about the gene expression levels and the specificity of the amplified products. Figure 1 displays the Ct values for the target gene *c-Jun*, while Figure 2 displays the Ct values for *NF-κB (p65)*. These results allow the assessment of the relative expression levels of these genes in various samples and determine any significant differences. Additionally, the melting curve analysis provides insights into the specificity of the amplification, ensuring that the detected signals are indeed derived from the target genes rather than any non-specific amplification or background noise, as seen in (Figure 3).

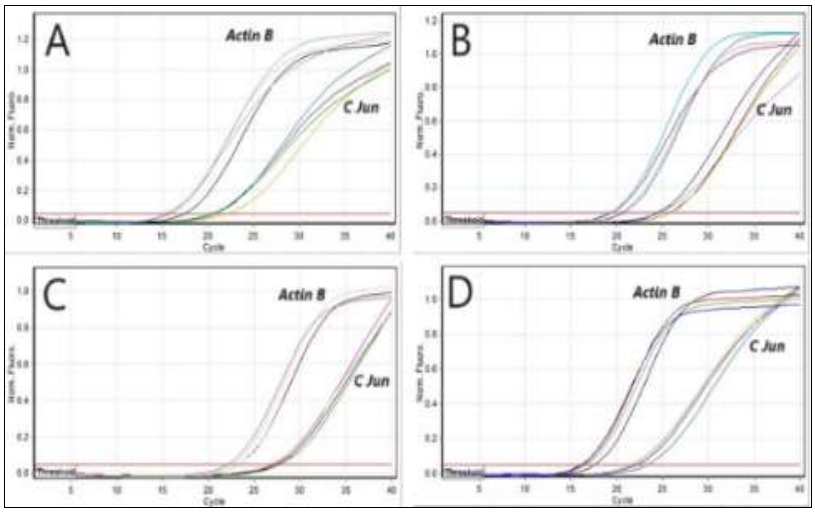


**Figure (1): Real-time amplification of *c-Jun*. A: Gastritis. B: Peptic ulcer. C: Gastric cancer. D: Healthy control.**

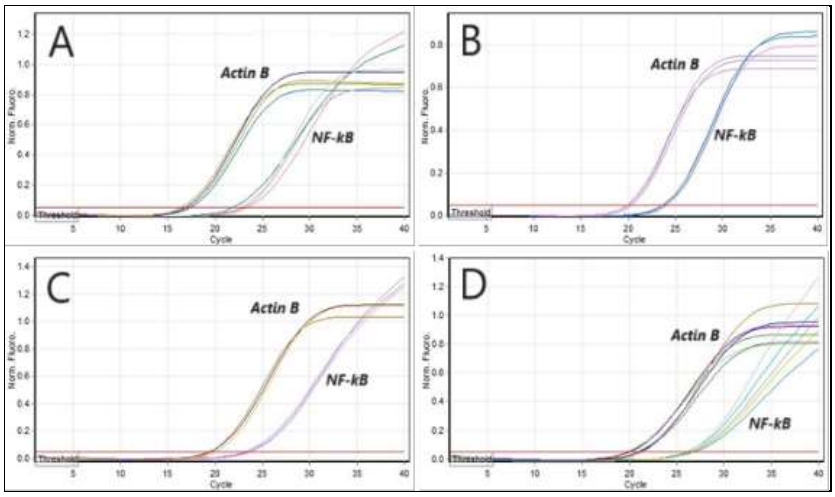


**Figure (2): Real-time amplification of *NF-kB*. A: Gastritis. B: Peptic ulcer. C: Gastric cancer. D: Healthy control.**

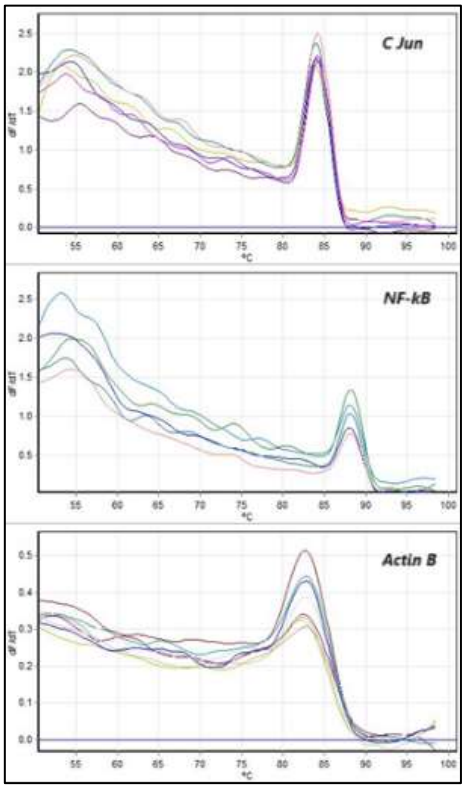


**Figure (3): Melt analysis for target and reference genes.**

According to the study results, there was a significant increase in the expression of *c-Jun* and *NF-kB (p65)* among all patient groups compared with the healthy control group. The fold change of *c-Jun* was the highest among gastric cancer patients at 2.49745, followed by peptic ulcer patients at 1.81332, and the gastritis group at 1.64770. Similarly, the highest fold change for *NF-kB (p65)* was observed among peptic ulcer patients at 4.87057, followed by gastric cancer patients at 3.34667, and the gastritis group at 1.19371. Figure 4 provides a visual representation of these findings. For a more detailed overview of the fold change values for each patient group, refer to Table 1 (*c-Jun*) and Table 2 (*NF-kB p65*).

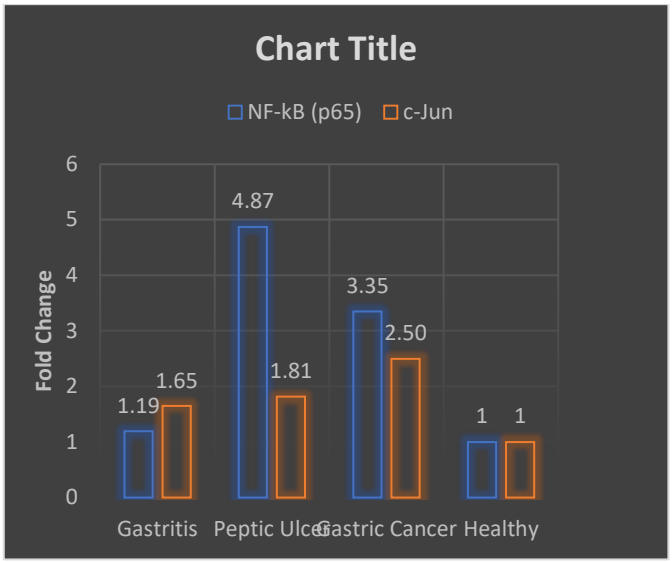


**Figure (4): Fold change in expression of c-Jun and NF-*kB (p65)* among gastroduodenal disorders.**

**Table (1): Fold change in gene expression of *c-Jun* gene normalized to *β-actin* gene in gastroduodenal diseases calculated by Livak method. Whereas Δ Ct Calibrator is the highest Δ Ct value of control samples.**

| Group | Mean Δ Ct ± SD (*c-Jun* Ct – *β-actin* Ct) | Δ Ct Calibrator | ΔΔ Ct | $2^{-\Delta\Delta Ct}$ | Fold difference in gene expression |
|---|---|---|---|---|---|
| **Healthy** | 5.9859 ± 0.17303 | 6.23 | -0.24409 | 1.18435 | 1 |
| **Gastritis** | 5.2655 ± 0.21826 | 6.23 | -0.96455 | 1.95145 | 1.64770 |
| **Peptic ulcer** | 5.1273 ± 0.21294 | 6.23 | -1.10273 | 2.14760 | 1.81332 |
| **Gastric cancer** | 4.6655 ± 0.28000 | 6.23 | -1.56455 | 2.95784 | 2.49745 |
| **Independent-Samples Kruskal-Wallis Test** | | **P-value** | | **0.000**** | |

**Table (2): Fold change in gene expression of *NF-kB (p65)* gene normalized to *β-actin* gene in gastroduodenal diseases calculated by Livak method. Whereas Δ Ct Calibrator is the highest Δ Ct value of control samples.**

| Group | Mean Δ Ct ± SD (*NF-kB* Ct – *β-actin* Ct) | Δ Ct Calibrator | ΔΔ Ct | $2^{-\Delta\Delta Ct}$ | Fold difference in gene expression |
|---|---|---|---|---|---|
| **Healthy** | 6.0477 ± 0.29382 | 6.54 | -0.49227 | 1.40666 | 1 |
| **Gastritis** | 5.7923 ± 0.24506 | 6.54 | -0.74773 | 1.67915 | 1.19371 |
| **Peptic ulcer** | 3.7636 ± 0.22321 | 6.54 | -2.77636 | 6.85123 | 4.87057 |
| **Gastric cancer** | 4.3050 ± 0.48326 | 6.54 | -2.23500 | 4.70763 | 3.34667 |
| **Independent-Samples Kruskal-Wallis Test** | | **P-value** | | **0.000**** | |

The study shows that *c-Jun* is upregulated in patient groups, which suggests that *c-Jun* may play a crucial role in the pathogenesis of gastric diseases, particularly in response to *H. pylori* infection. The significant fold change values observed in gastric cancer patients further suggest that *c-Jun* may play a role in the development and progression of gastric cancer. Recent research by Miao (11) also observed an increase in *c-Jun*

expression in gastric cancer cells, suggesting the involvement of KIAA1429 methyltransferases. Additionally, Cui (12) found that the inactivation of *c-Jun* and its downstream targets in the noncanonical Bone Morphogenetic Proteins (BMP) signaling pathway can restrain the progression of gastric cancer. These findings highlight the potential role of *c-Jun* in the development and progression of gastric disorders.

One possible mechanism by which *c-Jun* may contribute to gastric diseases is its role in regulating inflammatory responses. Chronic inflammation of the gastric mucosa, caused by *H. pylori* infection, can lead to gastritis and potentially gastric cancer. A Study has shown that *c-Jun* is involved in activating pro-inflammatory genes, including IL-2 and TNF-α, both of which have been linked to the development of gastric diseases (9).

Additionally, *c-Jun* has been found to interact with other transcription factors, such as *NF-κB*, to enhance the inflammatory response. Furthermore, bile acid is known to be a trigger for gastric carcinogenesis. It enhances the protein interaction of NF-κB and SHP, resulting in an increase in *c-Jun* expression and the production of the inflammatory cytokine TNF. This interaction between *c-Jun* and *NF-κB* can result in the upregulation of multiple inflammatory mediators, including cytokines, chemokines, and adhesion molecules, further exacerbating the inflammatory process(13).

Moreover, *c-Jun* has been shown to play a role in the recruitment and activation of immune cells, such as macrophages (14) and T cells (15), which contribute to the amplification of the inflammatory response. Overall, the involvement of *c-Jun* in the regulation of inflammatory responses highlights its importance as a potential therapeutic target for the treatment of inflammatory diseases. Targeting *c-Jun* could help mitigate the excessive production of inflammatory mediators and prevent the recruitment and activation of immune cells, ultimately reducing inflammation and its associated tissue damage.

Besides its role in regulating inflammatory responses, *c-Jun* is known to be involved in cell proliferation, differentiation, and apoptosis, and its dysregulation has been implicated in the pathogenesis of gastric cancer, peptic ulcers, and gastritis. For example, *c-Jun* participates in cell cycle control and has the ability to regulate cell proliferation. Its activity is associated with cell cycle transitions and progression through cell cycle phases (16). Moreover, apoptosis regulation has been linked to *c-Jun*. The activity of *c-Jun* can potentially impact the equilibrium between cellular survival and cellular death. The abnormal function of *c-Jun* may potentially play a role in cancer pathogenesis. *C-Jun* is also involved in the cellular stress response mechanism and can be triggered by a range of stress-inducing stimuli such as oxidative stress, DNA damage, and other forms of cellular harm (17).

Recently, it was found that *H. pylori* infection stimulates *c-Jun* expression, which increases STYX pseudophosphatase expression (18). This enzyme contributes to oncogenic processes in gastric cancer by inhibiting FBXO31, a protein involved in the degradation of cell cycle regulatory proteins (19), resulting in increased CyclinD1 and Snail1 levels in cancer cells. High STYX expression is linked to a poor prognosis in gastric cancer patients, and overexpression of STYX promotes cancer cell proliferation and migration.

Comparing *NF-kB* expression levels in different gastroduodenal disease groups, which have never been studied before, can provide valuable insights into the underlying mechanisms of each condition. The upregulation of *NF-kB* in *H. pylori*-positive patients suggests its potential role in the pathogenesis of gastroduodenal disorders. This is supported by previous studies that have shown *NF-kB*'s involvement in regulating inflammatory responses and immune system activity (20, 21). Ali (20) found that *NF-kB* plays a pivotal role in the progression of atrophic changes in *H. pylori*-associated gastritis. Similarly, Jiang *et al.* (21) studied mice with chronic atrophic gastritis (CAG). They showed that an inhibitor of the *NF-kB* signaling pathway (PDTC) could protect the mice by decreasing inflammatory factors, abnormal growth of the gastric mucosa, and improving the pathological state of the gastric mucosa. These findings suggest that targeting *NF-kB* could be a promising approach for treating gastroduodenal disorders, and further research in this area is warranted.

Interestingly, the peptic ulcer patients exhibited the highest fold change in *NF-kB* expression, indicating a more pronounced activation of this transcription factor in this particular group. This could be linked to the increased inflammation and tissue damage observed in peptic ulcer patients, as described by Dong (22). The data from this study reveal that *NF-kB* expression is elevated not only in the peptic ulcer group but also in the cancer group. This finding is significant because it demonstrates the involvement of *NF-kB* in both conditions. *NF-kB* has a multifactorial role, not only directly affecting cancer cells but also potentially influencing immune cells. These effects on immune cells can have implications for tumor growth, as they may either promote or inhibit it (23).

Multiple facts explain the elevation of *NF-kB* in gastric cancer. Jiao *et al.* (24) conducted a study investigating the effects of attenuating the *NF-kB* signaling pathway by targeting the metadherin gene. They observed a significant decrease in cancer cell proliferation and metastasis. Similarly, Xu (25) found that upregulation of *NF-kB* leads to increased antiapoptotic gene expression, which gives cancer cells a survival advantage. Verzella *et al*. (26) also supported these findings in their respective studies by demonstrating the role of *NF-kB* in promoting metastasis and inhibiting apoptosis in cancer cells.

Furthermore, oxidative stress, which is commonly observed in cancer cells, can lead to the production of reactive oxygen species (ROS). These ROS molecules have been shown to activate *NF-kB*, further enhancing its activity. This activation of *NF-kB* by ROS can have various consequences in cancer, such as the upregulation of genes associated with cell proliferation, inhibition of apoptosis, and metastasis. Understanding this interplay between oxidative stress and *NF-kB* can provide valuable insights into the mechanisms driving cancer progression.

The upregulation of *NF-kB* during gastroduodenal diseases associated with *H. pylori* infection is of great interest due to its potential clinical implications. By understanding how upregulated *NF-kB* signaling contributes to disease pathogenesis, researchers can develop targeted therapies or interventions that specifically target this pathway. These interventions could help reduce inflammation and improve patient outcomes in individuals affected by *H. pylori*-associated gastroduodenal diseases.

## Conclusion

The upregulation of both *c-Jun* and *NF-kB* in gastroduodenal disorders, particularly those associated with *H. pylori* infection, underscores the intricate involvement of these transcription factors in disease pathogenesis. This dysregulation in gene expression, as demonstrated in this study, contributes to chronic inflammation and tissue damage seen in conditions such as peptic ulcers and gastric cancer. Understanding these molecular pathways is crucial for unraveling the underlying mechanisms of these diseases.

## References


1. Malfertheiner, P.; Camargo, M. C.; El-Omar, E.; Liou, J.-M.; Peek, R.; Schulz, C.; *et al*. (2023). *Helicobacter pylori* infection. Nature Reviews Disease Primers 9 (1): 19.
2. Mustafa, A. J. and Ismail, P. A. (2022). Association of potent inflammatory Cytokine and Oxidative DNA Damage Biomarkers in Stomach cancer patients. Baghdad Science Journal 19 (6): 1313-1325.
3. Shehab, M. J.; Mahdi, B. M.; Al-Tabra, R. H. and Namaa, D. S. (2023). Evaluation of serum levels of Proinflammatory Cytokines IL-8, IL-17, and IL-22 in *Helicobacter pylori* infection and their association with the degree of gastritis histopathology in a sample of Iraqi patients. Baghdad Science Journal 20 (3): 0937-944.
4. Rzooqi, W. H. and Al-Mossawei, M. T. (2017). Study the relation of gastric cancer with *H. pylori* infection among dyspeptic patients. Iraqi Journal of Biotechnology 16 (3): 159–168.
5. Qasim, H. S. and Aboud, R. S. (2023). Impact of *Helicobacter pylori* in some Blood Parameters Change of Iraqi Patients with Gastritis Disease. Iraqi Journal of Biotechnology 22 (1): 25–30.
6. Lanas, A. and Chan, F. K. L. (2017). Peptic ulcer disease. The Lancet 390 (10094): 613–624.
7. Moss, S. F. (2017). The Clinical Evidence Linking *Helicobacter pylori* to Gastric Cancer. Cellular and Molecular Gastroenterology and Hepatology 3 (2): 183–191.
8. Dincă, A. L.; Meliț, L. E. and Mărginean, C. O. (2022). Old and New Aspects of *H. pylori*-Associated Inflammation and Gastric Cancer. Children 9 (7): 1083-1099.
9. Papavassiliou, A. G. and Musti, A. M. (2020). The Multifaceted Output of c-Jun Biological Activity: Focus at the Junction of CD8 T Cell Activation and Exhaustion. Cells 9 (11): 2470-2496.
10. Liu, T.; Zhang, L.; Joo, D. and Sun, S.-C. (2017). NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy 2 (1): 17023-17032.
11. Miao, R.; Dai, C.; Mei, L.; Xu, J.; Sun, S.; Xing, Y.;,*et al*.. (2020). KIAA1429 regulates cell proliferation by targeting c-Jun messenger RNA directly in gastric cancer. Journal of Cellular Physiology 235 (10): 7420–7432.
12. Cui, Y.; Zhang, F.; Jia, Y.; Sun, L.; Chen, M.; Wu, S., *et al*.. (2019). The BMP antagonist, SOSTDC1, restrains gastric cancer progression via inactivation of c-Jun signaling. American Journal of Cancer Research 9 (11): 2331–2348.
13. Hokello, J.; Lakhikumar Sharma, A. and Tyagi, M. (2021). AP-1 and NF-κB synergize to transcriptionally activate latent HIV upon T cell receptor activation. FEBS Letters 595 (5): 577–594.
14. Yung, J. H. M. and Giacca, A. (2020). Role of c-Jun N-terminal Kinase (JNK) in Obesity and Type 2 Diabetes. Cells 9 (3): 706-737.
15. Lai, B.; Wu, C.-H. and Lai, J.-H. (2020). Activation of c-Jun N-Terminal Kinase, a Potential Therapeutic Target in Autoimmune Arthritis. Cells 9 (11): 2466-2483.
16. Li, B.; Zhou, P.; Xu, K.; Chen, T.; Jiao, J.; Wei, H., *et al*. (2020). Metformin induces cell cycle arrest, apoptosis and autophagy through ROS/JNK signaling pathway in human osteosarcoma. International Journal of Biological Sciences 16 (1): 74–84.
17. Singh, R. R.; Mohammad, J.; Orr, M. and Reindl, K. M. (2020). Glutathione S-Transferase pi-1 Knockdown Reduces Pancreatic Ductal Adenocarcinoma Growth by Activating Oxidative Stress Response Pathways. Cancers 12 (6): 1501-1523.
18. Liu, J.; Zang, Y.; Ma, C.; Wang, D.; Tian, Z.; Xu, X., *et al*.. (2022). Pseudophosphatase STYX is induced by *Helicobacter pylori* and promotes gastric cancer progression by inhibiting FBXO31

function. Cell Death & Disease 13 (3): 268-280.

19. Choppara, S.; Malonia, S. K.; Sankaran, G.; Green, M. R. and Santra, M. K. (2018). Degradation of FBXO31 by APC/C is regulated by AKT- and ATM-mediated phosphorylation. Proceedings of the National Academy of Sciences 115 (5): 998–1003.
20. Ali, A. I. (2013). Molecular and Immunopathological Role of Nuclear Factor K B Detected by Insitu Hybridization in Pathogenesis of Chronic Atrophic Gastritis in Iraqi Patients. Tikrit Medical Journal 6 (3): 179–208.
21. Jiang, J.-Y.; Liu, D.-J. and Liu, M.-X. (2021). The protective effect of NF-κB signaling pathway inhibitor PDTC on mice with chronic atrophic gastritis. Scandinavian Journal of Gastroenterology 56 (10): 1131–1139.
22. Dong, S. X. M.; Chang, C. C. Y. and Rowe, K. J. (2018). A collection of the etiological theories, characteristics, and observations/phenomena of peptic ulcers in existing data. Data in Brief 19: 1058–1067.
23. Pires, B.; Silva, R.; Ferreira, G. and Abdelhay, E. (2018). NF-kappaB: Two Sides of the Same Coin. Genes 9 (1): 24-48.
24. Jiao, Y.; Yang, H.; Qian, J.; Gong, Y.; Liu, H.; Wu, S., *et al*.. (2019). miR-3664-5P suppresses the proliferation and metastasis of gastric cancer by attenuating the NF-κB signaling pathway through targeting MTDH. International Journal of Oncology 54 (3): 845-858.
25. Xu, D.; Li, J.; Li, R.; Lan, T.; Xiao, C. and Gong, P. (2019). PD-L1 Expression Is Regulated By NF-κB During EMT Signaling In Gastric Carcinoma. OncoTargets and Therapy 12: 10099–10105.
26. Verzella, D.; Pescatore, A.; Capece, D.; Vecchiotti, D.; Ursini, M. V.; Franzoso, G., *et al*.. (2020). Life, death, and autophagy in cancer: NF-κB turns up everywhere. Cell Death & Disease 11 (3): 210-224.