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Estimation of some immunological markers and Expression genes in Gastritis patients

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Abstract

Background: Gastritis is an Inflammation of the lining of the stomach. The inflammation of gastritis is most often the result of infection with the same bacterium that causes most stomach ulcers or the regular use of certain pain relievers. Drinking too much alcohol also can contribute to gastritis. This study aims to estimate the levels of immunological markers Interleukin-8 (IL-8) and Interleukin-6 (IL-6), and to analyze the expression of specific genes(*ClpB* and *BspA*) in patients with gastritis. By comparing these markers and gene expressions in gastritis patients with healthy controls, we seek to better understand the immunological and genetic factors involved in the disease process. **Objectives:**To estimate the levels of IL-8 and IL-6 in the gingival crevicular fluid (GCF) and serum of gastritis patients.To analyze the expression of

genes related to inflammation and tissue degradation in the gastritis tissues of gastritis patients.To compare these markers and gene expressions between gastritis patients and healthy controls.To investigate the correlation between cytokine levels, gene expression, and clinical parameters of gastritis. **Materials and Methods:** This study was carried out in Hilla Teaching Hospital in Babylon from (November 2023 to March 2024), patients with gastritis (60 patients 55 male and 5 female) with range age (25-60) years.Sample Collection(Gingival crevicular fluid (GCF) samples from gastritis patients .Blood samples for serum analysis. gastritis tissue samples for gene expression analysis.Quantification of IL-8 and IL-6 levels in GCF and serum using ELISA (Enzyme-Linked Immunosorbent Assay).quantitative real-time PCR (qRT-PCR) for analyzing the expression of target genes.**Results:** In this study, 120 samples were detected for present of *Helicobacter pylori* by q RT-PCR technique, the specific *ClpB* and *BspA* primers genes were used for the amplification of a fragment these genes for identification of *Helicobacter pylori* .**Conclusion :** The study highlight the potential of using immunological markers and gene expression profiles as diagnostic tools for early detection and progression monitoring of gastritis. Furthermore, understanding the molecular mechanisms underlying these changes can inform the development of targeted therapeutic strategies aimed at modulating the immune response, promoting tissue regeneration, and ultimately improving gastritis health outcomes.Future research should focus on longitudinal studies to validate these biomarkers and explore their utility in clinical practice. Integrating molecular data with clinical parameters will enhance our ability to personalize treatment approaches and improve prognosis for patients suffering from gastritis.

Keywords: ClpB and BspA, Helicobacter pylori, q RT-PCR, IL8, IL6.

* Introduction

Gastritis is an inflammation, irritation, or erosion of the lining of the stomach. It can occur suddenly (acute) or gradually (chronic). The condition is often caused by the same bacteria that lead to most stomach ulcers, namely Helicobacter pylori. Additionally, excessive alcohol consumption,

chronic vomiting, stress, or the use of certain medications such as aspirin or other anti-inflammatory drugs can also contribute to gastritis(Elseweidy *etal.,*1017). If left untreated, gastritis can lead to complications such as stomach ulcers and an increased risk of stomach cancer(Yury,2023). Therefore, seeking medical advice and adhering to treatment plans is crucial for managing and alleviating gastritis symptoms(Ghanem *etal*.,2019). Causes of Gastritis,Helicobacter pylori Infection: A common bacterial infection of the stomach lining,Regular Use of Pain Relievers: Nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and aspirin can irritate the stomach lining,Excessive Alcohol Consumption: Can irritate and erode the stomach lining,Stress: Severe stress due to major surgery, injury, burns, or severe infections,Autoimmune Conditions: Where the body attacks its own stomach lining,Other Diseases and Conditions: Such as Crohn's disease and sarcoidosis.Understanding these immunological markers can provide insight into the disease's progression and potential therapeutic targets(Pandey ,2020). ClpB and BspA are proteins associated with bacterial pathogens in gastritis. ClpB, a stress response protein, and BspA, a surface protein involved in bacterial adhesion and invasion, contribute to the inflammation and tissue destruction characteristic of this gastritis disease(Safira *etal.,*2021). Elevated levels of these proteins in patients with gastritis highlight their role in the disease's progression and severity.Advances in molecular biology have facilitated the study of gene expression profiles in gastritis patients. Altered expression of genes involved in immune regulation, tissue remodeling, and microbial defense has been observed, highlighting the molecular mechanisms driving periodontal inflammation and tissue destruction. These genetic insights can aid in identifying biomarkers for early diagnosis and monitoring of disease progression(Ashcheulova,2021).The estimating the levels of specific immunological markers and analyzing the expression of key genes in gastritis patients. By correlating these molecular findings with clinical parameters, we aim to identify potential biomarkers that can serve as diagnostic tools and therapeutic targets. Understanding the immunological and genetic landscape of gastritis will contribute to the

development of personalized treatment strategies, ultimately improving patient outcomes and gastritis health(Stinson *etal*.,2019).

*** Material and method**

This study was conducted at the Hilla teaching Hospital in Babylon, from November 2023 to March 2024. The participants included 60 patients with gastritis (55 males and 5 females), aged 25 to 60 years. Samples collected included blood sample and tissue of ulcer from gastritis, blood for serum analysis, and gastritis tissue for gene expression analysis. IL-8 and IL-6 levels in serum were quantified using ELISA, while quantitative real-time PCR (qRT-PCR) was used to analyze the expression of target genes.Genetic Detection of Mechanisms .patients with chronic gastritis (60 subject 55 male and 5 female) with range age (25-60)years.. The Control group study included 10 people apparently healthy that included (2 males and 8 females).

*** DNA Extraction and PCR Test**

The DNA extraction kit (DNeasy® Micro Genomic DNA Kit) was used for the bacterial DNA extraction. The manufacturing protocol was as follows: the samples were thawed for 20-30minutes and placed into a vortex for 30seconds and spun in a micro centrifuge for 10seconds. The paper points were then removed from the microfuge tubes and pelleted by refrigerated centrifuge at 13.2000rpm for 30 minutes at 4 °C and then the supernatant was discarded. After that 180|al of tissue lysis buffer (ATL) and 20|al of proteinase K were added to the pelleted cells followed by vortexing for 10 seconds and spinning for 10 seconds after which it was placed in a thermo mixer at 56 °C for 3hours for incubation to take place. After the samples were removed from the thermo mixer they were spun for 10 seconds. A 200|al of buffer (AL) was added to the tubes followed by vortexing for 10 seconds and spinning for 10 seconds and then incubated in the thermo mixers at 65 °C for 10 minutes' samples then were spun for 10 seconds after being removed from the thermo mixer. 200|al of cold absolute ethanol was added to each of the samples and the mixture was transferred to the DNeasy mini spin column and centrifuged at 8000rpm for 1 minute and then the flow was discarded. A 500|al of washing buffer 1 (AW1) was added and centrifuged at 8000rpm for 1 minute and then the flow was discarded. A 500|al of washing buffer 2 (AW2) was then

added and centrifuged at 8000rpm for 1 minute and the flow was then discarded. A new collection tube was placed and centrifuged at 13.2000rpm for 3 minutes for drying. The total bacterial DNA was then transferred to a new micro centrifugal tube and eluted with 35|ul of elution buffer (AE), and then the DNA concentration and quality were measured using NANODROP 2000 spectrophotometer device and the total product was stored at -20 °C until it was time to do the real-time polymerase chain reaction analysis.

*** Bacterial quantification by real-time PCR**

Primer Design™ Ltd Genesig kits were used for the quantification of *H.pylori,* in which the head shock protein 60 genes were used to detect and quantify the *H.pylori* genome(ATP-dependent chaperone (**ClpB**)(F 5' GAAGCCATCCGACGTAAACC '3/ R 5' GTCTTCGTCTCGGCAATCAC '3)(leucine-rich- repeat family virulence factor (**BspA**) F 5' TCACTATTGTGTCTCGCTG '3/ R 5' TCTCTCCGATTGTGGTTA '3). The Fast-RealTime PCR System (Applied Biosystems, Foster City, CA, USA) was used for the quantification of the bacterial genomic DNA according to the manufacturer protocol. The reaction mixes which include sufficient reactions for the negative control and standard curve wells was prepared. Then a 15|al of this mix was pipetted into each well according to the real-time PCR plate set up. PCR was performed in a total volume of 20|al consisting of 5|al of genomic DNA, Ten |ul of lyophilised Master mix (Primer Design Ltd Oasig™ 2X), 4|ul of RNAse/DNAes free water and 1|al of each bacterial species primer/probe mix. Five |ul of RNAse/DNAes free water was added to the negative control well making the final volume become 20|ul in each well. Finally, the quantitative PCR was done on the Fast Real-Time PCR using the manufacturer PCR conditions as follows: initial denaturation for enzyme activation for 15 minutes at 95 °C, followed by 50 cycles for denaturation (10 seconds at 95 °C) and data collection (60 seconds at 60 °C). The quantity of *H.pylori* DNA was generated by the Fast Real-Time PCR System.

*** ELISA**

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has

been pre-coated with an antibody specific to IL-6,IL-8. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for IL-6,IL-8 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain IL-6,IL-8, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of IL-8. You can calculate the concentration of IL-6,IL-8 in the samples by comparing the OD of the samples to the standard curve.

*** Statistical analysis**

All statistical analysis are calculated by using SPSS version 26 software.

Continuous data were expressed as means±SD. Kolmogorov-Smirnov test is used to determine the normality distribution of data. Comparison of continuous paired variables were analyzed using Paired sample T test for normally distributed data, and Wilcoxon signed ranks test for not normally distributed data. The correlation between continues variable are measured by spearman test if the data is not normally distributed. P value of less than 0.05 was considered significant.

*** Results and Discussion**

*** Detection of *ClpB* and *BspA* genes in *H.pylori* by qRT- PCR technique**

In this study, 160 samples collected from 80 subjects to detect the presence of *H.pylori* by qRT-PCR technique, the precise *ClpB* and *BspA* primers genes were used for the amplification of these genes for identification of copy number of *H.pylori*. The results showed that, there have been present of *ClpB* and *BspA* in all gastritis patients (100%). These results were in agreement with a previous study of Curtis *et al*., (2020) who found that, *H.pylori* is capable of inducing characteristic gastritis disease, it was the main microbes responsible for gastritis .qRT-PCR may be a DNA amplification technique that permits precise determination of macromolecule levels by observation

fluorescent signals at a cycle-to-cycle rate, transfer vital contribution to the detection of infective microorganism in gastritis infections (Dioguardi *et al*., 2020).As a diagnostic method, it was used molecular biology (PCR) for the identification of bacterial DNA (Kalcioglu *et al*., 2018). Stinson *et al*., (2019) has been shown that, PCR will offer up to 41-fold more sensitivity in comparison with colony numeration for best anaerobic species, providing 36% to 51%, will increase within the explicit cases of *H.pylori.*Molecular detection and quantification of oral microorganism are principally achieved by analysis of the ribosomal sequences (Jin *et al*., 2022). *H.pylori* is closely related to gastritis disease. Its presence is mainly found in the easily accumulated ulcer in patients with fixed appliances. It is essential to diagnose it for a treatment that can avoidharm to the patient. It was been considered as the common gastritis pathogens for the progression of gastritis disease (Contaldo *et al*., 2021).it has been shown that, variations in ribosomal DNA copy numbers among species and strains could impair correct determination of cell levels. During this sense, the *bspA* and *ClpB* protein-encoding genes has been pressed as a replacement marker for microorganism ecology research, providing biological process resolutions similar to those of the *16S rRNA* sequence at numerous compartmentalization levels, the *clpB* and *bspA* single-copy super molecule encryption genes are used as different markers for quantification of *Hpylori* that providing satisfactory detection sensitivities in samples (Saito *et al*., 2009).

*** The difference of specific *BspA* and *ClpB* genes according to study groups**

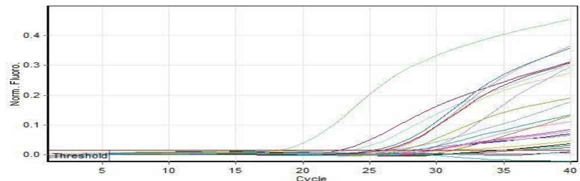
The mean differences in specific BspA and ClpB genes were analyzed across various study groups (Group A, Group B, and Group C) in comparison to a control group. The results indicated that there was no significant difference in BspA levels among the study groups; however, a significant difference was observed in the ClpB gene levels. These findings align with those reported by Jin et al. (2022), who found no significant difference in the prevalence of H. pylori BspA genotypes among the study groups compared to the control group, but did find a significant difference in the ClpB gene. Conversely, these results conflict with those obtained by

Highfield (2009).This study underscores the importance of analyzing specific genetic markers in understanding the pathogenesis and prevalence of H. pylori-related conditions. The significant variation in ClpB gene levels suggests a possible role in disease progression that warrants further investigation. The discrepancy with Highfield's findings highlights the complexity of genetic influences in different populations or study designs, suggesting that additional research is needed to reconcile these differences and gain a more comprehensive understanding of the genetic factors involved.Further studies should aim to clarify the mechanisms by which ClpB contributes to disease and explore the potential for these genetic markers to serve as diagnostic or therapeutic targets. Additionally, the lack of significant difference in BspA among study groups suggests that it may not play a critical role in the same contexts, though its functions in other aspects of H. pylori pathology should not be entirely ruled out. Understanding these dynamics is crucial for developing more effective strategies to manage and treat H. pylori-related diseases.

*** The difference of IL6,IL-8 according to study groups**

The study analyzed the mean difference in matrix metalloproteinase-8 across various groups, revealing a significant difference (P<0.001) in IL-6 and IL-8 levels between them. These results are consistent with Nardi et al. (2020), who also found significant differences in IL-6 and IL-8 levels in the SRP group. In contrast, Hendiani *et al.* (2021) did not observe significant differences in these cytokines in the SRP group.Despite therapy, IL-8 levels in gastritis patients remained higher than in the control group, contrary to Leppilahti *et al*. (2014), who found comparable IL-8 levels in gastritis and control groups. Komala *et al*. (2019) reported that non-surgical periodontal treatment led to a significant and rapid reduction in IL-8 concentrations in patients with gastritis.Helicobacter pylori (H. pylori) secretes IL-8, though in smaller quantities than host cells, but it enhances host-derived IL-8 activity, playing a pathological role in gastritis progression (Emingil *et al*., 2012). H. pylori bspA/ClpB genotypes were found in significantly higher concentrations in gastritis sites than in control sites. H. pylori is linked to multiple diseases, underlining its

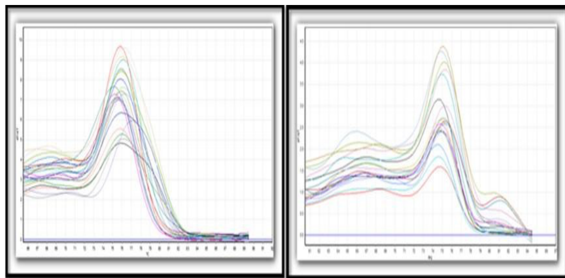
medical importance (Muñoz-Carrillo *et al*., 2019). The high odds ratio of H. pylori in gastritis strongly suggests its role in the disease, and the prevalence of bspA/ClpB genotypes serves as a useful marker for gastritis (Byrne *et al.,* 2009).

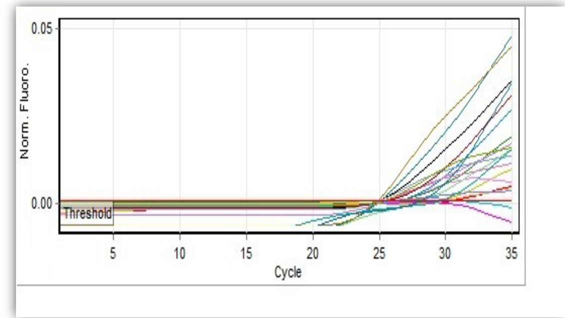


*** ClpB gene amplification plots by qPCR. Samples included all samples. The photograph was taken directly from qPCR machine**

*** Detection of *BspA* and *ClpB* genes in *H.pylori* by RT-PCR technique**

In this study, 80 samples were detected for present of ***H.pylori*** from by q RT-PCR technique, the specific *BspA* and *ClpB* genes were used for the amplification of a fragment, these genes were used for identification of ***H.pylori*** . The results showed that, there were present of *BspA* and *ClpB* genes in all 80(100%) samples as shown in Figure (4- 2, 4-3).





*** BspA gene amplification plots by qPCR. Samples included all samples. The photograph was taken directly from qPCR machine**

*** Conclusion**

The study successfully detected *Helicobacter pylori* in 120 samples using the qRT-PCR technique. The specific ClpB and BspA primer genes were effectively utilized to amplify fragments of these genes, ensuring accurate identification of *Helicobacter pylori.* This method proves to be a reliable and precise approach for the detection and identification of Helicobacter pylori in clinical samples, contributing to better diagnostic practices and potential treatment strategies for infections caused by this pathogen.

*** Conflicts of interest**

The authors report no conflict of interest.

*** Declarations of interest**

None.

*** Acknowledgement**

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