Original Article

Investigated the Link between Some Cytokines and Gene Expression of Gastritis in Type 2 Diabetic Patients in Iraq

Zahraa Maad Abdul-Sahib[1], Najah Ali Mohammad[2], Abdul Razzaq Nema[3] and Issam Abdul-Karim Selman[4]

ABSTRACT

Objective: To investigate immunological and molecular features of gastritis in individuals with diabetes mellitus.
Study Design: Comparative study
Place and Duration of Study: This study was conducted at the Gastroenterology & Hepatology Teaching Hospital, Medical City, Baqubah Teaching Hospital, and Al-Kazemi Teaching Hospital, Department of Gastroenterology, from 1st January 2024 to 30th April 2024.
Methods: A total of 138 participants including 67 H. pylori gastritis with type 2 diabetic (GHp+ve DM) group, 21 with gastritis without diabetes mellitus (G group), and 50 control group.
Results: The GHp+ve, diabetes mellitus had reduced levels. Regarding IL-10 in comparison to G and control, IL-18 levels increased in GHp+ve. In addition, discovered GHp+ve diabetes mellitus had lower levels of IL-10 than G ($p<0.05$). Moreover, found IL-18 more highly expressed in GHp+ve DM than in gastritis ($p<0.05$).
Conclusion: The gastritis by H. pylori infection to type 2 diabetes and shows IL-10 levels reduced in GHp +ve diabetes mellitus contrast with gastritis group and healthy controls and inversely correlated with high-sensitivity C-reactive protein, IL-18 levels raised in GHp +ve.

Key Words: Gastritis, Gene expression of IL-10 and IL-18, Helicobacter Pylori, RT-qPCR, Type 2 diabetic

Citation of article: Abdul-Sahib ZM, Mohammad NA, Nema AR, Selman IAK. Investigated the Link between Some Cytokines and Gene Expression of Gastritis in Type 2 Diabetic Patients in Iraq. Med Forum 2025;36(6):72-77. doi:10.60110/medforum.360615.

INTRODUCTION

Gastritis mean inflammation mucosalining of stomach. Major etiological agent's Helicobacter pylori infection and nonsteroidal anti-inflammatory medications.[1] Helical bacteria (Helicobacter pylori) common causes of gastritis has many feature which is gram-negative, microaerophilic and S-shaped bacteria.

[1.] Pathological Analysis Laboratory, Jazani Al-Imam Health Centre, Al-Khalis Sector for Primary Health Care, Diyala Health Department, Iraq.
[2.] Medical Technical Institute, Baghdad.
[3.] Department of Medical Laboratory Technology, College of Health & Medical Technique, Middle Technical University, Baghdad.
[4.] Department of Gastroenterology and Hepatic Teaching Hospital, Medical City Baghdad.

Correspondence: Zahraa Maad Abdul-Sahib, Pathological Analysis Laboratory, Jazani Al-Imam Health Centre, Al-Khalis Sector for Primary Health Care, Diyala Health Department, Iraq.
Contact No: 07726220065
Email: zahraaharbi97@gmail.com

Received: January, 2025
Reviewed: February, 2025
Accepted: March, 2025

The organism's exceptional motility attributed to a solitary cluster of enclosed flagella located at one end.[2] Due to inhabit mucosal layer of stomach's surface epithelium, stimulating persistent gastritis that may progress to cancer of stomach.[3,4] The interaction between H. pylori and superficial mucosa releasing of pro- and anti-inflammatory cytokines. These cytokines subsequently recruit polymorph nuclear cells and initiate inflammatory response.[5]

Type 2 diabetic is chronic metabolic disorder characterized by persistently elevated blood glucose levels. T2DM caused by defect in insulin function, either due to insulin resistance, inadequate insulin secretion, or due to a combination of both factors. [6,7] Infection with H. pylori can cause inflammation and production of inflammatory cytokines, leading to onset of diabetes.[8] Because chronic inflammatory response of H. pylori infection the neutrophils, T-cell and B-lymphocytes, macrophages, and plasma cells recruitment to gastric mucosa which promote continuous and localized inflammation.[9]

Activation and migration these inflammatory cells to stomach mucosa linked to an increased release proinflammatory cytokines and a reduction in anti-inflammatory cytokines, such as IL-10 and IL-18, subsequent to H. pylori infection.[10] IL-10, an

inflammatory mediator, secreted and generated by stomach epithelial cells in response to infection caused by H. pylori.[11] It plays a crucial role as an immunosuppressive cytokine with immune regulatory and antigenic effects.[12] IL-10 synthesized by a range of cell types, including innate lymphocytes, T cells, dendritic cells (DC), macrophages, and B cells.[13] As IL-10 gene affects inflammation, any change in IL-10 gene leads to increased production proinflammatory cytokines, which affects insulin action and causes type 2 diabetes.[14] The IL-18, belonging to IL-1 superfamily of cytokines, has a function in controlling both adaptive and innate immune responses. More precisely, it plays a role in stimulating synthesis IFN-γ in natural killer cells and CD4 T helper 1 lymphocytes.[15] Furthermore, IL-18 inhibits activity of Th2 and Th17 cells and regulates function of CD8 cytotoxic cells.[16] The evidence links genetic variations in IL-18 gene to increased susceptibility to H. pylori infection. This study examined serum concentration IL-18 and IL-10 in patients with type 2 diabetes and H. pylori-associated gastritis compared to those with gastritis and a healthy control. Blood glucose, HbA1c, BMI, H. pylori IgG, and hs-CRP that act as systemic inflammatory marker estimated in all three groups. Additionally, expression both IL-18 and IL-10 genes quantified using RT-qPCR.[17,18]

METHODS

The study involved 138 Iraqi participants, including 88 patients with gastritis (62 males, 26 females aged 16-77 years) and 50 healthy controls matched for age and sex, with no symptoms gastritis or diabetes and not on medications. Diagnosis gastritis in patients confirmed by doctors based on clinical symptoms and result gastric biopsy reports patients who underwent gastroscopy in one of hospitals: Gastroenterology and Hepatology Teaching Hospital, Medical City, Baqubah Teaching Hospital, and Al-Kazemi Teaching Hospital, Department of Gastroenterology, from January 2024 to April 2024. Diabetes was diagnosed based on fasting blood sugar (FBS) ≥120 mg/dl and HbA1c ≥6.5%. Subjects with neoplastic, autoimmune, inflammatory bowel, type 1 diabetes, or severe kidney and liver diseases were excluded.

Blood samples of 3 ml were collected in gel tubes, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 minutes to obtain serum, which was stored at -20°C until analysis. Serum samples were used to quantitatively measure H. pylori IgG levels using a specific ELISA kit (Elabscience). Additionally, levels of hs-CRP, IL-10, and IL-18 were also measured using ELISA kits following the manufacturer's instructions. The results were read using a semi-automated human reader. All serological and biochemical assessments were performed on both patients and healthy controls.

Diabetes screened by estimating FBS ≥120mg/dl and HbA1c ≥6.5 %, immediately after sample collection by Roche kit using a fully automated analyzer"INTEGRA-400 plus" and determine body mass index (BMI)kg/m^2normal range (18.5–24.9).

Gene expression analysis for IL-10 and IL-18 involved extracting RNA from blood samples of 38 participants - comprising 12 with H. pylori gastritis and type 2 diabetes mellitus, 13 with gastritis only, and 13 healthy individuals. Blood (2 ml) was collected in EDTA tubes, transferred into Eppendorf tubes with Trizol reagent, and stored at -20°C. RNA was isolated using the TransZol up plus RNA kit, and its concentration and purity were measured with a nanodrop device, ensuring acceptable purity ranges specific to each group. The extracted RNA was reverse transcribed into cDNA using the EasyScript® SuperMix kit. RT-PCR was performed to assess gene expression levels of IL-10, IL-18, and GAPDH (internal control) using SYBR Green dye. Primer sequences in Table 1 were designed accordingly, and qPCR reactions were conducted at an optimal annealing temperature of 64°C. The relative gene expression was analyzed using the 2-ΔΔCT method to determine changes in IL-10 and IL-18 expression levels among the groups. Statistical analysis performed using IBM SPSS statistics software (version 26.0) with Graph Pad Prism (8.4.3).

RESULTS

There were 89 male participants (64.5%) and 49 female subjects (35.5%) out of total 138 subjects. Percentage Ghp+ve. DM was (48.6%), percentage G was (15.2%), and Control was (36.2%). H. pylori IgG and hs-CRP levels: The IgG levels highly elevated at 247.74±3.38 in GHp+ve. DM compared to G and control ($P<0.05$). Control found to be 98.33±.96 and a slight elevation could be seen in G group at 99.44±1.48 suggesting non-significant in gastritis contrasting to control ($P>0.05$). In addition to IgG, levels of hs-CRP exhibit a substantial increase in average (10.87±0.27) for GHp+ve.DM participants ($p<0.05$), compared to average level of hs-CRP (5.46±0.29) for G subjects and control (5.31±0.14) [Fig. 1)

Level of HbA1c shown significantly elevated among patients in GHp +ve and DM from 4.58±.04 (control) to 10.11±.24 ($p<0.05$). On other hand, gastritis patients showed a slight increase (4.82±.07) compared to control. This likely confirms relationship between diabetes and gastritis. A similar trend was observed with FBS levels, where their levels were abnormally high among GHp+ve.DM patients by 172.70±4.01 ($P<0.05$) compared to group G 79.57±1.32 and control 82.12±0.99. As compared to mean level BMI(kg/m2) (22.56±0.23) for control, no significant changes seen among subjects.GHp+ve.DM 24.21±0.27 and G 24.64±0.33 ($P>0.05$). This same level was seen among

gastritis subjects also which clearly states that BMI is not influenced in study (p>0.05) [Fig. 2].

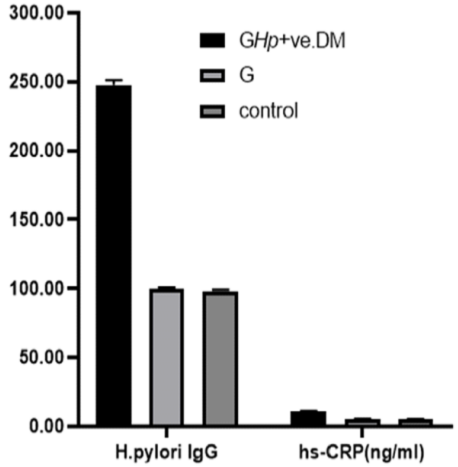

Figure No. 1: Comparative mean levels of H .pylori IgG level and hs-CRP between studied groups

After measuring levels IL-10 and IL-18 by ELISA and comparing levels of interleukins between GHp+ve.DM, G, and control, IL-10 found abnormally lowered to 9.16±0.14 and 24.36±2.48 for GHp+ve.DM and G groups respectively (P<0.05) from 44.73±2.39. On the other hand, IL-18 was found to be elevated to 327.30±12.58 and 124.41±3.54 respectively for GHp+ve.DM and G from 81.33±1.72 (control) result of ANOVA P-values (P<0.05 for both IL-10 and IL-18) [Fig. 3].

Result gene expression IL-10 and IL-18 between studies groups performed by using RT-qPCR and quantitative $2^{-\Delta\Delta CT}$. Relative expression ratio of each gene member to control gene used to show expression. Relative expression of IL-10 was found to be down regulated in subjects when compared to control (p<0.01). The GHp+ve.DM was found to be down regulated to 0.63 fold when compared to 1.00 control (P<0.05) [Fig. 4 & 5]. Similarly, in case of G, level found to be down regulated to 0.75 from 1.00 (control).

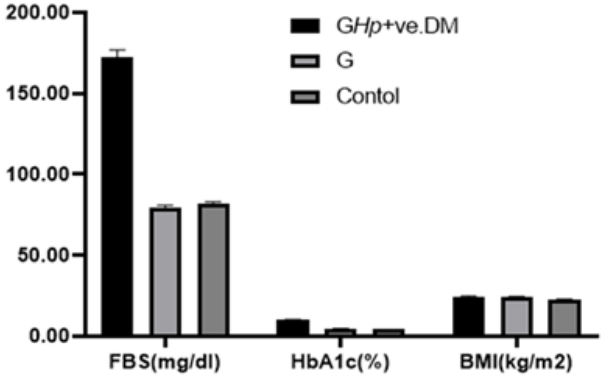

Figure No. 2: Comparative mean levels of FBS, HbA1c and BMI between studied groups

Table No.1: Real time PCR Primers sequence for IL-10 and 1L-18 Gene

Gene	Primer sequence (5′–3′)	Base pairs	References
IL-10	TCTCCGAGATGCCTTCAGCAGA	22	19
	TCAGACAAGGCTTGGCAACCCA	22	
IL-18	GATAGCCAGCCTAGAGGTATGG	22	20
	CCTTGATGTTATCAGGAGGATTCA	24	
GAPDH	GTCTCCTCTGACTTCAACAGCG	22	21
	ACCACCCTGTTGCTGTAGCCAA	22	

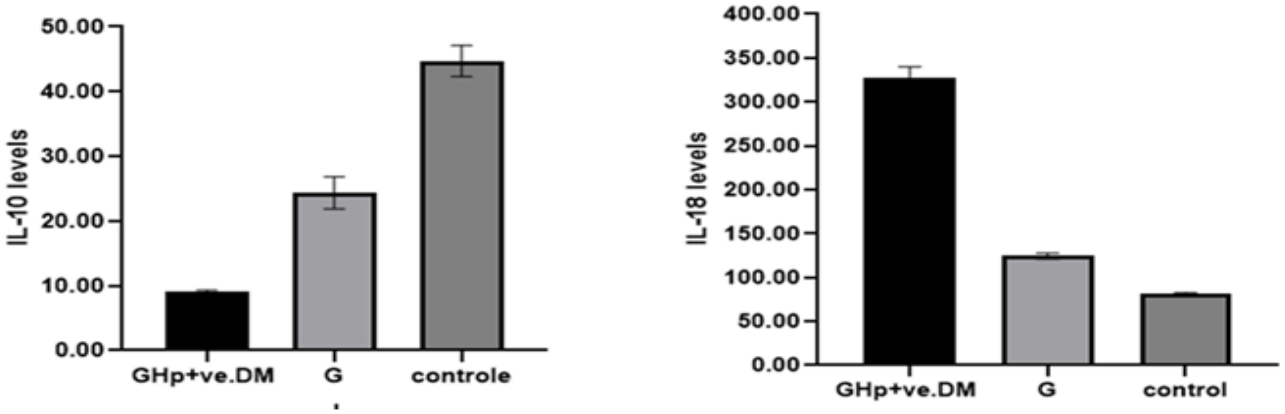

Figure No. 3: Comparative mean levels of IL-10 pg/ml (left) and IL-18 pg/ml (right) between studied groups

A similar trend was seen with cytokine level expression by ELISA method (P<0.05). Conversely, it was found participants' GHp+ve.DM relative expression IL-18 (2.6432) was increased in comparison to control (1.0025) (p<0.01). A similar trend seen among G group also demonstrating an increase IL-18 levels (1.245)

from 1.0025 (control) were shown in Figures 4 and 5. Association between T2DM and cytokine expression: Appeared important link between GHp +ve. DM and G group about cytokines (r=0.5515, P=0.014). Nevertheless, no statistically significant difference was seen between male and female participants (p<0.01). Spearman rank correlation analysis showed that T2DM was directly proportional to Cytokine levels within the plasma (r = 0.6132743, P < 0.001). We found IL-10 levels to be down regulated in H. pylori-associated gastritis than gastritis (p<0.01). At the same, found IL-18 to be upregulated in H. pylori-associated gastritis than gastritis (p<0.01).

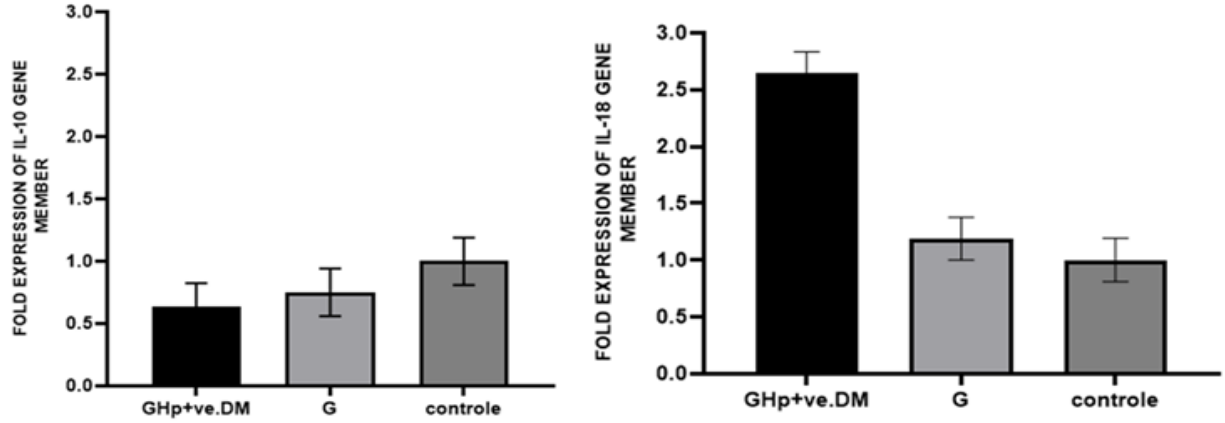

Figure No. 4: Graph showing relative fold expression of cytokine gene members (IL-10 and IL-18) of both subjects and control

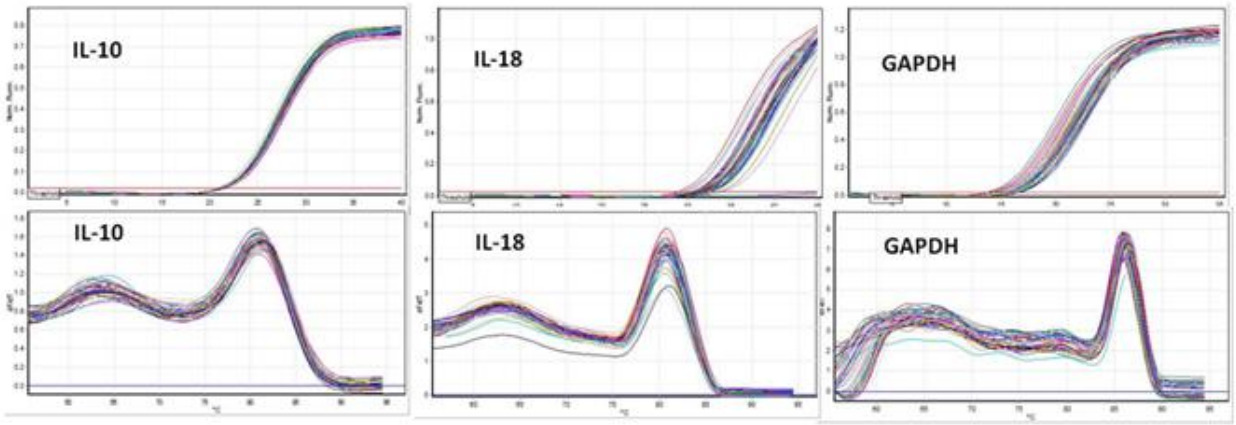

Figure No. 5: qPCR Ct and melt curves. Top: Ct curve; Bottom: Melt curves of respective gene members

DISCUSSION

Correlation between gastritis, specifically in H. pylori infection, and diabetes is intricate and diverse. Research has been conducted found individuals diagnosed with diabetes type 2, are more likely to have H. pylori infection. One study showed that 71.4% of diabetic patients tested positive for IgG antibodies against bacterium, indicating a strong bond between H. pylori infection and diabetes.[19] This study revealed link between IL-10 and IL-18 within GHp+ve.DM, which shows IL-10, a cytokine recognized for its anti-inflammatory characteristics, plays essential function in inflammatory response, associated with diabetes. Furthermore levels decreased in GHp+ve.DM, In agreement with research conducted by Novianti and others[20], a study revealed reduced level in individuals with diabetes, in particular patients with oral problems, in comparison to healthy controls.This suggests that inflammation may inhibit IL-10 production, indicating an insufficient anti-inflammatory response and explaining development of inflammation in these patients.[21] Interleukin-18 is a pro-inflammatory protein released by stimulated macrophages, epithelial cells, bone marrow cells keratinocytes, and malignant cells. It is one of cytokines involved in inflammation.[22] Through this research, elevated IL-18 levels were observed in both gastritis and diabetes., which is consistent with Abdel-Moneim et al[23] that indicating a heightened inflammatory response. Multiple investigations have demonstrated a distinct association between H. pylori infection with both type 2 diabetes mellitus (T2DM) and insulin resistance.[24] Root cause of insulin resistance and type 2 diabetes is chronic, mild inflammation that persists over time. Aggregation of active innate immune cells in metabolic organs

ultimately results in secretion of pro-inflammatory cytokines, including IL-10 and IL-18. Subsequently, these cytokines cause harm to β-cells and lead to emergence of insulin resistance.[25] H. pylori intestinal lipopolysaccharides found to be associated with activation of Toll-like receptors. This activation, in turn, leads to fat precipitation, energy harvesting, and stimulation of innate immunity. These factors all contribute to insulin resistance.[26] For level of H .pylori IgG, results of investigation indicated a significant elevated in GHp+ve.DM (247.74±3.38) which aggregated with Al-Mamari et al[27], while no statistical significance between G (99.44±1.48) and control (98.33±.96). The C-reactive protein: main source of human CRP is hepatocytes, and its production is controlled by inflammatory cytokines TNF-α and IL-10. Previous studies have mostly examined correlation between risks of diabetes with quantity of high-sensitivity CRP (hs-CRP), in our study noticed significant increase (10.87±0.27) in GHp+ve.DM participants. Compared to G participants (5.46±0.29) and control (5.31±0.14) related with study of Li et al[28], that found 65.28% of diabetic patients H. pylori positive, with mild increases hs-CRP levels noted.

Regarding present study, direct association observed between H. Pylori infection in diabetes patients with HbA1c% levels, which is a measure of their glycemic status. The GHp+ve.DM had significantly elevated HbA1c levels compared to control (4.58±.04 vs. 10.11±.24, $p<0.05$). Nevertheless, individuals who only had gastritis experienced a slight rise (4.82±.07) compared to control. This is likely to confirm connection between diabetes and gastritis. The FBS readings exhibited a comparable pattern.[21] While BMI among three groups non-significant ($p>0.05$) due to anorexia, difficulty swallowing, and chronic gastritis.[29] Expression levels of both cytokines (IL-10 and IL-18) measured using serum samples obtained from both participant and control by a real-time PCR technique. When compared to control, participants' relative expression of IL-10 found to be downregulated ($p<0.01$). Compared to 1 (control), GHp+ve.DM shown to be downregulated by 0.63 fold ($P<0.05$) enhanced by Mitra et al.[30] Comparably, in instance of G, level was seen to have been downregulated from 1 (control) to 0.75. By using ELISA approach, a similar trend in cytokine level expression was observed ($P<0.05$). On reverse, when evaluating to control (1.0025) ($p<0.01$), participants' (GHp+ve.DM) relative expression of IL-18 (2.6432) was shown to be higher (Fig. 4). A similar pattern was observed in G group, which similarly showed an increase IL-18 levels from (1.0025) control to (1.245).

CONCLUSION

The gastritis by H. pylori infection to type 2 diabetes and shows IL-10 levels reduced in GHp+ve. DM contrast with gastritis group and healthy controls and inversely correlated with high-sensitivity C-reactive protein, IL-18 levels raised in GHp+ve. IL-10 significantly downregulation in GHp+veDM, in contrast, IL-18 expression significantly upregulated for GHp +ve diabetes mellitus confirming gene's influence on H. pylori and diabetes.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zahraa Maad Abdul-Sahib, Najah Ali Mohammad
Drafting or Revising Critically:	Abdul Razzaq Nema, Issam Abdul-Karim Selman
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.7739 Dated 13.01.2023

REFERENCES

1. Lian DW, Xu YF, Ren WK, Fu LJ, Chen FJ, Tang LY, et al. Unraveling the novel protective effect of patchouli alcohol against helicobacter pylori-induced gastritis: insights into the molecular mechanism in vitro and in vivo. Frontiers Pharmacol 2018;9:1347.
2. Allahverdiyev AM, Bagirova M, Caliskan R, Tokman HB, Aliyeva H, Unal G, et al. Isolation and diagnosis of Helicobacter pylori by a new method: microcapillary culture. WJG 2015; 21(9):2622.
3. Suzuki H, Mori H. World trends for H. pylori eradication therapy and gastric cancer prevention strategy by H. pylori test-and-treat. J Gastroenterol 2018;53:354-61.
4. Hussaen DYK, Muhammaed DNA, Al-Satar SA. New staining solution for identification of helicobacter pylori in the gastric tissue. J Techniques 2009;22(2).
5. Bockerstett KA, DiPaolo RJ. Regulation of gastric carcinogenesis by inflammatory cytokines. Cellular Molecular Gastroenterol Hepatol 2017;4(1):47-53.
6. Alaa Abed M, Walaa Ismael J, Ahmed Abdul Hussein M, Ahmed MS. Concentration of asprosin associated with poor control of type 2 diabetes mellitus in Diyala Province. J Techniques 2023; 5(2):123-8.
7. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017;389(10085):2239-51.
8. Nodoushan SAH, Nabavi A. The interaction of Helicobacter pylori infection and type 2 diabetes mellitus. Advan Biomed Res 2019;8(1):15.

9. Kim N. Immunological reactions on H. pylori infection. Helicobacter pylori: Springer; 2024; 39-59.
10. Nguyen PM, Putoczki TL. Could the inhibition of IL-17 or IL-18 be a potential therapeutic opportunity for gastric cancer? Cytokine 2019; 118:8-18.
11. Bagheri N, Salimzadeh L, Shirzad H. The role of T helper 1-cell response in Helicobacter pylori-infection. Microbial Pathogenesis 2018;123:1-8.
12. Ma X, Yan W, Zheng H, Du Q, Zhang L, Ban Y, et al. Regulation of IL-10 and IL-12 production and function in macrophages and dendritic cells. F1000 Res 2015;4.
13. Comi M, Amodio G, Gregori S. Interleukin-10-producing DC-10 is a unique tool to promote tolerance via antigen-specific T regulatory type 1 cells. Frontiers Immunol 2018;9:682.
14. Al-Mansoori L, Al-Jaber H, Prince MS, Elrayess MA. Role of inflammatory cytokines, growth factors and adipokines in adipogenesis and insulin resistance. Inflammation 2022;1-14.
15. Yasuda K, Nakanishi K, Tsutsui H. Interleukin-18 in health and disease. Int J Molecular Sci 2019;20(3):649.
16. Techatanawat S, Surarit R, Chairatvit K, Khovidhunkit W, Roytrakul S, Thanakun S, et al. Salivary and serum interleukin-17A and interleukin-18 levels in patients with type 2 diabetes mellitus with and without periodontitis. PLoS One 2020; 15(2):e0228921.
17. fadil ma, Maroof RE, Fadil MA. Studying the role of C-reactive protein in patients with sleeve gastrectomy in Baghdad. J Techniques 2021; 3(1):55-60.
18. Del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. Brain Behavior Immunity 2018;70:61-75.
19. Mohammadzadeh A, Eivary SHA, Khajavian N, Hajavi J. Investigating the Relationship between Type 2 Diabetes and Helicobacter Pylori. J Shahid Sadoughi Univ Med Sci 2023[31(8): 6955-64.
20. Novianti Y, Nur'aeny N. Exploring interleukin-10 levels in diabetes patients with and without oral diseases: a systematic review. J Inflamm Res 2024;541-52.
21. Maluf S, Salgado JV, Cysne DN, Camelo DMF, Nascimento JR, Maluf BVT, et al. Increased glycated hemoglobin levels in patients with helicobacter pylori infection are associated with the grading of chronic gastritis. Frontiers Immunol 2020;11:2121.
22. Bebernitz CE. IL-12 and IL-18 combination improves activation of adoptively transferred T cells in solid tumors: Weill Cornell Med 2021; 13:65.
23. Abdel-Moneim A, Abd El-Twab SM, Nabil A, El Kazafy SA. Effect of antidiabetic therapy on TNF-α, IL-18, IL-23 and IL-35 levels in T2DM patients with coincidental Helicobacter pylori infection. J Taibah Univ Sci 2020;14(1): 1377-85.
24. Man S, Ma Y, Jin C, Lv J, Tong M, Wang B, et al. Association between helicobacter pylori infection and diabetes: a cross-sectional study in China. J Diabetes Res 2020;2020(1):7201379.
25. Sharif S, Van der Graaf Y, Cramer MJ, Kapelle LJ, de Borst GJ, Visseren FL, et al. Low-grade inflammation as a risk factor for cardiovascular events and all-cause mortality in patients with type 2 diabetes. Cardiovasc Diabetol 2021; 20:1-8.
26. Frauenlob T, Neuper T, Regl C, Schaepertoens V, Unger MS, Oswald AL, et al. Helicobacter pylori induces a novel form of innate immune memory via accumulation of NF-κB proteins. Frontiers Immunol 2023;14:1290833.
27. Al-Mamari A, Almiyah S. Estimate the prevalence of helicobacter pylori infection among diabetes & non-diabetes mellitus patients and its correlation with malignant gastritis patients attending in lower Shabelle Region (Somalia). J Biosci Med 2024;12(4):38-48.
28. Li W, Kashif MR, Yuping Y, Xia C, Hui X, Qili J. Association of Helicobacter pylori infection with metabolic and inflammatory profile in type 2 diabetes mellitus. Int J Diabetes Developing Countries 2020;40:47-51.
29. Li Y, Su Z, Li P, Li Y, Johnson N, Zhang Q, et al. Association of symptoms with eating habits and food preferences in chronic gastritis patients: a cross-sectional study. Evid Based Complement Alternat Med 2020;2020(1):5197201.
30. Mitra P, Suri S, Saxena I, Shukla RK, Shukla KK, Sharma P. Downregulation of interleukin-10 receptor (IL-10R) along with low serum IL-10 levels in newly diagnosed type 2 diabetes mellitus patients. Gene Reports 2021;24:101251.