Archives of Biological Sciences

ACCEPTED MANUSCRIPT

This article can be cited before page numbers have been issued; to do this please use:
Riezzo G, Orlando A, Clemente C, D'Attoma B, Valentini AM, Armentano R, Giorgio P, Pisani A, Russo F. Tissue transglutaminase is involved in the inflammatory processes of active chronic gastritis. Arch Biol Sci. 2021;https://doi.org/10.2298/ABS210412026R.

Tissue transglutaminase is involved in the inflammatory processes of active chronic gastritis

Giuseppe Riezzo[1], Antonella Orlando[1], Caterina Clemente[1], Benedetta D'Attoma[1], Anna Maria Valentini[2], Raffaele Armentano[2], Pietro Giorgio[3], Antonio Pisani[3] and Francesco Russo[1,*]

[1]*Laboratory of Nutritional Pathophysiology, National Institute of Gastroenterology Research Hospital, IRCCS "Saverio de Bellis"*, Castellana Grotte (BA), Italy
[2]*Department of Pathology, National Institute of Gastroenterology Research Hospital, IRCCS "Saverio de Bellis"*, Castellana Grotte (BA), Italy
[3]*Department of Gastroenterology, National Institute of Gastroenterology Research Hospital, IRCCS "Saverio de Bellis"*, Castellana Grotte (BA), Italy

***Corresponding author:** francesco.russo@irccsdebellis.it

Received: April 12, 2021; **Revised:** May 28, 2021; **Accepted:** June 9, 2021; **Published online:** June 14, 2021

Abstract: Since tissue transglutaminase-2 (TG2) can represent a marker of inflammation for some gastrointestinal (GI) diseases, we aimed to evaluate TG2 and inflammatory markers' mucosal content in gastric antrum biopsies to shed light on the histological and biochemical background of chronic gastritis inflammation. Fifty-one of 78 patients who underwent upper GI endoscopy (UGIE) for dyspeptic symptoms, had a gastric biopsy. The symptom profile was assessed by a GI symptom rating scale (GSRS) score. Thirty-five patients (69%) showed chronic gastritis. TG2, interleukin-6 (IL)-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, lipopolysaccharides (LPS) and toll-like receptor (TLR)-4 were evaluated in serum and the culture medium of gastric biopsies. TG2, IL-8, IL-10, TLR-4 and TNF-α were significantly higher in active chronic gastritis than in the inactive one and were linked to macrophage concentration. IL-6 was significantly lower in the active form of chronic gastritis than in the inactive one and negatively correlated with TG2. Lastly, IL-10 significantly correlated with the macrophage score. TG2 can exert an active role in chronic gastritis pathogenesis by cooperating with different markers of inflammation. It seems that TG2 can represent a possible therapeutic target for modulating inflammation and disease progression.

Keywords: chronic gastritis; inflammation; interleukins; transglutaminase

INTRODUCTION

Human tissue transglutaminase, also known as transglutaminase 2 (TG2), belongs to the transglutaminase family and can perform many different biological functions [1]. For example, TG2 can catalyze the deamidation or transamidation of specific proteins and peptides. Despite the variety of activities undertaken by TG2, the biological role of these interactions has not yet been completely clarified [1]. TG2 is abundantly expressed in endothelial cells, macrophages and smooth muscle cells, and is known to be involved in several physiological activities, including immune processes, apoptosis, angiogenesis, wound healing, cellular differentiation, neuronal regeneration, bone development [2], and in several inflammatory, autoimmune, and neoplastic diseases [3]. TG2 is in a closed conformation in normal conditions, maintained by guanosine-5'-triphosphate and integrin bindings. However, the physical and chemical injuries can open the conformation and activate the enzyme resulting in the most active enzyme in terms of functions, structures and regulatory mechanisms [4].

The best-known role of TG2 is in celiac disease (CD) where this enzyme exerts at least two critical functions. As a deamidating enzyme it increases the immune-stimulating effect of gluten, and as an autoantigen target TG2 mediates the immune response. Since glutamine-rich gliadin peptides are excellent substrates for the enzyme and the resulting deamidated and negatively

charged peptides have a much higher affinity for HLA-DQ2/HLA-DQ8 molecules, the activity of TG2 is considered a crucial step in CD pathogenesis [5].

The presence of anti-TG2 in the culture of the supernatant of gastrointestinal (GI) biopsies in patients suffering from pathologies other than CD has been demonstrated, including normal/negative circulating anti-TG2 in esophageal and gastric diseases associated with *Helicobacter pylori* (Hp) along with extraintestinal disorders. All these conditions are characterized by chronic inflammation, which opens the way for a field of study not yet explored [6-7].

Chronic gastritis is a widespread disorder with severe sequelae as peptic ulcer or gastric cancer [8]. Hp infection is thought to be the leading cause of chronic gastritis, although new information suggests it may even be part of the human microbiome composition, hypothesizing for this bacterium a role as a commensal or symbiont [9]. At present, more than half of the people in the world suffer from chronic gastritis, and a very long-lasting and aggressive inflammation in the stomach can determine the destruction of the gastric mucosa (atrophic gastritis) over decades [10].

Atrophic gastritis progression impairs gastric functions, causing a permanent, acid-free condition that is a significant risk factor for gastric cancer [11]. Its diagnosis requires histological examination from biopsies taken during upper GI endoscopy (UGIE), although a non-invasive test is currently available based on the serum concentration of gastric function markers [11]. Its progression has been demonstrated in both the presence and absence of Hp, which could explain the increase in gastric cancer cases despite the decline in Hp infection rates [12]. Additionally, gastric cancer risk remains in precancerous conditions such as atrophic gastritis and intestinal metaplasia even after Hp eradication [13]. Non-Hp gastritis is typically mild and more focal than Hp gastritis but tends to progress toward atrophic gastritis and cancer. As a result, mucosal changes can actively participate in the progression of gastric inflammation more than the Hp status alone does.

Gastric mucosa has an immune function involving innate and adaptive immunity and the recruitment of different immune cells and cytokines [14]. Understanding the specific structure of gastric immune tissue and its role in inflammatory responses can prevent the onset and progression of gastric diseases and open the way to their treatment with immunological therapies. Several researchers have demonstrated that TG2 and other markers of inflammation, such as tumor necrosis factor (TNF)-α [15], are more expressed in active chronic gastritis than in the inactive form, probably because they are linked to macrophage concentration [16]. The primary source of TNF-α are macrophages, T cells and lymphoid follicles; in turn, macrophages and monocytes express TG2 that is upregulated by TNF-α together with several other interleukins [17].

More research on animal models and clinical trials should be performed to establish satisfactory answers regarding the precise role of TG2. To our knowledge, no studies have been carried out aimed explicitly at evaluating the correlation between TG2 and inflammation indexes in chronic gastritis. The present study aimed to assess the concentrations of TG2 in the serum and mucosa in patients who had undergone UGIE for dyspeptic symptoms. By placing these values in relation to a series of inflammation indexes, such as TNF-α, interleukin (IL)-6, IL-8, IL-10, lipopolysaccharides (LPS) and toll-like receptor (TLR)-4, this research should shed light on the biochemical background of chronic gastritis inflammation. Since TG2 and other inflammation markers seem to be produced by macrophages during inflammation, histochemical evaluation of macrophages was also performed on the gastric biopsies.

MATERIALS AND METHODS

Patient recruitment

This study was approved by both the local Scientific Committee and the Institutional Ethics Committee of IRCCS Ospedale Oncologico di Bari – Istituto Tumori Giovanni Paolo II, Bari, Italy (n. prot C.E. 83 24.03.2015) and was performed following the Declaration of Helsinki. All patients were compliant and willing to participate in the study. Written informed consent was obtained from all participants for laboratory investigations and clinical data recording. Patients undergoing UGIE

for dyspeptic symptoms at the Digestive Endoscopy Service of the National Institute of Gastroenterology Research Hospital, IRCCS "Saverio de Bellis" were recruited from February to June 2017. Serum samples and gastric biopsies were obtained and all the relevant data, including demographic information, digestive symptoms, treatments, comorbidity, and any previous Hp treatments, were recorded. Patients who underwent UGIE for reasons other than dyspepsia (e.g. alarm symptoms, bleeding, celiac follow-up), cirrhosis, severe heart or kidney diseases, pregnancy, or lactation, active cancer and those taking medication, were excluded from the study.

Symptom profile

The symptom profile was investigated by administering the GI symptom rating scale (GSRS) questionnaire [18]. The GSRS is a validated GI questionnaire that utilizes a 7-level Likert scale (1-7) based on the intensity and frequency of GI symptoms experienced during the previous seven days. A higher score is representative of the main symptoms presented by the patients. The 7-level scores were merged to obtain a 4-level intensity and frequency score: absent, mild, moderate and severe. The combination scores among the GSRS items identified four clusters: "abdominal pain syndrome", "indigestion syndrome", "diarrhea syndrome" and "constipation syndrome". In this study, 2 of 4 clusters were calculated: (i) "abdominal pain syndrome" comprising abdominal pain (epigastric, colic, and continuous pain), gastric hunger pain and nausea scores; b) "indigestion syndrome", which includes abdominal distension, borborygmi, burping and flatulence scores.

Histology evaluation

Four biopsy samples were used for routine histological evaluation [19], and two samples were grown for 72 h at 37°C. The gastric biopsies were evaluated for the location, the intensity of mononuclear inflammatory cellular infiltrates, inflammatory activity (neutrophilic infiltrations), glandular atrophy, metaplasia, reparative atypia and dysplasia [20]. Chronic gastritis was categorized as active when chronic infection was histologically characterized by polymorphonuclear leukocytes (neutrophils) in a background of chronic inflammation composed of mononuclear cells, predominantly lymphocytes, plasma cells and macrophages. Lymphoid follicle formation and prominent germinal centers could also be seen [21]. Based on the report from trained GI pathologists, active vs inactive chronic gastritis, as well as the chronic inflammation grade, atrophy, intestinal metaplasia (IM) and bacteria on gastric samples were assessed.

Identification of macrophages in the biopsy

Since TG2 is extensively involved in monocyte- and macrophage-mediated physiological and pathological processes, immunohistochemical recognition of macrophages in the biopsies was performed [17]. Formalin-fixed, paraffin-embedded sections (4μm thick) were cut for each antrum sample and mounted on positively charged slides. Immunostaining for CD68 (MAb KP1 Flex, Dako, Denmark) was performed from deparaffinization to counterstaining using the Dako Omnis fully automated staining platform, following the manufacturer's instructions. Positive controls were included for each staining run. For evaluating CD68 positive cells, four fields (x20) with the most positive cells were selected for each slide. Two authors independently counted the number of positive cells and the mean value of each sample was recorded with a three-tiered score as follows: score 1=<20 positive cells; score 2=20-60 positive cells; score 3=60-100 positive cells; score 4>100.

Biochemical analyses

A 10-h fasting blood sample was obtained from all patients. Patients did not receive anti-secretory treatment, including proton-pump inhibitors (PPIs) 2 weeks before the evaluation. EDTA tubes were centrifuged at 2000×g for 15 min, and blood samples were stored at -20°C until the assay was performed. Serum levels of TNF-α, IL-6, IL-8, IL-10, LPS and TLR-4 were measured in duplicate using commercially available sandwich enzyme-linked immunosorbent assay kits (Human IL-6 ELISA, Human IL-8 ELISA, Human IL-10 ELISA, and Human TNF-α ELISA, BD Biosciences, Milan, Italy. LPS and TLR-4 ELISA Cloud-Clone Corp, Katy, TX, USA). Serum levels of human TG2 were detected by an enzyme-linked immunosorbent ELISA kit following the manufacturer's instructions (Human Transglutaminase 2, Sigma-Aldrich, Milan, Italy). Finally, the concentration of

anti-Hp IgG antibodies was calculated using an ELISA test (Hp IgG antibodies, Biohit Oyj, Helsinki, Finland).

Biopsy culture and biochemical evaluations in culture medium

Each antrum biopsy sample was first washed in physiologic saline solution (9 g/L NaCl) and then cultured in complete culture medium (13 mL of Trowell T8 medium, 4 mL of NCTC 135 medium, 15% fetal calf serum (FCS), penicillin 10 000 kU/L, streptomycin 10 000 mg/L, L-glutamine 200 mmol/L and gentamicin 10 g/L) for 48 h at 37°C in an environment of 95% O_2 and 5% CO_2 [22]. Culture supernatants were collected and stored at -80°C until use. TNF-α, IL-6, IL-8, IL-10, LPS, TLR-4 and TG2 levels in undiluted supernatants were determined using the same ELISA kit for serum evaluation.

Statistical analysis

All results were expressed as median and 5°-95° percentiles or mean±SEM unless otherwise specified. Nonparametric tests were performed to avoid violation of the assumption of normal distribution. The Mann-Whitney sum rank test assessed the differences between the groups. Spearman's correlation analysis was performed to determine the putative correlation among TG2, inflammation markers and the macrophage score. Fisher's exact test was used to determine if there were nonrandom associations between categorical variables. All the differences were considered significant at a 5% level. A specific statistical package for exact nonparametric inference (2005 Stata Statistical Software Release9; Stata Corp., College Station, Texas, USA) was used.

RESULTS

Seventy-eight patients with dyspeptic symptoms participated in the study; biopsy sampling was not done on 27 as normal mucosa was found on UGIE examination. Fifty-one patients underwent gastric biopsies, and 35 patients had chronic gastritis (Table 1). The remaining 16 patients showed esophagitis, cardias incontinence and hiatal hernia. The presence of Hp at biopsies was observed in 8 of 11 patients with active chronic gastritis. None of the patients with inactive gastritis showed the presence of Hp in their biopsies. Seven out of 11 (63.63%) patients with active chronic gastritis and 5 out of 24 (20.83%) patients with inactive chronic gastritis were found to be positive for anti-Hp IgG antibodies (cut-off 30 EIU) (Fisher's exact test = 0.022). Therefore, there was a statistically significant association between a history of Hp infection and active gastritis.

Table 2 describes the GSRS score as single items and cluster items. The score of the item "gastric hunger pain" was significantly higher in patients with active gastritis. In contrast, the item "abdominal distension" score was significantly higher in patients with inactive gastritis. No differences were found regarding the cluster GSRS scores.

The serum concentrations of TG2, IL6, IL8, IL10, LPS, TLR-4 and TNF-α did not exhibit any differences between active and inactive forms of gastritis (data not shown). Interestingly, the mean concentrations of TG2 and the inflammation markers in the culture medium were significantly different in the two groups; TG2, IL-8, IL-10, TLR-4 and TNF-α were significantly higher in the active form, whereas IL-6 showed a reduced concentration in active gastritis compared to inactive gastritis (Fig. 1 and Fig. 2).

According to the presence of macrophages in the biopsies, the scores for CD68 were significantly more represented in the active gastritis group than in the inactive one [3.0(1.0-4.0) vs 2 (1.0-4.0), P=0.016]. Spearman's correlation revealed that TG2 negatively correlated with IL-6, while a positive correlation was present between the macrophage score and IL-10 levels; IL-8 negatively correlated with IL-6 (Table 3). The small number of patients with Hp infection did not provide a demonstration of the relationship between the presence of Hp and TG2 and ILs in the culture medium.

DISCUSSION

Gastric mucosal inflammation is a preneoplastic condition that can promote stem cell transition into cancer cells. By discontinuing the inflammatory cascade, a window of opportunity is provided that can prevent neoplastic evolution. The presented results confirm the intriguing role for TG2 in the pathophysiology of chronic gastritis, introducing a specific link between TG2 and different markers of mucosal inflammation as well as a possible block of inflammation via the inhibition of TG2.

According to the type of chronic gastritis, the patients' symptom profile analysis showed that those with active chronic gastritis had a significantly higher score of "gastric hunger pain" and a significantly lower "abdominal distension" score than patients with inactive gastritis. However, it is difficult to conceive a practical use for this in the clinical management of chronic gastritis, likewise as to symptom clusters that differentiate between postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) in functional dyspepsia. Unfortunately, all these scores largely overlap, impeding a distinction based solely on symptoms [23].

It is known that TG2 regulates several physiological processes, including cell differentiation, inflammation, fibrosis and apoptosis [3-6]. Consequently, alterations in the activity of TG2 can have a role in the pathogenesis of several chronic disorders such as inflammatory bowel disease, liver disease, and liver and kidney fibrosis [7,24].

Our data confirm the role of macrophages in the pathophysiology of chronic gastritis. Hp activates macrophages and induces them to secrete nitric oxide synthase, proinflammatory cytokines (i.e., TNF-α, IL-6, IL-1, IL-β) and other chemokines causing inflamed and damaged gastric mucosae [25]. An experimental study in mice with achlorhydria and gastrin deficiency demonstrated bacterial overgrowth of other species other than Hp that are involved in the development of atrophic gastritis and progression to gastric cancer. These bacteria and their metabolites can be directly or indirectly responsible for gastric damage [26].

Macrophages and monocytes express TG2, which in turn is upregulated by inflammatory mediators such as TNF-α and several other interleukins [17]. Many cytokines and growth factors are secreted during the initial phases of inflammation, regulating the expression of TG2. Interferon (IFN)-γ and TNF-α have already been described as acting synergistically to activate TG2 [27], indicating the existence of an autocrine loop in which interleukins such as IL-1 and IL-6 could reinforce TG2 expression [28]. TG2 promotes the clearance of apoptotic cells (efferocytosis), and growing evidence suggests that impaired efferocytosis contributes to the long-lasting consequences of inflammation-associated diseases. [29]. As a result, TG2 seems to modulate inflammation and several other inflammation markers, providing evidence for a possible blockage of inflammation via the modulation of TG2. Besides, a recent paper has highlighted how deletion of TG2 diminished airway inflammation, Th2 responses, profibrotic gene expression and leukotriene level in a murine asthma model [30].

LPS is a crucial component of the outer membrane of gram-negative bacteria, and Hp LPS seems to be crucial in activating inflammatory pathways in monocytes and macrophages [31]. Hp LPS, along with other Hp-related molecules, may contribute to TLR-dependent responses, contributing to bacterial elimination, persistence or pathological reactions [32]. Hp LPS can engage gastric mucosal TLR4 [33], and the Hp virulence factor CagA is a potent factor inducing nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and IκB kinases (IKKs) activation via transforming growth factor β–activated kinase 1 (TAK1), a key regulator of inflammation cascades [34]. TLR-4 has been demonstrated in chronic active gastritis [35], precancerous cells and cancerous gastric cells [36]. In our study, TLR-4 concentrations, but not LPS, were significantly higher in the culture medium of biopsies from active gastritis than in those from inactive ones.

The culture medium concentrations of different inflammation markers highlighted the clear involvement of IL-6, IL-8, IL-10 and TNF-α. Specifically, IL-8, IL-10 and TNF-α increased in active gastritis, whereas IL-6 was reduced significantly, probably facilitating a persistent inflammatory condition. IL-6 significantly affects the host defense since it is promptly produced by monocytes and macrophages, thus contributing to removing infectious agents and restoring

damaged tissues [37]. In the culture medium, a negative correlation was observed between IL-8 and IL-6, and between TG2 and IL-6. The latter correlation further supports the link between TG2 and inflammation. [14]. IL-10 is currently considered an antiinflammatory peptide implicated in the downregulation of human intestinal immune responses. However, enhanced secretion of IL-10 has been demonstrated in gastric mucosae from Hp-infected patients more than in uninfected individuals, confirming the multifaceted functions of IL-10 [38]. The correlation between IL-10 and macrophage score found in our study suggests the cellular origin of IL-10 in chronic gastritis and considers multiple effects of IL-10 on other proinflammatory cytokine secretion.

A relatively new stimulatory aspect is that of the role of TG2 and IL-6 and the spread and metastasis of human cancer cells [39]. Even if the part played by TG2 in cancer is still controversial, a close relationship exists between increased expression of TG2, the inflammatory response and promotion, as well as invasion and spread of several extraintestinal neoplasms [23,40].

Inhibition of TG2 could help to reduce drug resistance and the invasion capacity of several tumors, representing a possible therapeutic target for treating or blocking tumor progression, as demonstrated by the novel peptide GX1 that inhibits angiogenesis by directly binding to TG2 [41-42]. Consequently, as for those extraintestinal inflammatory processes, the same mechanisms could also be hypothesized at the GI level. Recently, TG2 inhibitors have been developed that target the enzyme's active site, but clinically relevant inhibitors are not yet available [43].

The present work has some limitations. First, the small number of patients enrolled in the study could have increased false-positive results and exaggerated effects. The small number of patients did not allow us to compare Hp-positive and negative patients. One must keep in mind that the role of TG2 has already been described in previous studies conducted with a similar number of patients (14). Second, the main interest was identifying inflammation markers and their possible interactions with the histological characteristics of chronic gastritis (active vs inactive) and macrophage activity irrespective of Hp status.

Therefore, histologically, the group with chronic active gastritis included both Hp-positive and Hp-negative forms. A significant association between a history of Hp infection and active gastritis was demonstrated. The negativity may depend on several factors such as the administration of PPI together with previous Hp infection and a form of gastritis not associated with an existing or past Hp infection. However, it is worth noting that not all gastric cancer patients necessarily have a Hp-positive status. Some patients may have extensive gastric atrophy and achlorhydria not associated with an existing or past Hp infection. The histological score of the lesions is milder than that shown in Hp-positive patients and lesions are usually located in the antrum. In contrast, lesions can be equally distributed in the body and antrum of patients with Hp infection [44]. Based on the data shown, pathophysiological mechanisms in chronic active gastritis could be linked to the increased presence of TG2 and proinflammatory ILs, as well as the macrophage score. The epithelial changes due to active chronic gastritis intrinsically affect the progression in gastric inflammation. The activation of macrophages may depend not only on Hp but also on other factors. Further studies are needed to demonstrate what the actual causative triggers for this mechanism are.

CONCLUSIONS

TG2 may have a role in the pathophysiology of chronic gastritis, specifically in its active form. The next step is to establish how and when the enzyme could be a beneficial or a detrimental factor in gastritis, and to investigate the enzymatic and nonenzymatic activities of TG2, together with the multiple roles of concurrent inflammatory mediators.

Funding: The research was funded by the Italian Ministry of Health (IRCCS De Bellis code CQ018).

Author contributions: Conceptualization, F.R., G.R.; methodology, A.O. C.C.; formal analysis, A.O., F.R.; investigation, G.R., A.O.; Laboratory activities B.D.A.; Histology preparation and analyzation A.M. V, R.A.; endoscopy and biopsy P.G., A.P.; data curation, G.R., F.R.; writing/original draft preparation, G.R., F.R.; writing,

review and editing, F.R., G.R.; supervision, F.R.; project administration, F.R. All authors have read and approved the final manuscript.

Conflict of interest disclosure: The authors have no interests to declare.

REFERENCES

1. Zhuang R, Khosla C. Substrates, inhibitors, and probes of mammalian transglutaminase 2. Anal Biochem. 2020;591:113560. https://doi.org/10.1016/j.ab.2019.113560
2. Lee CS, Park HH. Structural aspects of transglutaminase 2: functional, structural, and regulatory diversity. Apoptosis. 2017;22(9):1057-68. https://doi.org/10.1007/s10495-017-1396-9
3. Szondy Z, Korponay-Szabó I, Király R, Sarang Z, Tsay GJ. Transglutaminase 2 in human diseases. Biomedicine (Taipei). 2017;7(3):1-13. https://doi.org/10.1051/bmdcn/2017070315
4. Lee CS, Park HH. Structural aspects of transglutaminase 2: functional, structural, and regulatory diversity. Apoptosis 2017;22(9):1057-68. https://doi.org/10.1007/s10495-017-1396-9
5. Di Sabatino A, Vanoli A, Giuffrida P, Luinetti O, Solcia E, Corazza GR. The function of tissue transglutaminase in celiac disease. Autoimmun Rev. 2012;11(10):746-53. https://doi.org/10.1016/j.autrev.2012.01.007
6. Maglio M, Ziberna F, Aitoro R Discepolo V, Lania G, Vassi V, Miele E, Not Tarcisio, Troncone R, Auricchio R. Intestinal production of anti-tissue transglutaminase 2 antibodies in patients with diagnosis other than celiac disease. Nutrients. 2017;9(10):1050. https://doi.org/10.3390/nu9101050
7. Das P, Rawat R, Verma AK, Singh G, Vallonthaiel AG, Yadav R, Gahlot GPS, Dinda AK, Ahuja V, Datta Gupta S, Agarwal SK, Makharia GK. Immunohistochemical expression of antitissue transglutaminase 2 in tissue injuries: an interpretation beyond celiac disease. Appl Immunohistochem Mol Morphol. 2018;26(6):425-30. https://doi.org/10.1097/pai.0000000000000430
8. Sipponen P, Maaroos HI. Chronic Gastritis. Scand J Gastroenterol. 2015;50(6):657-67.
9. Reshetnyak VI, Burmistrov AI, Maev IV. Helicobacter pylori: Commensal, symbiont or pathogen? World J Gastroenterol. 2021;27(7):545-60. https://doi.org/10.3748/wjg.v27.i7.545
10. Şenocak Taşçı E, Akbaş T. The Relationship between the Sydney Classification and the First-Line Treatment Efficacy in Helicobacter-Associated Gastritis. Med Princ Pract. 2020;29(6):551-7. https://doi.org/10.1159/000508248
11. Botezatu A, Bodrug N. Chronic atrophic gastritis: an update on diagnosis. Med Pharm Rep. 2021;94(1):7-14.
12. Vannella L, Lahner E, Annibale B. Risk for gastric neoplasias in patients with chronic atrophic gastritis: a critical reappraisal. World Gastroenterol. 2012;18(12):1279-85. https://doi.org/10.3748/wjg.v18.i12.1279
13. Michigami Y, Watari J, Ito C, Nakai K, Yamasaki T, Kondo T, Kono T, Tozawa K, Tomita T, Oshima T, Fukui H, Morimoto T, Das KM, Miwa H. Long-term effects of H. pylori eradication on epigenetic alterations related to gastric carcinogenesis. Sci Rep. 2018;8(1):14369. https://doi.org/10.1038/s41598-018-32717-3
14. Nie S, Yuan Y. The Role of Gastric Mucosal Immunity in Gastric Diseases. J Immunol Res. 2020;2020:7927054.
15. Pina Dore M, Pes GM, Errigo A, Manca A, Realdi G. Tissue transglutaminase activity in human gastric mucosa according to Helicobacter Pylori Infection. Exp Biol Med. 2018;243(15-16):1161-4. https://doi.org/10.1177/1535370218819423
16. Nordenstedt H, Graham DY, Kramer JR, Rugge M, Verstovsek G, Fitzgerald S, Alsarraj A, Shaib Y, Velez ME, Abraham N, Anand B, Cole R, El-Serag HB. Helicobacter pylori-negative gastritis: prevalence and risk factors. Am J Gastroenterol. 2013;108(1):65-71. https://doi.org/10.1038/ajg.2012.372
17. Chrobok NL, Sestito C, Wilhelmus MM, Drukarch B, Van Dam AM. Is monocyte- and macrophage-derived tissue transglutaminase involved in inflammatory processes? Amino Acids. 2017;49(3):441-52. https://doi.org/10.1007/s00726-016-2334-9
18. Kulich KR, Madisch A, Pacini F, Piqué JM, Regula J, Van Rensburg CJ, Ujszászy L, Carlsson J, Halling K, Wiklund IK. Reliability and validity of the Gastrointestinal Symptom Rating Scale (GSRS) and Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire in dyspepsia: a six-country study. Health Qual Life Outcomes. 2008;6:12. https://doi.org/10.1186/1477-7525-6-12
19. Rugge M, Pennelli G, Pilozzi E, Fassan M, Ingravallo G, Russo VM, Di Mario F; Gruppo Italiano Patologi Apparato Digerente (GIPAD); Società Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP). Gastritis: the histology report. Dig Liver Dis. 2011;43(Suppl. 4):S373-84. https://doi.org/10.1016/s1590-8658(11)60593-8
20. Rugge M, Savarino E, Sbaraglia M, Bricca L, Malfartheiner P. Gastritis: The clinico-pathological spectrum. Dig Liver Dis. 2021; https://doi.org/10.1016/j.dld.2021.03.007
21. Magen E, Schlesinger M, Ben-Zion I, Vardy D. Helicobacter pylori infection in patients with selective immunoglobulin E deficiency. World J Gastroenterol. 2015;21(1):240-5.

22. Carroccio A, Di Prima L, Pirrone G, Scalici C, Florena AM, Gasparin M, Tolazzi G, Gucciardi A, Sciumè C, Iacono G. Anti-transglutaminase antibody assay of the culture medium of intestinal biopsy specimens can improve the accuracy of celiac disease diagnosis. Clin Chem. 2006;52(6):1175-80. https://doi.org/10.1373/clinchem.2005.061366
23. Russo F, Chimienti G, Clemente C, Riezzo G, D'attoma B, Martulli M. Gastric activity and gut peptides in patients with functional dyspepsia: postprandial distress syndrome versus epigastric pain syndrome. J Clin Gastroenterol. 2017;51(2):136-44. https://doi.org/10.1097/mcg.0000000000000531
24. Tatsukawa H, Takeuchi T, Shinoda Y, Hitomi K. Identification and characterization of substrates crosslinked by transglutaminases in liver and kidney fibrosis. Anal Biochem. 2020;604:113629. https://doi.org/10.1016/j.ab.2020.113629
25. Lu Y, Rong J, Lai Y, Tao L, Yuan X, Shu X. The degree of helicobacter pylori infection affects the state of macrophage polarization through crosstalk between ROS and HIF-1α. Oxid Med Cell Longev. 2020;2020:5281795. https://doi.org/10.1155/2020/5281795
26. Zavros Y, Eaton KA, Kang W, Rathinavelu S, Katukuri V, Kao JY, Samuelson LC, Merchant JL. Chronic gastritis in the hypochlorhydric gastrin-deficient mouse progresses to adenocarcinoma. Oncogene. 2005;24(14):2354-66. https://doi.org/10.1038/sj.onc.1208407
27. Bayardo M, Punzi F, Bondar C, Chopita N, Chirdo F. Transglutaminase 2 expression is enhanced synergistically by interferon-γ and tumour necrosis factor-α in human small intestine. Clin Exp Immunol. 2012;168(1):95-104. https://doi.org/10.1111/j.1365-2249.2011.04545.x
28. Oh K, Lee OY, Park Y, Seo MW, Lee DS. IL-1β induces IL-6 production and increases invasiveness and estrogen-independent growth in a TG2-dependent manner in human breast cancer cells. BMC Cancer. 2016;16(1):724. https://doi.org/10.1186/s12885-016-2746-7
29. Eligini S, Fiorelli S, Tremoli E, Colli S. Inhibition of transglutaminase 2 reduces efferocytosis in human macrophages: Role of CD14 and SR-AI receptors. Nutr Metab Cardiovasc Dis. 2016;26(10):922-30. https://doi.org/10.1016/j.numecd.2016.05.011
30. Lee HS, Park DE, Bae B, Oh K, Jung JW, Lee DS, Kim IG, Cho SH, Kang HR. Tranglutaminase 2 contributes to the asthmatic inflammation by modulating activation of alveolar macrophages. Immun Inflamm Dis. 2021; https://doi.org/10.1002/iid3.442
31. Wong JH, Ho KH, Nam S, Hsu WL, Lin CH, Chang CM, Wang JY, Chang WC. Store-operated Ca2+ Entry Facilitates the Lipopolysaccharide-induced Cyclooxygenase-2 Expression in Gastric Cancer Cells. Sci Rep. 2017;7(1):12813. https://doi.org/10.1038/s41598-017-12648-1
32. Nemati M, Larussa T, Khorramdelazad H, Mahmoodi M, Jafarzadeh A. Toll-like receptor 2: An important immunomodulatory molecule during Helicobacter pylori infection. Life Sci. 2017;178:17-29. https://doi.org/10.1016/j.lfs.2017.04.006
33. Slomiany BL, Slomiany A. Role of LPS-elicited signaling in triggering gastric mucosal inflammatory responses to H. pylori: modulatory effect of ghrelin. Inflammopharmacology. 2017;25(4):415-29. https://doi.org/10.1007/s10787-017-0360-1
34. Lamb A, Chen J, Blanke SR, Chen LF. Helicobacter pylori activates NF-κB by inducing Ubc13-mediated ubiquitination of lysine 158 of TAK1. J Cell Biochem. 2013;114(10):2284-92. https://doi.org/10.1002/jcb.24573
35. Bagheri N, Azadegan-Dehkordi F, Sanei H, Taghikhani A, Rahimian G, Salimzadeh L, Hashemzadeh-Chaleshtori M, Rafieian-kopaei M, Shirzad M, Shirzad H. Associations of a TLR4 single-nucleotide polymorphism with H. pylori associated gastric diseases in Iranian patients. Clin Res Hepatol Gastroenterol. 2014;38(3):366-71. https://doi.org/10.1016/j.clinre.2013.12.004
36. Tsai CC, Chen TY, Tsai KJ, Lin MW, Hsu CY, Wu DC, Tsai EM, Hsieh TH. NF-κB/miR-18a-3p and miR-4286/BZRAP1 axis may mediate carcinogenesis in Helicobacter pylori-associated gastric cancer. Biomed Pharmacother. 2020;132:110869. https://doi.org/10.1016/j.biopha.2020.110869
37. Tanaka T, Narazaki M, Masuda K, Kishimoto T. Regulation of Il-6 in immunity and diseases. Adv Exp Med Biol. 2016;941:79-88.
38. Nahid-Samiei M, Rahimian G, Shafigh M, Taheri F, Karami-Hurestani M, Sanaei MJ, Heshmati M, Bagheri N. Enhanced Frequency of CD19+IL-10+B Cells in Human Gastric Mucosa Infected by Helicobacter pylori. Am J Med Sci. 2020;359(6):347-53. https://doi.org/10.1016/j.amjms.2020.03.019
39. Oh K, Moon HG, Lee DS, Yoo YB. Tissue transglutaminase-Interleukin-6 axis facilitates peritoneal tumor spreading and metastasis of human ovarian cancer cells. Lab Anim Res. 2015;31(4):188-97. https://doi.org/10.5625/lar.2015.31.4.188
40. Lee HT, Huang CH, Chen WC, Tsai CS, Chao YL, Liu SH, Chen JH, Wu YY, Lee YJ. Transglutaminase 2 Promotes Migration and Invasion of Lung Cancer Cells. Oncol Res. 2018;26(8):1175-82. https://doi.org/10.3727/096504018x15149761920868
41. Tabolacci C, De Martino A, Mischiati C, Feriotto G, Beninati S. The Role of Tissue Transglutaminase in Cancer Cell Initiation, Survival and Progression. Med Sci (Basel). 2019;7(2):19. https://doi.org/10.3390/medsci7020019

42. Lei Z, Chai N, Tian M, Zhang Y, Wang G, Liu J, Tian Z, Yi X, Chen D, Li X, Yu P, Hu H, Xu B, Jian C, Bian Z, Guo H, Wang J, Peng S, Nie Y, Huang N, Hu S, Wu K. Novel peptide GX1 inhibits angiogenesis by specifically binding to transglutaminase-2 in the tumorous endothelial cells of gastric cancer. Cell Death Dis. 2018;9(6):579. https://doi.org/10.1038/s41419-018-0594-x
43. Kim SY. New Insights into Development of Transglutaminase 2 Inhibitors as Pharmaceutical Lead Compounds. Med Sci (Basel). 2018;6(4):87.
44. Shiota S, Thrift AP, Green L, Shah R, Verstovsek G, Rugge M, Graham DY, El-Serag HB. Clinical Manifestations of Helicobacter pylori-Negative Gastritis. Clin Gastroenterol Hepatol. 2017;15(7):1037-1046.e3. https://doi.org/10.1016/j.cgh.2017.01.006

Table 1. Detailed description of patients according to active/inactive gastritis

Variables	**Active gastritis**	**Inactive gastritis**
Patient number	11	24
Sex (M/F)	3/8	7/17
Age	56.64±3.47	54.33±2.47
BMI	24.21±1.20	24.55±0.67
Location (proximal/distal)	0/11	3/21
Inflammation activity (neutrophilic infiltration)		
Grade 1	8	//
Grade 2	3	//
Grade 3	0	//
Presence of atrophy (yes/no)	4/7	16/8
Presence of intestinal metaplasia (yes/no)	2/9	8/16
Hp presence at biopsy (yes/no)	8/3	0/24

Age and BMI are expressed as the mean±SEM. BMI – body mass index; Hp – *Helicobacter pylori*. Proximal location – cardias, fundus, corpus. Distal location – distal – antrum, pylorus.

Table 2. Gastrointestinal symptom rating scale (GSRS) items according to active/inactive gastritis**.**

Parameters	**Active gastritis**	**Inactive gastritis**	**P**
GSRS single items			
Nausea/vomiting	1 [1-2.9]	1 [1-3]	P>0.05
Epigastric pain	2 [1-4]	2 [1-3.3]	P>0.05
Colic pain	1 [1-3.9]	1.5 [1-3.3]	P>0.05.
Continuous pain	1 [1-3.9]	1 [1-3]	P>0.05
Indefinite pain	2 [1-3]	2 [1-3]	P>0.05
Gastric hunger pain	2 [1-3]	1 [1-3]	0.037
Abdominal distension	2 [1-3]	3 [1.7-4]	0.038
Burping	1 [1-3]	2 [1-3.3]	P>0.05
Borborygmi	2 [1-3]	2 [1-3]	P>0.05
Flatulence	2 [1-3]	2 [1-3]	P>0.05
GSRS clusters			
Abdominal pain	9 [6-14.8]	8 [5-12.3]	P>0.05
Indigestion syndrome	8 [4-9.9]	8 [5.7-12]	P>0.05.

Data are expressed as median and 5°-95° percentiles. The Mann-Whitney sum rank test was used to assess the differences between the groups.

Table 3. Spearman correlation coefficient among TG2, macrophage score and inflammation markers at biopsy level.

	TG2	**M score**	**IL-6**	**IL-8**	**IL-10**	**LPS**	**TLR-4**	**TNF-α**
TG2	1.00							
M score	0.151	1.00						
IL-6	**-0.48***	-0.10	1.00					
IL-8	0.37	0.19	**-0.53*****	1.00				
IL-10	0.06	**0.42****	0.06	0.11	1.00			
LPS	0.11	0.003	0.18	-0.17	0.06	1.00		
TLR-4	0.20	0.03	0.01	-0.03	0.05	0.05	1.00	
TNF-α	0.03	0.14	-0.11	0.07	-0.08	-0.17	0.13	1.00

TG2 – Transglutaminase 2; IL – interleukin; LPS – lipopolysaccharides; TLR – toll-like receptor; TNF-α – tumor necrosis factor-α. M score – Macrophage score *P=0.003; **P=0.013; ***P=0.001

FIGURE LEGENDS

Fig. 1. The concentrations of TG2 (**A**), IL-6 (**B**), and IL-8 (**C**) according to active/inactive gastritis. TG2 – transglutaminase 2; IL – interleukin. Data are expressed as the mean±SEM. The Mann-Whitney sum rank test was applied to assess differences between groups; *P<0.05

Fig. 2. The concentrations of IL-10 (**A**), TLR (**B**) and TNF-α (**C**) according to active/inactive gastritis. IL – interleukin; TLR – toll-like receptor; TNF-α – tumor necrosis factor-α. Data are expressed as the mean±SEM. The Mann-Whitney sum rank test was applied to assess differences between groups; *P<0.05

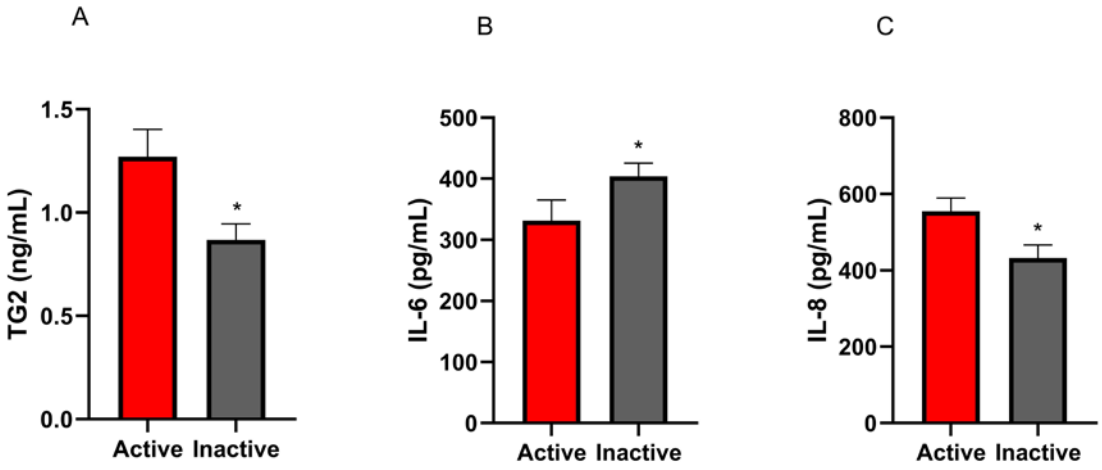
A
TG2 (ng/mL)
1.5
1.0
0.5
0.0
*
Active Inactive
B
IL-6 (pg/mL)
500
400
300
200
100
0
*
Active Inactive
C
IL-8 (pg/mL)
800
600
400
200
0
*
Active Inactive

Fig. 1.

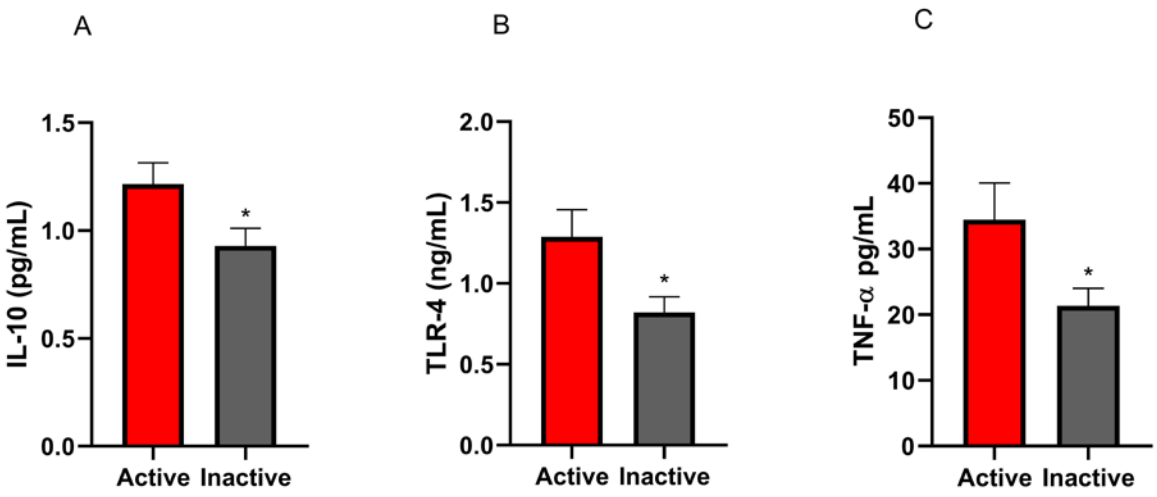
A
B
C
IL-10 (pg/mL)
1.5
1.0
0.5
0.0
*
Active
Inactive
TLR-4 (ng/mL)
2.0
1.5
1.0
0.5
0.0
*
Active
Inactive
TNF-α pg/mL
50
40
30
20
10
0
*
Active
Inactive

Fig. 2.