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# Research Article
# Interleukin-6 Expression in Serum, Gastric Juice and Gastric Mucosa of *Helicobacter pylori* Positive Gastric Cancer Patients

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## Abstract

**Background and Objective:** *Helicobacter pylori* infection is positively correlated with an increased frequency of gastric carcinoma. To investigate whether the pro-inflammatory cytokine interleukin-6 (IL-6) is a suitable molecular marker in the early diagnosis of gastric cancer in patients with concomitant *H. pylori* infection. **Materials and Methods:** A total of 177 patients were enrolled in this study and divided into the following groups: Gastric cancer group (n = 90), precancerous lesion group (n = 54) and a control group (n = 33). These patients were further subdivided into *H. pylori*-negative gastric cancer (n = 13), *H. pylori*-negative precancerous lesions (n = 9), *H. pylori*-negative control (n = 27), *H. pylori*-positive gastric cancer (n = 77), *H. pylori*-positive precancerous lesions (n = 45) and *H. pylori*-positive control (n = 6) groups. Enzyme-linked immunosorbent assay was used to quantify the expression levels of IL-6 in serum and gastric fluid. Immunohistochemistry was performed to assess the expression of IL-6 in gastric mucosa biopsy samples. **Results:** The levels of IL-6 in serum samples from gastric cancer patients, regardless of *H. pylori* infection status, were significantly higher than those in other groups ($p<0.05$). The serum levels of IL-6 were significantly higher in *H. pylori*-positive gastric mucosa cancer patients than in *H. pylori*-negative patients diagnosed with the same cancer condition and in the control group ($p<0.05$). The levels of IL-6 in the gastric mucosa of patients with precancerous lesions were lower than those in patients with gastric cancer ($p<0.05$). **Conclusions:** Both IL-6 and *H. pylori* infection contributed to the occurrence and development of gastric cancer. IL-6 and *H. pylori* may contribute to this process synergistically or alternatively, *H. pylori* may specifically promote the elevated expression of IL-6. In summary, it is believed that the detection of serum IL-6 levels in patients with gastric cancer has important clinical significance in the early diagnosis of gastric cancer.

Key words: IL-6, *Helicobacter pylori*, gastric cancer, precancerous lesions, early diagnosis, precancerous lesions

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Gastric cancer is considered the predominant malignant cancer affecting the digestive tract and typically has a very high mortality rate in the Chinese population. The high mortality rate in patients with gastric cancer is mainly thought to be linked to the lack of appropriate screening methods and thus effective early diagnosis[1]. To date, all potential gastric tumor markers have proved ineffective in the clinical diagnosis of gastric cancer. A tumor marker exhibiting robust sensitivity and specificity for use in clinical settings in the diagnosis of pre-cancer and early gastric cancers is very much still needed. The clinical significance of suitable candidate markers for the early detection of gastric cancers would be substantial[2,3]. *Helicobacter pylori* infection is positively correlated with the occurrence of gastric carcinoma in patients. *H. pylori* infection can cause inflammatory lesions in the stomach and atypical hyperplasia through a variety of mechanisms, which in turn significantly increase the likelihood of gastric carcinoma development[4]. The eradication of Hp infection can effectively reduce the risk of gastric cancer, which in turn confirms the contribution of *H. pylori* in the progression and development of new gastric cancers[4]. Interleukin-6 (IL-6) is predominantly secreted by monocyte-macrophages[5] and promotes the development and progression of tumor cells[6-9]. In this study, the potential use of IL-6 has been discussed as a tumor marker in the diagnosis of gastric cancer in the clinical setting. A significant was found correlation between increased IL-6 expression in gastric fluid, serum and biopsy samples and Hp infection, as well as significantly higher IL-6 expression in patients diagnosed with gastric cancer compared with those that were not. To contribute toward the early diagnosis of gastric cancer, the results of this study promote high-risk screening of Hb-positive patients using IL-6, thus reducing the incidence and mortality of gastric cancer.

## MATERIALS AND METHODS

### Case selection

**Inclusion criteria:** All patients were selected between December, 2013 and December, 2017 and were affiliated with the Department of Gastroenterology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan province, China. Patients were only selected if their primary health concern was specifically voiced as a digestive disorder. Gastric mucosal biopsies were collected during in or out-patient endoscopy procedures on site. Following biopsy, patients with pre-cancerous lesions or cancerous lesion were diagnosed and categorized. All patients signed informed consent.

**Exclusion criteria:** (1) Patients older than 75 years of age, (2) Patients with any history of surgery, radiotherapy treatment, endoscopic stunting or chemotherapy in the last 4 weeks and (3) Patients with the following conditions: A history of anorexia nervosa, infectious diseases, inflammatory bowel disease, endocrine disease, immune disease, liver failure (Child grade C), heart function higher than level 3 or renal failure, as well as those prescribed medications that may seriously affect metabolism or weight. Patients in the control group were selected from the patient cohort admitted to the hospital for gastrointestinal discomfort from December, 2013 to December, 2017. Patients in the control group were between 16 and 70 years of age, with no evidence of mucosal lesions and negative for Hepatitis B virus[9,10].

**Experimental grouping:** According to biopsy pathological diagnosis results, patients were further divided into the following groups: Control group (n = 33), pre-cancerous lesion group (n = 54) and a gastric cancer group (n = 90). Patients were further sub-divided into *H. pylori* -negative control (n = 27), precancerous lesion (n = 9) and gastric cancer (n = 13) groups; and *H. pylori* -positive control (n = 6), precancerous lesion (n = 45) and gastric cancer (n = 77) groups. *H. pylori* infection status in the entire study cohort was confirmed.

**Blood sample collection:** In the gastric cancer patient group, 2-3 mL of fasting venous blood was extracted at the start of the day before commencement of any treatment. Serum was collected from whole blood by centrifugation of the sample at 3,000 rpm for 20 min at 4°C and then aliquoted into a sterile container and stored at –80°C until further analysis.

**Collection and preservation of gastric fluid samples:** Informed consent for an endoscopic examination and collection of samples was obtained from all participating patients or their family members. A 10 mL syringe was connected to an improvised gastric fluid collection tube, with the tube inserted into the stomach through the biopsy channel and used to collect 3-5 mL gastric fluid during the endoscopy procedure. Collected gastric fluid samples were stored at -80°C until further analysis.

**Collection of gastric mucosal specimens:** Following the collection of gastric fluid samples, biopsy forceps were used to

sample lesioned tissue via the endoscopic biopsy channel. Biopsied tissue was then immediately fixed with formalin and subjected to histopathological examination.

**Instrumentation:** Gastroscope: Olympus, Japan; low temperature centrifuge (KDC-2046): USTC CHUANGXIN CO., LTD; medical research centrifuge (82-0 type): Surgical equipment factory, Shanghai, China; electric thermostat $CO_2$ incubator (YZB/Shanghai 0938-41-2005): SANFA Scientific Instrument Co. Ltd., Shanghai, China; suspension oscillator: Kou Hing Hong Scientific Supplies Ltd., Shanghai, China; high precision electronic analytical balance (TG3288): Balance instrument factory, Shanghai, China; temperature controller (QP-B1 type): Anhui Electric Power Research Institute; medical tissue dehydration machine (ZT-12P): Xiaogan Yaguang Medical-electronic Technology Co., Ltd., Hubei, China; medical body tissue embedding machine (YB-6 type): Xiaogan Yaguang Medical-electronic Technology Co., Ltd.; pathology slicing machine (Leica RM2235): Leica Instrument Co., Ltd., Germany; medical binocular optical microscope (BH-2): Olympus.

**Reagents and resources:** IL-6 anti-human mouse monoclonal antibody, anti-mouse rabbit secondary antibody, DAB color kit, ready-to-use SABC kit, 5% BSA blocking solution, 0.01 M citrate buffer and 0.02 M PBS buffer were purchased from Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China; 30% $H_2O_2$ was purchased from Fuzhou Maixin Biotech. Co., Ltd.; an hydrous ethanol and xylene were purchased from Damao Chemical Reagent Factory, Tianjin, China; low melting point paraffin was purchased from Great Wall Chemical Reagent Factory, Beijing, China; formalin was purchased from East China reagent factory, Shenyang, China; and anti-off slides were from Boster, Wuhan, China.

### Detection of IL-6 in serum, gastric fluid and gastric mucosal biopsy tissues

**Detection of IL-6 in serum and gastric fluid specimens:** Stored specimens were equilibrated at room temperature for at least 1 h. Following this, the levels of IL-6 in the specimens were detected using a fully automated analyzer[11]. A commercial IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Beijing Biotechnology Co., Ltd) was used for the detection and quantification of IL-6, according to the manufacturer's instructions.

**Detection of IL-6 in gastric mucosal biopsy tissue: HE staining:** Only endoscopic biopsy samples of gastric mucosal tissue from a lesion-containing area were used for further analyses. Biopsied tissue was immediately fixed in 10% formaldehyde and washed with water followed by gradient alcohol dehydration of the sample. Following this, the samples was kept at room temperature and soaked in paraffin. Paraffin-embedded fixed tissues were sectioned into 4 μm slices and stained with hematoxylin and eosin (HE)[12].

**Immunohistochemical staining:** The sterilized glass slides were subjected to pepsin antigen repair: One drop of type IV collagen plus pepsin was added to each slide and incubated at 37°C for 10 min. A drop of goat serum blocking agent A was added to each slide followed by one drop of mouse anti-human IL-6 monoclonal antibody (1:200 dilution) to each section, then incubated overnight at 4°C. The PBS was used as the blank control instead of primary antibody[13]. A drop of biotin-labeled rabbit anti-mouse IgG reagent B was mixed and incubated for 15 min. Finally, one drop of horseradish peroxidase-labeled streptavidin working solution reagent C was added and the slice was incubated for 15 min.

**Observation of immunohistochemical staining results:** Cells with brown particles in the cytoplasm were positive cells (the extracellular matrix was not included). A total of 100 cells from each field of view at a magnification of 10× were randomly selected to calculate the positive rate. A positive cell count of more than 10% was treated as a gastric cancer-positive tissue sample.

### Detection of *H. pylori* in gastric mucosal biopsy tissue

**Determination of *H. pylori*-positive status:** A modified Giemsa staining method and a silver staining protocol were performed on each endoscopic biopsy sample[14-16]. Tissues with positive signals from both staining methods were counted as *H. pylori*-positive tissues, whereas tissues with negative results from both staining methods were counted as *H. pylori*-negative tissue. Biopsy tissues with a positive signal from just one of the staining methods were excluded from further analysis.

**Giemsa staining:** Samples mounted on slides were then baked at 75°C for 30 min. Following this, a xylene dewaxing procedure and gradient alcohol hydration were performed. Following Giemsa staining for 15-30 min, samples were washed with water and dehydrated using a gradient of alcohol and xylene. After drying, the samples were sealed with neutral resin for observation under an oil lens.

**Silver staining:** Samples were embedded in the slides and baked at 75°C for 30 min. Then xylene dewaxing procedure and gradient alcohol hydration were performed. The slides were immersed in 1% silver acid solution at 60°C for 30 min. Developer was added and the reaction was stopped approximately 1 min later following the development of a brown color. After washing with water at 56°C, the samples were dehydrated using a gradient of alcohol and xylene. After drying, the samples were sealed with neutral resin for observation under an oil lens.

**Statistical analysis:** All data were processed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). The data was expressed as the Mean±Standard deviation and the count data are expressed as the constituent ratio.

## RESULTS

**Serum IL-6 levels measured by ELISA:** The levels of serum IL-6 in the seven *H. pylori*-positive gastric cancer patients were significantly higher than those in patients in the control group ($p<0.05$). Serum levels of IL-6 in the 45 pre-cancerous patients were significantly lower ($p<0.05$) than the levels in patients diagnosed with gastric cancer. Serum levels of IL-6 in the 45 pre-cancerous patients were significantly higher ($p<0.05$) than those in the control patients (Table 1).

Serum levels of IL-6 in the 13 *H. pylori*-negative gastric cancer patients were significantly higher than those in the control group ($p<0.05$). Serum IL-6 levels of the 9 patients with precancerous lesions were lower than those of patients diagnosed with gastric cancer ($p<0.05$) but were still higher than those of the control group ($p<0.05$) (Table 1). IL-6 serum levels in *H. pylor*-positive patients were significantly higher than those in *H. pylori*-negative patients ($p<0.05$) (Table 1).

**Gastric juice IL-6 levels detected by ELISA:** In the *H. pylori*-positive group, the average level of IL-6 in gastric fluid samples from patients diagnosed with gastric cancer was significantly higher ($p<0.05$) than that in patients in the control group. The average gastric fluid level of IL-6 in the precancerous lesion group was also significantly higher than that in the control group ($p<0.05$) but significantly lower than that in the gastric cancer group ($p<0.05$) (Table 2).

**IL-6 expression in gastric mucosal biopsy tissues detected by immuno-chemistry:** The expression levels of IL-6 in the *H. pylori*-positive control group were higher than those in the *H. pylori*-negative control group. The IL-6 levels in the *H. pylori*-positive precancerous lesion group were also elevated when compared with the levels in the *H. pylori*-negative precancerous lesion group. The gastric cancer group exhibited tumor cells that were observed to be formed by monoclonal hyperplasia, subsequently leading to a loss of function of normal gland cells. These tumor cells also showed a significant increase in IL-6 expression, particularly in the cytoplasm and cell membrane. The positive expression rate of IL-6 in the Hp-positive gastric cancer group differed significantly from that of the *H. pylori*-negative group with the same cancer diagnosis ($p<0.05$) (Table 3). In all groups used in this study, the expression of IL-6 in *H. pylori*-positive cohorts was significantly higher than that in the *H. pylori*-negative groups.

Table 1: Serum IL-6 levels in each group

| | *H. pylori* -negative group | | *H. pylori*-positive group | | | |
|---|---|---|---|---|---|---|
| Group | n | IL-6 (ng $L^{-1}$) | n | IL-6 (ng $L^{-1}$) | T-value | p-value |
| Control group | 27 | 56.28±5.23 | 6 | 62.33±3.23 | 2.25 | 0.034 |
| Precancerous lesions group | 9 | 65.43±6.52 | 45 | 78.44±7.33 | 3.12 | 0.028 |
| Gastric cancer group | 13 | 122.33±5.43 | 77 | 138.44±6.58* | 3.24 | 0.021 |
| F-value | - | 1.28 | - | 4.32 | - | - |
| p-value | - | 0.011 | - | 0.008 | - | - |

Table 2: IL-6 levels in gastric juice in each group

| | *H. pylori*-negative group | | *H. pylori*-positive group | | | |
|---|---|---|---|---|---|---|
| Groups | n | IL-6 (ng $L^{-1}$) | n | IL-6 (ng $L^{-1}$) | T-value | p-value |
| Control group | 27 | 11.6±3.44 | 6 | 16.60±6.38 | 2.28 | 0.032 |
| Pre-cancerous lesions group | 9 | 31.8±5.17 | 45 | 37.80±9.68 | 2.18 | 0.029 |
| Gastric cancer group | 13 | 45.5±8.37 | 77 | 50.11±10.37 | 3.18 | 0.029 |
| F-value | - | 2.37 | - | 1.88 | - | - |
| p-value | - | 0.011 | - | 0.012 | - | - |

Table 3: Expression of IL-6 in gastric mucosal biopsy tissues of each group

| | *H. pylori*-negative group | | *H. pylori*-positive group | | | |
|---|---|---|---|---|---|---|
| Groups | n | IL-6 (%) | n | IL-6 (%) | T-value | p-value |
| Control group | 27 | 9.10 | 6 | 13.30 | 2.11 | 0.038 |
| Pre-cancerous lesions group | 9 | 33.10 | 45 | 38.50 | 2.19 | 0.035 |
| Gastric cancer group | 13 | 65.20 | 77 | 84.00 | 3.21 | 0.032 |
| F-value | - | 1.87 | - | 2.75 | - | - |
| p-value | - | 0.001 | - | 0.017 | - | - |

## DISCUSSION

The results of this study demonstrated that serum levels of IL-6 and Tumor Necrosis Factor (TNF) in patients diagnosed with recurrent gastric cancer were significantly elevated, indicating that IL-6 may be involved in the development of gastric cancer. As reported by Tang *et al.*[6] and other similar reports, serum IL-6 levels were closely associated with tumor TNM staging, degree of infiltration, lymphatic metastasis and vascular infiltration. They concluded that detecting IL-6 expression levels presented clinical value in assessing the prognosis of patients with gastric cancer and could also be used to predict the degree of tumor metastasis. IL-6 is thought to promote tumor cell proliferation by regulating anti-cell apoptosis and promoting angiogenesis[7]. It has previously been reported[8,9] that serum levels of IL-6 combined with levels of C-reactive protein showed significantly higher sensitivity as a tumor marker in the diagnosis of gastric cancer[17,18]. In patients diagnosed with gastric cancer, the mean serum IL-6 level decreased following interventional surgery but increased again following cancer recurrence. An important point to consider is that previous studies have also confirmed that gastric cancer can subsequently lead to increased serum IL-6 levels[19]. IL-6 has broad biological effects on mononuclear cells, including B-cell and T-cell differentiation and activation of macrophages[20]. These biological properties may be relevant to the pathogenesis of gastroduodenal inflammation. We have previously shown that there were some factors other than the cag pathogenicity island which induced IL-6 production in *H. pylori* infection[21].

The serum levels of IL-6 in patients with gastric precancerous lesions were lower than those in patients with gastric cancer but still higher than those in the control group. In the *H. pylori*-negative group, serum IL-6 levels in patients with gastric cancer were higher than those in the control group. The average serum level of IL-6 in patients with gastric pre-cancerous lesions was lower than that in patients with gastric cancer but still higher than that in the control group. Our results also showed that both IL-6 and *H. pylori* infection contributed toward the occurrence and development of gastric cancer. IL-6 and Hp may be synergistically involved this process or *H. pylori* may promote the expression of IL-6. The *H. pylori* infection has been clearly correlated with gastric carcinogenesis. White *et al.*[22] concluded that the *H. pylori* infection strongly stimulates gastric mucosal inflammation and both the innate and acquired immune response. The usual consequence of *H. pylori* infection is chronic asymptomatic gastritis, probably because the bacteria have adapted to evade and suppress the immune response. The inflammatory response is important in the development of gastric adenocarcinoma; however, there is growing evidence that other aspects of the local and systemic response are also central to disease pathogenesis. It may ultimately be possible to develop prognostic tests based on these parameters, along with bacterial virulence types, to predict who is at risk of developing gastric cancer.

A study by Conteduca *et al.*[23] supports our conclusions and further concluded that the strongest support for a link between *H. pylori* infection and gastric cancer development comes from a decrease in gastric cancer diagnoses following *H. pylori* eradication associated with gastric ulcerative disease.

Tanahashi, *et al.*[24] concluded that the human gastric epithelial cells were sensitive to *H. pylori* urease and participated in the production of proinflammatory cytokines, such as IL-6 and TNF-α. These results suggested that the gastric epithelial cells play a role in the mucosal inflammation that accompanied *H. pylori* infection. The above mentioned study also favored the results and analysis of current study.

An important remaining challenge in the field of gastroenterology is to reduce the high mortality rates associated with gastric cancers and to increase median survival times following diagnosis. This can be achieved by identifying patients at high risk for gastric cancer, such as those with atrophic gastritis, intestinal metaplasia, dysplasia of the stomach and in rare cases, those with hyperplastic gastric polyps or germ-line mutations in CDH1, responsible for hereditary diffuse gastric cancer and increasing out patient screening regimes for these patients. A number of recent studies on gastric pre-cancerous lesions have offered the promise of novel biomarkers enabling the early detection of gastric cancer, whereas others have reported improvements

in invasive and non-invasive diagnostic tests for pre-malignant stages of gastric cancer.

## CONCLUSION AND CLINICAL IMPLICATIONS

This study concluded that the expression of IL-6 in gastric mucosa tissue can, to a certain extent, inform on the development of gastric cancer. Detection of IL-6 levels in cancer tissues is important for the diagnosis of gastric cancer associated with *H. pylori* infection and also highlights potential therapeutic avenues that could be explored to mitigate the progression and spread of early gastric cancers. It believe that the detection of serum IL-6 levels in patients with gastric cancer has important clinical significance in the early diagnosis of gastric cancer. Early diagnosis of gastric cancer or even diagnosis at a pre-cancerous stage could significantly reduce the progression of malignancy, thus reducing mortality rates.

The expression of IL-6 in gastric mucosa tissue can, in *H. pylori*-associated cases, be used to predict the likelihood of the development of gastric cancer. Detection of IL-6 levels in cancerous tissues is important for the diagnosis of gastric cancer and could be used to inform new treatment avenues.

## SIGNIFICANCE STATEMENT

IL-6 and Hp both contribute toward the occurrence and development of gastric cancer. IL-6 and Hp may be synergistically involved in this process or alternatively, *H. pylori* infection may promote the expression of IL-6. IL-6 could be used as a potential biomarker in the early diagnosis of gastric cancer associated with *H. pylori* infection. Early diagnosis of cases of gastric cancer potentially enables diagnosis at a precancerous stage and may prevent progression to a malignant stage of the disease, thus reducing mortality rates.
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## REFERENCES

1. Fock, K.M., N. Talley, P. Moayyedi, R. Hunt and T. Azuma *et al*., 2008. Asia-Pacific consensus guidelines on gastric cancer prevention. J. Gastroenterol. Hepatol., 23: 351-365.
2. Yu, J., S. Miehlke, M.P. Ebert, J. Hoffmann and M. Breidert *et al*., 2000. Frequency of TPR-MET rearrangement in patients with gastric carcinoma and in first-degree relatives. Cancer, 88: 1801-1806.
3. Colotta, F., P. Allavena, A. Sica, C. Garlanda and A. Mantovani, 2009. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. Carcinogenesis, 30: 1073-1081.
4. Allavena, P., C. Garlanda, M.G. Borrello, A. Sica and A. Mantovani, 2008. Pathways connecting inflammation and cancer. Curr. Opin. Genet. Dev., 18: 3-10.
5. Aggarwal, B.B., S. Shishodia, S.K. Sandur, M.K. Pandey and G. Sethi, 2006. Inflammation and cancer: How hot is the link? Biochem. Pharmacol., 72: 1605-1621.
6. Tang, L.P., C.H. Cho, W.M. Hui, C. Huang and K.M. Chu *et al*., 2006. An inverse correlation between interleukin-6 and select gene promoter methylation in patients with gastric cancer. Digestion, 74: 85-90.
7. Salgado, R., S. Junius and I. Benoy, P. van Dam and P. Vermeulen *et al*., 2003. Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. Int. J. Cancer, 103: 642-646.
8. Lukaszewicz-Zajac, M., B. Mroczko and M. Szmitkowski, 2010. The role of interleukin-6 and C-reactive protein in gastric cancer. Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego, 29: 382-386.
9. Lukaszewicz-Zajac, M., B. Mroczko, M. Gryko, B. Kedra and M. Szmitkowski, 2011. Comparison between clinical significance of serum proinflammatory proteins (IL-6 and CRP) and classic tumor markers (CEA and CA 19-9) in gastric cancer. Clin. Exp. Med., 11: 89-96.
10. Mehmet, N., M. Refik, M. Harputluoglu, Y. Ersoy, N.E. Aydin and B. Yildirim, 2004. Serum and gastric fluid levels of cytokines and nitrates in gastric diseases infected with *Helicobacter pylori*. New Microbiol., 27: 139-148.
11. Robati, M., A. Ranjbari, M.G. Boroujerdnia and Z. Chinipardaz, 2011. Detection of IL-4, IL-6 and IL-12 serum levels in generalized aggressive periodontitis. Iran. J. Immunol., 8: 170-175.
12. Michalkiewicz, J., A. Helmin-Basa, R. Grzywa, M. Czerwionka-Szaflarska and A. Szaflarska-Poplawska *et al*., 2015. Innate immunity components and cytokines in gastric mucosa in children with *Helicobacter pylori* infection. Mediat. Inflamm., Vol. 2015. 10.1155/2015/176726.
13. Lee, J.Y. and N. Kim, 2015. Diagnosis of *Helicobacter pylori* by invasive test: Histology. Ann. Transl. Med., Vol. 3, No. 1. 10.3978/j.issn.2305-5839.2014.11.03.
14. Ashton-Key, M., T.C. Diss and P.G. Isaacson, 1996. Detection of *Helicobacter pylori* in gastric biopsy and resection specimens. J. Clin. Pathol., 49: 107-111.
15. Riba, A.K., T.J. Ingeneri and C.L. Strand, 2011. Improved histologic identification of *Helicobacter pylori* by immunohistochemistry using a new Novocastra monoclonal antibody. Lab. Med., 42: 35-39.

16. Gisbertz, I.A.M., D.M.A.E. Jonkers, J.W. Arends, F.J. Bot, R.W. Stockbrugger, L.W. Vrints and H.C. Schouten, 1997. Specific detection of *Helicobactor pylori* and non-*Helicobactor pylori* flora in small-and large-cell primary gastric B-cell non-Hodgkin's lymphoma. Ann. Oncol., 8: S33-S36.
17. Kinoshita, T., H. Ito and C. Miki, 1999. Serum interleukin 6 level reflects the tumor proliferative activity in patients with colorectal carcinoma. Cancer, 85: 2526-2531.
18. Wu, C.W., S.R. Wang, M.F. Chao, T.C. Wu, W.Y. Lui, F.K. P'eng and C.W. Chi, 1996. Serum interleukin-6 levels reflect disease status of gastric cancer. Am. J. Gastroenterol., 91: 1417-1422.
19. Wang, D., 1997. Serum interleukin 6 level can reflect the changes of gastric cancer. Avant-Garde Med. Inform., 13: 96-98.
20. Akira, S., T. Hirano, T. Taga and T. Kishimoto, 1990. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB J., 4: 2860-2867.
21. Yamaoka, Y., M. Kita, T. Kodama, N. Sawai, K. Kashima and J. Imanishi, 1997. Induction of various cytokines and development of severe mucosal inflammation by *cagA* gene positive *Helicobacter pylori* strains. Gut, 41: 442-451.
22. White, J.R., J.A. Winter and K. Robinson, 2015. Differential inflammatory response to *Helicobacter pylori* infection: Etiology and clinical outcomes. J. Inflamm. Res., 8: 137-147.
23. Conteduca, V., D. Sansonno, G. Lauletta, S. Russi, G. Ingravallo and F. Dammacco, 2013. H. pylori infection and gastric cancer: State of the art (Review). Int. J. Oncol., 42: 5-18.
24. Tanahashi, T., M. Kita, T. Kodama, Y. Yamaoka and N. Sawai *et al*., 2000. Cytokine expression and production by purified *Helicobacter pylori* urease in human gastric epithelial cells. Infect. Immun., 68: 664-671.