Gastric mucosal interleukine-8 (IL-8) and interleukine-1beta (IL-1ß) levels in atrophic gastritis and gastric carcinoma patients.

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Summary

Background: Cytokines are the messengers of the immune system.They are mostly secreted by macrophages and lymphocytes and their production is induced in response to injury or infections.
Objective: Biopsy speciemens from the middle body of the stomach were obtained from 18 patients with gastric carcinoma, 32 patients with atrophic gastritis, 50 patients with chronic gastritis and 20 healthy subjects and IL-8 and IL-1B levels were detrmined.
Methods: Sandwich-type enzyme-linked immunosorbent assay kit was used to determine the IL-8 and IL-1B levels.
Results: IL-8 and IL-1B levels were significantly higher in gastric carcinoma patients than patients with atrophic gastritis and both significantly higher than healthy subjects.
Conclusions: IL-8 and IL-1B levels were recommended to be used in differentiation between gastric carcinoma and atrophic gastritis.
Key words: IL-8, IL-1B, atrophic gastritis, gastric carcinoma.

Introduction

Gastric cancer is ranks second as cancer-related deaths (1). Many epidemiologic studies have revealed a strong association between *Helicobacter pylori* infection and gastric cancer (2, 3). However, there are distinct differences in the extent of gastric inflammation among *H.pylori* infected patients, and only a small group of them develop as a gastric cancer indicating that gastric carcinogenesis may be under the combined influence of bacterial pathogenicity, host genetics, and environmental factors (4).

The cytokine response in gastric mucosa is thought to be T helper cells (Th) 1- predominant, characterized by the accumulation of IFN-γ, not IL-4 expressing T lymphocytes (5). Chronic inflammation with a Th-1 predominant immune response in the gastric mucosa and has been reported to cause gastric atrophy, whereas Th2 cytokines are protective against inflammation (6). In addition, proinflammatory cytokines play an important role in cellular proliferation and gastric mucosal damage (7). s one candidate for the host genetic factors, recent reports have revealed that pro- and anti-inflammatory cytokine [interleukin (IL)-1ß, tumor necrosis factor (TNF-α), and IL-10)] are associated with a risk for atrophic gastritis and gastric cancer (8). Proinflammatory cytokines such as IL-1ß, TNF-α, and IL-8 are playing a crucial role in inflammation of gastric mucosa. In addition, T helper cell 1 phenotype–predominant immune response is possibly associated with the development of cancer (9).

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Interleukine -1ß is an important proinflammatory cytokine with profound effect on gastric physiology. It is acid inhibitory properties and that higher level of IL-1ß correlate with increase risk of hypochlorhyderia and gastric atrophy (10).

The aim of this study is to (1) elucidate the relationship between gastric mucosal IL-8 and IL-1ß and the risk of atrophic gastritis and the development of gastric cancer, (2) to evaluate the effects of IL-8 and IL-1ß production on histological degree of atrophic gastritis in the non-cancerous gastric mucosa adjacent to cancer of surgical specimens.

Patients and Methods

Seventy patients (Age range; 23-77 years, mean; 49.3 years) were examined. The study was carried at the gastrointestinal unit at Al-Yarmook Teaching Hospital, and informed consent was obtained from all patients. Five biopsies specimens were obtained from the greater curvature of middle body of the stomach were homogenized, and mucosal IL-8 and IL1ß levels then measured by ELISA.

Gastric mucosal IL-8 and IL-1 ß levels:

Three biopsies tissue were homogenized in 1ml of phosphate buffered saline (pH 7.4) using a homogenizer and then centrifuged at 1800 rpm for 10 min. The supernatants were kept at 20˚C until the assay. The IL-8 and IL-1ß levels in the biopsy supernatants were determined using a sandwich - type enzyme - linked immunosorbent assay (ELISA) kit. Protein contents were determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, CA). Results are expressed as pg/mg protein. This ELISA has two immunological steps. In the first step, the cytokine is captured by monoclonal antibody bound to the wells of a microtiter plate. In the second step a monoclonal antibody linked to abiotinylated

monoclonal antibody is add together with streptavidine-peroxidase conjugate. The solid phase antibody-antigen complex and in turn, binds the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic substrate. The intensity of the color developed is directly related to the specific mAb concentration of the sample.

Histological evaluation:Two biopsies specimens from the greater curvature of the middle body of the stomach were fixed in 10% buffered formalin. The neutrophil infiltration, mononuclear cell infiltration and atrophy were assessed by the pathologist according to the Updated Sydney System and scored from zero to three (none, mild, moderate, or severe) (11).

Statistical analysis:Differences among groups in the gastric mucosa levels of IL-8, IL-1ß protein and in the histological score of gastritis were determined using ANOVA test to determine whether the means were equal among three groups – i.e. gastric carcinoma, atrophic gastritis and controls, $P < 0.05$ was considered statistically significant.

Results

Gastric mucosal levels of IL-8 and IL-IB:The patients profiles are listed in Table 1.

Table 1: characteristics of 120 patients used in the current study.

Variable	No.	(%)
Gastric carcinoma	18	15
Atrophic gastritis	32	26.67
Chronic gastritis	50	41.66
Healthy subjects	20	16.67

IL-8 level was significantly (p<0.01) higher in gastric carcinoma patients than patients with atrophic gastritis and both significantly (p<0.01) higher than healthy subject (Fig.1). In addinon IL-IB level also increased significantly in patients with gastric carcinoma (p<0.05) than in atrophic gastritis patients and both significantly (p<0.01) higher than healthy subjects (Fig.2).

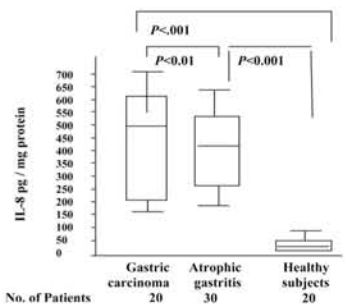

Figure 1: Gastric mucosal IL-8 levels in patients with gastric carcinoma.

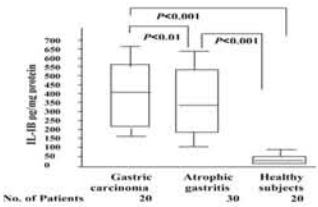

Fig 2: Gastric mucosal IL-1B levels in patients with gastric carcinoma, atrophic gastritis and healthy subjects .

IL-8 and IL1B production and histological findings: The mucosal levels of the IL-8 and IL-1B were higher in patients with severe neutrophil and mononuclear cell infiltration than those without infiltration. Furthermore, these two cytokines levels were increased with severe mononuclear cell than mild infiltration (Fig. 3A, 3B & Fig. 4A, 4B). Interestingly, both cytokines correlated with the severity of the atrophy (Fig. 3C & Fig. 4C).

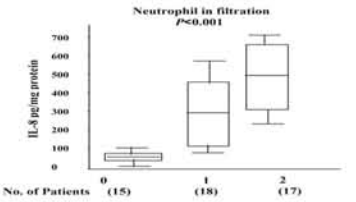

Fig 3A: Relationship between IL-8 and histological gastritis in the neutrophil filtration.

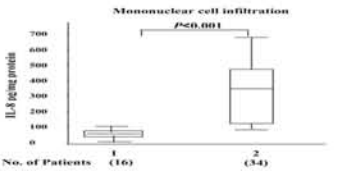

Fig 3B: Relationship between IL-8 and histological gastritis in the inflammation.

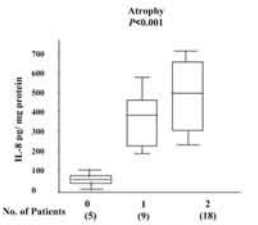

Fig 3 C: Relationship between IL-8 and histological gastritis in the atrophy.

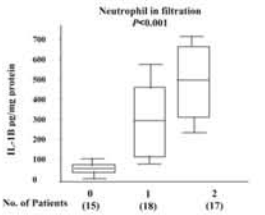

Figure 4 A: Relationship between IL-1B and histological gastritis in the neutrophil infiltration.

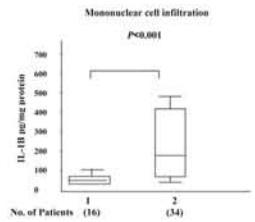

Figure 4 B: Relationship between IL-1B and histological gastritis in the mononuclear cell filtration.

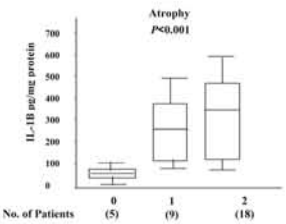

Figure 4C: Relation ship between IL-1B and histological gastritis in the atrophy.

Discussion

Interleukine -8, a member of the CXC chemokine family, was originally identified as a potent chemoattractant for neutrophils and lymphocytes (12). Subsequent studies confirmed that IL-8 could also induce cell proliferation and migration, as well as angiogenesis (13, 14, 15).

Gastric mucosal IL-8 play a significant role in the pathogenesis of gastritis (16, 17), and increased in parallel with the histological severity of gastritis (18, 19, 20). Prolonged production of IL-8 by gastric epithelial cells could result in the recruitment of leukocytes to gastric tissues (21). Infiltrated leukocytes would produce a number of proinflammatory cytokines, reactive oxygen and chemical mediators, which would further contribute to the progression of inflammatory processes (22).

This study shows IL-8 is associated with increased risk of both atrophic gastritis and gastric cancer, suggesting that high producer of IL-8 may induce a Th1- predominant immune response, lead to more sever gastric atrophy, and be more susceptible to gastric cancer than a low producer of IL-8 (4).

Interleukine-8 is shown to decrease expression of the epithelial cell adhesion molecule E–cadherin by autocrine mechanisms (23). In gastric cancer, low or absent E–cadherin expression is associated with disintegration of tissue architecture and leads the progression of diffuse type of gastric cancer, thus, a high IL-8 producer may be associated with elevated risk of adenocarcinoma, frequently developing in the upper third location (24).

It has been reported that the IL-8 level in gastric cancer directly correlated with the vascularity of the tumors and transfection of gastric carcinoma cells and enhanced their tumorigenesis in the gastric wall (25, 26). IL-8 induces metastasis by autocrine mechanisms, exogenous IL-8, derived from macrophages or neutrophils, also mediated cell migration (14, 15). Moreover, associated with more severe neutrophil infiltration in noncancerous gastric mucosa adjacent to cancer, therefore, these results suggest that IL-8 increases the metastatic potential of gastric cancer cells by both autocrine and paracrine mechanisms, affect the prognosis of gastric cancer (27).

Interleukin -1ß which can act as a potent inhibitor of acid production may cause hypochlorhydria witch result hypergastrinemia, suggest that hypergastrinemia is not only a result of inflammation but also may promote gastric inflammation synergistically with the Inflammatory cytokine and had increased risk for developing intestinal type of gastric cancer (8, 10). Therefore, suppression of acid secretion leads to *H. pylori* re-distribution and hence gastric atrophy (28). Some opposite studies have recently reported that no association between IL-I ß and gastric cancer in the Japanese population, two studies in Japan reported that IL-I ß decreased the risk of gastric atrophy and intestinal metaplasia and was not associated with increased risk of gastric cancer (29, 30). Since IL-I ß is a potent inhibitor of gastric acid secretion, may enhance IL-8 production, the mucosal level of IL-8 is correlated with IL-I ß (31). In conclusion, the level of IL-8 and IL-1 ß were higher in patients with sever neutrophil and severe mononuclear cell infiltration than in those without infiltration in gastric mucosa and play a major role in the development of atrophic gastritis and gastric cancer.

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