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Study of T-lymphocyte subsets, nitric oxide, hexosamine and *Helicobacter pylori* infection in patients with chronic gastric diseases

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INTRODUCTION

Chronic gastritis (CG) and peptic ulcer (PU) are frequently-occurring diseases. It is now well recognized that Helicobacter pylori (Hp) is a major fact or that leads to CG and PU[1-8]. In order to study the relationship among T lymphocyte subsets, NO, Hexosamine and *Hp* infection in patients with chronic gastric diseases, the levels of blood T lymphocyte subsets, plasma NO and hexosamine in gastric mucosa were measured respectively in 30 patients with CG and 32 patie nts of PU+CG.

MATERIALS AND METHODS

Clinical materials

Thirty-two patients with PU and CG (23 males and 9 females, aged 20 to 68 years , mean age 43.2)and 30 patients with CG (18 males and 12 females, aged 24 to 66 years, mean age 44. 3) were enrolled in the study. Twenty healthy people with comparable ages acted as control. All subjects were excluded of hepatic and other diseases.

Methods

Vein blood 2 mL from each fasted patient was obtained for blood T lymphocyte su b sets and NO determinations. Four gastric biopsies were taken from gastric antrum 3 cm from pylorus (2 from curvatura ventriculi minor and the other 2 from ante -wall) for *Hp*, pathology and hexosamine determinations. T-lymphocyte subsets were determined by FCM methods (FACS 420, by Becton-Dickinson Company, U.S.A.). Laser light source was 2 Wargon ionic laser, wavelength 488 nm. The emerging gr een fluorescence by FITC was used for fluorimetry by 520 nm long filter disc. The data were processed by an *HP*-300 computer programme. *Hp* infection was dia gnos ed if any two of the following methods were positive: (1) rapid urase test (test kit procured from San Qiang Company); (2) gastric mucosa smear with gram's stain. The Hp density gradients were according to Monshoy staging grades: 0, free; I, a few; II, obvious (in all fields of microscopy); III, plenty or piled; (3) Warthin-Starry stain of gastric mucosa.

Pathology

HE stain was used for observing mucosal inflammation. The severity of gastritis was graded as: mild: infiltrated inflammatory cells only at gastric pit or inte stinal villas; moderate: infiltrated cells in gland lamina propria; and heavy: infiltrated inflammatory cells in muscular layer of mucosa. Determination of hexo samine: two gastric biopsies were ground into 1 mL liquid, centrifuged it, and supernatant was kept at -20℃ for measuring. Protein content in supernatant was m easured by taking 20 μL sample and 500 μL Coomassie brilliant blue G250 reagent at the wavelength of 600 nm by automatic analysis appa ratus. 0.5 mL of the supernatant and 0.5 mL enriched hydrochloric acid were mixe d together at high pressure (147 KPa) for 30 min, put into 0.5 mL NaOH (8.3 mo l/L) and acetyl acetone solution, boiled for 15 min and cooled, and then analy zed. The results were expressed as mg/mL. Hexosamine was calculated as following: hexosamine = supernatant hexosamine (mg/mL)/supernatant protein (g/L). NO measureme n t: (1) pre-measurement: Took 200 μL plasma and the same amount of a cetonitrile, shook them, and centrifuged for 10 min (5000 r/min), repeating once again as above, taking 10 μL of supernatant overflow for measurement. Baseline 810 high effect liquid phase apparatus, made by Waters Company, U.S .A. and 486 outer purple detector were used. Conditions of chromatostrip includ ed: m obile phase 2.5 mmol/L LiOH water solution +50 g/L acetonitrile, velocity of flow 1.2 mL/min, column temperature 40℃, detection wavelength 214 nm, analysis cl oumn IC-Pak Anion, 4.6 mm × 5 cm (Waters Company, U.S.A.), detection time 10 min .

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RESULTS

T-lymphocyte subsets in CG patients

Total lymphocyte (CD_3^+), helper lymphocyte (CD_4^+), suppressor lymphocyte (CD_8^+), CD_4^+/CD_8^+ in CG patients were significantly lower than thos e of normal control ($P < 0.05$-0.01, Table 1). Eighteen out of 30 CG patients had *Hp* infection (60%). CD_3^+, CD_4^+ in Hp^+ group were significantly decreased ($P < 0.01$, < 0.05), but CD_4^+/CD_8^+ was not significantly changed (Table 2). In addition, the study of the relationship between different pathological changes and T lymphocyte subsets indicated that: CD_3^+, CD_4^+, CD_4^+/CD_8^+ in severe CG were significantly decreased than those in mild to moderate CG ($P<0.01$, <0.05, <0.05 respectively, Table 3).

Table 1 T-lymphocyte subsets in CG patients ($\bar{x} \pm s$, %)

Lymphocyte	CG (n = 30)	Normal control (n = 20)
CD_3^+	60.7 ± 2.4[b]	68.3 ± 3.9
CD_4^+	35.8 ± 2.5[b]	43.0 ± 3.8
CD_8^+	25.2 ± 2.4[a]	26.4 ± 1.7
CD_4^+/CD_8^+	1.43 ± 0.15[a]	1.64 ± 0.18

[a]$P < 0.05$, [b]$P < 0.01$, *vs* normal group

Table 2 T-lymphocyte subsets in CG patients with *Hp* infection ($\bar{x} \pm s$, %)

lymphocyte	*Hp* (+) (n = 18)	*Hp* (-) (n = 12)
CD_3^+	59.7 ± 2.5[b]	62.2 ± 0.9
CD_4^+	34.8 ± 2.6[a]	36.9 ± 2.0
CD_8^+	25.3 ± 2.5	25.0 ± 2.3
CD_4^+/CD_8^+	1.42 ± 0.17	1.45 ± 0.12

[a]$P < 0.05$, [b]$P < 0.01$, *vs Hp* (-)

Table 3 Pathological stages and T-lymphocyte subsets in CG ($\bar{x} \pm s$,%)

Lymphocyte	Mild-moderate (n = 13)	Severe (n = 17)
CD_3^+	62.3 ± 1.7	59.5 ± 2.1[b]
CD_4^+	37.4 ± 1.8	35.4 ± 2.7[a]
CD_8^+	25.5 ± 2.1	24.9 ± 2.6
CD_4^+/CD_8^+	1.50 ± 0.13	1.40 ± 0.13[a]

[a]$P < 0.05$, [b]$P < 0.01$, *vs* mild-moderate

Hexosamine levels

The hexosamine levels in patients with severe lesions (38.0 mg/g ± 3.8 mg/g) were significantly lower than those with mild and moderate CG (47.0 mg/g ± 7.6 mg/g, $P<0.01$). The hexosamine levels in Hp^+ group were significantly lower than those in Hp^- group ($P < 0.05$, Table 4). In addition, 24 out of 32 PU+CG patie nts (75.0%) had *Hp* infection. The hexosamine levels in Hp^+ group w ere significantly lower than those in Hp^- group ($P<0.01$, Table 4).

Table 4 Changes in hexosamine levels in CG, PU+CG patients with *Hp* infectio n ($\bar{x} \pm s$, mg/g)

	CG		PU + CG	
	Hp (+) n = 18	*Hp* (-) n = 12	*Hp* (+) n = 24	*Hp* (-) n = 8
Hexosamine	40 ± 6	45 ± 7	39 ± 8	51 ± 7

[a]$P < 0.05$, [b]$P < 0.01$, *vs Hp* (-)

Plasma NO levels

In CG, PU+CG patients, the levels of plasma NO (2514 μg/L ± 364 μg/L, 2824 μg/L ± 673 μg/L) were significantly higher than those of normal control (2228 μg/L ± 214 μg/L, $P < 0.05$, $P < 0.01$), and the level of plasma NO in PU+CG patients (2824 μg/L ± 673 μg/ L) was higher than those of CG patients (2514 μg/L ± 364 μg/L, $P < 0.05$). The levels o f NO in CG, PU+CG *Helicobacter pylori* positive group were significantly hi gher t han in Hp^- group ($P < 0.01$, $P < 0.05$, Table 5). The study of the relat ions hip between different pathological changes and NO levels indicates that the levels of NO in both groups with severe lesion were significantly higher than those in mild to moderate lesions ($P < 0.05$, Table 5).

Table 5 Changes in plasma NO, pathological stages and *Hp* infection in CG an d PU+CG ($\bar{x} \pm s$, μg/L)

	CG	PU+CG
Hp (+)	2671 ± 258	3071 ± 398
Hp (-)	2282 ± 387	2579 ± 668
Mild-moderate	2328 ± 413	2403 ± 284
Severe	2656 ± 251	2880 ± 802

[a]$P < 0.05$, [b]$P < 0.01$, *vs Hp* (-); [c]$P < 0.05$, *vs* severe.

DISCUSSION

As we all know, CG is closely related to *Hp* infection [9-12], but so far any report regarding the relationship between CG and T lymphocyte has not bee n observed. Our results showed that CD_3^+, CD_4^+, CD_8^+, CD_4^+/CD_8^+ T cells in CG patients were observed to be significantly lower than in normal control. This indicated that the functioning of cellular immunity was impaired in CG patients. In order to explore the relationship between T lymphocyte subse ts and *Hp* infection, this experiment divided CG patients into Hp^+ grou p and Hp^- group. The results showed that CD_3^+, CD_4^+ T cells in Hp^+ group wer e significantly decreased showing that *Hp* infection is related to the decrease in the function of cellular immunity. In addition, the majority of the severe cases of CG (71%) had *Hp* infection, and the levels of CD_3^+, CD_4^+, CD_8^+, CD_4^+/CD_8^+ T cells were significantly decreased than in mild to moderate CG patients. This implied that the impaired function of cellular immunity in CG patients especially in severe cases, may possibly be related to *Hp* infection.

Gastric mucoitin-bicarbonate barrier is one of main constituents of gastric mu cosal barrier[13,14]. Gastric mucoitin contains polymer glucoproteins, electrolytes, peptides, lipides, etc. The present study showed that: the orientat ion of *Hp* is related to pathogenic factors, such as lipopolysaccharide, urea enzyme[15-18], vacuole toxin[19-21] and adhesiveness. Hexosamine[22] reflects the glucoprotein. So, the purpose of this study was to evaluate the lesion of gastric mucosal barrier by measuring the levels of hexosamine in gastric mucosa. The results showed that in CG, and PU+CG patients, the levels of hexosamine in Hp^+ group were significantly decreased than in Hp^- group. The more severely the gastric mucosa was impaired, the higher the *Hp* infection rate was (moderate 60.7%, severe 74.2%). *Hp* infection leads to the decrease i n hex osamine levels in gastric mucosa especially in severe cases. It is thus indicate d that *Hp* is one of main pathogenic factors for endogastritis or severe gast ritis.

NO is a multifunctional regulatory substance of the body[23-30]. It has many biological activities. It participates in many physiological functions and pathogenesis in digestive tract. Endogenous NO is an important transmitter maintaining the blood flow of gastric mucosa, and has a role in inhibiting platelet aggregation in gastric mucosa microcirculation. The results in this study showed that the levels of plasma NO in CG and, PU+CG patients were increased to varying extents as compared to normal control, and the levels of plasma NO in PU+CG patients were significantly increased than those in CG patients. In addition, th e plas ma NO levels in Hp^+ group in CG and PU+CG patients were significantly increased as compared to Hp^- group. According to the pathological stages, the more the gastric mucosa was impaired by *Hp* infection, the higher the levels of NO were. It is suggested by our results that *Hp* infection in gastric mucosa may induce an increase in NO production by inflammatory cells[31]. It might be possibly an immune response of our body to resist *Hp* infection, but the exact pat hogenesis needs to be further confirmed.

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