Original Article

Circulating basic fibroblast growth factor in serum of gastric ulcers patient as a biomarker of wound severity

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Abstract

***Background*:** Basic fibroblast growth factor (bFGF) is a glycoprotein with stimulating ability of angiogenesis. In addition, bFGF plays an important role in wound healing process in some tissues for example gastrointestinal tract, but its ability for discrimination of various stages of wound severity in these diseases was not reported. Therefore, we aimed to determine the bFGF levels in gastric ulcer patients compared with healthy controls as a biomarker for staging the severity of wound.

Methods: The study group consisted of 33 patients with gastric ulcer and 27 healthy controls. The diagnosis of patients was based on standard clinical, endoscopic, and histological criteria. Serum levels of bFGF were analyzed by an Elisa kit.

Results: According to the histological findings, 19 (57.6%) patients were in moderate stage of ulcer and 14 (42.4%) were in the severe stage of gastric ulcer. The mean bFGF serum levels in patients group (7.8±1.3pg/ml) were lower than the healthy group (8.2±1.4pg/ml) in crude data, but in statistical analysis the differences were not significant ($p=0.082$). The mean bFGF serum levels in patients with severe stage of gastric ulcer were greater than the patients with moderate gastric ulcer (8.4±1.3 vs. 7.4±1.2 pg) and the differences were statistically significant ($p<0.05$).

Conclusion: The differences in serum bFGF levels in patients with severe stage of gastric ulcer vs. moderate gastric ulcer was significant. Therefore, serum bFGF level measurements can be used as a useful clinical tool for discrimination of patients with severe stage of gastric ulcer vs. moderate gastric ulcer, when endoscopic and histological examination are not possible to perform.

Key words: Basic Fibroblast Growth Factor, Gastric ulcer, Wound severity.

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Basic fibroblast growth factor (bFGF) is a potent stimulator of angiogenesis (1, 2) that is produced by endothelial cells and mast cells. This protein can be found in the extracellular matrix of tissues. BFGF has some important roles among them are stimulation of blood vessel growth, neovascularization, and fibroblast proliferation considered to be the most important roles (3). Some studies revealed that this protein is detectable in normal human colon and small intestine, and also in colonic tumor cell lines (4-6). In some diseases for example, rheumatoid arthritis and sarcoidosis, increased tissue levels of bFGF which have been reported (7, 8).

Also, there are some reports that pointed out that this protein is able to promote intestinal epithelial restitution in wounded epithelial cell line and ulcer healing in animal models (9, 10). Basic FGF that is expressed in tissues is ultimately released into the blood and can be measured in serum. It is suggested that serum bFGF determination is a useful marker for revealing the severity of pediatric Crohn's disease (11, 12).

Peptic ulcer disease is the result of an imbalance between offensive factors (such as acid and pepsin that produced in gastrointestinal tract) and defensive gastric factors like mucosal barrier. Others factors such as lipid per-oxidation, nitric oxide and also life style can contribute in this process (13, 14). Nowadays, endoscopy is essential for accurate diagnosis and differential diagnosis of peptic ulcer disease and ulcer complications and this technique is the gold standard for diagnosis of these ulcers (13, 15). This method needs advanced instruments to go with but is not present in all clinics. In addition, this method's acceptability in some patients is poor, because it is an invasive method. Therefore, we aimed to determine the bFGF levels in gastric ulcers patients compared with healthy controls as a biomarker for studying the severity of wound.

Methods

Study participants: Among the outpatients that referred to Ayatollah Rouhani Hospital, 33 patients with gastric ulcer (17 men and 16 women, age 52.5±18.7 years old) were selected. Cases were subjects that referred to the hospital because of some GI tract disorders from July 2009 to December 2010 and agreed to participate in the study. All of the referred individuals underwent upper endoscopy in the hospital.

The details of the diagnostic procedures are presented elsewhere (16). We selected 27 controls (14 men and 13 women, age 47.0±16.1 years old) from among the outpatients with normal endoscopy who referred to our hospital and according to the endoscopy results they did not have gastrointestinal tract ulcers. The eligible participants with a history of any other diseases such as cancer and chronic liver were excluded.

The data on demographic characteristics and habits, including cigarette smoking and lifestyle were collected using structured questionnaires which were administered by trained interviewers in a one on one interview.

Blood sample collection: Fasting blood samples (5 ml) were obtained from all the participants. Within two hours of following sample collection, the blood samples were centrifuged (at 2500 g for 5 minutes) and the serum samples were stored in -70°C freezers. Written informed consent was obtained from all the participants. The study was reviewed and approved by the Ethics Committee of Islamic Azad University, Damghan branch, Iran.

BFGF assay: Serum levels of bFGF were analyzed by Elisa kit (USCN life company, catalogue number: E90551HU). The bFGF Elisa assay employed the competitive inhibition enzyme immunoassay technique. A polyclonal antibody specific for human bFGF was pre-coated onto a microplate. A competitive inhibition reaction was launched between horse raddish peroxidase (HRP) labeled human bFGF and unlabeled human bFGF (standards or samples) with the pre-coated antibody specific for human bFGF. The concentrations of standard that were used in this assay were 1000, 333.3, 111.1, 37.0 and 12.3 pg bFGF/ml, and the standard diluent was used as the blank (0 pg/ml of bFGF).

The more the amount of human bFGF in samples, the less the HRP labeled human bFGF bounded by pre-coated antibody.

The substrate solution was added to the wells respectively, and the color developed opposite to the amount of human bFGF bound in the initial step. The color development was stopped and the intensity of the color was measured.

Statistical analysis: All statistical analyses were done by SPSS version 16.0 for Microsoft Windows (SPSS Inc.) and a p-value less than 0.05 was considered statistically significant. Numerical data were expressed as mean±SD. Mean of serum bFGF levels of patients as a whole and various stages of ulcers and control group were compared using parametric test (student t-test). Pearson's correlation coefficients (r) were calculated to assess the relationship between the histological degrees of peptic ulcer with the concentrations of serum bFGF and also with age, gender, height, weight and ultimately body mass index (BMI, ht/wt^2).

Results

The characteristics of the included patients and control group are presented in table 1. In addition, the comparison of these demographic parameters between these groups, student t-test was presented.

It was clear that the differences between age, weight, height, BMI and blood pressure were not significant both in patient and control groups. In addition, a statistical difference between the mean serum bFGF levels were not observed in the two groups, but, some differences were observed in crude data.

Table 1. Comparison of demographic and laboratory tests between patient and control groups

Variable	Patient (n=33) Mean±SD	Control (n=27) Mean±SD	P-value
Age (years)	52.5±18.7	47.0±16.0	0.23
Wt (Kg)	70.5± 8.2	72.6±10.1	0.36
Ht (m)	1.66± 0.1	1.67±0.1	0.56
BMI (kg/m^2)	25.5± 3.1	25.8±2.3	0.62
BP_{max} (mm/Hg)	11.9±1.8	12.0±1.3	0.64
BP_{min} (mm/Hg)	7.0± 0.9	7.2± 0.9	0.29
BFGF (pg/ ml)	7.83±1.3	8.2±1.5	0.34

With endoscopy, 19 patients revealed a moderate wound severity and 14 patients had severe wound in their stomach. Mean concentration of serum level of bFGF according to the wound severities (moderate, severe and control group) were 7.4±1.2, 8.4±1.2 and 8.8±2.7pg/ml, respectively. The mean bFGF serum levels in patients group (7.8±1.3pg/ml) were lower than the healthy group in crude data, but in statistical analysis, the differences were not significant (8.2±1.4pg/ml, p=0.082). Interestingly, the mean bFGF serum levels in patients with severe stage of gastric ulcer were greater than the patients with moderate gastric ulcer (8.4±1.3 vs. 7.4±1.2pg) and the differences were statistically significant (p <0.05).

In table 2, the correlation analysis of the various parameters in patients groups are presented. The correlations between these parameters were calculated by the Pearson correlation coefficient test (r). In addition, the net P-values are presented. It is clear in this table that age, weight and BMI with serum bFGF levels in patients group had a negative and significant correlation (p <0.05).

Table 2. Correlation analysis of the various parameters in patients group. Correlations between these parameters were calculated by the Pearson correlation coefficient (r).

Variables	Age (years)	Wt (Kg)	Ht (m)	BMI (kg/m^2)	BFGF (pg/ ml)
Age (years)	r =1	r =0.538	r =0.148	r =0.587	r =-0.370
	-	P =0.001	P =0.413	P =0.000	P =0.034
Wt (Kg)	r =0.538	r =1	r =0.202	r =0.842	r =-0.528
	P =0.001	-	P =0.260	P =0.000	P =0.002
Ht (m)	r =0.148	r =0.202	r =1	r =-0.356(*)	r =0.037
	P =0.413	P =0.260	-	P =0.042	P =0.838
BMI (kg/m^2)	r =0.587	r =0.842	r =-0.356	r =1	r =-0.525
	P =0.000	P =0.000	P =0.042	-	P =0.002
BFGF (pg/ ml)	r =0.370	r =-0.528	r =0.037	r =-0.525	r =1
	P =0.034	P =0.002	P =0.838	P =0.002	-

Discussion

Basic fibroblast growth factor is a heparin-binding protein that is present in the brain, heart, kidney, adrenal gland, colon, placenta, endothelial cells and macrophages (7, 17). The exact mechanism of bFGF released by cells had not been elucidated but it was proposed that bFGF was released by dead or injured cells, but might also be exocytosed by intact cells (11, 18). In this study, the serum bFGF levels in peptic ulcer patients were compared with control group. We tried to select two ages, sex and BMI matched groups and as it is shown in table 1. According to the statistical test the differences between these parameters between the two groups were not significant. In addition, in this study, we did not observe statistical differences between mean serum bFGF levels in patients group compared with the control, but in crude data, some differences were observed.

As we are aware, there is not a report about serum bFGF levels and peptic ulcer, but there are some studies that reported serum bFGF levels in various diseases.

In a study by Bousvaros et al. serum bFGF levels were measured in 64 children with Crohn's disease, 44 children with ulcerative colitis, 20 children with functional abdominal pain, and 29 children with documented inflammatory disease (11). They reported that mean bFGF level did not significantly differ between the children with Crohn's

disease and other conditions. In another study by Przewratil et al. on 52 children with infantile hemangioma, 14 with vascular malformations and 36 healthy patients, serum peripheral bFGF concentrations in children with proliferating hemangiomas were lower than in healthy controls (p=0.03), but serum bFGF levels in children with hemangiomas were higher than peripheral blood (p=0.022) (19).

Interestingly, there are some studies that have been reported with higher serum bFGF levels in patients compared with control. For example, in a study by Bilgic et al. serum bFGF levels were measured in 30 controls and 30 gastric cancer patients before surgery. Preoperative serum bFGF levels in patients with gastric cancer were significantly higher than the control patients and the differences were statistically significant (p=0.027) (20).

We did not detect any differences in bFGF serum levels between the patients group and the healthy group. It is possible that there may be higher local bFGF concentrations in the tissues of stricture-forming patients. However, since bFGF inhibits synthesis of collagen, patients who form strictures may paradoxically have decreased local bFGF concentrations. Further investigation is necessary to evaluate tissue bFGF concentrations in these patients (11).

In addition, patients who had severe wound compared with the moderate one, had higher serum bFGF levels and the differences were statistically significant (p=0.03). It is postulated that bFGF could potentially be released by activated cells of the immune system during periods of acute inflammation and these patients - because their wound - had inflammatory responses. In addition, bFGF may be released by the damaged lamina propria and muscularis layers as a result of direct tissue injury (11) and in this condition, the serum bFGF levels ultimately increased.

According to the correlation analysis - as it is presented in table 3- a negative and significant correlation was observed between age, BMI and serum bFGF levels. It seems that the patients in lower age bracket had higher serum bFGF levels and vice versa. This issue needs to be clarified in a larger group in the future. Also, we observed a negative correlation between BMI and serum bFGF levels. There was a report by Seida that indicated a negative correlation between serum bFGF levels and BMI which was similar to our study. It seems that higher body fat can alter the accurate metabolism of bFGF and such a negative correlation was reported earlier (21).

The difference in serum bFGF levels in patients with severe stage of gastric ulcer vs. moderate gastric ulcer was significant. Therefore, serum bFGF level measurements could be used as a useful clinical tool for discrimination of patients with severe stage of gastric ulcer vs. moderate gastric ulcer when endoscopic and a histological examination was not possible to perform.

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