Serum VEGF and bFGF in patients with inflammatory bowel diseases

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AIM: *The role of angiogenesis in inflammatory bowel diseases (IBD) remains controversial. We investigated the role of serum concentration levels of VEGF and bFGF in IBD patients and assessed their potential association to disease activity. Patients and Methods: Blood samples were obtained from 40 IBD patients with moderate to severe attack of the disease and 40 healthy controls. VEGF andbFGF serum levels were assessed. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured as markers of disease activity and correlated to VEGF and bFGF.*
RESULTS: *The demographic characteristics of both patients and controls were homogenous, in regard of age, sex, smoke and concomitant diseases. VEGF serum levels were significantly higher in IBD patients compared to controls (1158.5±845.4 pg/ml vs 464.6±283.1 pg/ml, p<0.001). Serum concentration levels of bFGF did not differ between groups. Linear regression analysis showed no direct association between VEGF or bFGF and CRP or ESR.*
CONCLUSION: *VEGF, but not bFGF, may have a prominent role in patients with IBD, without though direct association to disease activity.*

KEY WORDS: bFGF, Inflammatory bowel disease, VEGF

Introduction

Inflammatory bowel disease (IBD) comprise of two forms of chronic intestinal inflammation, ulcerative colitis (UC) and Crohn's disease (CD). The pathogenesis of IBD remains obscure, and IBD is considered an heterogeneous group of disorders of multifactorial cause involving both hereditary and environmental factors. As ulceration and regeneration of the intestinal epithelium occurs during the course of the disease, angiogenesis is undoubtedly an integral part of the IBD pathology [1]. However it is still unclear whether the angiogenic process is a secondary event triggered during the reparative process or if there is an association between pathological angiogenesis and IBD.

There have been documented increased serum levels of VEGF in both active UC and CD patients but not in inactive patients [2,3]. Other authors have documented reduced serum VEGF levels in patients with IBD [1]. It is also well known that pyoderma gangrenosum is a non-infectious skin disease often associated with UC or CD. There have been reported increased bFGF and VEGF levels in various collagen diseases with skin manifestations. Therefore its speculated that bFGF might also be the result of dysfunctional immunoregulation in IBD[4].

Assessment of disease activity in IBD, is done using clin-

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ical parameters and various biological disease markers. Traditional disease activity markers include erythrocyte sedimentation rate, acute phase proteins (especially orosomucoid and CRP), leukocyte and platelet counts, albumin, neopterin, and β2-microglobulin[5].
The aim of our study was to identify the association between these angiogenetic factors and inflammatory bowel diseases and their potential role as predictors of IBD activity.

Patients and Methods

The study included 22 patients with CD and 18 with UC (Table I) admitted to the Department of Gastroenterology at the General Hospital of Athens, "G.Gennimatas". Forty hospital employees with no history of gastrointestinal disease were used as controls.
All patients had a definitive diagnosis of UC or CD confirmed by radiological, endoscopic, and histological studies and they were all admitted to the hospital with symptoms of active disease. CD patients had a disease activity index (CDAI) >220. Patients with UC were classified according to Truelove and Witts' classification[6] of severity of ulcerative colitis, as moderate or severe attack of the disease.
Peripheral blood samples were obtained from patients and controls. General blood tests including CRP and ESR were assessed. Blood samples were centrifugedat 1000 *g* for 10 min, and the serum was collected and stored at–80°C until assay. Serum VEGF and bFGF were measured using enzyme-linked immunosorbent assays kits (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions.
The study was approved by the Institutional Review Board of the "G.Gennimatas" General Hospital, Athens, Greece. All participants gave their informed consent.
Quantitative variables are expressed as mean values ±SD. Qualitative variables are expressed as absolute and relative frequencies. For the comparison of median values between groups, Mann-Whitney tests were used. Spearman correlation coefficients were used in order to explore the association of continuous variables. For the comparison of proportions chi-square tests were used. Statistical significant level was set at .05 and analysis was conducted using SPSS 17.0 Statistical Software.

TABLE I - *Patients' and Control's characteristics*

	Patients (n=40)	Healthy Controls (n=40)	p-value
Sex (male)	20	22	p=0.823
Age(years± SD)	44±19	50±12.4	p=0.378
Smoking	18	16	p=0.566
Concomitant diseases*	25	23	p=0.82

*Hypertension, stroke, diabetes, acute myocardial infarction, pancreatitis, rheumatoid arthritis, cholecystectomy, prostatectomy, coronary heart disease, breast cancer.

Results

The demographic characteristics of both patients and controls were homogenous, in regard of age, sex, smoke and concomitant diseases (Table I). Among patients those with UC were significantly older (52.3±20.5 vs38.0±15.1, p=0.015), while no other differences were noticed, in respect of age, sex, smoke and concomitant diseases compared to those with CD.

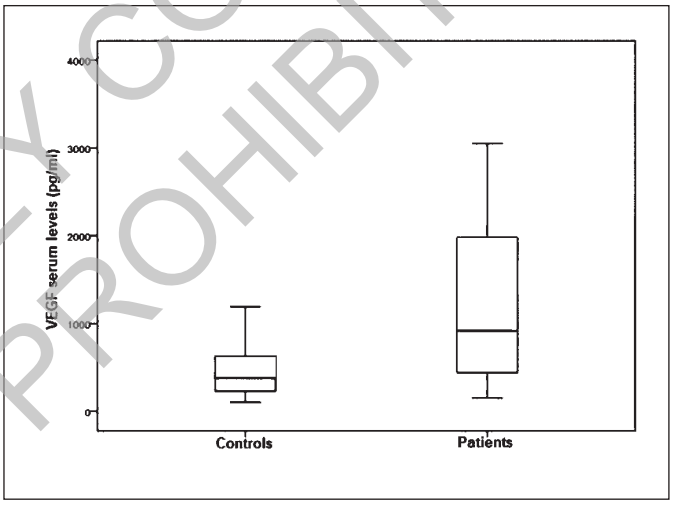

Fig. 1: Serum levels of VEGF in patients and healthy controls (p<0,001).

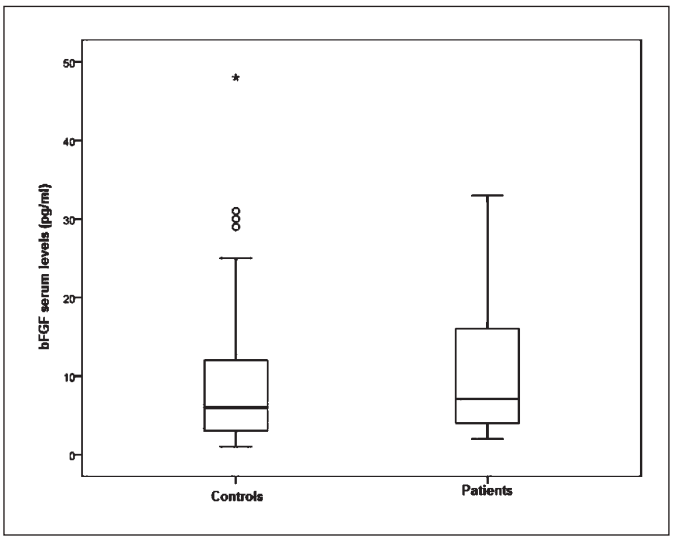

Fig. 2: Serum levels of bFGF in patients and healthy controls (p<0,362).

Serum levels of VEGF ranged from 153 to 3048 pg/ml in patients (1158.5±845.4 pg/ml) and from 104 to 1191 in controls (464.6±283.1 pg/ml).Serum levels of VEGF in patients were significantly higher than healthy controls ($p<0.001$) (Fig. 1).
Serum bFGF levels ranged from 2 to 33 pg/ml in patients (11±9.2pg/ml) and from 1 to 48 pg/ml in healthy controls (mean±SD 10.1±10.5 pg/ml). There wasn't any significant difference between patients and healthy controls (Fig. 2).
CRP levels were abnormal (>5mg/dl) in 97.5% of patients (70.6±38.8 mg/dl) and all patients had increased levels of ESR (58.1±19.1 mm/h). Among controls mean CRP and ESR levels were 4.7±6.0 and 12.2±3.7 respectively, both significantly lower than the patient cohort ($p<0.001$).
Between patients, linear regression analysis revealed that there was not a direct association between the angiogenetic factors VEGF or bFGF and CRP or ESR (Table II).

Discussion

Our study confirmed the association of angiogenetic factor VEGF, but not that of bFGF, with IBD. However, no correlation was found with disease activity.
Angiogenesis is the process of new capillary formation from preexisting vasculature in adult tissues. Angiogenesis is a fundamental constituent of many complex biological processes, including growth, development, and repair. In reality, the importance of angiogenesis extends well beyond cancer biology and has been shown to be an integral component of non-neoplastic chronic inflammatory and autoimmune diseases as diverse as atherosclerosis, rheumatoid arthritis, diabetic retinopathy, psoriasis, airway inflammation, peptic ulcers, and Alzheimer's disease [7].
The cause and pathogenesis of IBD remains elusive. Microvascular changes associated with angiogenesis are key contributors to the tissue injury and remodeling process that inevitably accompanies chronic inflammation [8,9]. Therefore angiogenesis probably has a role in the IBD pathology [8]. Several studies suggested that serum levels of the major angiogenetic factor VEGF are increased in patients with IBD [3,4,11]. Kapsoritakis et al [1] suggested that sVEGF levels are lower in IBD patients and that such a detected elevation is not of intestinal origin but rather derives from platelets aggregation.
In the present study, patients with IBD had significantly elevated serum levels of VEGF compared with the controls in line with the majority of the available literature.
Previous reports suggest that VEGF acts as an angiogenic mediator [12] and that b-FGF leads to healing and regeneration [13] in collagen diseases such as rheumatoid arthritis, systemic sclerosis, and dermatomyositis. It's speculated that bFGF might also be the result of dysfunctional immunoregulation in IBD.In contrast to previous studies [4], serum levels of bFGF were not elevated in our patients.
CRP and ESR levels were elevated in IBD patients. However linear regression analysis revealed that there was not a direct association between serum levels of the angiogenetic factors VEGF and bFGF and CRP, ESR. This result is reported for the first time to the best of our knowledge.

Table II - *Linear regression analysis for the correlation between VEGF, bFGF, CRP and ESR*

		VEGF	bFGF
CRP	r	-0.004	0.204
	P value	0.983	0.219
ESR	r	-0.112	0.001
	P value	0.503	1.000

Conclusions

Concluding, our results identify VEGF as a molecule intimately involved in IBD pathogenesis. Interestingly bFGF does not seem to have a direct role in the process of inflammation but further studies are needed. Furthermore our findings seem to support that VEGF and bFGF cannot be used as predictors of the disease's activity.

Riassunto

Il ruolo dell'angiogenesi nelle malattie infiammatorie croniche intestinali rimane ancora controverso. Abbiamo studiato il ruolo dei fattori di crescita VEGF e bFGF ai pazienti con MICI, e la loro associazione con l'attività della malattia. I nostri risultati confermano l'importante ruolo del fattore VEGF nella patogenesi delle malattie infiammatorie croniche intestinali ma non abbiamo verificato nessuna associazione dei valori nel plasma di questo fattore con l'attività della malattia.

References

1. Kapsoritakis A, Sfiridaki A, Maltezos E, Simopoulos K, Giatromanolaki A, Sivridis E, et al: *Vascular endothelial growth factor in inflammatory bowel disease.* Int J Colorectal Dis, 2003; 18:418-22.

2. Griga T, Tromm A, Spranger J, May B: *Increased serum levels of vascular endothelial growth factor in patients with inflammatory bowel disease.* Scand J Gastroenterol, 1998; 33:504-08.

3. Griga T, Voigt E, Gretzer B, Brasch F, May B: *Increased pro-*

duction of vascular endothelial growth factor by intestinal mucosa of patients with inflammatory bowel disease. Hapatogastroenterology, 1999; 46:920-23.

4. Kanazawa S, Tsunoda T, Onuma E, Majima T, Kagijama M, Kichuki K: *VEGF, basic FGF and TGF-b in Crohn's disease and Ulcerative colitis: A novel mechanism of chronic intestinal inflammation.* Am J Gastroenterol, 2001; 96:822-28.

5. Nielsen OH, Vainer B, Madsen SM, Seidelin JB, Heegaard NH: *Established and emerging biological activity markers of inflammatory bowel disease.* Am J Gastroenterol, 2000; 95:359-67.

6. Dinesen LC, Walsh AJ, Protic MN, Heap G, Cummings F, Warren BF, et al.: *The pattern and outcome of acute severe colitis.* J Crohns Colitis, 2010:4:431-37.

7. Danese S, Sans M, De La Motte C, Graziani C, West G, Phillips M, et al.: *Angiogenesis as a novel component of inflammatory bowel disease pathogenesis.* Gastroenterology, 2006; 130:2060-73.

8. Bagli E, Xagorari A, Papetropoulos A, Murphy C, Fotsis T: *Angiogenesis in inflammation.* Autoimmun Rev, 2004; 3(Suppl 1):S26.

9. Szekanecz Z, Koch AE.: *Vascular endothelium and immune responses: Implications for inflammation and angiogenesis.* Rheum Dis Clin North Am, 2004; 30:97-114.

10. Beck PL, Podolsky DK.: *Growth factors in inflammatory bowel disease.* Inflamm Bowel Dis, 1999; 5:44-60.

11. Bousvaros A, Leichtner A, Zurakowski D, Kwon J, Law T, Keough K, et al.: *Elevated serum vascular endothelial growth factor in children and young adults with Crohn's disease.* Dig Dis Sci, 1999; 44:424-30.

12. Kikuchi K, Kubo M, Kadono T, Yazawa N, Ihn H, Tamaki K: *Serum concentrations of vascular endothelial growth factor in collagen disease.* Br J Dermatol, 1998; 139:1049-51.

13. Kadono T, Kikuchi K, Kubo M, Fujimoto M, Tamaki K: *Serum concentrations of basic fibroblast growth factor in collagen diseases.* J Am Acad Dermatol, 1996; 35:392-97.