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CLINICAL STUDY

Imbalance of VEGF family serum levels and receptors in patients with inflammatory bowel disease

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Abstract: The aim of this study was to analyze the serum levels and prognostic significance of vascular endothelial growth factor (VEGF)-A, -C, and –D, and their receptors, VEGFR-1 and -2 in inflammatory bowel disease (IBD).
The serum levels of VEGF family members were measured in 80 control subjects and 200 patients with inflammatory bowel disease using an enzyme linked immunosorbent assay (ELISA). These measurements were evaluated with regard to the levels of inflammatory markers, such as C reactive protein (CRP) and serum amyloid A (SAA) and the clinical characteristics of patients, so that potential correlations could be recorded. A correlation between VEGF and their receptors serum levels is present in IBD patients. These new findings open the question on the potential role of VEGF and their receptors in IBD *(Tab. 2, Ref. 9)*. Full Text in free PDF *www.bmj.sk.*
Key words: inflammatory bowel disease, vascular endothelial growth factor, receptor.

Abbreviations: VEGF-A – vascular endothelial growth factor-A; VEGF-C – vascular endothelial growth factor-C; VEGF-D – vascular endothelial growth factor-D; VEGFR-1 – vascular endothelial growth factor receptor-1; VEGFR-2 – vascular endothelial growth factor receptor-2; IBD – inflammatory bowel disease; HC – healthy control; UC – ulcerative colitis; CD –Crohn's disease; CRP – C reactive protein; SAA – serum amyloid A; IL-6 – interleukin-6; IL-1α – interleukin-1α; IL-10 – interleukin-10.

Inflammatory bowel diseases (IBD) are characterized by an inflammatory cascade of mediators capable of degrading and modifying bowel wall structure as well as inducing the formation of chronic inflammatory lesions of the digestive tract. The inflammatory cell infiltrate observed in chronic mucosal inflammation is associated with changes in epithelial proliferation and migration and accompanied by intensive remodeling of the subepithelial connective tissue. There is a growing recognition of the central role that the vascular endothelial growth factor (VEGF) family plays in inflammation. The VEGF family consists of seven members-VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placental growth factor (PIGF)-which share eight cysteine residues in a VEGF homology domain (1, 2). The members act through specific tyrosine kinase receptors, VEGFR-1, -2 and -3. VEGF-A acts through VEGFR-1 and -2 receptors, VEGF-C and –D act through VEGFR-2 and -3 (1, 3).

Some endothelial components in the mucosa and plasma of IBD patients have been shown to be elevated in previous studies (4, 5). To our knowledge, no data regarding VEGF and VEGFR in IBD have been previously presented. On the other hand, the assessment of serum VEGF and their receptors levels has not been performed to date in IBD patients. The aim of the present study was to evaluate serum levels of VEGF-A, -C and –D and their receptors, VEGFR-1 and -2 in IBD patients and to examine possible correlations with known inflammatory markers as well as with the clinical characteristics of patients.

Methods

Patients

Two hundred consecutive IBD patients monitored at the Department of II. General Surgery, Haseki Training Hospital, Istanbul, participated in this study. Details on the clinical characteristics of the patients included in the study are shown in Table 1. All patients had a definitive diagnosis ulcerative colitis (UC) or Crohn's disease (CD) confirmed by clinical, endoscopic, radiological and histological work-up. Disease activity in CD patients was determined by the Crohn's Disease Activity Index (CDAI) (6). The cut-off point for active disease was a score higher than 150. Disease activity in patients with UC was assessed by the Clinical Colitis Activity Index (CCAI) (7). The score higher than 4 on a scale of 0-16 was required for the disease to be considered active. Evaluation of disease activity was performed at the time of serum collection. These patients were compared to the control group, which consisted of 80 healthy blood donors

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Tab. 1. Clinical characteristic of the patients with inflammatory bowel disease (mean±SD).

	UC	CD
Patients no	101	99
Age	47.2 ± 10.3 (29–66)	45.3 ± 11.6 (21–60)
Duration of disease, months	90.6 ± 35.7 (29–66)	87.2 ± 41.5 (1–300)
Male	59	46
Female	42	53
Current smokers	12	20
Ex smokers	56	47
Never smokers	33	32
Disease extent (UC)		
Proctitis	17	
Left-sided colitis	36	
Extensive colitis	48	
Disease location (CD)		
Ileum		21
Colon		9
Ileum + colon		69
Disease behavior (CD)		
Inflammatory		72
Strictures		7
Penetrating		20
Active disease	54	51
Inactive disease	47	48
Extraintestinal manifestations	28	32
Current treatment*		
5-ASA	98	97
Steroids	23	51
Enemas (5-ASA and/or steroid)	29	7
Immunosuppresive agents	16	18
Infliximab	5	16
Parenteral nutrition	3	1

* Some patients received >1 drug.

(healthy controls, HC), 41 of them were men, with a mean age (±SD) of 48.1 (±7.2) years. Apart from routine laboratory parameters, serum amyloid A (SAA) and C reactive protein (CRP) were evaluated in all patients. Patients with myeloproliferative disorders, malignancies, or autoimmune diseases were excluded from the study.

Serum VEGF family level assay

Peripheral venous blood samples were obtained prior to any treatment and allowed to clot at room temperature and centrifuged at 2000 g for 10 min. Sera were separated, aliquoted, and stored at -70 °C until assay. Serum VEGF concentrations were determined using a commercially available enzyme-linked immunoassay (ELISA) designed to measure VEGF-A (VEGF 165), VEGF-C, VEGF-D, VEGFR-1, and VEGFR-2 levels (Quantikine, R&D Systems Europe, Abingdon, UK). Assays employed the quantitative sandwich enzyme immunoassays and were performed according to the manufacturer's procedure.

Serum CRP and SAA measurements were performed by immunonephelometry with the Behring Nephelometer Analyzer, using the N High Sensitivity kit and the N Latex SAA kit, respectively. The appropriate control and standard sera were provided by the same company, and used according to the manufacturer's instructions.

Statistical analysis

All results are expressed as mean ±SD. Comparisons between two groups were made by the Mann-Whitney U test. A linear regression or a Spearman r-test was used to assess correlation. All analyses were two-tailed. A level of $p<0.05$ was considered statistically significant.

Results

Mean±SD serum VEGF-A levels were 612.8±105.6 pg/ml (median: 589.1; range: 358.5-836.2) in UC patients, 673.9±98.3 pg/ml (median: 637.2; range: 426.7-831.6) in CD patients and 143.7±75.1 pg/ml (median: 98.5; range: 74.2-226.8) in HC. UC and CD patients had significantly higher serum VEGF-A levels compared to HC ($p<0.0001$) in both groups). The difference observed in VEGF-A levels between UC and CD patients ($p<0.001$). The mean serum VEGF-A levels were significantly higher in patients with active disease (598.3 pg/ml) as compared to the inactive disease (523.7 pg/ml, $p=0.03$). Moreover, mean serum VEGF-A levels proved to be significantly higher in IBD males (501.8 pg/ml), compared to IBD females (436.2 pg/ml, $p=0.01$). Male gender was associated in all groups, HC included, with significantly higher mean serum VEGF-A levels (149.4 pg/ml) compared to female gender (117.6 pg/ml, $p=0.03$).

Mean serum VEGF-C and VEGF-D levels were 1771.9±308.6 pg/ml (median: 1128.9, range: 987.3-2194.2), 167.1±64.1 pg/ml (median: 139.5, range: 109.6-362.4) in UC patients, respectively; these levels were 1576.3±275.7 pg/ml (median: 1210.4, range: 1075.3-1989.2), 181.5±58.2 pg/ml (median: 110.4, range: 97.2-349.3) in CD patients , respectively; these levels were 1684.3±291.5 pg/ml (median: 1214.7, range: 843.6-1927.1), 149.8±37.2 pg/ml (median: 127.4, range: 96.3-276.3) in HC, respectively. No statistically significant difference was recorded between IBD patients and HC and this was also true for patients with active and inactive disease and for male and female patients ($p>0.05$).

Mean serum VEGFR-1 levels were 425.9±190.2 pg/ml (median: 248.2, range: 209.2-516.7) in UC patients, 401.4±185.2 pg/ml (median: 272.5, range: 245.2-578.1) in CD patients and 414.7±94.2 pg/ml (median: 286.1, range: 200.4-537.2) in HC.

No statistically significant difference was recorded between UC and CD patients; between IBD patients and in HC ($p>0.05$), also no statistically significant difference was recorded between patients with active and inactive disease. Also, no statistically difference was recorded between male patients and female patients ($p>0.05$).

Mean serum VEGFR-2 levels were 7362.5±254.9 pg/ml (median: 6583.2 range: 5947.2-8547.3) in UC patients, 7412.6±658.3 pg/ml (median: 6234.7, range: 5478.4-8621.5) in CD patients, 7219.5±601.3 pg/ml (median: 6098.3, range:

Tab. 2. Serum levels of VEGF family and their receptors in patients with inflammatory bowel disease and controls (mean±SD).

	UC	CD	HC
VEGF-A	612.8±105.6	673.9±98.3	143.7±75.1
VEGF-C	1771.9±308.6	1576.3±275.7	1684.3±291.5
VEGF-D	167.1±64.1	181.5±58.2	149.8±37.2
VEGFR-1	425.9±190.2	401.4±185.2	414.7±94.2
VEGFR-2	7362.5±254.9	7412.6±658.3	7219.5±601.3

5129.5-8291.6) in HC. No statistically significant difference was recorded between IBD patients and HC and this was also true for patients with active and inactive disease and for male and female patients ($p>0.05$).

Analyses of other subgroups did not reveal any statistically significant difference in the serum levels VEGF family and their receptors when smokers and never smokers were compared, and in patients with our without extraintestinal manifestations ($p>0.05$ in all cases). Treatment with 5-ASA alone or with additional corticosteroids, inflixmab or immunosuppressive agents, did not appear to influence VEGF family and their receptors serum levels. Moreover, VEGF family and their receptors serum levels did not vary significantly among the subgroups, where patients where classified with regard to disease location and behavior of CD, or the extent of UC.

Patients with active IBD had statistically significant higher SAA levels, (9.52±2.16, range: 0.91-24.5), compared to the patients with inactive disease (5.78±1.98, range: 0.82-20.6, $p=0.001$). Similarly, patients with active IBD had statistically significant higher CRP levels, (7.8±1.5, range: 4.0-9.6), compared to the patients with inactive disease (4.2±1.4, range: 2.9-8.1, $p=0.001$).

The CRP levels were found to be significantly correlated to the levels of VEGF-A ($r=0.417$, $p=0.01$) and VEGFR-1 ($r=0.475$, $p=0.01$). The SAA levels were found to be significantly correlated to the levels of VEGF-A ($r=0.328$, $p=0.006$) and VEGFR-1 ($r=0.312$, $p=0.03$). A statistically significant correlation between VEGF-A and VEGFR-1 levels ($r=0.372$, $p=0.01$) was also observed. No correlations were evident between VEGF-C, -D and VEGFR-2 serum levels and the age of patients or disease duration, as seen in the Table 2.

Discussion

Our study provides new data on the tissue remodeling process in IBD with regard to serum VEGF family and their receptors. We confirmed an increased production of VEGF-A and decreased VEGFR-1 levels in IBD patients, especially in active disease, and showed that this increase correlated to the production of the well-known inflammatory markers CRP and SAA. What seemed to be of particular interest was the observation that males, compared to females, tend to show an increased the levels of serum VEGF-A, accompanied by decreased levels of serum VEGFR-1.

It has been shown that an uncontrolled and sustained inflammatory cascade, observed in IBD, gives rise to the VEGF-A and VEGFR-1 which in turn can induce tissue degradation and lesion development. We agree that the evaluation of serum VEGF family and their receptors would increase the impact of this study. Unfortunately this was not included in the initial study design. The sera from these 200 patients are not available anymore, as they have been utilized. On the other hand, it would be biased to obtain new sera because the inflammation status of these patients has changed.

An increased production of VEGF-A in the mucosa was first described in IBD patients. It has been demonstrated that in IBD, VEGF-A is expressed by inflammatory cells, fibroblasts and vascular smooth muscle cells most prominently in actively inflamed areas of ulcer bases. VEGF showed a strong correlation with proinflammatory cytokines (IL-6, IL-1β and IL-10) (8). An increased expression of VEGF in the inflamed and especially ulcerated colon mucosa of IBD patients was evident. Increased levels of VEGF could be associated with the presence of fibrotic strictures in CD. CD and especially its stenotic form are characterized by increased fibrosis. In our study, a trend towards higher VEGF-A levels was observed in CD compared to UC patients, which was statistically significant. Maybe, VEGF-A levels in UC patients are associated with a scored endoscopic degree of mucosal injury, disease activity indices, clinical activity indices and CRP concentration, thus implying that VEGF-A serum concentration may be a possible biomarker of disease activity. External stimuli such as growth factors, phorbol esters and cytokines (IL-6, IL-1 and IL-1β) are all well-known triggers of VEGF expression in various cell types (9).

No statistically significant difference in VEGF-C and -D and VEGFR-2 levels was recorded between IBD patients and HC, in our study. The expression of VEGF-C and –D and VEGFR-2 in not inflamed mucosa in IBD patients was evident. However, these subjects require further studies.

One major finding presented in this study was that VEGFR-1 serum levels were decreased in IBD patients. The decreased VEGFR-1 levels in IBD may be of significant importance, as they are key players in angiogenesis and re-epithelization, as well as in the regulation of the epithelial barrier function of the intestine. Additionally, VEGF-A and VEGFR-1 may inhibit platelet aggregation, thus implying VEGF-A and VEGFR-1 involvement in the regulation of platelet aggregation and recruitment. These platelet aggregation responses proved to be enhanced in IBD patients, even in inactive disease. These findings suggest that the increased VEGF-A and decreased VEGFR-1 serum levels observed in IBD patients, even in those with inactive disease, have an aggravating influence on the platelet aggregation in IBD. The imbalance of VEGF-A and VEGFR-1 serum levels observed herein may reflect the paradoxical effects of these inhibitors, which may affect important pathophysiological processes involved in inflammation in IBD.

We have also showed that gender had an influence on VEGF family and their receptors levels. This gender-related difference might be proved interesting but should be the subject of differing lines of research.

In summary, our data may reflect the continuous endothelial turnover occurring in IBD, a process strongly associated with an imbalance of VEGF-A and VEGFR-1 serum levels. The increase of VEGF-A serum levels in active disease and their significant correlation with the production of known inflammatory markers such as CRP and SAA indicate that VEGF-A levels could be used not only for diagnostic purposes but also for the activity assessment in IBD. These findings open the question on the potential role of VEGF in the pathogenesis, activity and therapy of IBD, thus emphasizing the need for further studies on this subject. Perhaps in future, the modulation of VEGF-A and VEGFR-1 activities as well as epithelial turnover may become part of a more rational, mechanism-based therapeutic manipulation in IBD.

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