Study of Endothelin-1 and Vascular Endothelial Growth Factor in Patients with Cancer Colon

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ABSTRACT

**Purpose:** The levels of endothelin-1 and VEGF were evaluated in the sera of newly diagnosed patients with cancer colon and were compared with the routinely used tumor markers; CEA and CA19.9. Their relations with some prognostic factors of cancer colon were also investigated.

**Subjects and Methods:** The study included 48 patients with cancer colon and 20 apparently healthy volunteers as a control group. Patients were 23 males and 25 females with age range from 18 to 71 years (mean = 47±1.8). Both serum and plasma samples were obtained from patients and controls.

**Results:** Six percent of patients had grade 1 tumors, 77% had grade 2 and 17% had grade 3 disease. As regard to the stage, 52% of patients were stage II, 35.5% were stage III, while 12.5% were stage IV. Liver metastasis was present in 12.5%, while 35% showed lymph node metastasis. The VEGF, endothelin-1, CA19.9 and CEA were significantly higher in the cancer colon patients than in control groups (p-value <0.001, 0.006, <0.001 and <0.001; respectively). Plasma level of endothelin-1 and serum level of VEGF showed significantly higher levels in advanced stages of the disease (p value < .001 and in presence of liver metastasis (p value <0.001 & 0.002 respectively), while VEGF showed significant result when compared with the grade (p value=0.032). In this study, when comparing the levels of plasma endothelin-1 and serum VEGF between the metastatic, non-metastatic liver patients of the cancer colon group and the control group, the comparison was statistically significant for both markers (p<0.001). Endothelin-1 and VEGF showed significant positive correlation (r=0.77 and p-value <0.0001). Serum VEGF and CA19.9 showed good sensitivities which were not different (97.9% and 87.5%; respectively), while there was no significant difference between VEGF, CA19.9 and CEA with respect to specificities (100%, 90% and 100% respectively).

**Conclusion:** Both endothelin-1 and VEGF may be used for early detection of liver metastasis in cancer colon and VEGF may be used as a potential new marker for the diagnosis of cancer colon.

Further studies with larger number of patients are recommended to establish the value of VEGF and endothelin-1 as potential diagnostic and prognostic markers for cancer colon.

**Key Words:** Cancer colon – VEGF – Endothelin-1 – Angiogenesis.

INTRODUCTION

Endothelins have numerous potential roles in tumors, including modulating angiogenesis, inducing mitogenesis and invasion of tumor cells and protecting cells from apoptosis. Endothelin-1 modulates tumour angiogenesis, both directly through stimulation of endothelial cells, and indirectly through the induction of vascular endothelial growth factor (VEGF) [1].

The whole Endothelin-1 system has been identified in the human normal colon; its distribution suggests that it is secreted as a neuropeptide and a vasopeptide in this tissue [2].

It is a known fact that tumor growth and metastasis are highly dependent upon neoangiogenesis, the formation of capillary sprouts. These may arise either from preexisting blood vessels, circulating endothelial cells, or bone marrow-derived endothelial precursor cells [3]. The VEGF is one of the principal regulators of pathological and physiological angiogenesis [3,4].
The VEGF-A expression is up-regulated in numerous solid tumors [5,6]. In colon cancer, evidence from preclinical and clinical studies has shown the necessity of angiogenesis for tumor growth and metastasis [7].

**Aim of work:**

The aim of this work is to determine the levels of endothelin-1 and VEGF in the sera of newly diagnosed patients with cancer colon and to compare them with the routinely used tumor markers; CEA and CA19.9. Also to elucidate the relation between the serum levels of endothelin-1 and VEGF and some prognostic factors of cancer colon.

**PATIENTS AND METHODS**

**Patients:**

This study was conducted on 48 newly diagnosed patients with cancer colon, presented to the outpatient clinic in the National Cancer Institute (NCI), Cairo University; over a period of four consecutive months from March to June during the year 2006 were studied. Patients were 23 males (48%) and 25 females (52%). Their age ranged from 18 to 71 years (47±1.8). The study also included 20 apparently healthy volunteers as controls. Consent was obtained from all patients according to ethical committee.

**Methods:**

After careful history taking and clinical examination, the diagnosis was confirmed by biopsy and pathological examination. Chest X-ray and CT scan of abdomen and pelvis were performed for the patients to detect metastasis. Both serum and plasma samples were obtained from both patients and controls.

The following tests were then carried out:

- Routine laboratory tests: Liver function tests and kidney function tests using Beckmann CX-9 and complete blood count using coulter counter.
- Determination of plasma endothelin-1 by use of quantitative enzyme immunoassay technique (ELISA) using a kit supplied by (R and D system, Minneapolis, MN) [8].
- Determination of serum levels of VEGF by accucyte ® assay system [9].
- Determination of serum levels of tumour markers routinely used for the diagnosis of cancer colon (CEA and CA19, 9), using AX-SYM assay which is a Microparticle Enzyme Immunoassay (MEIA) [10]. The parameters studied were correlated with stage, grade, age, presence of liver metastasis and lymph node metastasis.

**Statistical analysis:** [11]

Data management and analysis were performed using Statistical Analysis System (SAS). Kruskal Wallis non-parametric analysis of variance was used to compare medians of the 3 independent groups and ANOVA was used to compare means of the 3 independent groups. Groups with respect to numerical variables were compared using the student’s t-test and Mann-Whitney non-parametric t-test. Pearson’s correlation coefficient was used to measure the strength of association between 2 numerical variables. All p-values were two sided. p-values ≤0.05 were considered significant. Sensitivity, specificity and diagnostic accuracy were the validity measures used for testing the studied parameters as diagnostic tools for cancer colon.

**RESULTS**

Patients with cancer colon were classified according to grade into: Three out of 48 patients (6%) with grade 1, 37/48 (77%) with grade 2 and 8/48 (17%) with grade 3. As regards the stage, 25/48 patients (52%) were stage II, 17/48 (35.5%) were stage III, while 6/48 (12.5%) were stage IV. Six out of 48 patients (12.5%) showed liver metastasis, while 17/48 (35%) showed lymph node metastasis.

Table (1) shows comparison of the serum level of VEGF, plasma level of endothelin-1, serum CA19.9 and serum CEA between the cancer colon group in relation to the control groups, the comparison was statistically significant for all the studied markers (p-value <0.001, 0.006, <0.001 and <0.001; respectively).

Table (2) shows comparison of the plasma level of endothelin-1 with the grade, stage, liver metastasis and lymph node metastasis in the cancer colon patients, the comparison was statistically significant for the stage and liver metastasis (p value <0.001). As regards the stage, endothelin-1 plasma level was lowest in
stage II and highest in stage IV, it showed statistically significant results between stage II & stage III, between stage II & IV and between stage III and stage IV.

Table (3) shows comparison of the serum level of VEGF with the grade, stage, liver metastasis and lymph node metastasis in the cancer colon patients, the comparison was statistically significant for the grade and the stage and liver metastasis (p value of 0.032, <0.001 and 0.002 respectively). As regards the stage, VEGF serum level was lowest in stage II and highest in stage IV, it showed a statistically significant result between stage II and stage IV.

On performing correlation between serum endothelin-1 with age in the cancer colon group, the comparison was statistically non-significant (r 0.067 and p value 0.651).

Table (4) shows comparison of the levels of plasma endothelin-1 and serum VEGF between the metastatic, non-metastatic liver patients of the cancer colon group and the control group. The comparison was statistically significant for both markers (<0.001). Endothelin-1 levels showed a statistically significant difference between the control and the metastatic groups, and between the metastatic and the non-metastatic groups. As regards VEGF, a statistically significant difference was observed between the control and the metastatic groups, between the control and the non-metastatic group and between the metastatic and the non-metastatic groups.

Serum VEGF in the cancer colon group showed negative correlation with age which was not statistically significant (r=−0.098 and p value 0.491).

Serum endothelin-1 showed positive correlation with serum VEGF in cancer colon patients, which was statistically significant (r=0.7725 and p value <0.0001) (Fig. 1).

Table (5) shows the diagnostic performance for the 4 studied markers. Serum VEGF and CA19.9 have good sensitivities which are not different (97.9% and 87.5% respectively). There is no significant difference between VEGF, CA19.9 and CEA with respect to specificities (100%, 90% and 100% respectively).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Endothelin-1 (pg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&amp;2 (n=40)</td>
<td>8.3 (6.0-11.2)</td>
<td>0.115</td>
</tr>
<tr>
<td>3 (n=8)</td>
<td>14.6 (8.2-22.3)</td>
<td></td>
</tr>
<tr>
<td>Stage:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II (n=25)</td>
<td>6.5 (5.2-8.5)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>III (n=17)</td>
<td>10.3 (8.5-14.0)</td>
<td></td>
</tr>
<tr>
<td>IV (n=6)</td>
<td>24.0 (21.5-24.7)</td>
<td></td>
</tr>
<tr>
<td>Liver metastasis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence (n=6)</td>
<td>22.9 (22-24)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Absence (n=42)</td>
<td>8.2 (5.8-10.5)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence (n=17)</td>
<td>10.4 (8.5-17.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>Absence (n=31)</td>
<td>7.4 (5.2-11.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Significant.  
Values are medians and interquartile ranges in parenthesis.  
VEGF : Vascular endothelial growth factor.  
CA19.9: Carbohydrate antigen 19.9.  
CEA : Carcinoembryonic antigen.

Table (3): Comparison of serum VEGF with the some of the prognostic factors of cancer colon using Mann-Whitney test and Kruskal-Wallis test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>VEGF (pg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&amp;2 (n=40)</td>
<td>728.5 (612-842)</td>
<td>0.032*</td>
</tr>
<tr>
<td>3 (n=8)</td>
<td>939 (809.5-1120.5)</td>
<td></td>
</tr>
<tr>
<td>Stage:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II (n=25)</td>
<td>663 (523-784)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>III (n=17)</td>
<td>841 (753.3-898.8)</td>
<td></td>
</tr>
<tr>
<td>IV (n=6)</td>
<td>998 (919.0-1177.8)</td>
<td></td>
</tr>
<tr>
<td>Liver metastasis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence (n=6)</td>
<td>1134.5 (991-1231)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Absence (n=42)</td>
<td>744 (612-843)</td>
<td></td>
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<tr>
<td>Lymph node metastasis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence (n=17)</td>
<td>836 (745-984)</td>
<td>0.053</td>
</tr>
<tr>
<td>Absence (n=31)</td>
<td>693.5 (542-843)</td>
<td></td>
</tr>
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</table>

* Significant.  
VEGF : Vascular endothelial growth factor.  
Values are medians and interquartile ranges in parenthesis.  
Group medians showing same letters are not statistically significant.
DISCUSSION

Colorectal cancer is the second leading cause of mortality from neoplastic disease in the United States. However its incidence in the developed countries is almost double that in the developing countries [12].

Collectively, the endothelins and their receptors-referred to as the endothelin (ET) axis-have key physiological functions in normal tissue. The ET axis also functions in the growth and progression of various tumours [13,14,15]. Their activation seems to promote tumour progression by means of several mechanisms [15,16], including cell proliferation, inhibition of apoptosis, matrix remodelling and bone deposition in skeletal metastases through activation of osteoblasts.

Tumor microvessel density is an important determinant of colon cancer metastasis and patient mortality [17]. The level of VEGF expression is a major determinant of microvessel density in colon cancers and VEGF expression is also correlated with metastasis and mortality [18].

In this study, the pre-therapeutic levels of endothelin-1 and VEGF were determined in the sera and plasma of patients with cancer colon and were compared with the tumor markers routinely used for diagnosis and follow-up of cancer colon as CEA and CA19.9. The relation between the levels of endothelin-1 and VEGF with some prognostic factors of cancer colon were also studied.

On comparing the serum levels of the four studied tumour markers (VEGF, endothelin-1, CA19.9 and CEA) between the cancer colon and the control groups, the comparison was
statistically significant for the four studied markers ($p$-value <0.001, 0.006, <0.001 and <0.001; respectively).

Consistent with our results, Asham et al., [19], found in their study done on 39 patients with primary colon cancer that ET-1 plasma levels were significantly increased in patients with primary tumor compared to controls ($p<0.01$). It was also found in the study done by Arun et al., [20], on 60 patients with primary colorectal cancer that the median plasma concentration of endothelin-1 in both hepatic non-metastatic and metastatic groups was significantly elevated compared with the control group ($p$-value 0.002 and 0.0001; respectively). Similarly, Peeters et al., 2000 [21], found in their study done on 55 patients with cancer colon that the serum level of endothelin-1 was higher in the cancer group compared to the control group ($p<0.05$). Eberl et al., [22] suggested that ET-1 seems to protect colon cancer cells from FASL-induced apoptosis.

As regards VEGF, De Vita et al., [23], found in their study done on 81 patients with colon cancer that the preoperative VEGF serum levels were significantly higher in the group of patients with colon carcinoma compared with the control group ($p<0.001$) which is consistent with results of this study.

In accordance with our results on VEGF, a study by Ratchada et al., 2005 [24], done on 49 cancer colon patients revealed that patient group possessed a significantly higher level of circulating VEGF than a healthy volunteer group ($p<0.001$).

Also Mark et al., [25], found that both plasma and serum VEGF were significantly higher in colon cancer patients ($p<0.0001$) in his study done on 116 colorectal cancer and 116 control patients. Also patients with colorectal cancer had significantly higher levels of sVEGF, compared to healthy blood donors ($p<0.0001$) in a study done by Werther et al., [26].

Consistent with our results, the VEGF levels of cancer patients were found by Tsai et al., [27], to be significantly higher than those of controls in their study which was done on 279 patients with primary colorectal cancer and 20 patients with hemorrhoids, as a control ($p<0.005$). Several studies carried out by Kumar et al., [28], Cubo et al., [29] and Nicolaisen et al., [30], also reported that VEGF pretreatment levels in colon cancer patients were significantly higher than controls ($p=0.0001$, =0.008 and <0.001; respectively).

The liver is the most common and critical site for the development of colorectal cancer metastases. Most metastatic tumors in the liver are supplied by the hepatic artery; however, the portal blood flow may contribute to the tumor blood supply to varying degrees [31,32]. The liver represents a unique microenvironment for metastasis formation, not only because of its sinusoidal endothelial cell lining, but also because of the abundant expression of certain angiogenic and growth factors. In general, the expression of angiogenic factors results from complex interactions among tumor cells, smooth muscle cells, endothelial cells, pericytes, fibroblasts and cells of the immune system. Several cytokines that are not angiogenic in vitro, such as interleukin (IL)-1ß, IL-6, PDGF-BB (homo-dimer of PDGF-B), TGF-α, IGFs and hepatocyte growth factor, are angiogenic in vivo, presumably because of the co-induction of angiogenic factors such as VEGF [33].

It was also postulated by Bagnato et al., [34], that endothelin-1, by acting directly on endothelial cells via the ET(B) receptor, modulates different stages of neovascularization, including proliferation, migration, invasion, protease production and morphogenesis and also stimulates neovascularization in vivo. Endothelin-1 can also modulate tumor angiogenesis indirectly through the induction of vascular endothelial growth factor (VEGF).

In this study, serum levels of endothelin-1 showed significant difference when compared to different stages of colon cancer and to the presence or absence of liver metastasis ($p<0.001$). The VEGF showed significant difference when compared to grade, different stages of colon cancer and in the presence or absence of liver metastasis ($p=0.032$, <0.001 and 0.002; respectively).

Consistent with our results, Tsai et al., [27], found in his study done on 279 patients with primary colorectal cancer and 20 patients with hemorrhoids (as a control) that the preoperative plasma vascular endothelial growth factor levels were positively correlated with tumor stage,
distant metastasis ($p<0.01$), but were not associated with lymph node metastasis. The same results were obtained by Lee et al., [35], who found a direct correlation between VEGF expression and the development of metastatic disease in his study done on 52 human colon carcinoma specimen.

Also Mark et al., [25], found in his study done on 116 colorectal cancer and 116 control patients that both plasma and serum VEGF levels, increased with advancing disease stage.

Werther et al., [26] as well, found in his study done on 614 patients with colon cancer and 91 blood donors volunteers as controls that patients with Dukes >> stage D disease had significantly ($p=0.01$) higher VEGF values than patients with Dukes >> stage A, B and C disease, who had comparable values.

Multiple regression analysis demonstrated a significant correlation between preoperative VEGF serum levels and age ($p=0.013$) in a study done by Devita et al., [23] which is contrary the results of the present study.

In this study, when comparing the levels of plasma endothelin-1 and serum VEGF between the metastatic, non-metastatic liver patients of the cancer colon group and the control group, the comparison was statistically significant for both markers ($p<0.001$). For endothelin-1, statistically significant results were obtained when further comparing control group with the metastatic group and metastatic with non-metastatic groups and for VEGF when comparing the control with the non metastatic group and metastatic group ($p<0.05$).

Consistent with our results, Arun et al., [20], found in their study done on 60 patients with primary cancer colon and 45 patients with known colorectal liver metastasis that plasma concentration of endothelin-1 were higher than in the control group, but was higher in the metastatic group, so he suggested that plasma big ET-1 levels should be evaluated as a potential tumour marker for the identification of hepatic metastasis at an earlier stage.

VEGF stimulates critical events in angiogenesis including endothelial cell proliferation and migration into and invasion of the extracellular matrix [36]. Therefore, the regulation of its synthesis is a critical event in new capillary formation. It was shown that the two members of the ET family that circulate in plasma, ET-1 and ET-3, fairly comparably stimulate VEGF production 3-4-fold in cultured human vascular smooth muscle cells and are equipotent to hypoxia Bagnato et al., [14].

Salani et al., [37], investigated the role of ET-1 on human umbilical vein endothelial cell (HUVEC) phenotype related to different stages of angiogenesis. When tested in combination with vascular endothelial growth factor (VEGF), ET-1 enhanced VEGF induced angiogenic-related effects.

A statistically significant positive correlation between serum endothelin-1 and VEGF in cancer colon patients was found in this study ($r=0.7725$ and $p<0.0001$).

On comparing the diagnostic performance for the four studied markers, serum VEGF and CA19.9 have good sensitivities which are not different (97.9% and 87.5%; respectively). There is no significant difference between VEGF, CA19.9 and CEA with respect to specificities (100%, 90% and 100%; respectively).

Consistent with our results, Celen et al., [38], found that the diagnostic sensitivity of VEGF for colorectal carcinoma was higher than the sensitivity of CEA in his study done on thirty-three patients with colorectal adenocarcinoma and 10 healthy controls.

Conclusion:

We can conclude that VEGF may be considered a useful marker for the diagnosis of cancer colon due to its good sensitivity and specificity. Also serum endothelin-1 and VEGF may be used as potential factors to predict early metastasis to the liver in patients with cancer colon. We can also conclude that serum endothelin-1 and VEGF can be considered good prognostic markers for cancer colon, however, further studies with larger number of patients are recommended to establish the use of VEGF and endothelin-1 as potential markers for cancer colon.

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