Clinical Relevance of Serum Vascular Endothelial Growth Factor and Interleukin-6 in Patients with Colorectal Cancer

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ABSTRACT

Background/Aim: Some biological factors play a role in stimulation of malignant growth, metastasis and angiogenesis; however, their clinical relevance has not yet been well established for most of them. This work was aimed at studying the clinical relevance of serum vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6), in patients with colorectal cancer (CRC).

Materials and Methods: Preoperative serum levels of VEGF and IL-6 were measured by enzyme-linked immunosorbent assay in 35 CRC patients and in 30 healthy controls.

Results: CRC patients with or without metastasis had significantly higher VEGF and IL-6 levels than healthy controls (all \( P < 0.001 \)). Patients with advanced clinical stage had significantly higher levels of VEGF and IL-6 than those with early clinical stage (all \( P < 0.001 \)). Also, patients with metastatic disease had significantly higher VEGF and IL-6 levels than those with localized disease (all \( P < 0.001 \)). The diagnostic accuracy for invasiveness was 83% for VEGF (cut off value = 240 pg/ml) and 66% for IL-6 (cut off value = 6.7 pg/ml), with sensitivity 79% and 74% and specificity 68% and 59%, respectively.

Conclusion: In CRC patients, preoperative measurement of serum VEGF and IL-6 may prove useful non-invasive diagnostic indicators associated with advanced clinical stage and tumor metastasis that warrants further investigations.

Key Words: Clinical relevance, colorectal cancer, interleukin-6, vascular endothelial growth factor

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endoscopy unit of the Specialized Medical Hospital and from referrals of the Oncology Center, Mansoura University. The included patients comprised 26 males and 9 females with an average of age $49 \pm 13.2$ years. Thirty healthy subjects (23 males and 7 females) were selected on voluntary basis and included as the control group. Patients with history or clinical evidence of chronic infections, immunological disorders, recent pregnancy, trauma, surgery (within 1 month), or any other malignances were excluded.

The study protocol conforms to the Medical Sciences Ethics Committee of Mansoura Faculty of Medicine and all the included patients and selected controls had their written informed consent. Medical history included in addition to the demographic data, symptoms suggestive of colonic neoplasm (abdominal pain, bloody stools, fever, anemia and loss of weight), history of colonic troubles and family history of CRC. Clinical examination included both general and local abdominal examination with per rectal examination. A series of investigations were performed including laboratory tests (complete blood count, ESR, liver function tests, serum creatinine, fasting blood glucose, and carcinoembryonic antigen) and radiological assessments (abdominal ultrasound, barium enema, and abdominal computed tomography).

Elective colonoscopy was performed following routine preparation with recoding of the observed colonic lesions and obtaining mucosal biopsies from the tumor tissues and the adjacent normal mucosa for histopathological assessment. Tumors were graded according to the pathological features (TNM classification).[^15]

### Sample collection
Preoperative serum samples were obtained from the included 35 CRC patients once the diagnosis was established. Serum was also collected from 30 healthy volunteers using the same procedure as for the cancer patients. The control group comprised 7 females and 23 males with an age range of $59 \pm 10.9$ years. To avoid pre-analytical sample-to-sample variation due to blood collecting procedures, each blood sample was allowed to clot for at least 4 h before collecting the serum, which was immediately frozen and stored at -80 °C until used for the measurement of VEGF and IL-6 levels.[^16]

### Quantification
For quantitative measurements of serum VEGF and IL-6 levels, we used the Quantikine quantitative human VEGF and IL-6 sandwich enzyme-linked immunosorbent assay kits (R and D system, Abington, UK for VEGF and Minneapolis, Minnesota for IL-6), which are specific for VEGF and IL-6, respectively. All the analysis and calibrations were performed in duplicate according to manufacturer’s instructions. The calibration on each microtiter plate included recombinant human VEGF and IL-6 standards. Optical density was measured using a microtiter plate reader at 450 nm. The blank was subtracted from duplicate readings for each standard and sample. The VEGF and IL-6 concentration was reported in pg/ml.

### Statistical analysis
Statistical analysis was done using the SPSS 10.0 software package. The comparison of continuous variables in two different subgroups was performed by the using non-parametric Mann-Whitney U-test. Comparison of the continuous variables in three different subgroups was performed using the ANOVA test. The diagnostic performance of both VEGF and IL-6 was assessed by receiver operating characteristic curve. $P \leq 0.05$ was considered statistically significant.

### RESULTS
The results of patient’s characteristics showed that CRC patients with metastasis had significantly higher percentage of abdominal discomfort, ESR ($P = 0.02$, and 0.005, respectively) than CRC patients with localized lesions [Table 1]. Patients with advanced clinical stages had significantly higher levels of VEGF and IL-6 than those with early clinical stage ($P < 0.001$) [Table 2].

CRC patients with or without metastasis had significantly higher VEGF and IL-6 levels than controls (all $P \leq 0.001$). Patients with metastatic disease had significantly higher VEGF and IL-6 levels than those with localized disease ($P < 0.001$) [Table 3].

Using cutoff values 240 pg/ml for VEGF and 6.7 pg/ml for

### Table 1: Characteristics of the studied patients with colorectal cancer

|                            | Total (n = 35) | Localized (n = 12) | Metastatic (n = 23) | $P$ value |
|-----------------------------|---------------|--------------------|--------------------|-----------|
| **History**                 |               |                    |                    |           |
| Diarrhea                    | 13 (37)       | 3 (25)             | 10 (43)            | 0.28      |
| Blood in stool              | 31 (89)       | 9 (75)             | 22 (96)            | 0.06      |
| Abdominal discomfort        | 28 (80)       | 7 (58)             | 21 (91)            | 0.02      |
| Significant weight loss     | 17 (49)       | 5 (42)             | 12 (52)            | 0.55      |
| Smoking                     | 33 (94)       | 12 (100)           | 21 (91)            | 0.29      |
| Positive family history     | 8 (23)        | 3 (25)             | 5 (22)             | 0.82      |
| **Laboratory**              |               |                    |                    |           |
| HB (g/dl)                   | 10.3±0.9      | 10.8±1.6           | 9.9±2.1            | 0.2       |
| ESR (1st hour)              | 41±16         | 34±7               | 46±13              | 0.005     |
| **Colonoscopy**             |               |                    |                    |           |
| Proximal colon              | 13 (37)       | 5 (42)             | 8 (35)             | 0.68      |
| Distal colon                | 20 (57)       | 7 (58)             | 13 (57)            | 0.91      |
| Multiple                    | 2 (6)         | 0                  | 2 (9)              | 0.29      |

Figures in parentheses are in percentage.
IL-6, the diagnostic accuracy for invasiveness was found to be 83% and 66% respectively, with sensitivity 79% and 74% and specificity 68% and 59%, respectively [Table 4].

**DISCUSSION**

CRC patients had higher VEGF levels than healthy controls. Also, significantly higher serum VEGF was found in CRC patients with advanced clinical stages than in those with an early clinical stage, and in patients with metastasis than those with localized cancer. These findings run in parallel with previous reports where, the metastatic colon cancer expressed significantly more VEGF gene than localized tumors. The occasional discrepancies in the results of the different studies could be explained by certain factors, e.g. inflammation, tumor infiltrating cells, and number of platelets in the serum sample. Patients with high serum VEGF had high pathological tumor stage and regional lymph node metastasis. VEGF could modulate lymph vessel density and microvessel density that correlate with the malignant potential of tumors, patient survival and could be a useful tool for the selection of postoperative management and treatment strategies in patients with CRC. Therefore, high serum VEGF could be considered as an usher for advanced disease state and tumor metastasis in CRC patients. Similar to the findings of Ueda et al., who reported that an increase in the serum level of VEGF was shown to be a predictor of metastasis in gastrointestinal tumors.

There is a dynamic relationship among tumor cells, inflammatory cells, cytokines, and chemokines. Cytokines and chemokines within tumors can contribute to the progression of tumors to a more aggressive metastatic phenotype or play a role in tumor therapy. IL-6 is most often classified as a proinflammatory cytokine, although IL-6 and IL-6-regulated acute phase proteins were previously suggested as anti-inflammatory and immunosuppressive, and may negatively regulate the acute-phase response.

Our results proved higher levels of IL-6 in patients with CRC than healthy controls. Similarly, higher levels of IL-6 were found in metastatic than non-metastatic CRC patients. Our results are in agreement with the previous studies that reported increased circulating levels of IL-6 in patients with advanced pathological stages of CRC. Previous correlation between tumor tissue expression of IL-6 and serum concentration in CRC implies that increased systemic IL-6 might be a result of local tumor production. These findings have encouraged us to suggest the possible role of IL-6 as a trigger for the disseminated cells to develop into metastatic tumors. We ask for more clinical trials addressing the investigation of the novel genetic and pharmacologic agents including anti-angiogenic agents giving hope for future therapies which could be suitable for advanced CRC patients.

IL-6 can be released from tumor infiltrating leukocytes, but is produced to a large extent by tumor cells themselves. In human colon cancer, IL-6 expression parallels tumor progression, reaching a maximum in high grade cancerous lesions. In addition, IL-6 increases invasiveness of colon cancer cells and likely promotes secondary tumor formation through its angiogenic potency. The role of IL-6 in promoting progression and metastatic spread of colon cancer depends not only on the extent of basal but also, importantly, on the extent of inducible IL-6 expression at certain stages of tumor development. Thus, IL-6 could serve as autocrine and paracrine growth factor for CRC, and high serum level of this cytokine might correlate with the poor prognosis and the increased production of angiogenic factors.

| Group            | VEGF (pg/ml) | IL-6 (pg/ml) |
|------------------|--------------|--------------|
| Control (n = 30) | 78.1 ± 12.6  | 2.98 ± 0.7   |
| Localized (n = 12) | 108.7 ± 17.3 | 4.45 ± 1.9   |
| Metastasis (n = 23) | 518.6 ± 138.5 | 8.82 ± 2.4   |

**Table 2:** Comparison of serum VEGF and IL-6 between colorectal cancer patients and controls

| No. | Median VEGF pg/ml | P  | Median IL-6 pg/ml | P  |
|-----|-------------------|----|-------------------|----|
| Control | Total 30 | - | 0.21 | - | 0.52 |
| Male | 23 | 82±13.3 | 2.9±0.8 |
| Female | 7 | 75±10.5 | 2.7±0.3 |
| Colorectal cancer | Total 35 | 0.86 | - | 0.9 |
| Male | 26 | 299±176 | 7.6±2.7 |
| Female | 9 | 287±195 | 7.46±2.1 |
| Clinical stage | T1 16 | 213.4±32.2 | <0.001 | 5.17±2.1 | <0.001 |
| T2-T4 19 | 434.2±76.7 | 8.23±2.8 |

*Mann-Whitney U-test (P value), VEGF: vascular endothelial growth factor, IL-6: interleukin-6

**Table 3:** Comparison of serum VEGF and IL-6 levels between the studied groups

**Table 4:** Diagnostic accuracy, sensitivity, and specificity of serum VEGF and IL-6 in colorectal cancer patients

| Cut off value (pg/ml) | VEGF | IL-6 |
|-----------------------|------|------|
| Diagnostic accuracy (%) | 83   | 66   |
| Sensitivity (%)        | 79   | 68   |
| Specificity (%)        | 74   | 59   |

ROC curve with 95% CI
IL-6 has been shown to correlate with platelet count and platelet VEGF content. As circulating VEGF is mostly transported by platelet, IL-6 could be regarded as an indirect angiogenic factor that facilitates the production and distribution of VEGF to the metastatic sites. The importance of IL-6 on VEGF metabolism has been confirmed.[8] Both VEGF and IL-6 possibly have a role in stimulation of CRC growth and metastasis. An association between VEGF and IL-6 has been previously reported in carcinomas of the breast.[18] Although the biological mechanisms linking metastatic CRC with the increase in the serum levels of IL-6 and VEGF are not clearly understood, the findings of this study support a possible link between the advanced clinical disease and these cytokines.

In conclusion, in the CRC patients, raised serum VEGF and IL-6 may prove valuable non-invasive diagnostic indicators associated with advanced clinical stage and tumor metastasis that warrants further investigations.

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