

## Clinical Significance of Vascular Endothelial Growth Factor (VEGF) in Sera of Patients with Pediatric Malignancies

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### ABSTRACT

**Background:** Angiogenesis is essential for solid tumor growth. It is induced by tumor cells through stimulatory angiogenic peptides, one such peptide is vascular endothelial growth factor (VEGF).

**Purpose:** The ultimate aim of the work is to investigate the possible role of VEGF as an early biomolecule involved in the progression of pediatric malignant tumors with high metastatic potential.

**Patients and Methods:** Forty-five pediatric patients were studied. They included four groups with malignant solid tumors suffering from Ewing's sarcoma, osteosarcoma, neuroblastoma, and rhabdomyosarcoma. In addition, a healthy control group including fifteen age and sex matched children was included in the study. Serum VEGF levels were determined by ELISA technique.

**Results:** The level of VEGF was significantly higher in all types of solid tumors compared to normal healthy children. The mean values obtained for patients and controls were 429.44±258.55 pg/ml and 79.36±63.81 pg/ml, respectively. No significant difference was detected in the level of VEGF among males and females. Also, no statistically significant difference was detected among the different types of malignant tumors. However, a marked significant difference was elucidated between metastatic and non-metastatic cancer patients, the values recorded were 753.33±173.64 pg/ml and 267.5±75.54 pg/ml, respectively ( $p < 0.001$ ). Furthermore, the results showed that 207 pg/ml of serum level of VEGF is the optimal cut-off value (mean  $\pm$  2 SD of control) with sensitivity of 87% and specificity of 100%. Using the receiver operating characteristic (ROC) curve analysis, the area under the curve (0.917) indicated the validity of using serum VEGF level in the diagnosis of all different types of pediatric malignant solid tumors with high potentiality to metastasis.

**Conclusion:** VEGF is an angiogenic stimulatory peptide. Its serum level could be a reliable marker in assessing pediatric malignancies with high metastatic potentials.

**Key Words:** VEGF - Pediatric malignancy - Ewing sarcoma - Osteosarcoma - Neuroblastoma - Rhabdomyosarcoma.

### INTRODUCTION

Pediatric solid tumors represent about 60% of all pediatric malignancies. The groups of pediatric neoplasia are brain tumors (30%), neuroblastoma (15%), soft tissue sarcomas including rhabdomyosarcoma (10%), Wilms tumor (10%), bone tumors including osteosarcoma and Ewing's sarcoma (8%), retinoblastoma (5%) and miscellaneous tumors including hepatoblastoma, germ cell tumors and melanoma (17%) [1].

Angiogenesis is a prerequisite for tumor growth and metastasis. The tumor associated angiogenic factors could be produced by tumor cells or derived from inflammatory cells that infiltrate the tumor [2]. The most important biochemical factor is VEGF. It is expressed in a wide variety of tumor cells and elevated levels could be detected in sera of cancer patients such as pediatric cancer patients [3]. VEGF is a dimeric angiogenic growth factor. It is a key regulator of embryonic, physiological and pathological angiogenesis and is believed to be a control mediator of tumor angiogenesis [3]. The expres-

sion of VEGF in several tumors has been correlated with high vascularity, distant metastasis and a poorer prognosis than VEGF negative tumors [4]. The effects of VEGF are mediated mainly through two distinct receptors, namely FLT-1 (VEGF-1R) and (VEGF-2R) [5].

### PATIENTS AND METHODS

The study population included 45 pediatric patients with different types of solid tumors with age range 6 months to 17 years in addition to 15 normal healthy age- and sex-matched children. The group included the following:

*Group (1):* Included 15 healthy children used as a control group with age range of 6 months to 17 years.

*Group (2):* Included 15 newly diagnosed patients before chemotherapy and with no evidence of metastasis.

*Group (3):* included 15 patients under chemotherapy and with no evidence of metastasis.

*Group (4):* included 15 patients with evidence of metastasis. The most abundant malignant tumors were osteosarcoma, Ewings sarcoma, neuroblastoma and rhabdomyosarcoma.

#### *Determination of VEGF level:*

The assay was carried out by the ELISA technique using polyclonal antibody (Oncogene Research Products, USA) against VEGF conjugated to horseradish peroxidase with VEGF standard, measuring the absorbance at 450 nm.

#### *Statistical Analysis:*

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) was used for statistical analysis. Data were expressed as mean  $\pm$  standard deviation. Mean values of continuous variables were compared using analysis of variance (ANOVA) and/or t-test after transforming the data to logarithmic values. The significance level was set at *p*-values equal to or less than 0.05.

The receiver operating characteristic (ROC) curve was constructed by calculating the sensitivities and specificities of VEGF at several cut-off points. The area under the curve reflects the diagnostic efficiency of using the parameter in diagnosis [6].

### RESULTS

Table (1) shows the mean values of VEGF in patients and controls ( $429.44 \pm 258.55$  pg/ml and  $79.36 \pm 63.81$  pg/ml, respectively) as well as the effect of sex, age, and diagnosis of solid tumors on the level of VEGF in the patient groups. A marked significant increase in VEGF level can be observed in the patient groups collectively than in the control ( $p < 0.001$ , Fig. 1). In the patient group, no significant difference could be detected between males and females, also the difference among the different age categories was insignificant ( $p = 0.946$ ). As for the different types of pediatric tumors, Ewing sarcoma, osteosarcoma, neuroblastoma, and rhabdomyosarcoma, no significant difference could be observed among them although all showed significant differences from the control. With respect to metastasis, a highly significant increase was found in VEGF level in metastatic ( $753.33 \pm 173.44$  pg/ml) than non-metastatic ( $267.50 \pm 75.54$  pg/ml), patients with different pediatric tumors ( $p < 0.001$ , Fig. 2). The effect of treatment on the level of VEGF was highly significant since patients treated by chemotherapy showed significantly lower VEGF levels ( $236.07 \pm 84.63$  pg/ml) than those without treatment ( $524.36 \pm 265.13$  pg/ml,  $p < 0.001$ , Fig. 3)

Taking the cut-off value as mean + 2SD of the control group, 207 pg/ml was the optimal point. At this level, the sensitivity, specificity, diagnostic accuracy were all 100% since all patients without treatment recorded higher VEGF levels than the controls. With respect to the patients receiving chemotherapy, 10 out of 15 patients were higher than the selected cut-off level (sensitivity 66.67%). All the controls (15 cases) were under the cut-off value (specificity 100%) and so the diagnostic accuracy was 83.33%.

The receiver operating characteristic (ROC) curve constructed by drawing several sensitivity and specificity pairs at several cut-off values is shown in Fig. (4). The calculated areas under the curve were 1.0 and 0.917 for the comparison of the untreated and treated patient groups with the control group, respectively. The higher calculated area (more than 0.7) offers a good indication of the validity of using serum VEGF as a diagnostic tool for pediatric tumors.

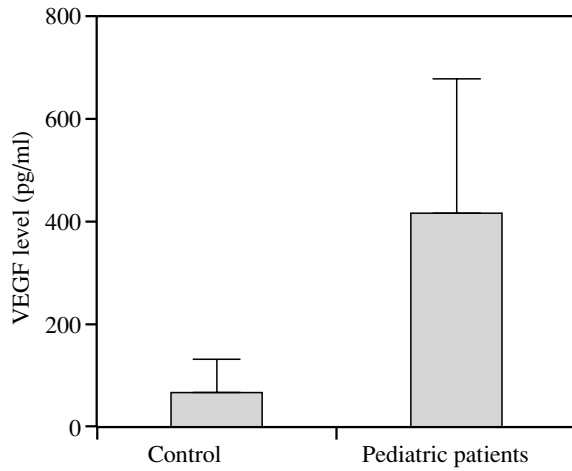


Fig. (1): Mean level of VEGF in the control and pediatric patient groups. [Bars represent the standard deviation. The difference is highly significant ( $p < 0.001$ )].

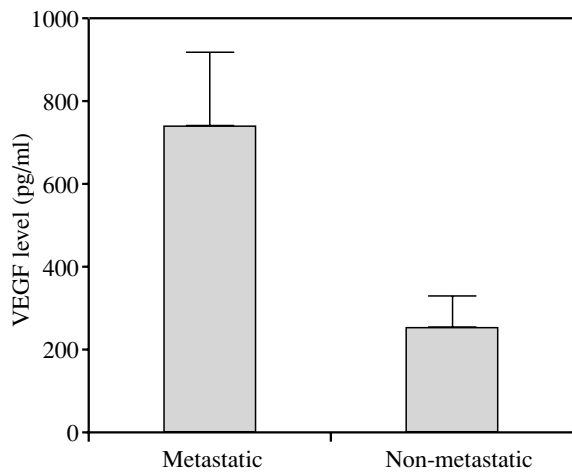


Fig. (2): Mean level of VEGF in the sera of metastatic and non-metastatic pediatric patient groups. [The difference is highly significant ( $p < 0.001$ )].

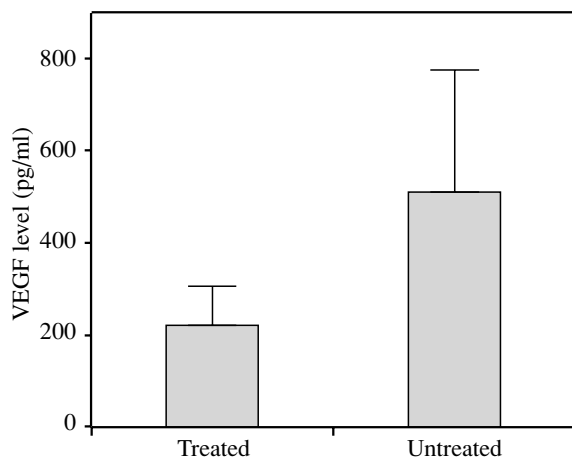


Fig. (3): Mean level of VEGF in the treated and untreated pediatric patient groups. [The difference is highly significant ( $p < 0.001$ )].

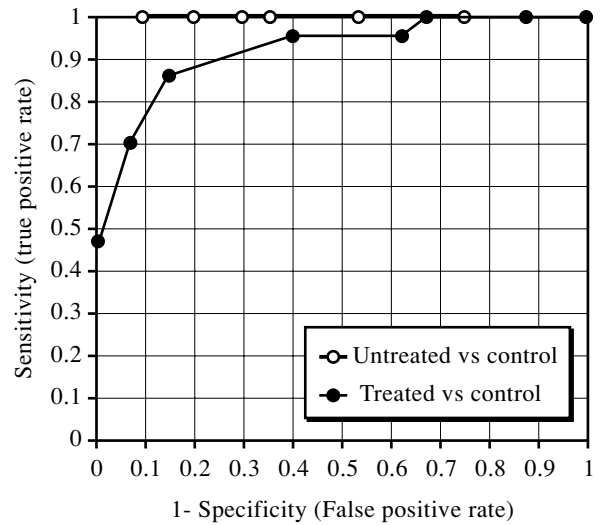


Fig. (4): The value of VEGF in the sera of pediatric tumors among 45 patients with different pediatric tumors and 15 controls as analyzed with the help of the receiver operating characteristic (ROC) curve. The area under the curve equals 1.0 and 0.917 for comparison of the untreated and treated patient groups with the control group, respectively.

Table (1): VEGF serum level (pg/ml) in the controls and in patients classified according to age, sex, tumor type and metastasis.

| Group                   | Mean±SD       | p-value |
|-------------------------|---------------|---------|
| Control (n=15)          | 79.36±63.81   | 0.001   |
| Patients (n=45)         | 429.44±258.55 |         |
| <i>Sex:</i>             |               |         |
| Males (n=29)            | 374.82±231.28 | 0.18    |
| Females (n=16)          | 528.43±283.4  |         |
| <i>Age (years):</i>     |               |         |
| < 5 (n=17)              | 473.82±284.81 | 0.946   |
| 5-10 (n=13)             | 313.07±167.32 |         |
| > 10 (n=15)             | 480.00±275.46 |         |
| <i>Tumor type:</i>      |               |         |
| Ewing sarcoma (n=11)    | 387.27±167.32 | 0.144   |
| Osteosarcoma (n=10)     | 495.00±310.21 |         |
| Neuroblastoma (n=13)    | 446.53±302.58 |         |
| Rhabdomyosarcoma (n=11) | 391.81±247.55 |         |
| <i>Metastasis:</i>      |               |         |
| Metastatic (n=15)       | 753.33±173.64 | 0.001   |
| Non-metastatic (n=30)   | 267.50±75.54  |         |
| <i>Treatment:</i>       |               |         |
| Treated (n=15)          | 236.07±84.63  | 0.001   |
| Untreated (n=30)        | 524.36±265.13 |         |

Table (2): Sensitivity, specificity, diagnostic accuracy and positive and negative predictive values of VEGF level.

| Cut-off value (pg/ml) | Sensitivity | Specificity | Diagnostic accuracy | Positive predictive value | Negative predictive value |
|-----------------------|-------------|-------------|---------------------|---------------------------|---------------------------|
| 207                   | 86.66%      | 100%        | 90%                 | 100%                      | 71.42%                    |

## DISCUSSION

Vascular endothelial growth factor (VEGF) is a dimeric angiogenic factor that is overexpressed by many tumors and stimulate tumor angiogenesis. VEGF initiates signaling by dimerizing the receptors, VEGF-1R and VEGF-2R.

The findings of the present work revealed a highly significant elevation of VEGF in sera of different types of pediatric malignancy in comparison with normal healthy children. The types of pediatric tumors included Ewing sarcoma, osteosarcoma, neuroblastoma, and rhabdomyosarcoma. Furthermore, there was no significant change in serum levels of the previously mentioned groups of pediatric tumors. However, a highly significant change was observed between the non-metastatic and the metastatic cases ( $p < 0.001$ ). The results indicated that VEGF level could be used as a useful marker for high potentiality to metastasis. The results of the current study are in agreement with what has been done with the results of serum levels of VEGF in patients with hematological malignancies that significantly exceeded those of the normal control [7]. This indicated that both solid and hematological tumors share a common biological phenomenon that the former show increase in vascularity and the latter reveal bone marrow angiogenesis.

The results of the present study might shed a light on the high serum levels of VEGF in neuroblastoma tumor as well as other types of pediatric tumors, the former has a neural crest origin which is biologically heterogenous. In a previous study, it has been shown that the high expression level of VEGF was associated with advanced tumor stage in human neuroblastoma [8]. This indicated that VEGF could play a crucial role for this tumor growth and its metastasis. The key role of VEGF in this pediatric solid tumor type could be applied to the other types of pediatric patients.

The results of the work also agreed with what has been reported previously that measuring angiogenesis regulators such as VEGF in serum or urine of patients with malignant solid tumor provided a new and sensitive marker for tumor progression, metastasis as well as prognosis [4].

For clinical decision making, the selected cut-off value of a laboratory test should provide the best diagnostic performance for either ruling out or ruling in the particular disease. The receiver operating characteristic curve (ROC) is a graphic method which can be used to determine this optimal cut-off level. In addition, it is a precise and valid measure of diagnostic accuracy [9]. Using ROC analysis, the area under the curve of VEGF was 0.976. When compared with the minimal acceptable area (0.7) [9] of a parameter to be considered as a good diagnostic tool, one can conclude that VEGF is a highly acceptable diagnostic marker for pediatric tumors with high potentiality of metastasis.

### Conclusion:

Evaluation of VEGF in sera of patients with pediatric malignancy could be of help in predicting patients at high risk for metastatic disease.

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