Circulating Adhesion Molecules In Relation To Angiogenesis Markers In Embryonal Malignancies

Safinaz A. Elhabashy, Mohamed Fawzy, Wafaa I. Rashid and Nagwa Abd EL-Ghaffar and Mona E. Khadr.

Abstract: Purpose: This study has been designated to assay the serum levels of a selected panel of soluble adhesion molecules (ICAM-1, V-CAM-1, and E-selectin) and correlate them to another group of serum angiogenic markers (B-FGF, and V-EGF) in a group of patients with embryonal malignancies. Subjects and methods: thirty pediatric patients with neuroblastoma, Wilms' tumor, and rhabdomyosarcoma were unselectively enrolled on to the study according to the inclusion criteria. Every patient had been evaluated and staging of his/her disease was done according to corresponding protocol guidelines after thorough history taking, clinical examination, radio imaging and laboratory investigations. Thirty age and sex-matched healthy children were included as controls. ELISA assayed serum levels of ICAM-1, VCAM-1, E-selectin, V-EGF, and b-FGF in patients before commencing any treatment, and controls. Results: 15 boys and 15 girls were evaluated, their ages ranged from 3 months to 9 years with a median of 36 months (mean: 36.2 months). We found that ICAM & VCAM were elevated above cutoff value in every individual patient, while E-selection, VEGF & b-FGF was only high in 10, 23 & 24 patients respectively. On comparison to the control group, mean ICAM, VCAM, b-FGF and V-EGF were significantly elevated (p<0.001 for each), whereas E-selection though higher in patients than controls, it didn't show any statistical significance (p=0.130). Loco-regional disease (stage 1, 2, and 3) versus distant metastatic spread (stage 4) revealed a statistically significant difference between the two groups regarding VCAM & E-Selection levels (p=0.007and p=0.04, respectively). The relationship between the two groups of study parameters showed a highly significant correlation between ICAM & VCAM, and V-EGF and b-EGF. Conclusion: the significance of adhesion molecules and angiogenesis markers in childhood malignancy can be extended with confidence to include a diversity of embryonal tumors indicating neuroblastoma, Wilms' tumor, and rhabdomyosarcoma. Serum levels of ICAM, VCAM, VEGF, and b-EGF are relevant pretreatment indicators of such diseases and both VCAM and E-selectin can represent indices of the extent and stage. However more trials are required for further adding the prognostic value of such parameters and their possible therapeutic potentials in immune-targeted therapy.

Key words: Adhesion molecules, angiogenic markers, embryonal malignancies

INTRODUCTION

The role of adhesion molecules expressed by malignant cells and their role in the dislodgement of tumor cells has been evaluated in several studies[1]. Movement or dislodgement of the neoplastic cells from its in situ position requires diminishing of adherence between cells as well as between cells and extra vascular matrices[2]. Adhesion molecules, which are distinct membrane surface receptors that participate in coordinating vital biological events such as morphogenesis, cell migration, and intercellular communication[3], can play a role in the adherence and penetration of the blood vessel wall by tumor cells allowing them to disseminate[4]. In addition, shedding of such molecules from surface of the cancer cells may represent an important mechanism for tumor cells to escape immunosurveillance[5].

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are cytokine-inducible glycoproteins belonging to the immunoglobulin supergene family; both are up
regulated by cytokines such as IL-1, TNF-alpha, and interferon-gamma[5]. ICAM-1 has shown to be expressed on malignant cells in a number of hematological and non-hematological neoplasms[7]. E-selectin (CD62E) which is detected on the surfaces of endothelial cells is another adhesion molecule mediating the initial binding of leucocytes to microvascular endothelium by carbohydrate ligands on corresponding target cells, while "E" stands for endothelial[8-9].

Angiogenesis, the formation of new blood vessels from pre-existing ones, is involved in growth, maintenance, and metastasis of most solid tumors[10]. During tumorigenesis, neoplastic lesions initially undergo avascular growth to a size not greater than 2-3 mm. This phase is followed by a second event that distinguishes a growing tumor from one that is dormant. The switch from avascular to vascular phenotype is known as "angiogenic switch". This initiates a cascade of events that result in the expansion of tumor volume and subsequent metastasis[11]. Angiogenic factors such as b-FGF, VEGF, and others, secreted by tumor, endothelial, and supporting cells are required for angiogenesis[12].

Several reports have demonstrated that neovascularization correlates with clinicopathological factors and patient's prognosis in a variety of tumors[13]. This role though considered by some authors as a matter of debate[14], yet the majority of studies confirms its importance[15]. Data in literature suggest that the circulating VEGF level is a useful marker of tumor status and prognosis in most types of human cancers[16].

MATERIALS AND METHODS

The current study has been designated to assay the serum levels of a selected panel of soluble adhesion molecules (ICAM-1, V-CAM-1, and E-selectin) as well as another group of serum angiogenic markers (V-FGF, and b-FGF) in a group of embryonal tumors among the pediatric population with cancer to clarify patterns of correlations and association. During the period from JUNE, 2005 to April, 2006 a total of thirty pediatric patients with different subtypes of embryonal malignancies (neuroblastoma, Wilms’ tumor, and rhabdomyosarcoma) who presented to the Pediatric Oncology Clinics of both NCI and Ein-Shams university Hospitals were randomly enrolled on to the study whenever fitting the inclusion criteria; age less than 18 years, previously untreated patients, with pathologically confirmed embryonal malignancy of any stage and without any past history of illness or other co-morbidity.

Every patient has been evaluated and staged according to corresponding protocol guidelines after thorough history taking, clinical examination, radio-imaging and relevant tumor markers.

Thirty healthy children matched for age and sex were included as controls. All subjects (newly diagnosed patients before any kind of treatment, and controls) were subjected to sampling of 5 ml of blood after an informed verbal consent from each study subject guardians. After clotting at room temperature for 2 hours, the aliquots were separated following centrifugation for 5 minutes at 2500 r.p.m. and stored at -30ºC until time of assay.

Assay of Soluble Adhesion Molecules: Serum levels of ICAM-1, VCAM-1, and E-selectin were measured with commercial sandwich ELISA assays based on dual monoclonal antibodies according to the manufacturer (R&D systems Europe, Abingdon, UK)[17].

Assay of Angiogenic Markers: The serum levels of V-EGF, and b-FGF were assayed with sandwich enzyme immunoassay methods (Quantikine: R&D systems, Minneapolis, MN)[17-18].

Statistical Analysis: Statistical analysis was performed using statistical package for social sciences (SPSS) version 13.0. Cut off levels of all studied parameters were defined as values above the 95th percentile of normal controls. P-values<0.05 were assigned to be significant.

RESULTS AND DISCUSSIONS

Results: Thirty patients with embryonal malignancies, 15 boys and 15 girls were evaluated. Their ages ranged from 3 months to 9 years at time of presentation with a median of 36 months (mean: 36.2 months). They were diagnosed pathologically as neuroblastoma (n=12), Wilms[5] and rhabdomyosarcoma[7].

Fig. (1) and table (1) show patients' criteria and disease status at time of presentation.

Fig. 1: Loco-regional disease versus distant metastasis
Adhesion Molecules and Angiogenic Markers: Among patients’ group, mean levels of ICAM, VCAM, V-EGF and b-FGF were significantly elevated compared to the control group (each, p=0.000), while E-selection though higher in patients, yet this was not of statistical significance (p=0.130), table (2).

Study Parameters According to Disease Entity and Stage: Comparing the difference between subgroups according to disease entities, E-selection was the only parameter showing a statistically significant difference between NBL and RMS (P<0.05) and NBL versus WT (P<0.05), table (2).

Table (4) shows patterns of different parameters among patients with loco-regional disease (i.e. stage 1, 2, and 3) against those with distant metastasis spread (i.e. stage 4). Only VCAM and E-Selection showed a statistically significant difference between the two groups (p=0.007 & 0.04, respectively).

Correlation Between Adhesion Molecules and Angiogenic Markers: The relationship between the two groups of study parameters was questioned. The answer is presented in table (5) with highly significant correlation between adhesion molecules and angiogenic markers, but E Selectin showed non-significant correlation with angiogenic markers.

Taking the 95th centile of the control group as a cut off point, ICAM & VCAM were found to be elevated in all patient individuals, while E-selection, VEGF & b-FGF were only high in 10, 23 &24 patients respectively, table (3).

Discussion: Adhesion proteins are involved in many of the intermediate steps of metastasis cascade and are likely to show pronounced changes in expression during malignant progression[12]. Tumor cells invade the surrounding connective tissue and are liberated away from their primary localization after disruption of connections between neighboring cells. Circulating tumor clusters adhere selectively to the micro vascular endothelium of the selected secondary target organ site. This process is mediated by organ specific adhesion molecules, which are expressed on the endothelial cells of the preferred site and which serve as "homing receptors". These adhesion molecules have also been shown to facilitate tumor cell motility and therefore enhance the invasion of tumor cells into the tissue parenchyma at metastasis site[19].

Increased angiogenesis has been demonstrated to be a significant prognostic factor in many solid tumors. It was reported with non-Hodgkin’s lymphoma, myelodysplastic syndromes (MDS), chronic myeloid leukemia, and acute lymphoid and myeloid leukemia[20]. Though the role of angiogenic factors was more extensively investigated in adults rather than children with cancer[20], yet many pediatric tumors found to express a polysialylated form of the neural cell adhesion molecule (NCAM). Serum concentration of such adhesion molecule has been shown to be tumor associated in children with rhabdomyosarcoma, neuroblastoma and Wilms tumor. It has been concluded that serum adhesion molecule level can serve as a useful marker for differential diagnosis during workup of such tumors. Moreover, the authors stated that it could be helpful as well in distinguishing between various embryonal tumors and other lesions suspicious of malignancy[21].

In 1993 Banks et al. reported their preliminary observation of high soluble ICAM, VCAM, and E-selectin concentrations in patients with a heterogeneous group of advanced cancers[17]. Many other studies that included a wide variety of malignant types have established the role of ICAM in the process of tumor

Table 1: Patient’s criteria and disease status at presentation

<table>
<thead>
<tr>
<th>Age in months (mean)</th>
<th>All patients N=30</th>
<th>NBL N=12</th>
<th>WT N=11</th>
<th>RMS N=7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36.2</td>
<td>41.66±27.68</td>
<td>32.09±25.92</td>
<td>33.28±8.92</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>15</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>15</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (3.3 %)</td>
<td>-</td>
<td>-</td>
<td>1 (14.3 %)</td>
</tr>
<tr>
<td>2</td>
<td>8 (26.7 %)</td>
<td>2(16.7 %)</td>
<td>2</td>
<td>2 (28.6 %)</td>
</tr>
<tr>
<td>3</td>
<td>12 (40 %)</td>
<td>5(41.7 %)</td>
<td>5(45.5 %)</td>
<td>2 (28.6 %)</td>
</tr>
<tr>
<td>4</td>
<td>9 (30 %)</td>
<td>5(41.7 %)</td>
<td>2(18.2 %)</td>
<td>2 (28.6 %)</td>
</tr>
<tr>
<td>NSE</td>
<td>547.27±1553.46</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. Ferritin</td>
<td>-</td>
<td>125.98±177.18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LDH</td>
<td>-</td>
<td>3124.100±5030.54</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

growth and metastasis. VCAM has also emerged as an important adhesion molecule in malignancy[22]. And serum levels of ICAM were elevated in association with advanced stage malignancy among children with Hodgkin's disease, ES, and WT[21]. Both ICAM and VCAM are predominantly involved in leucocytes-endothelial cell adhesion, with their ligands being implicated in the progression of malignant melanoma and myeloid malignancies possibly by mediating tumor cell-endothelial cell interaction[24].

Our results on a group of embryonal tumor patients of different subtypes (neuroblastoma, Wilms tumor, and rhabdomyosarcoma) revealed higher serum values of ICAM, VCAM, and E-selectin in comparison to the

---

**Table 2:** Mean values of Adhesion molecules and Angiogenic markers in patients versus controls

<table>
<thead>
<tr>
<th></th>
<th>Controls N=30</th>
<th>All patients N=30</th>
<th>NBL N=12</th>
<th>WT N=11</th>
<th>RMS N=7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ICAM (ng/ml)</strong></td>
<td>242.48 ± 21.9</td>
<td>665.45 ± 148.7</td>
<td>684.75 ± 167.7</td>
<td>673.436 ± 158.8</td>
<td>619.826 ± 100.3</td>
</tr>
<tr>
<td><strong>VCAM (ng/ml)</strong></td>
<td>791.47 ± 10.4</td>
<td>2021.88 ± 36</td>
<td>2084.308 ± 579.3</td>
<td>2015.927 ± 444.5</td>
<td>1924.214 ± 321.7</td>
</tr>
<tr>
<td><strong>E-selectin (ng/ml)</strong></td>
<td>41.192 ± 48.6</td>
<td>58.216 ± 44.8</td>
<td>71.95 ± 28.2</td>
<td>51.236 ± 28.2</td>
<td>45.642 ± 24.1</td>
</tr>
<tr>
<td><strong>V-EGF (pg/ml)</strong></td>
<td>186.8 ± 87.2</td>
<td>413.7* ± 193.6</td>
<td>359.35 ± 33.7</td>
<td>301.74 ± 52.9</td>
<td>395.557 ± 270.9</td>
</tr>
<tr>
<td><strong>b-FGF (pg/ml)</strong></td>
<td>14.676 ± 2.5</td>
<td>26.95* ± 7.2</td>
<td>27.283 ± 7.3</td>
<td>26.663 ± 6.1</td>
<td>26.814 ± 9.4</td>
</tr>
</tbody>
</table>

* = Significant difference when compared to control group.
** = Significant difference between the 3 patients' groups.

**Table 3:** Percentage of patients with elevated levels of adhesion molecules and angiogenic markers

<table>
<thead>
<tr>
<th></th>
<th>Cut off value</th>
<th>Normal level (%) N=30</th>
<th>Elevated (%) N=30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ICAM (ng/ml)</strong></td>
<td>281.6</td>
<td></td>
<td>30(100)</td>
</tr>
<tr>
<td><strong>VCAM (ng/ml)</strong></td>
<td>883.5</td>
<td></td>
<td>30(100)</td>
</tr>
<tr>
<td><strong>E-SELECTIN (ng/ml)</strong></td>
<td>59.8</td>
<td>20 (66.7)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td><strong>V-EGF (pg/ml)</strong></td>
<td>297.4</td>
<td>7 (23.3)</td>
<td>23 (76.66)</td>
</tr>
<tr>
<td><strong>b-FGF (pg/ml)</strong></td>
<td>17.4</td>
<td>6 (20)</td>
<td>24 (80)</td>
</tr>
</tbody>
</table>

**Table 4:** Adhesion molecules and angiogenic markers in loco-regional versus distant spread disease

<table>
<thead>
<tr>
<th></th>
<th>Loco- regional (N=21) Mean ±SD</th>
<th>Disseminated (N=9) Mean ±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ICAM (ng/ml)</strong></td>
<td>616.028 ± 85.1</td>
<td>780.777 ± 201.7</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>VCAM (ng/ml)</strong></td>
<td>1844.823 ± 198.9</td>
<td>2435.011 ± 653.5</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>E-selectin (ng/ml)</strong></td>
<td>48.191 ± 21.8</td>
<td>81.61 ± 51.1</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>V-EGF (pg/ml)</strong></td>
<td>398.485 ± 192.2</td>
<td>449.211 ± 203.6</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>b-FGF (pg/ml)</strong></td>
<td>26.9 ± 7.5</td>
<td>27.055 ± 6.7</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**Table 5:** Correlation between different parameters in patients’ group

<table>
<thead>
<tr>
<th></th>
<th>ICAM</th>
<th>VCAM</th>
<th>E-selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>V-EGF</strong></td>
<td>$r = 0.599$</td>
<td>$r = 0.501$</td>
<td>$r = 0.108$</td>
</tr>
<tr>
<td><strong>P &lt; 0.001</strong></td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.490$</td>
</tr>
<tr>
<td><strong>b-FGF</strong></td>
<td>$r = 0.688$</td>
<td>$r = 0.586$</td>
<td>$r = 0.001$</td>
</tr>
<tr>
<td><strong>P &lt; 0.001</strong></td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.996$</td>
<td></td>
</tr>
</tbody>
</table>
The importance of VEGF has been shown to modulate the production and activity of matrix metalloproteinases (MMPs) that have its role in the induction of the angiogenic process. Micro vascular endothelial cells are producing these MMPs, breaking down the extra cellular matrix. This is one of the earliest and sustained events in the process of new capillary formation\(^{(31)}\). As matter of feedback response activation of some MMPs (MMP9) induces up regulation of VEGF\(^{(32)}\).

Nor et al.,\(^{(33)}\) demonstrated that VEGF expressed by tumor cells up regulates expression of the antiapoptotic protein Bel-2 in endothelial cells in vitro as well.

Over expression of Bel-2 is sufficient to enhance endothelial cell survival and protect against apoptosis induced by growth factor deprivation\(^{(15)}\).

High level of serum VEGF in cancer patients is generally considered to be associated with unfavorable clinical parameters such as disease progression, lack of response to chemotherapy, and poor survival. Thus some authors stated that an increased serum VEGF level might be clinically useful for the prediction of increased tumor growth, recurrence, or metastatic spread in individual patients\(^{(14)}\).

Addressing the significance of serum angiogenic markers (V-EGF, and b-EGF) in our study, both were significantly elevated than controls in 23 and 24 patients respectively. However, no correlation between the levels of any of these parameters and extent or stage of the disease was observed. In consistence to that, serum concentration of b-FGF was elevated in different cohorts of patients as neuroblastoma, Wilms' tumor, Ewing's sarcoma, and osteosarcoma\(^{(35,36)}\) as well as PNET, non-Hodgkin's lymphoma, Langerhan's cell histiocytosis, and medulloblastoma\(^{(37)}\) when compared to normal subjects. As well VEGF was higher in children and adolescent patients with NHL than normal controls. However, serum VEGF level was found not correlated to stage of the disease, and its level had a tendency to drop to the normal standard after achieving remission\(^{(29)}\).

Several reports have suggested that serum levels of VEGF as well as IL-6 are independent indicators of long-term outcome in NHL, Multivariate analysis indicated that early changes of IL-6 and VEGF serum levels within the first 3 weeks after initiation of chemotherapy were independent predictors of clinical response even when corrected for the influence of clinical prognostic factors. The data indicate that serial measurements of serum IL-6 and VEGF may be early prognostic indicators\(^{(18)}\). In animal model study (lymphoma affected dogs), level of circulating VEGF significantly correlated with the WHO sub-stage "b" prognostic factor. VEGF value at presentation showed an independent influence on the length of the disease
free interval\cite{39}. Similarly, a direct relationship between VEGF and leukemic blasts has been established in acute leukemia\cite{30}, both pre-therapy and post-therapy VEGF levels found to be independently predictive of survival in patients with HD\cite{40}. In osteosarcoma, increased pretherapeutic levels of VEGF found to be correlated not only with MVD and metastasis\cite{41} but also, associated with decreased overall and disease free survival\cite{42}. The role of MVD as an indicator of angiogenesis and long-term outcome was denied in other reports for non-metastatic osteosarcoma\cite{43}.

The inter-correlation between different study parameters (adhesion molecules and angiogenesis markers) was questioned in our study. Each of ICAM, and VCAM showed a significant correlation with each other and with each of VEGF and b-EGF as well. Although E-selectin was not correlated to any of the other parameters, yet it was the only adhesion molecule that showed statistical difference between subgroups of the study (NB vs. RMS, and NB vs. WT).

In cohort of gastric carcinoma patients, apart from E-selectin and VCAM the circulating soluble adhesion molecules ICAM and E-cadherin were significantly correlated with each other with the exception of\cite{39}. Other investigators concluded that the levels of circulating ICAM were significantly correlated with those of VCAM and E-selectin in a study of 48 patients with colorectal cancer\cite{42}.

The importance of understanding the role of angiogenesis and adhesion molecules in different types of malignancy is clearly rising nowadays. In application to treatment current protocols with anti-VEGF agents in patients with hematological malignancies involve the use of monoclonal antibody, blockers of the VEGF-receptor tyrosine kinase pathway, thalidomide and its analogs, as well as cyclo-oxygenase inhibitors\cite{39}.

**Conclusion:** The significance of adhesion molecules and angiogenesis markers in childhood malignancy can include a diversity of embryonal tumors; i.e. neuroblastoma, Wiln's tumor, and rhabdomyosarcoma. Serum levels of ICAM, VCAM, VEGF, and b-EGF are relevant pretreatment indicators of such diseases. Both VCAM and E-selectin can be considered as representative indices for disease stage and extent. However, further research with recruitment of bigger patients samples may be required for stronger support of these results.

**REFERENCES**


38. Pedersen, LM, TW Klausen, UH Davidsen and HE Johnson, 2005. Early changes in serum IL-6 and
VEGF levels predict clinical outcome following first line therapy in aggressive NHL. Ann Hematol, 84(8): 510-516.