Evaluation and Clinical Correlation of Bone Marrow Angiogenesis and Levels of Serum Angiogenic Factors in Acute Leukemia

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Abstract

In this study serum angiogenic factors [vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and tumour necrosis factor a (TNF a)] and cellular angiogenic factors (VEGF and VEGF-R2) were studied in 50 newly diagnosed acute leukemia patients, they were 24 ALL and 26 AML patients. The correlations of the studied angiogenic factors to each other and to the patients' survival and disease outcome were studied. During the follow-up period of 6 months, 22 patients died and 28 patients remained alive from whom 11 patients were refractory and 17 patients achieved complete remission.

On comparison between pretreatment concentration levels of measured serum angiogenic factors (VEGF, TNF- a and HGF) in ALL, AML and the control group, all the comparisons were statistically significant (p<0.0001, <0.0001 and 0.021 respectively). All serum markers were higher in AML group than control group, but only VEGF showed statistically significant elevation (p<0.0001), while in ALL patients, all markers were significantly higher than control group (p=0.01).

When comparing ALL and AML cases according to cellular angiogenic factors detected by immunocytochemistry, cellular VEGF-R2 was slightly higher in ALL group, while cellular VEGF was slightly higher in AML group. The comparisons were statistically non-significant for both angiogenic factors.

As regards response to therapy, in ALL, cases with high sVEGF showed a statistically significant lower rate of complete remission than cases with low sVEGF (p=0.041). The same results were obtained for AML but the comparison did not reach a significant level (p=0.082).

Serum VEGF was the only reliable marker to predict relapse in ALL (p=0.009) and AML (p=0.049).

On comparing serum VEGF to the outcome in ALL, high sVEGF cases showed a statistically significant higher rate of death than low sVEGF cases (p=0.05), while in AML, the same results were obtained but the comparison did not reach a significant level.

As regards the survival time, cases with low sVEGF level showed higher mean survival and 6-month survival than cases with high sVEGF level (p=0.03).

A significant negative correlation was detected between serum VEGF and serum TNF- a (correlation coefficient (r) =-0.642, p<0.0001).

Conclusion: Serum angiogenic factors (VEGF, TNF- a and HGF) are markedly increased in cases of acute leukemia compared to normal controls. Cases with high sVEGF showed higher rate of death than cases with low sVEGF, so its targeting may provide a potent novel therapeutic approach in acute leukemias. VEGF may also be useful as a new prognostic factor and a predictor of relapse in different types of acute leukemia. Further studies with larger number of patients and longer duration of follow-up are recommended to throw more light on the significance of other angiogenic factors in relation to acute leukemia.

Key Words: Serum angiogenic factors – (VEGF) – Acute leukemia – Bone marrow angiogenesis.

Introduction

ACUTE leukemias are aggressive disorders characterized by accumulation of immature malignant cells in the bone marrow. The risk of relapse depends on many abnormalities and influences of growth factors [1]. In leukemia, the clonal population is characterized by a hierarchical organization similar to the normal hematopoiesis and a subset of the stem cells retains their undifferentiated "stem cell" morphology [2].

Angiogenesis is the formation of new vessels from an existing network of vasculature; it plays a significant role in a variety of physiologic and pathophysiologic processes [3]. Continuous supply of oxygen, growth factors and nutrients needed for progression, metastasis and invasion of the tumor tissue necessitates development of new vascular structures [4]. This process of neovascularization is regulated by the impact of competing influences between inhibitors and activators of angiogenesis [5].
It is well established that angiogenesis plays a critical role in tumor growth and development in solid tumors and hematologic malignancies [6,7]. Many studies have shown that patients with leukemia and lymphoma have increased microvascularization as well as increased levels of proangiogenic vascular growth factors, including VEGF-A [8,9].

Dysregulation of VEGF expression and signaling pathways may therefore play an important role in the pathogenesis and clinical features of hematologic malignancies [10].

All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation, although to different sites, times and extents. VEGF-A binds to VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF [11].

Hepatocyte growth factor (HGF), one of the pro-angiogenic factors, also known as a multiple function factor, protects cancer cells from cytotoxic agents, contributes to the development of chemoresistance and stimulates hematopoiesis [12]. Hepatocyte growth factor (HGF) has opposite biological activities in regulating apoptosis; although underlying molecular mechanisms are not clearly defined [13].

Tumor necrosis factor-alpha (TNF-alpha) is a proangiogenic factor which is a pro-inflammatory cytokine significantly elevated in all leukemias except for AML and myelodysplastic syndromes [14].

**Aim of work:**

The aim of this study is to evaluate some angiogenic factors in cases of acute leukemia and to detect their correlation to each other and their relation to the patients’ survival and disease outcome.

**Patients and Methods**

**Patients:**

This study was conducted on 50 newly diagnosed patients with acute leukemia (24 ALL-26 AML) who presented to the outpatient clinic of the Medical Oncology Unit of the NCI during consecutive 6 months from April to September during the year 2006. There were 32 males and 18 females. Their age ranged from 19 to 69. During the follow-up period of 6 months, 22 patients died, and 28 patients remained alive from who 11 were refractory and 17 patients achieved complete remission.

All patients were monitored regularly in the Medical Oncology outpatient clinic and treated with ordinary regimen of treatment. Ten apparently healthy volunteers were included as a control group. They were 6 males and 4 females, their age ranged from 22 to 83 years. A written consent was obtained from all patients according to institutional guidelines.

**Methods:**

1- The diagnosis of acute leukemia was made using WHO classification following conventional cytochemical stains (SBB) and surface markers analysis (IPT).

2- Both serum and whole blood samples were obtained from both patients and controls.

3- 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.
   - Determination of serum VEGF, TNF-α and HGF by use of quantitative enzyme immunoassay technique (ELISA) using a kit supplied by (R and D system, Minneopolis, MN) [15].
   - Immunocytochemical staining for VEGF-R2, and VEGF:

     Blast cells were separated from bone marrow using Ficoll-Hypaque sedimentation (Sigma-Aldrich, specific gravity 1077) according to Perper et al., 1989 [16]. Cell viability was determined using trypan blue exclusion test. Cyto-drops were then fixed on glass slides using acetone and kept at -80°C until stained.

     The method included 3 normal controls for both VEGF and VEGFR2.

     We used purified FLK-1 mouse monoclonal IgG1 (A-3) (sc-6251; Santa Cruz Biotechnology, Santa Cruz, CA, USA; working dilution 1:500) and VEGF rabbit polyclonal IgG (A-20) (sc-152; Santa Cruz Biotechnology, Santa Cruz, CA, USA; working dilution 1:500) [17]. The visualization system used was (Envision systems, DAKO) which is a 2 step immunohistochemical staining technique. It is based on horse radish peroxidase labeled polymer conjugated with secondary antibodies that doesn’t contain biotin or avidin [18]. The peroxidase enzyme and therefore the original antigens are then visualized with DAB as a chromagen. The slides were counterstained with light green.
**Interpretation of the results:** At least 500 cells were counted. Only the cells which were identified morphologically as blasts were counted and the results were expressed as the percentage of positive cells.

**Statistical analysis:** [19]

Data management and analysis were performed using Statistical Analysis System (SAS). Groups with respect to numerical variables were compared using the student’s t-test. ANOVA was used to compare means of 3 or more independent groups. Multiple comparison between pairs of alleles were performed using Bonferroni test (Post hoc range 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. The survival rate was expressed as the percentage surviving for 6 months calculated using the Kaplan-Meier method. Log rank test was used to test the difference between survival curves. A receiver operating characteristic (ROC) curve was used to illustrate the diagnostic properties of a test on a numerical scale.

Chi-Square test $X^2$ and Fisher’s Exact Test were used to test the difference in proportions.

Sensitivity, specificity and diagnostic accuracy were the validity measures used for testing the studied parameters as diagnostic tools for cancer colon.

**Results**

Leukemic patients were 32 males and 18 females. During the follow-up period of 6 months, 22 patients died and 28 patients remained alive from who 11 were refractory and 17 patients achieved complete remission.

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage %</th>
<th>Type</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL 24/50</td>
<td>48</td>
<td>AML 26/50</td>
<td>52</td>
</tr>
<tr>
<td>L1 1/24</td>
<td>4.20</td>
<td>M0 1/26</td>
<td>3.80</td>
</tr>
<tr>
<td>L2 18/24</td>
<td>75.00</td>
<td>M1 7/26</td>
<td>26.90</td>
</tr>
<tr>
<td>L3 5/24</td>
<td>20.80</td>
<td>M2 11/26</td>
<td>42.30</td>
</tr>
</tbody>
</table>

Table (1) shows percentages of different types and subtypes of acute Leukemia in patient study group.

<table>
<thead>
<tr>
<th></th>
<th>Control Mean±SD</th>
<th>ALL Mean±SD</th>
<th>AML Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVEGF (pg/ml)</td>
<td>28.3±1.7 (a)</td>
<td>151±27 (b)</td>
<td>308.7±88.5 (c)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>sTNF-α (pg/ml)</td>
<td>7.7±1 (a)</td>
<td>75±23 (b)</td>
<td>20±14.3 (a)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>sHGF (pg/ml)</td>
<td>313±271 (a)</td>
<td>3293±3166.5 (b)</td>
<td>2489.6±2602 (a,b)</td>
<td>0.021*</td>
</tr>
</tbody>
</table>

* Significant.

Groups with the same letter are not statistically significant.

VEGF : Vascular endothelial growth factor

TNF-α: Tumour necrosis factor-α.

HGF : Hepatocyte growth factor.

Table (2) shows Comparison between pretreatment concentration levels of measured serum angiogenic factors in the control, ALL and AML groups. There was a statistically significant differences between sVEGF, sTNF-α and sHGF when compared in the 3 groups ($p$=0.0001, <0.0001 and 0.021 respectively). Serum VEGF showed the highest concentration in AML group, while sTNF-α and sHGF both showed highest levels in ALL group.

<table>
<thead>
<tr>
<th></th>
<th>ALL Mean±SD</th>
<th>AML Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular VEGF-R2%</td>
<td>55.5±23</td>
<td>53.1±24.6</td>
<td>0.721</td>
</tr>
<tr>
<td>Cellular VEGF %</td>
<td>41.6±22.3</td>
<td>42.7±22.4</td>
<td>0.866</td>
</tr>
</tbody>
</table>

VEGF-R2: Vascular endothelial growth factor receptor-2.

VEGF : Vascular endothelial growth factor.

Table (3) shows comparison between ALL and AML according to cellular angiogenic factors detected by immunocytochemistry. The comparisons were statistically non-significant for both angiogenic factors.

<table>
<thead>
<tr>
<th></th>
<th>ALL Mean±SD</th>
<th>AML Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff of VEGF</td>
<td>≥149</td>
<td>≥290</td>
<td></td>
</tr>
<tr>
<td>Area under the curve</td>
<td>83</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.67-0.99</td>
<td>0.60-0.95</td>
<td></td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>78</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Specificity %</td>
<td>63</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Positive predictive factor %</td>
<td>70</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Negative predictive factor %</td>
<td>83</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Diagnostic accuracy %</td>
<td>75</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

$C$-value

0.009* 0.049*
Table (4) shows the diagnostic performance of serum VEGF as a prognostic factor in ALL and AML, the best cutoff levels of VEGF were $\geq 149$ in ALL and $\geq 290$ in AML (Figs. 1,2 respectively).

Table (5): Comparison between serum VEGF and response to therapy in ALL and AML cases using Chi-square test.

<table>
<thead>
<tr>
<th>(cutoff)</th>
<th>ALL</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum VEGF (pg/ml)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>$\geq 149$ pg/ml</td>
<td>14.3</td>
<td>7.5</td>
</tr>
<tr>
<td>$\geq 290$ pg/ml</td>
<td>71.4</td>
<td>3</td>
</tr>
</tbody>
</table>

Prognosis:
- Refractory: $x^2 = 6.65$, $p = 0.041^*$
- Died: $x^2 = 5.0$, $p = 0.082$

$^*$Significant.

VEGF: Vascular endothelial growth factor.

Table (5) shows comparison between serum VEGF and response to therapy in ALL and AML cases. There was a statistically significant difference between high and low serum levels of VEGF and prognosis in ALL cases, high sVEGF cases showed a statistically significant lower rate of complete remission than low sVEGF cases ($p=0.041$).

In AML, high sVEGF cases showed lower rate of complete remission than low sVEGF cases but the comparison did not reach a significant level ($p=0.082$).

Serum VEGF was the only reliable marker to predict relapse in ALL ($p=0.009$) (Fig. 1) and in AML ($p=0.049$) (Fig. 2).

Table (6): Comparison between serum VEGF and outcome in ALL and AML.

<table>
<thead>
<tr>
<th>(cutoff)</th>
<th>ALL</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum VEGF (pg/ml)</td>
<td>$\geq 149$ pg/ml</td>
<td>$\geq 290$ pg/ml</td>
</tr>
<tr>
<td>Alive</td>
<td>85.7</td>
<td>7</td>
</tr>
<tr>
<td>Died</td>
<td>14.3</td>
<td>10</td>
</tr>
</tbody>
</table>

$^*$Significant.

VEGF: Vascular endothelial growth factor.

Table (6) shows comparison between serum VEGF and outcome in ALL and AML. There was a statistically significant difference between high and low levels of sVEGF and outcome in ALL cases using a cutoff $\geq 149$. High VEGF cases showed a statistically significant higher rate of death than low sVEGF cases ($p=0.05$). In AML, high sVEGF cases showed higher rate of death than negative cases but it did not reach a significant level using a cutoff $\geq 290$ ($p=0.07$).

Figs. (1,2) show Receiver Operating Characteristic (ROC) curves to define the best cutoff to predict relapse in ALL and AML respectively. Serum VEGF was the only reliable marker to predict relapse in ALL ($p=0.009$) and in AML ($p=0.049$).
There was a statistically significant difference between cases with low and high sVEGF levels as regards the survival time ($p=0.03$). Cases with low sVEGF level showed mean survival of 194.7 days (95% CI 148-241) and 6-month survival of 71% (95% CI 70.8-71.2), while cases with high sVEGF level showed mean survival of 125.5 (95% CI 81-169) and 6-month survival of 17% (95% CI 16.8-17.2).

There was no statistically significant difference between AML and ALL as regards the survival time.

On studying correlation between serum and cellular studied parameters in ALL and AML all correlations were statistically non-significant.

On performing Pearson’s correlation between studied serum markers in leukemic patients, the only significant correlation was detected between serum VEGF and serum TNF-α (correlation coefficient ($r$) = -0.642, $p<0.0001$) (Fig. 1). While when performing Pearson’s correlation between studied serum markers and both the blast percentages in the bone marrow and TLC in leukemic patients, all correlations were statistically non-significant.
Angiogenesis is an important role in tumor progression, metastasis and invasion. It has been shown that there is an increase in vascular density of bone marrow in patients harboring hematological malignancies [20].

Angiogenesis is a complex and dynamic processes mediated by many pro-angiogenic and anti-angiogenic molecules. Disruption of the balance between pro-angiogenic and anti-angiogenic factors in favor of pro-angiogenic factors leads to formation of new vascular structures. Tumor cells, fibroblasts, monocytes and degradation of collagen matrix are the main sources of angiogenic factors.

From more than 20 proangiogenic and antiangiogenic agents identified, VEGF has been established as one of the most potent positive regulators of angiogenesis [21,22]. Besides its role as an essential regulator of physiologic and pathologic angiogenesis, VEGF triggers growth, survival and migration of leukemia and multiple myeloma cells; plays a pivotal role in hematopoiesis; inhibits maturation of dendritic cells and increases osteoclastic bone-resorbing activity as well as osteoclast chemotaxis [10].

Other cytokines-including tumor necrosis factor-α (TNF-α), hepatocyte growth factor (HGF) and others-have been reported to be involved as well, though their roles have not always been clearly defined [23].

In this study, there was a statistically significant difference between the 3 angiogenic factors VEGF, TNF-α and HGF when compared in the 3 groups (p=0.0001, <0.0001 and 0.021 respectively). In AML, when comparing serum VEGF, TNF-α and HGF to the control group, all markers were higher in AML group, but only VEGF showed statistically significant elevation (p<0.0001).

Consistent with our results, Wang et al. [24], found that VEGF plasma level was significantly higher in 107 AML patients when compared with normal controls (p<0.05) and Fuat et al. [25] reported high pretreatment serum VEGF in AML patients compared to the control group, but the comparison was not statistically significant.

Also, the HGF levels were higher in patients with AML than in controls in a study by Kim et al. [26] done on 30 AML patients, 10 ALL patients, and 14 CML patients.

Aguayo et al. [27], in his study done on 23 patients with CLL, 20 with ALL, 24 with CML, 30 with AML and 32 with MDS, measured levels of VEGF, bFGF, HGF, TGF-α and TNF-α in pretreatment plasma samples. All groups displayed a significantly higher level of VEGF and HGF compared with healthy controls.

In the present study, serum VEGF, TNF- α and HGF in ALL patients were all higher than controls (p=0.01).

In a similar study Fuat et al. [25] reported high serum VEGF levels that didn’t reach statistically significant levels in his study done on 45 patients with hematological malignancies.

Also, the pretreatment concentrations of plasma VEGF, bFGF, VEGFR1 and VEGFR2 were measured in 95 patients with newly diagnosed ALL. VEGF Level was significantly elevated (p<0.001) (Stefan et al. [28]).

Aguayo et al. [27], reported significantly high level of VEGF and HGF in their study on ALL patients.

VEGF and TNF-alpha were investigated in (B-ALL) and (B-CLL), in a study by Aref et al. [29]. In contrast to the results of the present study, in B-ALL patients, sVEGF, was significantly lower than control levels at diagnosis (p<0.001) and increased to near control levels in remission (p>0.05), while serum TNF-alpha levels showed no significant difference at diagnosis (p>0.05) and in remission (p>0.05) compared to control levels, this discrepancy may be attributed to their selection of only B-lineage phenotype.

In the present study, there was a significant difference between ALL and AML groups regarding serum VEGF and TNF-α (p<0.0001 and =0.01 respectively).

Contrary to these results, Fuat et al. [25], found no statistically significant difference between AML and ALL when he measured pretreatment serum VEGF (p>0.05).

Emerging data suggest that VEGF receptors are expressed by endothelial cells as well as hematopoietic stem cells. Therefore, it was hypothesized that functional VEGF receptors may also be expressed in malignant counterparts of hematopoietic stem cells such as leukemias. It was demonstrated that certain leukemias not only produce VEGF but also express functional VEGFR-2 in vivo and in vitro, resulting in the generation of an autocrine loop that may support leukemic cell survival and proliferation [30].
In the present study, when comparing ALL and AML according to measured parameters by immunocytochemistry, cellular VEGF-R2 was slightly higher in ALL group, while cellular VEGF was slightly higher in AML group.

The pretreatment concentrations of VEGF, bFGF, VEGFR1 and VEGFR2 were measured in the plasma of peripheral-blood samples obtained from 95 patients with newly diagnosed ALL in a study by Stefan et al. [28]. Levels of all factors were elevated in ALL patients compared to healthy controls ($p < 0.001$).

Agnieszka et al. [31], reported detectable levels of measured levels of sVEGF and sVEGFR-2 in all patients with AML and ALL as well as in all healthy control subjects in their study done on 39 AML and 15 ALL patients.

In this study, there was a significant difference between high expression and low expression of VEGF and outcome in ALL cases. High VEGF cases showed a significant higher rate of death than low VEGF cases ($p = 0.05$).

Consistent with these results, Sung et al. [32], examined bone marrow plasmas of 33 ALL patients at diagnosis for the presence of VEGF and bFGF. ALL patients were divided into 3 groups; one group is standard risk ALL, other group is high risk ALL who did not experience relapse and the other group is high risk ALL who have experienced relapse during treatment. Three patients who showed high levels of both angiogenic markers were relapsed and died due to disease progression. The remaining relapsed patients were alive without disease after salvage chemotherapy.

In the present work, in ALL cases, high VEGF cases showed a statistically significant lower rate of complete remission than low VEGF cases ($p = 0.04$). Also, in AML, high VEGF cases showed lower rate of complete remission than low VEGF cases but it did not reach a significant level ($p = 0.08$).

In a study by Wang et al. [24], done on 107 patients AML, VEGF level was found to be much lower in patients, who got complete remission (CR) after 2 cycles of chemotherapy, than that in patients without CR ($p < 0.05$). Furthermore, the higher the concentration of VEGF the shorter the survival was.

In the present study, only VEGF of all the studied angiogenic factors showed significant results when compared with survival time in leukemic patients. Cases with negative VEGF showed longer mean survival and 6-month survival than cases with positive VEGF ($p = 0.03$). There was no statistically significant difference between AML and ALL as regards the survival time.

Consistently, Aguayo et al. [14], found that elevated plasma levels of VEGF were associated with a reduced survival time and lower CR rates in acute leukemia cases.

Several studies in AML have indicated that higher levels of VEGF correlated with more aggressive disease [33,14].

Contrary to our results, Agnieszka et al. [31] found no significant difference in survival times in high and low expressers of VEGF, sVEGFR-1 and sVEGFR-2.

Also, in Stefan and colleagues’ study [28], on performing multivariate analysis, the likelihood of death was 8 times higher for patients with VEGF levels less than or equal to 19.1pg/mL (95% CI, 2.28-28.1) and 4 times increased for patients with VEGFR2 levels greater than 8222pg/mL (95% CI, 1.05-15.3), independently which is contrary to our results, Stefan et al. [28] postulated that although the follow-up in their study was short (maximum, 2.5 years), their findings indicated that angiogenic factors have different prognostic significance, depending on the biologic background in which they are analyzed. Those findings were in contrast to what has been described in other hematologic malignancies and they suggested significant biologic differences between ALL and AML and between adult and childhood ALL itself.

Many authors have shown that subsets of acute leukemia cells express VEGF and its receptors resulting in autocrine loops that modulate leukemia survival, proliferation and migration [34]. Thus, acute leukemia growth involves both autocrine and paracrine VEGF/VEGF receptor loops that regulate expansion of the leukemia clones within the BM microenvironment [35].

Pretreatment serum level of VEGF was negatively correlated with VEGFR1 ($r = -0.34; p = 0.013$) in a study by Stefan et al. [28] in his study done on 95 newly diagnosed ALL patients.

In the present study, when performing Pearson’s correlation between studied serum markers in leukemic patients, the only significant correlation was detected between serum VEGF and serum TNF-a (correlation coefficient ($r$) = -0.642, $p<0.0001$). While when performing Pearson’s cor-
relation between studied serum markers and the blast percentages in the bone marrow and TCL in leukemic patients, all correlations were statistically non-significant.

Consistently, VEGF serum levels were not significantly correlated with peripheral white cell count or bone marrow blast cell count in a study done by Aref et al. [29] on In B-ALL patients.

Conclusion:

We can conclude that angiogenic factors may play a significant role in the leukemic process. Understanding their roles may help in designing new therapeutic strategies for acute leukemias. The results of this study also contributed to better understanding of the prognostic significance of sVEGF levels in cases of acute leukemia and proposed that high levels of sVEGF may facilitate the disease progression and interfere with patients’ survival. Further studies with larger number of patients and longer duration of follow-up are recommended to throw more light on the significance of serum angiogenic factors in cases with acute leukemia.

References
23. FUHRMANN-BENZKEIN E., MA M.N., RUBBIA-BRANDT L., et al.: Elevated levels of angiogenic cytok-
Amira M. Khorshid, et al.


