RESEARCH ARTICLE

STUDY TO ASSESS THE PATHOPHYSIOLOGICAL ROLE OF VEGF IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML)

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ABSTRACT

Background: VEGF play a significant role in the pathogenesis of chronic myeloid leukemia.

Aims & Objective: (1) Assess the pathophysiological role of VEGF in patients with chronic myeloid leukemia (CML). (2) Study the effect of Hydroxyurea and Imatinib on serum VEGF level.

Material and Methods: A total of 40 cases of chronic myeloid leukemia (CML) and 20 age and sex matched healthy controls were included in this study. The patients were divided into 3 subcategories: Untreated cases (which did not receive any treatment); patients who were treated with Hydroxyurea and patients who were treated with Imatinib Mesylate. 5 ml of blood was collected, were centrifuged at 5000 rpm for 10 minutes and stored at - 20°C until assay.

Results: On comparing the various subgroups with control, the value was 726.61 ± 199.67 pg/ml in untreated group. It was 573.53 ± 213.423 pg/ml in Hydroxyurea group and 530.00±180.96 pg/ml in Imatinib group. Fresh cases had significantly elevated VEGF value (P value < 0.001). VEGF level significantly elevated in Untreated cases when compared to treated groups either hydroxyurea (p=0.02) or Imatinib (p=0.019). There was no significant difference between Hydroxyurea and Imatinib groups.

Conclusion: This study suggests that VEGF play a significant role in the pathogenesis of chronic myeloid leukemia. Understanding this may help in designing new therapeutic strategies (antiangiogenic agents) for this dreaded disease.

KEY-WORDS: VEGF; Imatinib; Hydroxyurea; CML

Introduction

Vascular endothelial growth factor (VEGF) is one of the potent angiogenic growth factor which is expressed in most of the malignancies.[1-6] The dependence of tumour growth and metastasis on blood vessels makes tumour angiogenesis a rational target for therapy .Antiangiogenic therapy has been tried in various solid malignancies with encouraging results but data is limited for haematological malignancy especially CML.[7-10] Imatinib is thought currently to be the most effective therapy in CML, but many patients on Imatinib show disease progression after variable duration.[11] Antiangiogenic therapy might have a role as an adjuvant therapy in such patients. Studies, aiming to explore the detailed angiogenic profile of CML may help in developing new therapeutic strategies for this myeloproliferative disorder.

Objectives: (1) Assess the pathophysiological role of VEGF in patients with chronic myeloid leukemia (CML). (2) Study the effect of Hydroxyurea and imitanib on serum VEGF level.

Materials and Methods

This study was conducted in Department of General Medicine, SS Hospital, IMS, BHU during the period of June 2008 to March 2009. There were 40 cases of chronic myeloid leukemia (both fresh and receiving treatment) and 20 age and sex matched healthy controls included in the study. The patients were divided into 3 subcategories: Untreated cases (which did not receive any treatment); patients who were treated with Hydroxyurea and patients who were treated with Imatinib Mesylate. Out of 40, 18 cases didn't receive treatments in past, rest 22 cases were receiving treatment, out of which, 15 receiving Hydroxyurea and 7 receiving Imatinib from variable duration. All patients in this group were asymptomatic and none was in blast crisis or accelerated phase of disease. Thorough history and clinical examination of the patients was done.

Diagnosis of chronic myeloid leukemia was done by GBP/ Bone marrow examination and detection of Philadelphia chromosomes. 5 ml of blood was collected from each subject, were centrifuged at 5000 rpm for 10 minutes and stored at - 20°C until assay. Measurement of the "free" form of cytokine, vascular endothelial growth factor (VEGF) was done by VEGF sandwich enzyme immunoassay (EIA) as per protocol. Study was approved by the ethics, committee of BHU. All patients gave written informed consent.

Statistical Analysis

Statistical analysis was performed using the SPSS16.0 for windows package. The level of significance was considered at 5% as cut off point. The significance level > 5% written as $p > 0.05$ was taken as statistically insignificant, similarly probability level such as $p < 0.05$; $p < 0.01$ and $p < 0.001$ were considered just significant, moderately significant and highly significant, respectively.

Results

Mean age of patients were 36.72 ± 11.99 years and the mean age in control group was 38.30 ± 11.61 years. There was no statistically significant age difference among cases and controls. The mean duration of treatment in Hydroxyurea group was 2.07 ± 1.69 (yr) and in Imatinib group was 2.51 ± 1.69 (yr) (Table-1).

Table-1: Characteristics of Patients

Characteristics	Untreated Cases (n=18)	Hydroxyurea (n=15)	Imatinib (n=7)
Male	11 (61%)	7 (46%)	5 (71%)
Female	7(39%)	8(54%)	2(29%)
Mean age (yr)	37.06 ± 13.03	33.40 ± 10.43	43.00 ± 11.24
Mean duration of symptoms (mon.)	3.4 ± 1.9	-	-
Mean duration of treatment (yr)	-	2.07 ± 1.69	2.51 ± 1.35
Mean spleen size (cm)	21.1 ± 3.5	-	-
Palpable spleen	-	5 (33%)	1 (14.2%)
Mean Hb (gm/dl)	10.41 ± 1.76	11.76 ± 1.24	11.68 ± 1.87
Mean Leukocyte count (per mm^3)	110355	8200	8185
Mean Cr (mg/dl)	0.90	0.80	0.84
Ph chromosomes (+)	16 (88%)	13(86%)	7(100%)

Table-2: Serum VEGF values in Major Groups (Independent T–Test)

Major Groups	Mean ± SD (pg/ml)	t - value	p - value
Control	467.75 ± 137.80	3.09	0.003*
Cases	634.80 ± 214.64		

* Statistically significant

Table-3: Mean VEGF Levels in Various Subgroups

Subgroups	VEGF Levels (pg/ml)
Untreated cases	726.61 ± 199.67
Hydroxyurea	573.53 ± 213.42
Imatinib	530.00 ± 180.96
Control	467.75 ± 137.80

Table-4: Comparison of S. VEGF Levels among Various Subgroups and Control

Subgroups	P value
Untreated Cases vs. Control	< 0.001*
Cases on Hydroxyurea vs. Control	0.10
Cases on Imatinib vs. Control	0.44
Untreated Cases vs. Cases on Hydroxyurea	0.02*
Untreated Cases vs. Cases on Imatinib	0.019*
Cases on Hydroxyurea vs. Cases on Imatinib	0.61

* Statistically significant

The control group has a mean serum VEGF value of 467.7 5± 137.80 pg/ml while cases of CML had a mean of 634.80 ± 214.64pg/ml. The t –value of test was 3.09 with p value of 0.003 (table-2). On comparing the various subgroups with control, the values was726.61 ± 199.67 pg/ml in untreated group, it was 573.53 ± 213.423 pg/ml in Hydroxyurea group and 530.00 ± 180.96 pg/ml in Imatinib group (table-3). Fresh cases had significantly elevated VEGF value (P value < 0.001). VEGF level significantly elevated in Untreated cases when compared to treated groups either hydroxyurea (p=0.02) or Imatinib (p=0.019). There was no significant difference between Hydroxyurea and Imatinib groups (table 4).

Discussion

Vascular endothelial growth factor (VEGF) is involved in tumour angiogenesis, an important process for the growth and metastasis of solid tumours.[12,13] Recently there are increasing data linking VEGF with pathogenesis of different haematological malignancy[6,14-17], but study on CML is still limited to draft any conclusion. The present study was undertaken in order to analyze the serum level of VEGF (a potent activator of angiogenesis), in different subgroups of patients

of chronic myelogenous leukemia (CML) to hypothesize any role of VEGF in the pathogenesis of CML. The mean duration of symptoms in untreated cases was 3.4 months. Serum Vascular endothelial growth factor (VEGF) level of control and various study groups were estimated. The level of VEGF was significantly higher in the case (all group combined) than in the control. In control group, the level of VEGF was found to be 467.75 ± 137.80 pg/ml. In study group its level was 634.80 ± 214.64 pg/ml (table 2). On subgroup analysis, we found that serum VEGF value was significantly elevated in untreated group when compared to controls. Mean VEGF level was 726.61 ± 199.67 pg/ml in untreated group while in control it was 467.75 ± 137.80pg/ml. In patients treated with hydroxyurea and imatinib it was 573.53±213.42 pg/ml and 530.00 ± 180.96 pg/ml respectively (table 3), although higher than control but was not statistically significant. On intergroup analysis of study group we found that serum VEGF was significantly higher in fresh group comparing to treated group. It has been shown in some studies that imatinib reduces the serum level of VEGF.[18,19] There was no significant difference between hydroxyurea and imatinib group. There were significant association between plasma VEGF levels and spleen size in Untreated patients with trends for higher VEGF values in patients with enlarged spleens (p=0.03), this finding is similar to the Peng L, Jian et al., which showed that Elevated plasma levels of vascular endothelial growth factor is associated with marked splenomegaly in chronic myeloid leukemia & Lymphoma.[20] Some studies have reported an increased secretion of GM-CSF from human endothelial cells after VEGF exposure.[21] Such findings reveal that VEGF can increase the expression for several growth factors with known stimulatory effects in hematopoietic malignancies. Lundberg et al[22] demonstrated that the chronic myeloproliferative diseases associated with an increased vascular density in the bone marrow compared to the bone marrow of healthy subjects. Alvaro et al.[23] evaluated the bone marrow vascularity and the plasma levels of angiogenic factors in patients with acute and chronic leukemia's including CML. Peng Liu et al[20], evaluated plasma levels of VEGF and FGF in chronic-phase CML patients and found significantly increased VEGF but not FGF in patients of CML. They also observed significant positive correlation between plasma VEGF level with spleen size and platelet count. Our study also had similar results obtained in above mentioned studies. Although we didn't directly measure bone marrow vascularity, we demonstrated increased VEGF in untreated patients which might lead to increase vascularity in bone marrow. Similarly we also found positive correlation between VEGF level and spleen size as reported by Peng Liu et al[20], but the present study found no correlation between VEGF level and platelet count. Recent investigations have shown that the production of VEGF in CML cells may be directly triggered by disease specific oncogene BCR/ABL.[24] John M.Ebos et al[19], found that the levels of VEGF expression in BCR-ABL positive cells were reduced in vitro by treatment with imatinib in a dose dependent fashion. Similarly Laurence Legros et al[18] demonstrated decreased VEGF in patients treated with Imatinib. In the present study, we found significantly low plasma VEGF levels among patients treated with imatinib, strengthening the observation made by above mentioned observer. We also found similar decrease in serum VEGF in patients treated with hydroxyurea. In literature we have not found any study regarding hydroxyurea and VEGF in CML. Our sample size studied is small and this observation will need further confirmation after large sample size study. These data suggest that the angiogenesis may have a role in the pathophysiology of leukaemias and that antiangiogenesis therapy could have an anticancer effect. Antiangiogenic therapy has been tried in various solid malignancies [8-10] with encouraging results, but data is limited for haematological malignancy especially CML. It has been observed that the patients with advanced CML are less sensitive to imatinib.[11] Responses to imatinib in patients with advanced disease are often transient, generally lasting less than 6 months. Further-more, the emerging problem of resistance in chronic phase CML, and particularly in advanced CML, may limit the long term treatment benefits of imatinib. These data signifies the need of newer effective therapy for CML and antiangiogenesis therapy may play a big role in future.

Conclusion

This study suggest that VEGF play a significant role in the pathogenesis of chronic myeloid leukemia, our sample size studied is small and this observation will need further confirmation after large sample size study. Understanding this may help in designing new therapeutic strategies (Antiangiogenic therapy) for this dreaded disease.

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