Prognostic Significance of Angiopoitein-2 in patients with Chronic Lymphocytic Leukemia

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Abstract: Introduction: Chronic lymphocytic leukemia is a lymphoproliferative disorder characterized by progressive lymphocytosis in the blood, bone marrow and lymphatic tissue. Angiopoietin-2 is a member of the angiopoietin family, which plays an important role in angiogenesis, the formation of blood vessels from pre-existing blood vessels. This study tried to throw light on the behavior of serum Ang-2 in newly diagnosed B-CLL searching for possible value for its estimation. **Patients and method:** The present study was conducted on 40 individuals. They were subdivided into two groups: Control group: Included 10 healthy individuals. Patient group: They were 30 newly diagnosed chronic lymphocytic leukemia patients. Patients and controls were subjected to history taking and clinical examination laying stress on splenomegaly, hepatomegaly and lymphadenopathy. Laboratory investigations including; complete blood picture, ESR, serum lactate dehydrogenase, kidney function tests, liver function tests, estimation of serum angiopoitein-2 by ELISA technique. Absolute lymphocyte count, bone marrow aspiration and Immunophenotypeing were performed in patients only. **Results:** There was statistical significant decrease in hemoglobin level and platelets counts in patients group as compared to control. However, TLC and ESR were increased significantly in patients as compared to control group. According to Rai classifications; ESR, Absolute lymphocytic count, TLC, and absolute lymphocytic count, the percentage of lymphocytes in BM, serum LDH, serum Ang-2 levels, and percentage of CD38 expression showed significantly progressive increase all through stages However Platelet count and Hb level showed significantly progressive decrease with the progress of the stages. There was a significant positive correlation between the level of Ang-2 and the stage of CLL, CD38, total leucocytic count, percentage of lymphocytes in BM and LDH in CLL patients. **In conclusion**, the present study shows that circulating levels of Ang-2 were higher in CLL patients at diagnosis in comparison with apparently healthy individuals

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Key words: Angiopoitein-2, Chronic lymphocytic leukemia (CLL).

1. Introduction:

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by progressive lymphocytosis in the blood, bone marrow and lymphatic tissue ***(Dighiero, 2003)***. In this disorder, the lymphocytic count is usually greater than or equal to 5,000/mm^3 with a characteristic immunophenotype (CD5+, CD19+, and CD23+ B cells) ***(Hallek et al., 2008)***.

CLL has a highly variable clinical course which spans from asymptomatic disease to a rapidly progressing course needing urgent and intensive treatment .There is a wide range of initial presenting features, most commonly painless lymphadenopathy, followed by splenomegally and or hepatomegally***(Jantus et al., 2008)***. Complications of pancytopenia including hemorrhage and infection represent a major cause of death in those patients ***(Rawstron et al., 2008)***. Treatment should be reserved for those with advanced symptomatic or progressive disease ***(Hallek et al., 2008)***. An evaluation of prognostic factors at the time of diagnosis can guide the timing and strategy of treatment **(Bertilaccio et al., 2010).**

Angiopoiteins are protein growth factors that promote angiogenesis, the formation of blood vessels from pre-existing blood vessels. The angiopoietin family has four members (Ang1, Ang2, Ang3, and Ang4) and two related receptors Tie1 and Tie2 ***(Shim et al., 2007)***.

Angiopoiteins cannot produce endothelial cell proliferation. They play an important role in the development of newly formed blood vessels. Ang-1 and Ang-2 are required for the formation of mature blood vessels. An Ang-1 act as maturation factor and promotes recruitment of pericytes and smooth muscle cells to the developing vessels ***(Tait and Jones, 2004)***.

Ang-1 and Ang-2 are reported to have many reciprocal effects. Ang-1 has an anti-apoptotic effect on endothelium, while Ang2 is reported to promote

apoptosis ***(Thurston et al., 2000)***. Ang-1 stabilizes vessels by promoting the association of the vascular endothelium with perivascular cells, while Ang-2 destabilizes vessels in the presence of vascular endothelial growth factor-A (VEGF-A) leads to robust angiogenesis while in the absence of VEGF-A or basic fibroblast growth factor, vessel regression results ***(***Yancopoulos , ***et al., 2000)***.

2. Subjects and Methods

The present study was conducted on 40 individuals. They were subdivided into two groups:

1-Control group: Included 10 healthy individuals. Seven males and 3 females with their ages ranged from (45-63) years with a mean age 52.1±5.95 years.

2- Patient group: They were 30 newly diagnosed chronic lymphocytic leukemia patients. Twenty males and 10 females with their ages ranged from (50-70) years with a mean age 60.9±5.71 years.

Patients and controls were subjected to thorough history taking. Thorough clinical examination laying stress on splenomegaly, hepatomegaly and lymphadenopathy.

Laboratory investigations including:

Complete blood picture, ESR, Serum lactate dehydrogenase, Kidney function tests, Liver function tests, Estimation of serum angiopoitein-2 by ELISA technique. Calculation of the absolute lymphocyte count, Bone marrow aspiration and Immunophenotypeing carried out in patients only.

3. Results

There was statistical significant decrease in hemoglobin level and platelets counts in patients group as compared to control. However, TLC and ESR were increased significantly in patients as compared to control group (table 1).

Rai classification showed that 53.4% of patients were in stage I and II while 20% were in stage IV. On the other hand 26.6% were in stages 0 and III. According to Rai classifications; ESR showed significantly progressive increase all through stages (P= 0.011). TLC and Absolute lymphocytic count showed significantly progressive increase with the progress of the stages (P= 0.005). The percentages of lymphocytes in BM, showed significantly progressive increase with the progress of the stages (p=0.017). Serum LDH levels, there was significantly increase in patients compared to control (P= 0.003). Serum ang-2 levels showed statistically significantly increase in patients compared to control (P= 0.001). Percentage of CD 38 expression in patients showed significantly progressive increase through the stages (P= 0.001). However Platelet count and Hb level showed significantly progressive decrease with the progress of the stages (table 2).

There was marked significance relation between CD38 and ang-2 in CLL patients, those patients with CD38 below 30% had ang-2 level lower than those with CD38 above 30% (table 3). There was a significant positive correlation between the level of Ang-2 and the stage of CLL (r = 0.635, p = 0.001), (fig. 1). There was a significant positive correlation between the Ang-2 serum level and total leucocytic count (r = 0.552 and p= 0.009), (Fig. 2). There was a significant positive correlation between the level of Ang-2 and percentage of lymphocytes in B.M. (r = 0.588, and p= 0.001), (Fig 3) There was a significant positive correlation between the level of Ang-2 and the level of LDH in CLL patients (r = 0.628 and p = 0.001), (Fig. 4).

Table (1): patients group as compared to control group

	Hb g/dL	TLC $x10^9$/L	Platelet count $x10^9$/L	ESR mm/h	CD 38	LDH (U/L)	Serum ang-2 (pg/ml)
Control	13.9 6±1.24	6.98±1.47	280.4 ±66.9	8.80 ±2.65	10.5±2.1	293.6 ±80.9	553.1±49.21
Patients	10.6 5±2.1*	102.2 ±6.98 *	147.8 ±51.3 *	59.2 ±21.4*	49.2+5.2	972.7 ±173.2*	2044.9 ±150.4 *

* Significant

Table (2): Rai classification of CLL staging

	Stage 0	Stage I	Stage II	Stage III	Stage IV	F	p
No(M:F)	4(2:2)	8(4:4)	8(4:4)	4(4:0)	6(6:0)		
ESR	36±6.89	41.5± 10.1	56.5±7.7	80±22.1	85.4± 10.7	3.258	0.011 *
TLC	75.2+ 6.98	85.2+ 6.2	98.19.7	107+12.5	120.8 +23.8	5.212	0.005 *
ALC	35+0.4	47+6.9	65.2+7.4	85.2+15	98.5+ 12.5	5.200	0.005 *
% of lymphocytes in BM	32%	35%	42%	50%	62%	5.588	0.001 *
Platelets count	196.5 ±27.71	174±22.31	168±12.88	145±13.91	61.50 ±27.2	6.325	0.003 *
LDH	637±12.9	739.5 ±56.8	822±37	884±65.8	1630.5±220	5.835	0.003 *
CD 38	15.5± 3.54	24.5± 4.24	33±4.12	50±3.77	72±7.31	8.362	0.001 *
Ang-2	504.5 ±78.8	845.5 ±168.4	1399.3 ±586.1	2444.2 ±767.3	5265.5±575	5.362	0.001 *

* Significant

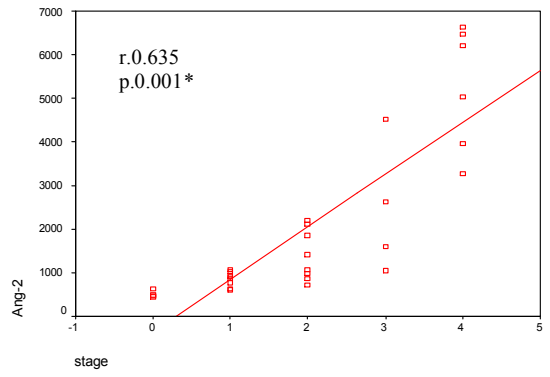

Fig. (1): Correlation between the level of Ang-2 and the stage of CLL patients.

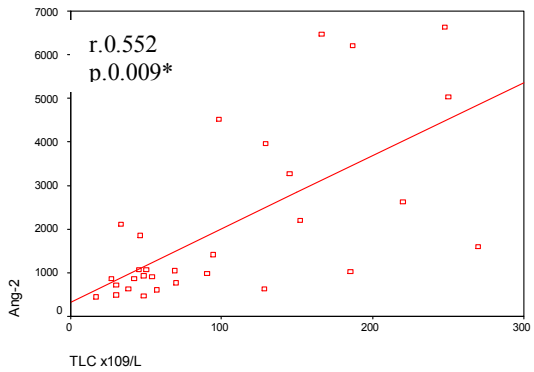

Fig. (2): Correlation between the level of Ang-2 and TLC $\times 10^9$/L in CLL patients.

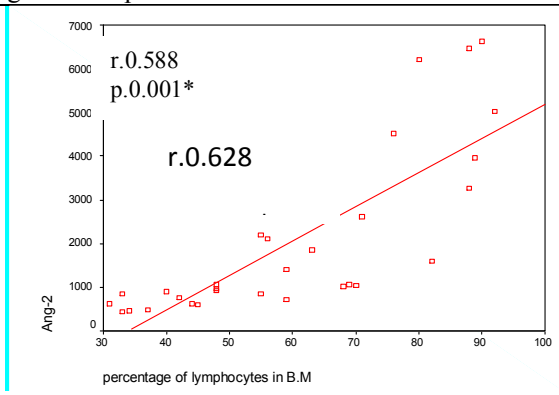

Fig. (3): Correlation between the level of Ang-2 and the percentage of lymphocytes in BM.

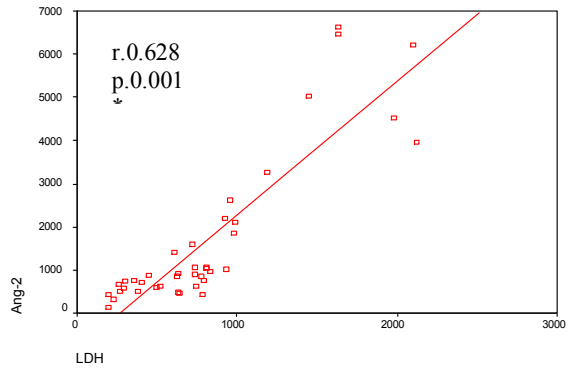

Fig. (4): Correlation between the level of Ang-2 and the level of LDH in CLL patients.

Table (3): Correlation between the percentage of CD38 expression and the ang-2 serum level

	CD 38 <30 % (13/30 = 43.3%)	CD 38 >30% (17/30 = 56.7%)
ang-2	730.6±202.6	3049.9±2041.4

4. Discussion

In the present study serum LDH levels were statistically significant increase in patients compared to control group .The increase was obvious in patients extending from stage 0 up to stage IV. This finding correlates with *Shen et al., (2007)* who demonstrated that serum LDH level in CLL patients in Binet C were significantly higher than those in Binet A. Binet C and high LDH level were associated with significantly shorter overall survival. The overall survival time in group of elevation of LDH level was shorter than that in group of normal levels of LDH. It is concluded that serum LDH level and Binet stage are important prognostic factor for CLL. Furthermore, *Krober et al., (2002)* reported that a high level of serum LDH, which is a measure of tumor burden and turnover, is associated with rapid disease progression and worse clinical prognosis in B-CLL.

In the present study, percentage of CD 38 expression in patients group showed significant increase through the stages of the diseases. CD38 expression was shown to predict the clinical course of the disease. Evaluation of CD38 expression allowed distinguishing the patients groups with the most favorable prognosis as well as those with the worst. It's recommend assessing CD38 protein expression for the definition of prognostic subgroups in patients with B-CLL *Hus et al., (2006)*.

Matrai, (2005) reported that cell surface expression of CD38 in CLL has been recognized recently as a marker of progressive disease and poor outcome. In contrast to traditional staging systems, CD38 is able to identify progressive cases at an early stage. Measurement of CD38, in conjunction with other novel prognostic factors such as p53 and ZAP-70 helps to identify patients who might benefit from early and more intensive therapy.

In the present study, serum Ang-2 level was found to be significantly higher in the serum of CLL patients in comparison to normal controls and it significantly increase with the progress of the stage.

This finding correlate with *Maffei,et al., (2007)* who found that ang-2 plasma levels detected in Binet B

and C (which represent late stages of CLL) exceeded those of Binet A cases (which represent early stages of CLL) suggesting correlation between ang-2 secretion and CLL progression with higher white blood cells and lower platelet counts which matched with the results of the study association between high Ang-2 levels and more advanced disease and shorter progression-free survival was assessed in small series of CLL patients and higher Ang-2 secretion seems to be a biologic characteristic of more aggressive CLL *(Huttmann et al., 2006)*.

Martinelli et al., (2008) had reported that Ang2 mRNA is differently expressed in CLL patients and its increased expression appears to be associated with poor prognostic features while, it was reported that CLL patients can be divided into 2 subgroups (Ang-2 +ve and –ve CLL) with 30% of them displaying Ang-2 RNA level above the cut off. A shorter progression free survival was observed in Ang-2 +ve CLL.

A similar result was reported for 33 untreated CLL patients, indicating that Ang-2 mRNA was differentially expressed in patients with CLL and that increased expression appears to be associated with poor prognostic features *(Vrbacky et al., 2010)*. Also, *Maffei et al., 2010(2)* measured the Ang-2 plasma level, an angiogenic cytokine, in 316 patients with CLL and found that a high Ang-2 level predicted a shorter time to first treatment and shorter overall survival. Also, significant associations were found between high levels of Ang-2 and advanced clinical stage, high β2M, un-mutated status of the immunoglobulin heavy chain variable (IgVH) gene segments, and adverse cytogenetic *(Maffei et al., 2010(2))*.

In this study there was marked relation between CD38 and ang-2 in CLL patients, that patient with CD38 below 30% had ang-2 level lower than those with CD38 above 30% And the difference was statistically significance this might show that ang-2 had a bad prognostic value in CLL

There was a significant positive correlation between the level of Ang-2 and the level of LDH in CLL patients this finding correlate with *Maffei et al., (2010(1))*, Who found that CLL patients expressing at diagnosis high levels of Ang-2 usually had more advanced clinical stage and a higher percentage of CD38+ cells, had un-mutated immunoglobulin status and unfavorable cytogenetic and had a shorter progression-free survival. These associations support the idea of the involvement of Ang-2 in the mechanisms of CLL disease progression.

Ang2 seems to be an interesting molecule among other angiogenesis-related factors. The immunohistochemical study of Ang2 expression in BM and LN compartments infiltrated by CLL cells revealed that Ang-2 preferentially originates from leukemic cells and not from activated endothelial or other surrounding cells. Moreover, abnormal Ang-2 production seems to be a feature acquired precociously by CLL and maintained during the disease course. Finally, high Ang2 shows strong and independent prognostic power *(Kanduri et al., 2010)*.

CLL cells are the principal source of elevated Ang-2 levels in CLL patients. Surprisingly and in contrast to patients with multiple myeloma, *Maffei et al., (2010(1))* did not find any correlation between the degree of marrow infiltration and Ang-2 levels, nor did they find any changes of Ang-2 levels in patients undergoing treatment, indicating that the disease burden may have no impact on Ang-2 levels. Studies about the regulation of Ang-2 expression by CLL cells, for example, the effect of co-culture of CLL cells with endothelium on Ang-2 expression, may help to clarify this important issue *(Jan Burger., 2010)*.

both circulating and BM-infiltrating Ig-un-mutated CLL B-cells are able to express higher levels of Ang-2 than Ig-mutated CLL and normal B cells, suggesting the presence of an intrinsic defect in Ang-2 expression that could be pathogenetically relevant in CLL marrow microenvironment and could be involved in the differential clinical behavior of Ig-mutated and Ig-un-mutated CLL cases *Maffei et al., (2010(1))*..

The pathways activated by Ang2 in CLL microenvironment deserve particular attention to identify new possible pathogenetic mechanisms and related therapeutic targets. Because several molecules targeting microenvironment and angiogenesis are being developed in clinical trials for CLL, it could be also intriguing to evaluate if such drugs may modulate Ang-2 secretion. *Blockade of Ang-2 using inhibitors of enzymes or using selective antibodies fusion proteins was shown to reduce vascularization and growth in mouse models of several solid tumors* ***(Villeneuve et al., 2008)***.

As such, this paper encourages more exploration of function and significance of angiogenic factors in CLL. Such studies will help to determine whether or not Ang-2 will play in the premier league of prognostic markers in CLL *(Jan Burger., 2010)*.

Our results regarding the adverse prognostic significance of high expression of Ang-2 by CLL cells are substantially in keeping with Hüttmann et al. Importantly, in our series *Maffei et al., (2010)*.

In conclusion, the present study shows that circulating levels of Ang-2 were higher in CLL patients at diagnosis in comparison with apparently healthy individuals. However, the number of patients in the present study was small, so, the present findings need further investigations of the prognostic significance of serum Ang-2 level done on a large scale with follow up patients for longer period to determine its relation with overall survival and disease free survival.

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