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Original Article

Haematology Section

The Significance of the Plasma L-Selectin Levels in Cases of Acute Myeloid Leukaemia: A Case Control Study

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ABSTRACT

Background: Acute Myeloid Leukaemia (AML) is a heterogeneous group of haematological malignancies which are characterized by malignant proliferation and the accumulation of immature myeloid progenitor cells in the bone marrow. A clinical history of myelodysplasia, the assessment of the morphology of the blast cells, cytogenetics and immunophenotyping are the important criteria which have been used in the recent WHO classification to prognosticate as well as to guide the therapy of AML. L-selectin is a cellular soluble adhesion molecule and it mediates the initial tethering of the normal leucocytes to the endothelial surface . An increase in the plasma L-selectin levels in cases of acute leukemia has been observed in many studies, but till date, this biochemical parameter has not been used as a marker in monitoring leukaemic patients. This study was conducted to compare the L-selectin levels in fresh and treated cases of AML and also with healthy controls. The assessment of the plasma L-selectin levels is expected to add more information about the disease activity and it could also serve as an adjunct to the existing immunological and genetic markers.

Aims, Settings and Design: This case control study aimed at estimating the plasma L-selectin levels in cases of acute myeloid leukaemia in comparison with those in normal healthy adults. Further, we assessed the plasma L-selectin levels during the active and the remission phases of AML.

Materials and Methods: 20 freshly diagnosed and 20 treated cases of AML were included in the study group from the Haemato Oncology Department of our institution. Forty healthy people who were the staff members of the hospital were included as the controls. The quantitation of L-selectin was done by a highly sensitive immunometric ELISA assay (Manufacturers; Bendermed systems, Vienna, Austria), by using monoclonal antibodies against L-selectin. The statistical significance was calculated by using the Bonferroni T-test and Pearson's correlation analysis.

Results: There was a statistically significant (p=<.001) increase in the plasma L-selectin levels in the freshly diagnosed AML patients as compared to the control group. The plasma L-selectin levels in the patients who were in remission matched with that of the controls.

Conclusions: Plasma L-selectin may be used as a marker of the disease activity in AML patients. It may also be used to assess the remission status of these patients.

Abbreviations: AML=Acute Myeloid Leukaemia

Key Words: AML, Plasma L-selectin, Prognostic marker, Remission

INTRODUCTION

Acute myeloid leukaemia is a group of haematological malignancies which predominantly involve the adult population. Despite intense therapies in the form of radiation and chemotherapy, relapses are common. A history of myelodysplastic syndromes or genotoxic therapy, age at diagnosis, the morphological features of the blast cells and cytogenetic aberrations are the accepted prognostic factors of AML [1, 2, 3,4]. The significance of the plasma L-selectin levels as a biochemical marker for acute leukaemias has been reported since long [5]. L-selectin is an adhesion molecule which is expressed in normal leucocytes as well as in leukaemic cells, which helps in the initiation of the leucocyte attachment to the activated endothelium [6,7].

Its proteolytic shedding facilitates the detachment of laeukocytes from the endothelial cells as they migrate through the endothelial layers. An increase in the shed L-selectin levels in leukaemic patients has been observed in a few studies [3,8] and its significance as a prognostic marker has been observed [9], but till date, this biochemical parameter has not been used as a marker for monitoring leukaemic patients. In our study, we attempted to quantitate the levels of plasma L-selectin in fresh and treated cases of AML in comparison with healthy normal controls, to understand the importance of this biochemical marker in the follow up of AML patients.

MATERIALS AND METHODS

20 freshly diagnosed cases of acute myeloid leukaemia, 20 cases of AML who had undergone treatment with two to three cycles of chemotherapy and were clinically remitted and 40 healthy controls were included in the study, based on the inclusion and exclusion criteria.

Exclusion criteria:

- Acute infection or inflammation
- Diabetes mellitus
- Connective tissue disorders
- Post-operative deep vein thrombosis

Inclusion criteria:

Group A (Fresh cases of AML)

The 20 patients who were selected were of the age group of 18-60 years and they were diagnosed as the cases of AML, based on the following criteria [10]:

- Complete blood count, differential count
- Peripheral smear study and cytochemistry
- Bone marrow aspiration cytology
- Cytogenetics
- Immunophenotyping

Among these, 50% were women.

Group B (Treated cases of AML)

20 patients (different set of cases from Group A) who had undergone treatment with two to three cycles of chemotherapy with Cytarabine + Daunorubicin (7+3 regimen) and were clinically remitted11 were included. The clinical remission was assessed by using the following criteria [11]:

- Normalization of the neutrophil counts (at least 1.5 X 109/L).
- Platelet counts of more than 100 X 109/L.
- Marrow aspirates that demonstrated at least 20% cellularity and less than 5% blasts with no Auer Rods.
- Absence of extra medullary leukaemia.

They were of the age group of 18-60 years. Among these, 50% were women.

Group C (Control)

40 age and sex matched healthy people were selected as the controls. The controls were chosen from among the staff of Sree Balaji Medical College.

A written consent was obtained from each subject. Ethical clearance and an approval to conduct the study were obtained from our institutional human ethical committee. The plasma samples (in EDTA) and the serum samples were collected.

A complete haemogram, liver function tests, renal function tests and serum uric acid tests were performed. As bone marrow aspiration was already done for all the fresh cases of AML, it was not repeated for the Group A cases, though the procedure was done for all the treated cases (Group B). The quantitation of L-selectin in the plasma samples was done by a highly sensitive immunometric ELISA assay (Manufacturers; Bendermed systems, Vienna, Austria) by using monoclonal antibodies (murine) against L-selectin.

RESULTS

The plasma L-selectin levels ranged from 963 ng/ml to 2500 ng/ml in the freshly diagnosed AML patients, from 98 ng/dl to 683 ng/ml in the treated cases of AML which were under remission and from 110 ng/ml to 423 ng/ml in the healthy controls.

[Table/Fig-1] gives the mean levels and the standard deviation of various parameters in the 3 groups and [Table/Fig-2] gives the statistical significance p(<.001) which was obtained from the comparison.

The significance of the differences in the values of the parameters among the Groups I, II and III was evaluated by using the Bonferroni T-test. The Pearson's correlation analysis was employed to find out the correlations between the L-selectin levels and the bone marrow blast percentage [Table/Fig-3].

[Table/Fig-4] and [Table/Fig-5] show the increasing levels of L-selectin from the controls to the treated cases to the fresh cases of AML. [Table/Fig-2]shows the statistical correlation of the plasma L selectin values among the three groups. The fresh cases of AML (Group A) showed a statistically significant increase in the plasma L-selectin levels as compared to those in the treated cases (Group

I Group	J Group	Mean Difference (I-J)	Standard Error	Significance
Fresh (Group A)	Treated	1184.90	89.543	.000
	Control	1302.85	77.547	.000
Treated (Group B)	Fresh	-1184.90	89.543	.000
	Control	117.95	77.547	.397

[Table/Fig-2]: Statistical correlation of plasma L-selectin values among the three groups

		Bone Marrow Blast %	L-Selectin
Blast Percentage In Bone Marrow	Pearson correlation	1.000	.705
	Sig (2 tailed)	-	.000
	N	80	80
Plasma L .Selectin Levels	Pearson correlation	.705	1.000
	Sig (2 tailed)	.000	-
	N	80	80

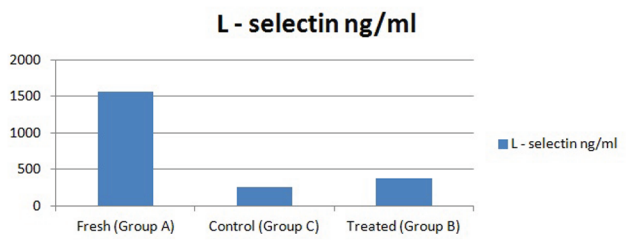
[Table/Fig-3]: Correlation Between L-Selectin And Bone Marrow Blast %

Parameter	Sex	Number	Mean	Std Deviation	Std Error Mean
L -SELECTIN	Female	37	590.62	649.47	106.77
	Male	43	638.89	599.12	91.36

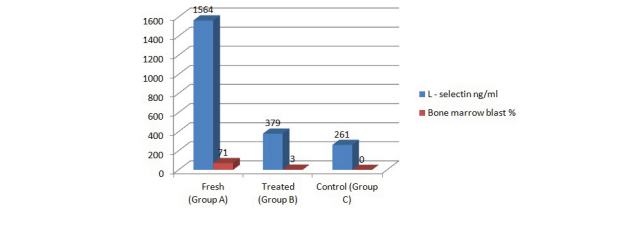
[Table/Fig-4]: Gender Based L-Selectin Levels

Parameters	Group					
	Fresh Cases Of Aml (Group A)		Treated Cases Of Aml (Group B)		Control Group (Group C)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Age	32	9	36	6	34	8
Total Wbc Count (Cells/Cu.Mm)	10000	7400	10355	2024	8549	1031
L-Selectin (Ng/Ml)	1564	528	379	186	261	75
Uric Acid(Mg/Dl)	5.90	1.46	5.07	0.99	4.49	0.72
Bone Marrow Blasts (%)	71	5	3	6	-	-

[Table/Fig-1]: Mean values and standard deviation for selected parameters taken up for studying in the respective groups.



[Table/Fig-5]: L-selectin levels in various groups



[Table/Fig-6]: Comparison between L-selectin levels and Bone marrow blast %

B) and in the control group (Group C), (p =000). There was no significant change in the values between the Groups B and C.

[Table/Fig-6] demonstrates that only the freshly diagnosed cases of AML showed an elevation in the blast cell count in the marrow. There was no significant overlap in the marrow blast cell count between the fresh and the treated cases. This could be reasoned by the fact that the patients in the treated category had undergone a clinical remission. There was a statistically significant correlation between the percentage blasts in the marrow and the L-selectin levels, as was noted in the Pearson's correlation analysis [Table/Fig-3].

[Table/Fig-4]highlights that there was no gender based difference in the levels of plasma L-selectin in the leukaemic patients.

DISCUSSION

Under normal conditions, L-selectin plays an important role in inflammation by mediating leucocyte rolling along the activated endothelium [12]. It also increases the recruitment of leucocytes into the tissues by mediating leucocyte rolling on the already adherent leucocytes [13]. Soon after the endothelial adhesion, this receptor gets shed from the white cell surface due to a proteolytic cleavage and it circulates in the plasma.

In acute leukaemias, it is observed that there is an increase is the shedding of L-selectin, which probably inhibits the binding of leukaemic cells to the vascular endothelium, thereby influencing the migration of the leukaemic blast cells. The shed L-selectin probably would protect the leukaemic cells from cell death by immune surveillance, by either interfering with the lymphocyte homing or by inhibiting the cytotoxic lymphocytes to interact with the blast cells [3]. On the other hand, the over expression of L-selectin in the leulaemic cells might help these cells in attaching to the vascular endothelium, leading to transmigration and organ infiltration [2].

The present study observed an increase in the plasma L-selectin levels in fresh cases of AML as compared to those in those who were treated. A statistically significant correlation was observed between the blast percentage in the marrow and the L-selectin levels in the fresh and treated patients. These findings added value to the aim of our study, of establishing L-selectin as a biochemical marker for the disease activity in AML. The findings of the study which was done by Exterman et al., [3]. were in agreement with our findings. The observed values of L-selectin and its correlative findings in comparison with the percentage blasts, were in concordance with the findings which were observed by Spertinii O, and Callegari P et al., [8].

CONCLUSION

Our study emphasized the fact that plasma L-selectin was a marker for the disease activity in AML patients. It can be used as an indicative marker for the remission status of the patient.

An important challenge in the management of AML is to distinguish the patients with a high risk of relapse, who may benefit from high dose chemotherapy, followed by BMT, from those cases in which such treatments can be delayed. Though cytogenetic aberrations can affect the prognosis of such cases in a greater way, it can be suggested that L-selectin can still be used as a marker for serially monitoring the disease activity and as a prognostic marker of AML in future.

FUTURE PERSPECTIVES AND RECOMMENDATIONS: A comparative study of the plasma L-selectin levels can be done with immuno phenotyping and cyto genetic analysis to ascertain whether L-selectin could be an adjunct to the other prognostic markers. The serial monitoring of the plasma L-selectin values in fresh cases of AML during the course of the treatment and following up their remission may also give therapeutic suggestions and options.

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