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Plasminogen Activator Inhibitor-1 in Acute Myeloid Leukemia: Is It Useful in Predicting Response to Induction Remission Therapy?

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Abstract

BACKGROUND: Coagulation and fibrinolytic abnormalities are common in patients with acute myeloblastic leukemia (AML). Plasminogen activator inhibitor (PAI) activity is believed to be elevated during the initial diagnosis and relapse of AML patients.

AIM: This study aimed to evaluate the levels of plasma PAI-1 activity in AML patients before and after remission induction.

METHODS: Thirty AML patients and 20 healthy volunteers were included in this study. The patients were classified according to AML-FAB subtypes. All patients received 7+3 induction chemotherapy. They were evaluated for complete remission after induction chemotherapy and followed up for 6 months.

RESULTS: PAI-1 activity was measured by ELISA immunoassay. PAI-1 activity was significantly higher in AML patients than the control group (P=0.016), whereas there was insignificant difference among patients in various AML subgroups (P>0.05). Before and after treatment, there was a significant difference in PAI-1 activity between patients with active disease and those at remission (P= 0.023 and <0.0001, respectively).

CONCLUSION: High PAI-1 activity in AML patients is found to be associated with poor response to treatment.

Introduction

Acute myeloblastic leukemia (AML) is a malignant clonal disorder characterized by the proliferation and accumulation of immature hemopoietic precursor cells in the bone marrow and peripheral blood [1]. Fibrinolytic and coagulation aberrations are common in patients with AML as seen in other forms of leukemia. Abnormal bleeding in AML may sometimes be relatively independent of platelet count and can reflect disordered mechanisms, leading to fibrin formation or its lysis [2]. The plasminogen activation system plays a key role in tissue remodeling, angiogenesis, proteolysis, mobility, chemotaxis, invasion, and metastasis [3], [4], [5]. Two types of plasminogen activators (PAs) have been identified; urokinase PA (uPA) and tissue type PA (tPA). They are serine proteases that convert plasminogen into plasmin. The fibrinolytic system consists of uPA, uPA receptor (uPAR), plasminogen, and plasminogen activator inhibitor (PAI). PAI-1 and PAI-2 can regulate plasminogen activation at different levels. The main regulator of tPA activity in plasma is PAI-1, whereas uPA activity is regulated by both PAI-1 and PAI-2 [6], [7], [8]. Several PAs and PAIs are present in leukemic cells and they are considered to be involved in hemostatic abnormalities and can be of prognostic value in patients with leukemia [9], [10]. PAs facilitate the leakage of immature myeloid cells from the bone marrow and can be detected on the surface of leukemic cells. UPA, UPAR, PAI-, and PAI-2 mRNAs are liberated by AML cells. The uPA-uPAR complex is thought to be involved in the drifting and invasive properties of the leukemic cells. It is suggested that the fibrinolytic imbalance observed in leukemic patients is due to inappropriate plasminogen activation [10].

The study aims to determine the impact of plasma PAI-1 activity on AML patients' response to induction remission therapy.

Materials and Methods

This prospective pre-post study included 40 adult *de novo* AML patients sequentially selected during a period of 5 months. Ten patients were excluded from the study; five patients chose to receive therapy in other hospitals and five patients died during or after induction therapy before assessing remission. The sample size

was estimated using the online formula Raosoft® sample size calculator [11]. Using a margin of error of 9.06%, a confidence level of 95%, a population size of 40, and a response distribution of 50%, the estimated sample size was 30. In addition, 20 healthy age- and sex-matched volunteers (11 males and nine females) were included in this study between April and December 2018. The diagnosis of AML was based on morphological, cytochemical, and flow cytometry examination of peripheral blood and bone marrow samples. The patients were divided into subtypes according to the French American British (FAB) criteria excluding promyelocytic subtype from this study due to different treatment protocol. All patients received intensive chemotherapy, following the "7+3" protocol that includes: Cytosine arabinoside for 7 days and anthracycline (daunorubicin or doxorubicin) for 3 days. All patients were evaluated for complete remission (CR) achievement between 21 and 35 days from the start of chemotherapy according to Döhner *et al.* definition [12]. Patients' status was followed up for 6 months.

Blood samples of the patients and control group were obtained and platelet poor plasma was collected within 30 min of collection after centrifugation of the K_3-EDTA tubes at 1000 g for 15 min. Plasma was immediately separated into small aliquots and stored at −40°C until assay was performed within a 6 weeks interval. The PAI-1 activity was measured employing the quantitative sandwich enzyme immunoassay technique (Quantikine® ELISA Human Serpin E1/PAI-1 Immunoassay, R&D Systems, Minneapolis MN, USA), according to manufacturer's instructions. PAI-1 levels were determined from the plasma samples of 20 healthy persons once and from the 30 patients twice; at diagnosis (before receiving any chemotherapy) and post-induction chemotherapy (after assessing remission status).

The research was approved by the Research Ethics Committee, College of Medicine, University of Baghdad, and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Statistical analysis was performed with the IBM-SPSS 22 statistical software program. Univariate data were summarized using standard descriptive statistics and tabulation of variables. The Mann–Whitney U-test (a non-parametric equivalent of the independent samples t-test) and the Wilcoxon signed-rank test (a non-parametric equivalent of the paired t-test) were used to compare means of continuous variables. Exact tests were used to calculate the p-value. $p < 0.05$ was considered statistically significant.

Results

Thirty patients were included in the study with a mean age of 33.76 ± 14.05 years with a range of 15–58 years. Fourteen (46.66%) were male and 16 (53.33%) were female with a male-to-female ratio of 1:1.14.

The two most frequent signs at presentation were pallor in 19/30 (63.33%) patients and fever in 13/30 (43.33%) patients followed by hepatosplenomegaly in 12/30 (40%) patients and bleeding tendency in 10/30 (33.33%) patients. Lymphadenopathy and gingival hypertrophy were the least frequent and were present in 6 (20%) and 2 (6.7%) patients, respectively.

According to AML FAB subtypes classification, 12 (40%) patients were in M2, 5 (16.7%) were in M1, 5 (16.7%) were in M4, 3 (10%) were in M5a and 3 (10%) were in M5b, and 2 (6.6%) were in M0. Two patients were in M6 and one patient in M7, those patients were excluded from the study because they were not available for re-evaluation after completion of induction chemotherapy, as shown in Figure 1.

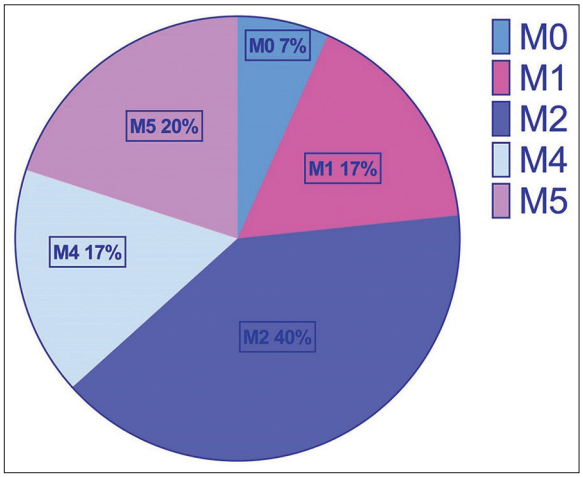

Figure 1: Distribution of patients according to FAB subtypes.

The baseline hematological parameters of the studied group are shown in Table 1. All values showed statistically significant differences from controls.

Table 1: Baseline hematological parameters of studied groups

Parameter	Control, n = 20 median (IQR) (range)	Patients, n = 30 median (IQR) (range)	p-value
WBC ($\times 10^9$/L)	6.75 (2.45) (4.6–9.3)	19.1 (108.11) (1.2–311)	0.020
ANC ($\times 10^9$/L)	4.1 (2.93) (2.5–6.9)	1.5 (3.65) (0.05–29.4)	0.003
Hb (g/dL)	13.1 (2.35) (11.8–15.8)	7.5 (2.85) (4.8–11)	<0.0001
Platelet ($\times 10^9$/L)	286 (168) (158–410)	58.5 (63.8) (13–387)	<0.0001
PB blast (%)	–	58 (43) (0–97)	
BM blast (%)	–	81 (30) (30–96)	

WBC: White blood cell, ANC: Absolute neutrophil count, Hb: Hemoglobin, PB: Peripheral blood, BM: Bone marrow.

In AML patients, the median PAI-1 level in plasma was 2.27 ng/mL (range 0.61–8.43 ng/mL) while in the control group, the median PAI-1 level was 1.77 ng/mL (range 0.66–2.81 ng/mL) and the difference proved to be statistically significant with $p = 0.016$.

At diagnosis, the median PAI-1 level in males was 2.191 ng/mL (range, 0.609–8.238 ng/mL) and in female patients 2.567 ng/mL (range, 1.027–5.834 ng/mL). There was no statistically significant difference in PAI-1 level in the patients' group according to gender ($p = 0.561$).

There was no statistically significant difference in the PAI-1 level before and after treatment between monocytic and non-monocytic groups of AML and also within each group (Table 2).

Table 2: Comparison of PAI-1 (before and after treatment) according to AML subtypes and the presence of extra-medullary manifestation in patients' group

PAI-1 (ng/mL) median (IQR) (range)	AML subtypes Non-monocytic, n = 19	Monocytic, n = 11	p-value*
Before treatment	2.281 (1.675) (0.609–5.834)	2.256 (1.352) (1.387–8.438)	0.914
After treatment	2.687 (3.29) (0.95–6.67)	2.33 (1.1) (1.18–4.41)	0.5
p value**	0.702	0.534	
PAI-1 (ng/mL) median (IQR) (range)	Presence of extra-medullary manifestations Yes, n = 17	No, n = 13	p-value*
Before treatment	2.853 (2.277) (1.027–8.438)	2.256 (1.575) (0.609–4.433)	0.233
After treatment	2.731 (2.27) (1.25–6.67)	1.835 (2.83) (0.95–6.04)	0.137
p value**	0.57	0.507	

*Mann–Whitney U-test, ** Wilcoxon rank test.

The median PAI-1 level before treatment was 2.853 ng/mL and 2.256 ng/mL in patients with and without extra-medullary manifestation (hepatosplenomegaly, lymphadenopathy, and gingival hypertrophy), respectively, while the median PAI-1 level after treatment was 2.731 ng/mL and 1.835 ng/mL in patients with and without extra-medullary manifestation, respectively. Both were statistically insignificant.

The median PAI-1 level before treatment did not differ significantly for patients after they completed their induction chemotherapy (median level was 2.277 ng/mL and 2.377 ng/mL, respectively) with p = 0.951.

Sixteen (53.33%) patients achieved remission CR, whereas 14 (46.66%) patients did not (no remission, NR). In the NR group, the PAI-1 median level before treatment was 3.265 ng/mL, which was significantly higher than that in the CR group (1.835 ng/mL) with p = 0.0002. Moreover, the NR group after treatment also presented a higher level of PAI-1 (3.484 ng/mL) than the CR group (1.873 ng/mL) with p = 0.038 (Figure 2).

After a follow-up period of 6 months, patients were divided into two groups: Those who were still alive (17 patients, 56.66%) and those who were deceased (13 patients, 43.33%). The median PAI-1 level before the start of treatment was found to be significantly higher in those who deceased (3.221 ng/mL) than in the patients who remained alive (2.183 ng/mL) with p = 0.023. After treatment, the median PAI-1 level continued to be significantly higher in patients who deceased (4.413 ng/mL) compared to patients who were still alive (1.708 ng/mL) with p < 0.0001 (Figure 3).

Discussion

The implication of a high PAI-1 concentration in malignant tumors is uncertain. Probably, it relates to the neovascularization of tumors which, in turn, has been associated with high PA activity. It is possible that PAI-1 functions to defend the tumor itself against degradation. The PAI-1 antigen can also be used as a diagnostic indicator for severity of veno-occlusive disease [13]. The lysis of extracellular matrix by tumor cells (colon carcinoma and fibrosarcoma) can be inhibited by PAI-1 [14].

The clinical and hematological parameters in this study were nearly similar to that reported by other authors [2], [15], [16], [17]. The PAI-1 was significantly higher in patients than in the control group. Similar results were reported by Yilmaz *et al*. [15]. Levels of uPA are found to increase in AML M4-M5 patients with extra-medullary permeation and organ involvement [17]. PAI-1 activity in the present study did not differ between AML M4-M5 group and the non-monocytic group, this could be attributed to the low number of AML patients or to the limited involvement of the spleen, liver, lymph nodes, and gingival tissues.

CR was achieved in 53.33% of patients which is similar to Yilmaz *et al.* study (50%) [15]. PAI-1 level did not differ significantly for patients after they completed their induction chemotherapy. Yilmaz *et al.* found that total PAI activity was significantly decreased after chemotherapy [15]. After chemotherapy, however, in patients of whom

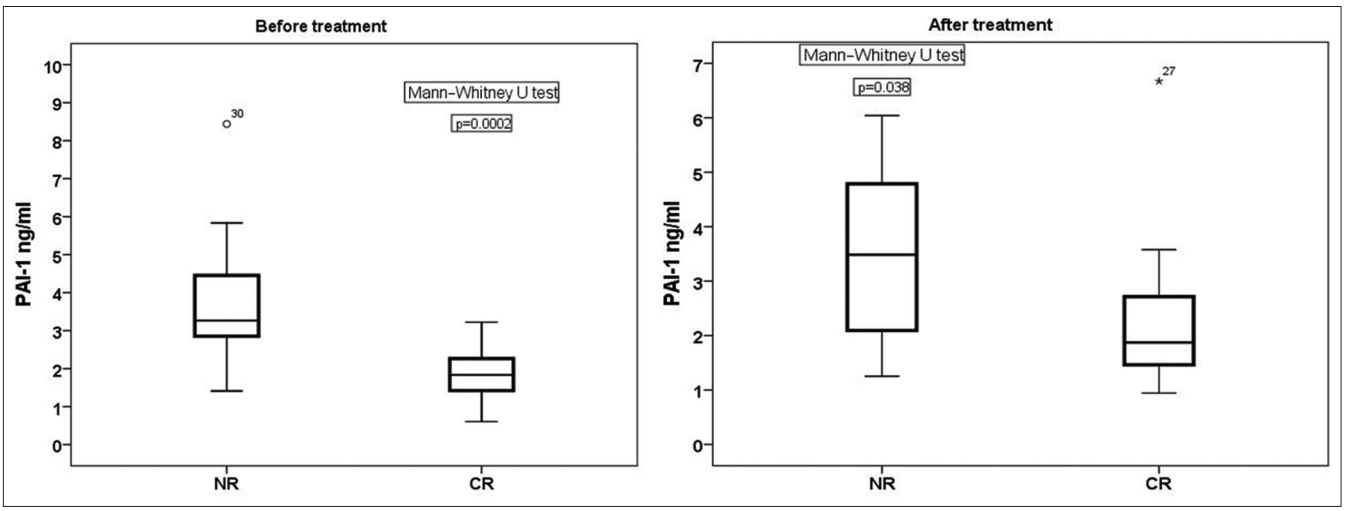

Figure 2: PAI-1 levels in AML patients according to treatment status

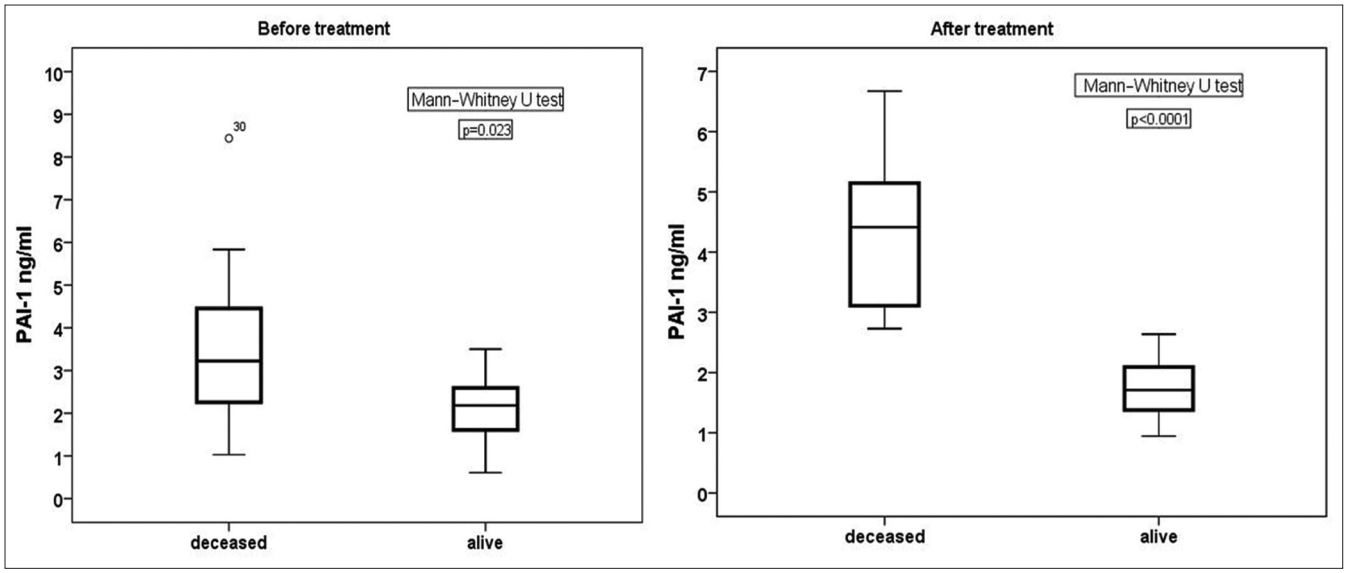

Figure 3: PAI-1 levels in AML patients after 6 months

remission was achieved, PAI-1 levels were found to be significantly reduced ($p < 0.038$). Similar results were reported by Yilmaz *et al*. [15]. At the same time, PAI-1 levels before treatment were significantly higher in the NR group than patients who achieved CR ($p = 0.0002$). High PAI-1 levels were associated with poor outcomes in patients with AML as the PAI-1 level was higher in the patients who were deceased than in the patients who were still alive at end of follow-up, before and after treatment, with $p = 0.023$ and <0.0001, respectively.

Although many studies examined the different components of the fibrinolytic system in acute leukemia, the main focus was on the impact of PA and their receptors, few studies addressed the impact of PAI-1 and PAI-2 in acute leukemia, although its role in cancer has long been established.

Many studies have established the association of high level of PAI-1 in different types of cancers such as gastric, ovarian, and breast cancer and poor outcome and have proved to have a potential prognostic significance in breast cancer [8]. In the present study, high levels were demonstrated in patients who failed to achieve CR as well as to patients who deceased when compared with patients with CR, it is believed that the PAI-1 activity is amplified secondary to increased amount of u-PA and uPAR, whether this can be the explanation for the adverse effect noted on treatment outcome or another yet undetermined factor this requires further studies. Inhibition of uPA and uPAR activity may give anticipation in the treatment of AML patients [15], [16], [17].

Conclusion

An increased PAI-1 level in AML patients was associated with poor outcomes in our study. However, further studies are required to assess the clinical relevance of PAI-1.

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