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Effect of remission induction therapy on the level of soluble urokinase plasminogen activator receptor in acute myeloid leukemia

Eman Qassim Ahmed, Haithem Ahmed Al-Rubaie[1]

Abstract:

BACKGROUND: Soluble urokinase plasminogen activator receptor (suPAR) in acute myeloid leukemia (AML) is associated with resistance to chemotherapy. The aim is to assess the effect of remission induction on plasma level of suPAR in AML patients and to correlate between plasma levels of suPAR with certain hematological parameters, AML subtypes, and extramedullary involvement.

MATERIALS AND METHODS: The study was conducted on 30 newly diagnosed adult AML patients and 20 controls. The peripheral blood and bone marrow smears were examined at diagnosis and after remission induction therapy to assess complete remission (CR). The plasma level of suPAR was measured by enzyme immunoassay which was done at diagnosis and after remission induction therapy.

RESULTS: Plasma suPAR level was higher in AML patients at presentation than the control group ($P < 0.001$). The plasma level of suPAR revealed a significant reduction in the 37% of patients who achieved CR or partial response ($P = 0.004$), whereas the differences were insignificant and remained high in patients who did not show response ($P = 0.573$). Before and after treatment, there were insignificant differences in suPAR level in patients with or without extramedullary involvement and in various AML subgroups ($P > 0.05$). The suPAR levels were high before starting treatment in both the alive and those who deceased ($P = 0.984$). After 5 months of follow-up, it showed a significant reduction among the alive group ($P = 0.001$). There were insignificant correlations between the level of uPAR and hematological parameters at presentation.

CONCLUSIONS: The reduction of plasma suPAR level is associated with a better response, and a high level is associated with a high risk of death. Before and after induction therapy, there is no association between plasma level of suPAR in monocytic group, nonmonocytic group, and in patients with or without extramedullary involvement. Plasma suPAR level is uncorrelated with hematological parameters at presentation.

Keywords:

Acute myeloid leukemia, French–American–British classification, soluble urokinase plasminogen activator receptor

Department of Hematology, Teaching Laboratories, Medical City, [1]Department of Pathology, College of Medicine, University of Baghdad, Baghdad, Iraq

Address for correspondence:
Dr. Haithem Ahmed Al-Rubaie, Department of Pathology, College of Medicine, University of Baghdad, Baghdad, Iraq.
E-mail: haithemalrubaie@yahoo.com

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Introduction

Acute myeloid leukemia (AML) is a malignant clonal disorder leading to proliferation and accumulation of blasts and immature cells in the hematopoietic system coupled with impairments in differentiation and inhibition of apoptosis in the bone marrow (BM). The blasts eventually suppress normal hematopoiesis leading to marrow failure and infiltrate other organs and tissues.[1,2] Accurate diagnosis and classification of AML are essential for treatment decisions and assessment of prognosis. Initial assessment requires a careful

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history, physical examination, complete blood count with peripheral blood (PB) smear and BM examination, flow cytometry, cytogenetics, and selected molecular genetic analyses.[3] Soluble urokinase plasminogen activator receptor (suPAR) is a glycoprotein attached to the plasma membrane via glycophosphatidylinositol-anchored protein.[4] The plasminogen activator (PA) system is composed of the serine protease urokinase type PA and its receptor (uPAR), tissue-type PA, plasminogen and its multiple receptors as well as three inhibitors (plasminogen activator inhibitors [PAI]-1, PAI-2, and protease nexin 1), these molecules are glycoproteins and found in most tissues and body fluids.[5] uPAR expression has been found most significantly in those leukemias of myeloid origin and only occasionally in those with lymphoid markers. A little expression is seen in T- or B-cell lymphomas or Hodgkin's disease. It therefore appears that uPAR expression by hematopoietic cells is normally confined to monocytic and myeloid cells.[6]

Materials and Methods

This cross-sectional study was approved by the ethical committee of the Iraqi council for medical specialization. All enrolled patients gave their written informed consent prior to study. The study conducted on 30 newly diagnosed adult AML patients (14 males and 16 females) and 20 healthy adults as a control group. All patients have to receive "7 + 3" protocol. They were diagnosed according to the French–American–British (FAB) classification based on cytomorphology, cytochemistry, and flow cytometric immunophenotyping of the PB and/or BM aspirate samples. Patients with AML-M3, other types of hematological malignancies, solid cancers, active inflammatory disease (including systemic lupus erythematous and rheumatoid arthritis), malaria, tuberculosis, leishmaniasis, AML patients who died before assessing remission, and those who were diagnosed as secondary or relapsed AML were all excluded from this study. For all AML patients, an examination of PB and BM aspirate samples was done at day (21–28) from the start of remission induction chemotherapy to assess the remission status. The patients were divided into two groups: the first group includes those with no response (NR) to treatment and a second group who responded to treatment whether complete response (CR) or partial response complete remission with incomplete count recovery (CRi). Patients were followed-up for 5 months from the diagnosis to assess disease outcome. Plasma suPAR was assessed once for control individuals and twice for AML patients; at time of diagnosis and after the patients had completed their remission induction therapy, using human SuPAR Quantikine ELISA kit (R&D, USA),[7] automated microplate washer system (Germany) and microplate reader (BioRAD, USA).

Statistical analysis

Microsoft Excel software 2016 and Statistical Package for the Social Sciences version 23 was used. The data were presented as median, interquartile range and range. Comparison between the median of the study groups was done by using Mann–Whitney *U*-test and Wilcoxon signed ranks test for unpaired and paired groups, respectively. Spearman correlation was done to show the relation between suPAR and different hematological parameters. $P < 0.05$ was considered as significant.

Results

The mean age of AML patients was 34.67 ± 12.65 years. About 53% (16/30) of AML patients were females and 47% (14/30) were males with an F: M ratio of 1.1:1. According to FAB subtypes, 3 were M1, 15 were M2, 3 were M4, 5 were M5a, and 4 were M5b subtypes. There was a statistically significant difference in the median of suPAR level between the patients and control, with $P < 0.001$ [Table 1].

Statistically insignificant difference was found between the median of suPAR level of the patients before and after remission induction therapy [Table 2].

AML patients who achieved a response (7/11 patients had CR and 4/11 had CRi) showed remarkable lowering in the median level of suPAR after treatment ($P = 0.004$), whereas the comparison of the median levels of suPAR before and after treatment in those who did not achieve response showed a statistically insignificant difference ($P = 0.573$). Comparison of suPAR levels between NR and response groups, before and after treatment, revealed statistically insignificant differences with $P = 0.866$ and 0.077, respectively [Table 3].

Table 1: Comparison between soluble urokinase plasminogen activator receptor levels in acute myeloid leukemia patients group and control group

Parameter suPAR (pg/ml)	Control (*n*=20)	Patients (*n*=30)	*P**
Median (IQR)	1854.8 (610.6)	3631.7 (5517.8)	<0.001
Range	1087.4-3169.9	969.4-21,187.5	

*Mann-Whitney *U*-test. suPAR=Soluble urokinase plasminogen activator receptor, IQR=Interquartile range

Table 2: Comparison between soluble urokinase plasminogen activator receptor levels in acute myeloid leukemia patients group before and after treatment

Parameter suPAR (pg/ml)	Before treatment	After treatment	*P**
Median (IQR)	3631.7 (5517.8)	2539.7 (3282.5)	0.162
Range	969.4-21,187.5	881.5-27,135.9	

*Mann-Whitney *U*-test. suPAR=Soluble urokinase plasminogen activator receptor, IQR=Interquartile range

In the monocytic and in the nonmonocytic AML groups, the suPAR level did not show a significant reduction after treatment (P = 0.239 and 0.085, respectively). Furthermore, before and after treatment, there were no significant differences in the levels of suPAR between the different AML subtypes with *P* values of 0.632 and 0.884, respectively [Table 4].

After 5-month duration of follow-up, it was found that the level of suPAR after treatment in patient group who died were remarkably higher than that of those who remained alive showing highly significant statistical difference [Table 5].

The mean PB blast percentage was 47.47 ± 26.63 with a range of 2%–90% and the mean BM blast percentage was 64.37 ± 20.34% with a range of 30%–93%. No statistically significant correlations were found between plasma suPAR level and the patients' age and hematological parameters [Table 6].

Discussion

The mean age of AML patients included in this study was comparable to other Iraqi studies in 2014, 2009, 2008, and 2012;[8-11] a Jordanian study in 2012;[12] and a Saudi study in 2006.[13] AML cases were observed more in females, this result is inconsistent with other reports from Iraq (2016);[14] Oman (2007);[15] and Western studies in 2005, 1999, and 1998,[16-18] whereas it is in agreement with one Turkish study in 2016.[4] Among the 30 cases studied, M2 was the most frequent and consistent with previous Iraqi studies.[9,19,20] However, this result disagrees with another Iraqi study done by Mohammed and Al-Rubaie and an Egyptian study done by Nassar *et al*.[21] where M4 was the most frequent AML subtype in both the studies.

The plasma level of suPAR was high in AML patients at presentation, this finding totally agrees with studies done by Erkut *et al*.[4] and Aref *et al*.[22] After remission induction therapy, plasma levels of suPAR showed insignificant difference than that before therapy, this finding may be explained by the presence of 19/30 (63.3%) AML patients who did not achieve response after first induction, whereas within patients group who achieve response (11/30 patients), there was a significant reduction of plasma level of suPAR after treatment. Mustjoki *et al*.[23] observed that plasma suPAR levels decreased dramatically shortly after the start of chemotherapy in AML patients, with the simultaneous disappearance of tumor cells. Nassar *et al*.[21] reported that the expression of uPAR (CD87) was inversely associated with response to treatment (*P* = 0.002). Moreover, Graf *et al*.[24] found that a high expression rate of uPAR on AML cells was significantly correlated with a lower remission rate (*P* = 0.03). Erkut *et al*.[4] reported that serum suPAR levels were lower in patients who achieved CR than those who did not (*P* < 0.001).

There was no association between suPAR level and various AML subtypes, this finding agrees with Erkut *et al*.,[4] whereas this finding is inconsistent to other studies[21,24,25] which may be attributed to a low number of patients enrolled in this study in comparison to the studies mentioned above. In respect to alive and deceased patients groups, the suPAR level revealed no significant change before treatment; however, after follow-up of

Table 3: Comparison between soluble urokinase plasminogen activator receptor levels before and after remission induction therapy in acute myeloid leukemia patients group according to their response

suPAR (pg/ml)	Before treatment	After treatment	*P**
No response (*n*=19)			
Median (IQR)	3522.8 (5524.2)	3241.9 (6000.0)	0.573
Range	969.4-21,187.5	881.5-27,135.9	
Response (*n*=11)			
Median (IQR)	4008.0 (5461.5)	2098.1 (1335.8)	0.004
Range	1545.2-13,923.0	947.4-3249.9	
*P***	0.077	0.866	

*Wilcoxon signed ranks test, **Mann-Whitney *U*-test. suPAR=Soluble urokinase plasminogen activator receptor, IQR=Interquartile range

Table 4: Comparison between soluble urokinase plasminogen activator receptor levels before and after remission induction therapy in acute myeloid leukemia patients group according to French-American-British classification

suPAR (pg/ml)	Before	After	*P**
Monocytic (*n*=12)			
Median (IQR)	3470.5 (3808.7)	2676.2 (3712.7)	0.239
Range	2059.4-13,923.0	881.5-27,135.9	
Nonmonocytic (*n*=18)			
Median (IQR)	4036.4 (5873.0)	2539.7 (3485.3)	0.085
Range	969.4-21,187.5	947.4-19,465.4	
*P***	0.632	0.884	

*Wilcoxon signed-rank test, **Mann-Whitney *U*-test. suPAR=Soluble urokinase plasminogen activator receptor, IQR=Interquartile range

Table 5: Comparison between soluble urokinase plasminogen activator receptor levels in acute myeloid leukemia patients groups according to the disease outcome

Parameter suPAR (pg/ml)	Alive (*n*=14)	Died (*n*=16)	*P**
Before			
Median (IQR)	3470.5 (4504.0)	3874.3 (5574.2)	0.984
Range	1461.8-13,923.0	969.4-21,187.5	
After			
Median (IQR)	1793.3 (793.1)	4571.1 (4925.1)	0.001
Range	881.5-3249.9	947.4-27,135.9	

*Mann-Whitney *U*-test. suPAR=Soluble urokinase plasminogen activator receptor, IQR=Interquartile range

Table 6: Correlations of soluble urokinase plasminogen activator receptor at presentation with other parameters in acute myeloid leukemia patients group

Parameter	suPAR	
	R	*P**
Age (year)	−0.195	0.301
Hb (g/dl)	0.108	0.568
WBC (×10⁹/L)	0.187	0.322
ANC (×10⁹/L)	−0.069	0.719
PLT (×10⁹/L)	−0.264	0.158
PB blast %	−0.023	0.906
BM blast %	0.106	0.577

*Spearman rank correlation. suPAR=Soluble urokinase plasminogen activator receptor, Hb=hemoglobin, WBC=White blood cell, PB: Peripheral blood, BM=Bone marrow, PLT=Platelet, ANC=Absolute neutrophil count

4 months duration after the remission induction therapy, there was a statistically significant difference between the two groups. The expression of uPAR can be associated with increased tumor cells and poor outcomes.[6]

No statistically significant correlation was found between the level of uPAR at presentation and the age of patients which agrees with Erkut *et al*.,[4] Nassar *et al*.,[21] and Atfy *et al*.[25] Furthermore, no significant correlation was shown with the total white blood cells count which is consistent with Erkut *et al*.'s study.[4] Furthermore, no significant correlation was found with PB blast percentages which disagrees with Mustjoki *et al*.[23] who reported that the increased amount of suPAR found in plasma from patients with AML associated with tumor-cell count in the circulation and with the level of uPAR found in tumor-cell lysates. This may be attributed to the time of assessment at the presentation where the PB blast percentages were high in most of our patients. There was insignificant correlation between uPAR level and BM blast in % at presentation which is consistent with Mustjoki *et al*.'s[23] study that showed no correlation between the BM leukemic infiltrate and plasma suPAR levels, even in some patients, a decrease in suPAR occurred, although the BM aspirate revealed the presence of leukemic cells, possibly because the suPAR produced by these tumor cells was insufficient to increase the suPAR level above the normal range.

Conclusions

A significant reduction in plasma suPAR level in AML patients after the first remission induction therapy is associated with a better response, and a high plasma level of suPAR is associated with a high incidence of death. There is no association between plasma level of suPAR in monocytic and nonmonocytic groups before and after remission induction therapy. There is no association observed between plasma levels of suPAR with hematological parameters at presentation.

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Conflicts of interest

There are no conflicts of interest.

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