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Comparison of circulating matrix metalloproteinase-2 levels in untreated acute myeloid leukemia patients with remission status

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Abstract:

BACKGROUND: Matrix metalloproteinases (MMPs) are proteases responsible for cleaving and rebuilding connective tissue components and also affect early carcinogenesis events, tumor development, growth, and neovascularization. The study aimed to evaluate the level of MMP-2 in acute myeloid leukemia (AML) patients in comparison with that in remission status, and healthy subjects, and to find its correlation with hematologic parameters.

PATIENTS, MATERIALS, AND METHODS: This study included sixty newly diagnosed AML patients. Remission status was assessed after induction chemotherapy. The overall survival (OS) was determined after 6 months. The plasma MMP-2 level was measured at diagnosis by enzyme immunoassay. Twenty-eight healthy individuals were recruited as a control group.

RESULTS: Plasma MMP-2 was higher in AML patients than in healthy individuals ($P = 0.005$). The level of MMP-2 was much higher in the M5 subtype than in the other subtypes ($P = 0.0001$). There was no statistically significant difference in the level of MMP-2 between patients who achieved complete remission and those who did not ($P = 0.113$). After 6 months, no significant difference in the initial MMP-2 levels was found between deceased and alive patients ($P = 0.174$). A positive correlation of MMP-2 level was found with white blood cell (WBC) count and hemoglobin ($P = 0.0001$ and 0.033, respectively) while insignificant with age, platelet count, and blast counts.

CONCLUSIONS: The high MMP-2 level in AML patients suggests a possible role in the pathogenesis. However, it does not show any association with remission status or OS. The elevation was significantly associated with marrow monocytosis (M5) and correlated with a higher WBC count.

Keywords:

Acute myeloid leukemia, enzyme-linked immunosorbent assay, matrix metalloproteinase-2

Introduction

Acute myeloid leukemia (AML) is characterized by the accumulation of leukemic blast cells in the marrow and impaired production of normal blood cells.[1] Recurrent acquired genetic abnormalities have established diagnostic and prognostic markers, suggesting an essential role in leukemogenesis.[2] Accurate diagnosis and classification in AML are essential for treatment decisions and assessment of prognosis.[3]

Matrix metalloproteinases (MMPs) function in the extracellular environment and degrade both matrix and nonmatrix proteins. They play central roles in morphogenesis, wound healing, tissue repair, and remodeling in response to injury.[4,5] MMPs not only facilitate the breakdown of the extracellular matrix but also affect early carcinogenesis events, tumor development, growth, and neovascularization.[6] A possible role for MMPs in acute leukemia has been hypothesized, because of the role of MMP-2

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in tumor angiogenesis.[7] Soluble MMP-2 is able to bind to the surface of invasive cells *in vitro* and *in vivo* by interaction with the integrin receptor αvβ3, thereby promoting the invasive capacities of these cells.[8] It can be hypothesized that leukemic cells have illicitly acquired the biochemical machinery for trafficking. Genetic alterations and regulatory dysfunctions in MMP production may facilitate leukemic cells to prematurely leave the bone marrow (BM) and invade peripheral tissues.[9]

The study aimed to compare MMP-2 levels between AML patients and healthy subjects and to find their correlations with other hematologic parameters.

Patients, Materials, and Methods

This cross-sectional study included sixty newly diagnosed untreated adult *de novo* AML patients who were sequentially selected. The diagnosis was based on cytomorphology assisted by flow cytometric analysis which was already done to confirm diagnosis and to determine lineage involvement. Patients with a coexistent solid tumor, other hematologic malignancies, or with secondary or relapsed AML have been excluded from the study. Twenty-eight healthy individuals were enrolled as a control group.

Patients received different types of treatment taking into consideration the AML subtype, the patient's age, and the general health status. Thus, the time of assessment of remission induction status also varied. For patients receiving the 3 + 7 protocol, assessment was done on days 21–28 from the start of remission induction chemotherapy, while for those having AML M3 (receiving all trans retinoic acid, daunorubicin, and arsenic trioxide) was done on days 28–36. The assessment of those aged >60 years who received decitabine and venetoclax was done after 4 cycles. The overall survival (OS) was determined after 6 months.

The patients were divided into two groups; according to response to treatment; the first "no remission group" included those who showed no response to treatment and the second "remission group" responded to treatment.

Plasma MMP 2 level was determined for patients and control groups by ELIZA kit. Plasma MMP-2 was assessed for patients at the time of diagnosis and for the control group using human MMP-2 enzyme-linked immunosorbent assay kit.[10]

The study was approved by the Ethical Committee of the Scientific Council of Pathology at the Iraqi Board for Medical Specializations and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Statistical analysis

Data were carried out using the available Statistical Package for the Social Sciences version 28. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range. The significance of the difference was tested using Student's *t*-test for the difference between two independent means or the ANOVA test for the difference between more than two independent means. The significance of the difference in percentages (qualitative data) was tested using the Pearson Chi-square test with the application of the Fisher's exact test. Pearson correlation was calculated for two quantitative variables. Statistical significance was considered whenever the $P \leq 0.05$.

Results

The mean age of the patients was 45.35 ± 18.69 years and that of the control group was 45.29 ± 19 years. The age range for patients were 15–78 and for control range was15–65 years. About 53.3% (32/60) of AML patients were male and 46.7% (28/60) were female with an M:F ratio of 1.14: 1. Patients were classified according to FAB subtypes as follows: 13 M0, 10 M1, 13 M2, 12 M3, 4 M4, and 8 M5. AML-M6 and M7 were not recorded at the time of the study. French-American-British (FAB) classification was used to find out if there is an association between MMP-2 level and specific AML subtype. Leukocytosis was present in 73.3% of patients. The hematological parameters in AML patients are shown in Table 1.

MMP-2 plasma levels were higher in patients than those in the control group with a statistically significant difference with $P = 0.005$ [Table 2].

MMP-2 level in AML patients according to FAB classification shows a significant difference between the M5 subtype (AML with marrow monocytosis) in comparison with other subtypes with $P = 0.0001$ [Table 3].

Of the sixty AML patients, 31.7% had extramedullary involvement (Extramedullary disease (EMD); lymphadenopathy and/or splenomegaly and/or hepatomegaly), whereas 68.3% had no EMD. The MMP-2 levels were higher in the presence of EMD but with a statistically insignificant difference [Table 4].

Table 1: The means and ranges of hematological parameters in acute myeloid leukemia patients

Parameters	Mean±SD	Range
Hemoglobin (g/dL)	8.17±1.97	3.6–15.0
WBC (×10^9/L)	44.63±53.38	0.9–195
Platelets (×10^9/L)	53.65±57.69	2–405
Peripheral blood blast cells count (%)	62.18±25.39	0–98
Bone marrow blasts (%)	69.13±21.30	20–96

SD=Standard deviation, WBC=White blood cell

In the assessment of remission status after induction chemotherapy, AML patients who achieved complete remission were 40 (66.7%), whereas those who failed to achieve remission were 20 (33.3%). All patients in the AML M5 subtype experienced complete remission and had a statistically significant difference when compared with the other subtype groups [Table 5].

The mean level of MMP-2 was higher in the remission group than that in nonresponders; however, no statistically significant difference (P = 0.113) was found [Table 5].

Concerning the OS after 6 months, no significant difference was found between AML-M5 and other subtypes (P = 0.174), and no statistically significant difference in the MMP-2 level was measured at diagnosis between deceased and alive patients; however, the mean MMP-2 level was higher in the alive patients than the mean level of the deceased individuals [Table 6].

The MMP-2 levels were found to have a moderately significant positive correlation with white blood cell (WBC) count and a significantly weak positive correlation with hemoglobin concentration [Table 7].

Discussion

In this study, there is a slight male preponderance in agreement with previous Iraqi studies.[11-13] The most common FAB subtype was M2 and M0, followed by M3; this agrees with other Iraqi studies.[12,14,15] The mean hemoglobin concentration is consistent with that reported by Tawfiq *et al*.[16] The mean WBC count was comparable to a Palestinian study.[17] Leukocytosis was present in 73.3% of patients which is higher than that reported in Al-Husseiny study[11] and Alwan *et al*.[14] The mean platelet count was lower than that reported by Al-Husseiny[11] and Alwan *et al*.[14] AML-M5 was found to have a higher WBC count than other subtypes which is consistent with Hasham *et al*.'s study.[18] The presence of extramedullary manifestations in 31.7% of patients is close to that reported by Chang *et al*.[19] with no significant difference in MMP-2 level in those with EMD and those without EMD. Regarding the response to treatment, 66.7% of patients achieved remission and have higher MMP-2 levels than those who are not but did not reach a statistically significant level; this finding is consistent with Travaglino *et al*.[20] who reported that MMP-2 level did not influence response to treatment. However, this finding disagrees with Ismail *et al*.'s study who reported that higher MMP-2 levels are associated with better response to treatment.[21]

Plasma MMP-2 was higher in AML patients than in the control group; this finding is consistent with Pirillo *et al*.[22] but disagrees with Aref *et al*.,[23] who reported higher level in the control group than the patient group. Much higher

Table 2: Comparison of matrix metalloproteinase-2 levels in acute myeloid leukemia patients and control group

MMP-2 (ng/mL)	Patients	Controls	*P**
Mean±SD	46.25±71.00	7.23±4.28	0.005
Range	1.09–338.24	2.70–17.00	

*Student's *t*-test. SD=Standard deviation, MMP-2=Matrix metalloproteinase-2

Table 3: Matrix metalloproteinase-2 level in acute myeloid leukemia FAB subtypes

FAB subtypes	*n* (%)	MMP-2 (ng/mL), mean±SD
M0	13 (21.7)	23.56±18.60
M1	10 (16.6)	26.84±22.85
M2	13 (21.7)	32.80±21.18
M3	12 (20.0)	26.08±28.76
M4	4 (6.7)	16.88±24.56
M5	8 (13.3)	174.16±131.05
*P**		0.0001
M0, M1, M2, M3, and M4	52 (86.6)	26.57±22.64
M5	8 (13.3)	174.16±131.05
*P***		0.0001

*ANOVA-test, **Student's *t*-test. SD=Standard deviation, MMP-2=Matrix metalloproteinase-2, FAB=French-American-British

Table 4: Matrix metalloproteinase-2 level according to extramedullary involvement

Extramedullary diseases	*n* (%)	MMP-2 (ng/mL), mean±SD	*P**
Present	19 (31.7)	71.93±96.89	0.056
Absent	41 (68.3)	34.34±52.46	

*Student's *t*-test. SD=Standard deviation, MMP-2=Matrix metalloproteinase-2

Table 5: Comparison of remission status after induction chemotherapy in acute myeloid leukemia patients with FAB subtypes and matrix metalloproteinase-2 levels

FAB subtype	Response to treatment		*P**
	Remission (*n*=40; 66.7%)	No remission (*n*=20; 33.3%)	
M1, M2, M3, and M4	32 (61.5)	20 (38.5)	0.032
M5	8 (100)	0	
MMP-2 (ng/mL), mean±SD	56.55±84.08	25.65±21.70	0.113

*Pearson's Chi-square test, **Student's *t*-test. SD=Standard deviation, MMP-2=Matrix metalloproteinase-2, FAB=French-American-British

Table 6: Comparison of the overall survival of acute myeloid leukemia patients with FAB subtypes and matrix metalloproteinases2 levels

FAB subtype	Overall survival		*P*
	Alive patients (*n*=50; 83.3%)	Deceased (*n*=10; 16.7%)	
M1, M2, M3, and M4	42 (80.8)	10 (19.2)	0.174*
M5	8 (100)	0	
MMP-2 (ng/mL) (Mean±SD)	50.06±76.55	27.17±25.55	0.174**

*Pearson Chi-square test, **Student's *t*-test. SD=Standard deviation, MMP-2=Matrix metalloproteinase-2, FAB=French-American-British

Table 7: Pearson's correlation of matrix metalloproteinase-2 level with age and various hematological parameters in acute myeloid leukemia patients group

Parameter	MMP-2 (*r*, *P*)
Age (years)	−0.165, 0.208
WBC (×10^9/L)	0.508, 0.0001
Hemoglobin (g/dL)	0.276, 0.033
Platelets (×10^9/L)	0.024, 0.858
Peripheral blast cells count (%)	0.166, 0.204
Bone marrow blasts (%)	0.124, 0.347

MMP-2=Matrix metalloproteinase-2, WBC=White blood cell

MMP-2 level in M5 subtype than in other subtypes; this may be due to a higher WBC count in the M5 subtype. For the OS after 6 months, there was no significant difference in plasma MMP-2 level between deceased and alive patients and also between the M5 subtype and the other subtypes. The significant correlation between the MMP-2 level at presentation with WBC count and the absence of significant correlation with age and blast count is consistent with Aref *et al*.'s[23] study.

Conclusion

The significant elevation in MMP-2 level in AML patients may play a possible role in the pathogenesis. The higher WBC count in the M5 subgroup is associated with higher plasma MMP-2 levels. There is no association between the level of MMP-2 and remission induction status or OS after 6 months. Studying MMP-2 in a larger sample size with a longer duration of follow-up is recommended.

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Conflicts of interest
There are no conflicts of interest.

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