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Gelatinases A and B (MMP-2 & MMP-9) and interleukin 18 (IL-18) gene in Adult Acute Myeloid Leukemia: Expression and Clinical Relevance

MANAR MOHMAD M. ISMAIL, M.D.[1]; ABEER MOSTAFA E.ASHMAWY, Ph.D.[2];
MANAR M. MONEER, M.D.[3] and AMANY M. HILAL, M.D.[4]

The Departments of Clinical Pathology[1], Molecular Biology and Biochemistry[2], Epidemiology and Biostatistics[3] and Medical Oncology[4], National Cance Institute, Cairo University.

ABSTRACT

Introduction: Acute myeloid leukemia is an aggressive heterogeneous disease which progresses quickly with unpredictable behavior and response to therapy. Previous studies suggested angiogenesis as one of the controlling mechanism for blast cell mobilization, the induction of which has been proposed to be through angiogenic factors, among these are gelatinases A and B. Interleukin-18, originally called IFN-γ inducing factor (IGIF), is a multifunctional cytokine. Recent data have suggested that inappropriate production of IL-18 contributes to the pathogenesis of cancer and may influence the clinical outcome of patients. The role of IL-18 in tumor progression and angiogenesis is an area of conflicting data.

Aim of the Work: This study was conducted to analyze the cellular expression of gelatinases A and B as well as IL-18 gene in adult AML, evaluate their clinical relevance regarding response to induction therapy and survival.

Material and Methods: A cohort of 42 newly diagnosed adult AML patients was analyzed for expression of gelatinases A and B (MMP-2, MMP-9) by flowcytometry as well as IL 18 gene expression by RT-PCR using gel documentation for semiquantitation.

Results: Gelatinase A was expressed in 26/42 (61.9%) of adult AML cases showing no difference among FAB subtypes (p=0.545), while gelatinase B was expressed in 14/42 (33.3%) showing significantly higher expression among M3 FAB subtype (p=0.001). The median expression level of IL18 in AML group was 1.5 (0.8-2) which was significantly higher than the control group (p<0.001). Within the AML group, it showed a higher expression level in M4 and M5 FAB subtype (p=0.003). Blast cell distribution ratio (BCDR) and splenomegaly were significantly higher in gelatinase A positive group (p=0.004 & <0.001 respectively). As regards to response to induction therapy, a higher incidence of CR was achieved within the gelatinase A positive group (p=0.018), while there was a significantly lower incidence of CR within both the gelatinase B positive group and in the group that expressed IL 18 >1.5 (p=0.002 & 0.021 respectively). There was no significant difference regarding gelatinases A or B expression and overall survival (OS) (p=0.078 & 0.082 respectively). However, the overall survival for patients who expressed IL 18 >1.5 was significantly lower (p=0.019). Regarding combined marker expression and response to induction therapy, 7/21 (33.3%) patients who achieved CR showed double or triple marker expression compared to 14/21 (66.7%) who failed to express any of the studied markers or expressed only one marker (p=0.038), but equivalent result could not be reached regarding OS.

Conclusion: The expression of gelatinase A (MMP-2) by AML blasts supports the hypothesis that, it may be a marker of the invasive phenotype. Gelatinase B (MMP-9) expression might carry poor prognosis in AML regarding response to induction therapy. IL18 over expression may be considered as a poor prognostic factor. Combined marker expression seems to have cumulative effect on response to induction. However, to validate these assumptions, a study on a larger number of patients is required.

Key Words: *Gelatinase A – Gelatinase B – MMP-2 – MMP-9 – IL-18 – AML.*

INTRODUCTION

Acute myeloid leukemia is an aggressive heterogeneous disease which progresses quickly with unpredictable behavior and response to therapy. Only about one-third of those between ages 18-60 years who are diagnosed as AML can be cured [1].

One of the prominent features of the heterogeneity of this disease is the difference in mobility of leukemic cells (blasts) from bone marrow to peripheral blood and various organs. This mobility is normally restricted to functionally mature leukocytes. Cells have to cross matrix-barrier and penetrate blood vessels wall, and this depends on the catalytic modifications of extra-cellular matrix (ECM) and basement membrane. Previous studies have suggested a role of angiogenesis as the controlling mechanism for blast cell mobilization [2].

Angiogenesis is the formation of new blood vessels from pre-existing endothelium-lined vessels. Angiogenesis is mediated by a balance of various positive [e.g. vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), interleukin-8 (IL-8), matrix metalloproteinases (MMPs)] and negative [e.g. platelet factor-4 (PF-4), tissue inhibitor of metalloproteinases (TIMPs)] angiogenic molecules released by tumor cells **[3]**. It has been recognized that migration of proliferating endothelial cells requires a regulated degradation of the basement membrane and surrounding ECM, which involves the production of ECM-degrading proteinases such as MMPs **[4]**.

MMPs, a family of zinc-dependent endopeptidases that degrade all proteins in the ECM, are categorized according to their substrate specificity and domain structure into collagenases, gelatinases, stromelysins and membrane-type (MT) MMPs **[5]**. Gelatinases A and B (MMP-2/72 KDa and MMP-9/92 KDa); play important roles in angiogenesis, inflammation and cancer metastasis owing to their ability to degrade components of basement membrane such as gelatin (denaturated collagen), native collagen, fibronectin, vitronectin, elastin and aggrecan **[6]**. Gelatinases are synthesized as latent proteins that are activated by caspases and other secreted MMPs. They exist in three forms: cytoplasmic, membrane-bounded and free soluble form **[4]**, and produced by many cell types including endothelial, epithelial, normal and malignant haematopoietic cells **[7]**.

Interleukin-18, originally called IFN-γ inducing factor (IGIF) is a multifunctional cytokine consisting of a single 18.3 KD peptide synthesized by Kuppfer cells and activated macrophages **[8]**. It is an immunoregulatory cytokine, which is known to be involved in many pathological processes such as autoimmune diseases as well as infections. Recent data have suggested that inappropriate production of IL-18 contributes to the pathogenesis of cancer and may influence the clinical outcome of patients **[9]**. The role of IL-18 in tumor progression and angiogenesis is an area of conflicting data. It has an angiogenic role through increase in vascular cell adhesion molecules, vascular endothelial growth factor **[10,11]** and gelatinase A **[6]**. On the other hand, IL-18 has an antiangiogenic role via increasing antiangiogenic factor (IL-10) and decreasing both angiogenine and fibroblast growth factor-2 that stimulate proliferation of capillary endothelial intima. This suggests that IL-18 may be a malignancy associated protein **[12]**.

Aim of the work:

The aim of the present work was to evaluate the cellular expression of gelatinases A and B as well as IL-18 gene expression in leukemic cells from newly diagnosed adult acute myeloid leukemia and correlate data with their significance in extramedullary invasion, disease progression, response to treatment and survival as well as their potential use as target molecules in therapy.

SUBJECTS AND METHODS

Forty-two newly diagnosed adult patients with acute myeloid leukemia (AML) who presented to the National Cancer Institute, Cairo University in the period from April to September 2006 were included in this study following an informed consent. AML was diagnosed according to the standard criteria including morphological and cytochemical examination. French-American-British (FAB) subtype was determined **[13]**. Immunophenotyping was done to verify the leukocyte differentiation antigens which reflect commitment to the myeloid lineage as well as the level of differentiation **[14]**.

Significant organ infiltration at initial diagnosis was defined by ultrasonographic extension of the liver edge 2 cm below the right costal margin in the right mid-clavicular line or the spleen edge 2 cm below the left costal margin in the left mid-clavicular line or multiple lymph node enlargements **[13]**.

Blast cell distribution ratio (BCDR) which is the ratio between peripheral blood blasts and bone marrow blasts was used to measure the potential capacity of the blasts to disseminate from BM to PB **[4]**.

Twelve apparently normal adults of comparable age and sex not suffering from malignant diseases were used as a control.

Methods:

A- *Flowcytometric detection of gelatinases:*

Purified monoclonal antibodies (MoAbs) against gelatinases A and B (MMP-2 & 9) were purchased from Zymed Invitrogen (Cat No.35-

1300) and Santa Cruze (Cat No.SC-21733) respectively. Suitable isotype control (IgG1 Kappa) was used.

Procedures of the indirect intracellular staining:

1- Bone marrow or peripheral blood EDTA samples were used provided they have malignant myeloid cells more than 20%, the cell count was adjusted to 10^4 and 50 µl were added to 100 µl of DAKO intra stain reagent A (fixative) (Cat No 60659) into 3 separate tubes, mixed gently and incubated at room temperature for 15 minutes.

2- The tubes were washed with 3mL PBS (0.01mol/L), centrifuged at 1500g and the supernatant was discarded.

3- One hundred µl of reagent B (permeablising solution) were added to all tubes and 5 µl of each monoclonal and isotype control were added to corresponding tubes, labeled and incubated for 30 minutes.

4- Step 2 was repeated, 50 µl of diluted (1:10) FITC labeled rabbit antimouse immunoglobulin (RAM) (DAKO. Cat. No. F0313) were added and incubated in the dark for 20 minutes. Step 2 was repeated, and cells were suspended in 500µl PBS for acquisition.

5- Samples of the control group were subjected to the same procedures.

Flowcytometric analysis:

Gelatinases expression was measured by (FACS Vantage: Becton Dickinson, San Diego, USA) using the Cell Quest software program (Becton Dickinson).

1- Full alignment procedures were performed each time using the standard Nile Red Beads (BD. Cat. No.347240) for adjusting forward scatter, side scatter and photomultiplier tubes.

2- Ten thousand events were passed in front of the laser for each sample tube and twenty thousand events for the control tube.

3- Analytical gates were set on the desirable viable cells based on forward and side light scatters combined with exclusion of normal cells using a CD45 tube. Threshold for positivity was based on isotype negative control. Antigen expression was determined as percent positivity of stained cells within the blast population.

4- The control sample: Lymphocytes were selected by their bright expression for CD 45 and analyzed for the expression of gelatinases A & B.

B- *RT-PCR for detection of IL-18 gene:*

1- Total RNA was extracted from 10^6 cells from B.M. EDTA sample using QIA Gen kit (Cat. No.52304).

2- One step RT-PCR QIA Gen kit (Cat. No. 210212) that combines cDNA synthesis from RNA with PCR amplification to provide a rapid, sensitive method for analyzing gene expression was used.

3- A pair of IL-18 specific primers: sense: 5'-GATGGCTGCTGAACCAGTAG-3' anti-sense 5'-GCTAGTCTTCGTTTTGAA-CAGTG-3' was designed to amplify 584 bp fragment of human IL-18.

4- An internal control, human glyceraldehydes 3-phosphate dehydrogenase (GAPDH) was also amplified using its specific primer: sense: 5'-GAAGGTGAAGGTCGGAGTC-3' anti-sense: 5'- GAAGATGGTGATGG-GATTTC-3', that amplifies 1020 bp fragment of human GAPDH.

5- The total reaction volume was 50µl containing 2µg RNA, 400µM of each dNTP, 20pMol from each primer (forward and reverse primer for both IL-18 & GAPDH) and the enzyme Mix included in the kit (reverse transcriptases and hot start Taq polymerase) in 1 X RT reaction buffer.

6- The thermal cycle program included a step for reverse transcription (30min.50ºC), initial PCR activation step (15min.95ºC), 30 cycles consisted of denaturation (1 min. 94ºC), annealing (1 min. 58ºC) and extension (1 min.72ºC) and a final extension step (10 min.72ºC).

7- Ten microliters of the PCR products were subjected to electrophoresis on 2% agarose gel containing ethidium bromide. A molecular weight marker (200bp) was used to asses the positions of the defined DNA band. The gels were visualized under UV light.

8- Semi-quantitative determination of the PCR products was performed using complete gel documentations and analysis system (Biometra, Germany). Relative expression of IL-18 gene (R) was calculated as follow: R= Densitometrical Units of IL-18 / Densitometrical Units of GAPDH **[9]**.

9- Control samples were subjected to the same amplification procedures and analysis.

C- *Response to induction chemotherapy:*

All patients received induction chemotherapy; their response was evaluated after 2 weeks. Complete remission (CR) was defined as normocellular marrow with 5% blasts, no circulating blasts, no evidence of extramedullary leukemia and recovery of granulocytes to 1500/µl and platelets to 100.000/µl [13]. Unfavorable outcome includes refractory cases (did not achieve complete remission) and early death (death within 14 days after first diagnosis).

D- *Chemotherapy protocol:*

Patients with newly diagnosed AML (except APL/M3) received induction chemotherapy with combination of 7 days of cytosine arabinoside (100mg/m^2) and 3 days of adriamycin (45 mg/m^2). Patients who achieved CR and had favorable cytogenetics[t (8; 21) and inv 16] received consolidation chemotherapy with combination of high dose Ara-C (1gm/m^2 IV infusion over 3 hours/12 hours for 3 days) and mitoxantron (12mg/m^2 IV short infusion days 3-5) for total of 3-4 cycles. Patients with high risk cytogenetics (monosomy 7 or 5, deletion of 5q and abnormalities of 3q and those with a complex karyotype) or intermediate risk cytogenetics (those with normal cytogenetics and other changes not associated with high risk or favorable groups) were transferred for allogenic bone marrow transplantation if they had matched sibling donor after achieving CR. Patients who did not have matched donor received consolidation chemotherapy as in favorable group, then they were transferred for autologous bone marrow transplantation (ABMT). Patients who relapsed after conventional chemotherapy or failed to achieve CR despite optimal induction treatment received second induction and then were transferred for Allogenic BMT if they had matched sibling donor or ABMT if they did not have a donor. Patients who relapsed after bone marrow transplantation received palliative chemotherapy (as HAM, AVVV or FLAG).

Patients with acute promyelocytic leukemia (APL) received induction treatment with ATRA (45mg/m^2 p.o daily until CR or maximum of 90 days) and adriamycin (45mg/m^2 IV days 1-3 for 3 courses every 28 days). These patients received maintenance treatment after achieving CR with ATRA (45mg/m^2 p.o daily for 2 weeks every 3 month), 6-mercaptopurin (60mg/m^2 daily) and methotrexate (20mg/m^2 IV once weekly) for 2 years.

Statistical methods:

Data were analyzed using SPSSwin. version15. Numerical data were expressed as median and range because the data were not normally distributed. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. Comparison between 4 groups of FAB classification was done using Kruskal-Wallis test followed by post-Hoc comparison test "Schefe test" on the rank of variables to compare pairs of groups. Survival analysis was done using Kaplan-Meier method. Comparison between two survival curves was done using Log-rank test. Receiver Operating Characters (ROC) curve was used to determine the prognostic cut off for IL18 gene expression. *p*-value 0.05 was considered significant.

RESULTS

Clinical criteria of the studied AML patients are shown in Table (1).

Cellular expression of gelatinases A & B (MMP-2 & MMP-9) and IL-18 gene:

The median and range of gelatinase A and B expression levels in the lymphocytes in the control samples were 8 (2-19%) and 2.6 (0.3-9.5%) respectively. Accordingly, 20% and10% was considered the cut off for the expression of gelatinase A & B respectively.

Gelatinase A (20%) was expressed in 26/42 (61.9%) and gelatinase B (10%) in 14/42 (33.3%) of the studied cases. The median expression of gelatinase A was not significantly different among different AML FAB subtypes (p=0.545). However, gelatinase B expression was significantly higher in M3, where all the studied five M3 cases were highly expressing it (p=0.001) (Table 2 and Figs. 1, 2).

The median expression level of IL18 gene in the AML group was 1.5 with a range of (0.8-2) which was significantly higher than the control group (median=1.0, range 0-1.2) (p<0.001). Within the different FAB subtypes, the expression level of IL-18 gene showed a significantly higher expression in M4 and M5 subtypes (p= 0.003) (Table 2 and Figs. 3, 4).

Relation between gelatinases A & B (MMP-2 & MMP-9) expression and hematological data at presentation:

There was no significant association between gelatinase A & B expression and any of the hematological findings except for a significantly higher blast cell distribution ratio (BCDR) in gelatinase A positive group (p=0.004) and a significantly lower hemoglobin in the gelatinase B positive group (p=0.012) (Table 3).

Relation between IL-18 gene expression and initial TLC:

The studied group was risk stratified regarding TLC at presentation into TLC 50x10^9/L and >50x10^9/L. The median expression level of IL-18 in the former group was 1.4 (0.8-2) and 1.6 (1.3-2) in the latter group; the difference was not statistically significant (p=0.081).

Relation between gelatinases expression and extramedullary infiltration:

There was a statistically significant higher incidence of splenomegaly among gelatinase A positive group regardless of the FAB subtype [22/26 (84.6%)] compared to 4/16 (25%) in the gelatinase A negative group (p<0.001). However, there was no statistical difference regarding hepatomegaly or lymphadenopathy (p=0.130 & 0.397, respectively).

On the other hand, there was no significant association between gelatinase B expression and splenomegaly, hepatomegaly or lymphadenopathy (p=0.261, 0.060 & 0.266, respectively).

Relation between the studied markers expression and response to induction therapy:

To examine the influence of gelatinases expression on remission probability, we compared their expression in patients who achieved complete remission with the unfavorable responders (refractory & early death). CR was significantly higher among the gelatinase A positive group (p=0.018), while it was significantly lower among the gelatinase B positive group (p=0.002).

As regards IL-18, a ratio of 1.5 was considered a prognostic cut-off. Accordingly, the studied group was classified into 2 groups: group that expressed IL18 1.5 [23/39 (58.9%)] and group that expressed IL18 >1.5 [16/39 (41.1%)]. CR was significantly lower in the latter group (p=0.021) (Table 4 & Fig. 5).

To verify the effect of combined marker expression on response to induction, the patients were classified into two groups: a group that expressed 2 or 3 of the studied markers (19/39) and a group that failed to express any of the studied markers or expressed only one marker (20/39), showing a significantly lower incidence of CR with double or triple marker expression (p=0.038) (Table 4).

NB. Three cases were excluded from this analysis as their data were missing.

Survival analysis:

The median follow-up duration was 16 weeks (range 1.3-64 weeks). The overall survival (OS) was 55.6%. There was no significant difference regarding gelatinase A or B expression and OS (p=0.078 & 0.082 respectively). However the overall survival was significantly lower in patients with IL-18 ratio >1.5 (p= 0.019). Regarding combined marker expression and OS, the median survival was 7 week with combined marker expression compared to 32 weeks in the other group without a statistically significant difference between the two group (p=0.120) (Table 5).

N.B. Leukemia free survival (LFS), defined as the period lasts from achievement of CR till relapse, could not be studied in this work as relapse was documented only in two cases at 8 & 12 weeks after CR.

Table (1): Clinical criteria of the studied AML patients at diagnosis.

Number of patients	42
Male / Female (ratio)	20/22(0.9)
Age (years) median (range)	35 (18-60)
WBC (x 10^9) median (range)	25 (3-103)
FAB morphology (no., % of total):	
M1	12 (28.6)
M2	13 (31.0)
M3	5 (11.9)
M4	10 (23.8)
M5	2 (4.8)
Hepatomegaly (no., % of total)	27 (64.3)
Splenomegaly (no., % of total)	26 (61.9)
L.N enlargement (no., % of total)	8 (19.0)
Hb (gm/dl) median (range)	7.7 (4.5-10)
Platelets(103/µl) median (range)	44 (10-142)
P.B blasts median (range)	33 (0-89)
B.M blasts median (range)	61 (20-98)
BCDR* median (range)	0.7 (0-1)
*Response to induction chemotherapy (no., % of total)**:*	
Complete remission	21 (50)
Refractory	14 (33.3)
Early death	4 (9.5)

* Blast cell distribution ratio (BCDR): PB blasts/BM blasts.
** Three cases were missing.

Table (2): The median expression of the studied markers in relation to FAB subtypes in 42 AML cases.

	M1	M2	M3	M4+M5	*p* value
Gelatinase A (%)	24 (5-57)	31 (12-47)	18 (2.9-29)	17.5 (7-61)	0. 521
Gelatinase B (%)	2 (0.1-19) [a]	9 (0.9-37) [a]	47 (34-51) [b]	6 (0.3-11) [a]	0. 001*
IL-18 gene/GAPDH ratio	1.4 (0.8-1.5) [a]	1.6 (1.3-2) [a]	1.6 (1.5-1.9) [a]	1.8 (1.3-2) [b]	0.003*

Data as median (range). Groups with different letters are statistically significant. *Significant.

Table (3): Relation between gelatinases A & B (MMP 2 & MMP 9) and hematological data at presentation in 42 AML cases.

	Gelatinase A expression		*p* value	Gelatinase B expression		*p* value
	Positive	Negative		Positive	Negative	
TLC	25 (3-69)	44 (23-60)	0.151	25 (3-79)	33 (5-103)	0.679
Hb	7.8 (4.5-9.8)	7.6 (4.7-10)	0.726	6.1 (4.5-9.8)	8.1 (5.6-10)	0.012*
Platelets	41 (10-142)	69 (23-119)	0.143	34 (11-109)	45 (10-142)	0.262
P.B. blasts	41 (12-89)	16 (0-85)	0.060	40 (6-86)	33 (0-89)	0.915
B.M. blasts	61 (20-98)	67 (40-96)	0.182	68 (51-97)	61 (20-98)	0.069
BCDR	0.8 (0.2-1)	0.3 (0-0.9)	0.004*	0.6 (0.1-1)	0.7 (0-1)	0.749

Data as median (range). *Significant.

Table (4): Relation between studied markers expression and complete remission in 39 AML cases.

		Complete remission (CR) (n=21)	Non responders (n=18)	*p* value
Gelatinase A	positive	17 (81.0%)	8 (44.4%)	0.018*
	negative	4 (19.0%)	10 (55.6%)	
Gelatinase B	positive	2 (9. 5%)	10 (55.6%)	0.002*
	negative	19 (90.4%)	8 (44.4%)	
IL-18 gene/GAPDH ratio	1.5	16 (76.2%)	7 (38.9%)	0.021*
	>1.5	5 (23.8%)	11 (61.1%)	
Combined marker expression	0/1marker	14 (66.7%)	6 (33.3%)	0.038*
	2/3markers	7 (33.3%)	12 (66.7%)	

Table (5): Impact of the studied marker expression on survival in 39 AML cases.

		Cumulative survival %	Median±SE	95% confidence interval	*p* value
Gelatinase A	positive	71.2	31.0±8.3	14.8-47.2	0.078
	negative	28.5	4.0±0.6	2.8-5.2	
Gelatinase B	positive	18.7	5.0±0.8	3.5-6.5	0.082
	negative	70.3	32.5±4.1	24.4-40.6	
IL-18 gene/GAPDH ratio	1.5	73.2	32.5±1.1	30.2-34.7	0.019*
	>1.5	31.2	4.0±0.5	2.9-5.0	
Combined marker expression	0/1marker	39.3	7.0±1.5	3.9-10.0	0.120
	2/3markers	70.0	32.5±1.0	30.4-34.5	

SE: Standard error. *Significant.

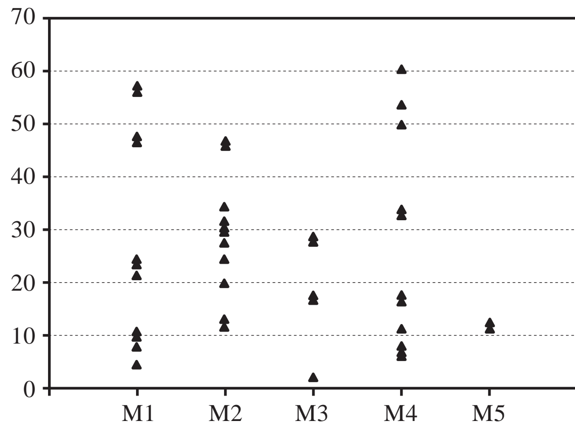

Fig. (1): Gelatinase A expression in relation to FAB subtypes in 42 AML cases

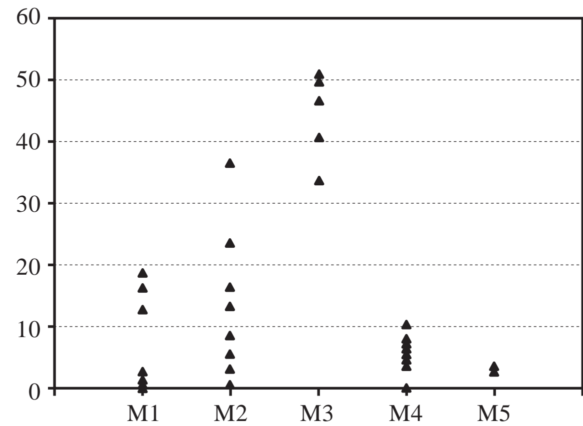

Fig. (2): Gelatinase B expression in relation to FAB subtypes in 42 AML cases

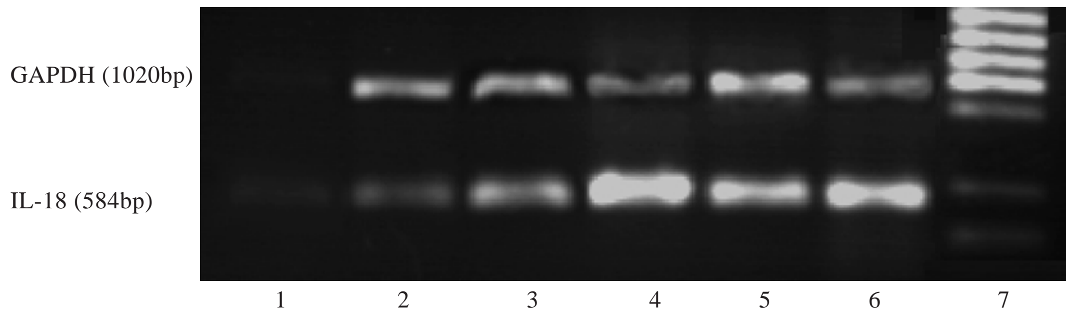

Fig. (3): IL-18 expression in bone marrow of patients with AML and controls.
Lane 1: negative control; 2: normal control; 3: M1; 4: M4; 5: M2; 6: M5 & 7: molecular weight marker.

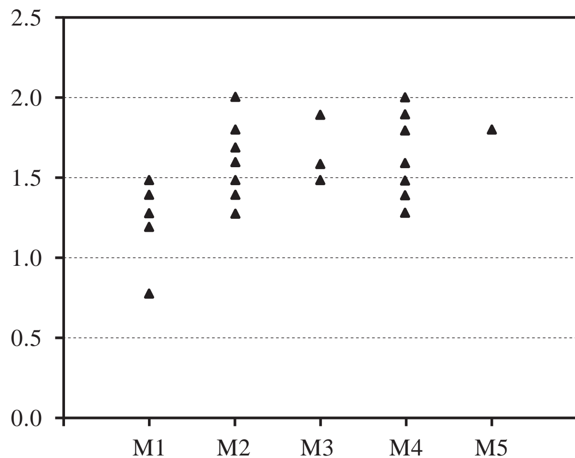

Fig. (4): IL18 gene expression in relation to FAB subtypes in 42 AML cases.

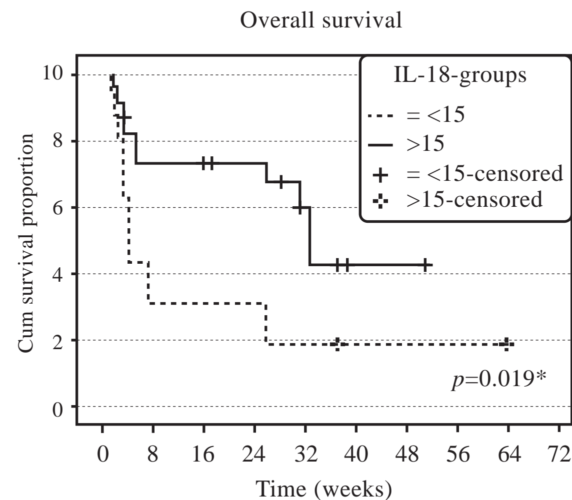

Fig. (5): IL18 gene expression in relation to overall survival in 39 AML cases

DISCUSSION

Knowledge about the biological and clinical role of gelatinases A & B (MMP-2 and MMP-9) in solid malignancies is rabidly increasing. However, in the field of hematological malignancies, the data are scarce **[4]**.

In the present work, expression of gelatinases A & B as well as IL 18 gene in blast cells from de novo adult AML cases was studied. Also, their significance in extramedullary invasion, disease progression, response to induction therapy and survival was evaluated.

In the current study, gelatinase A (MMP-2) was expressed in 26/42 (61.9%) in adult AML cases with no significant difference in its expression among different FAB subtypes (p= 0.545). Our data regarding the level of expression of gelatinase A are comparable to several published reports which ranged from 71-83% [15,16,17]. In agreement with our results Aref et al. found no correlation between pretreatment soluble gelatinase A (sMMP-2) levels and different FAB subtypes [4].

In this study, gelatinase B (MMP-9) was expressed in 14/42 (33.3%) in adult AML cases with a significantly higher expression in M3 subtype (p=0.001). Our results regarding the level of expression of gelatinase B is higher than that reported by Kuittinen et al. [15] who detected MMP9 in only 1/35 (2.8%) of cases using immuocytochemistry (IHC). Our higher results could be explained by the fact that we measured expression of gelatinase B using flow-cytometry which is known to be a more sensitive technique compared to IHC. However, our percentage of gelatinase B expression (33.3%) is lower than Janowska et al who detected MMP-9 mRNA by PCR in 4/6 (66.6%) of their cases [17]. Their higher percentage may be related to the small sample size which could not represent the studied group fairly or alternatively to using a different method.

In our work, gelatinase B (MMP-9) showed a significantly higher expression among FAB-M3 subtype and this supports what was previously reported by Ries et al. who found MMP-9 expressed predominantly in promyelocytic HL-60 cell line, which may be explained by the fact that gelatinase B normally secreted from the neutrophilic series specially the more mature stages [18]. However, other reports regarding expression of MMP-9 among AML FAB subtypes were conflicting with either no correlation [19] or a significantly higher expression among FAB-M5 [20].

In this study, the incidence of extramedullary infiltration was significantly higher among gelatinase A (MMP-2) positive group as seen by the higher BCDR (a measure of blasts to mobilize peripherally) (p=0.004) as well as, the higher incidence of splenomegaly (p<0.001). However such correlation between extramedullary infiltration and gelatinase B (MMP-9) expression could not be obtained. Our results are in agreement with the previously published data by Sawicki et al. [21] who found that MMP-2 but not MMP-9 is involved in the invasive process of AML blasts by matrigel assay. However, our finding is not in accordance with that reported by Aref et al. [20] who found that serum level of MMP-9 correlated significantly with PB blasts, BCDR and extramedullary infiltration and Yang et al. [22] who found that both MMP-2 and MMP-9 participated in the extramedullary leukemic invasion of AML patients. Our results regarding the relation between gelatinase A expression and extramedullary infiltration support the suggestion that MMP2 might enhance the degrading function of leukemic cell, thus making the crossing of them through the bone marrow barrier easier with their subsequent release into blood.

In this study, the incidence of CR was significantly higher among gelatinase A (MMP-2) positive group (p=0.018). However, the overall survival (OS) did not differ significantly among gelatinase A positive and negative groups (p=0.078). Literature reports regarding correlation between OS and MMP-2 expression are conflicting. While some reports showed better OS among MMP-2 positive AML patients than among MMP-2 negative patients [15,21,23,24]; others showed that high serum levels of MMP-2 was associated with short OS in AML patients [4]. This discrepancy between results regarding MMP-2 protein expression and good prognosis in AML may be related to measuring the MMP protein or mRNA levels instead of measuring the enzyme activity.

In this work, CR was significantly higher among gelatinase B (MMP-9) negative group (p=0.002). However, no correlation between gelatinase B expression and OS was found (p=0.082). Lin et al. [19] found that lower marrow levels of MMP-9 was significantly associated with both higher CR and better OS rates (p=0.001 & 0.012 respectively). Also, Aref et al. [20] found that a lower serum level of MMP-9 is associated with higher incidence of CR and better OS. Absence of statistical significance regarding OS and gelatinase B expression may be due to the small sample size.

In the present work, IL18 gene expression in AML group was significantly higher than the control group (p<0.001). All FAB subtypes had

significantly higher values than the control, with highest expression detected in M4 and M5 FAB subtypes (*p*=0.003). Similar results were obtained by many authors [9,12]. The possible source of augmented IL-18 gene expression may be the leukemic cells or the stromal cells or both. The higher levels obtained in monocytic leukemia may be due to the fact that IL 18 is mostly expressed in activated macrophages and monocytes derived cells [9].

While Zhang et al. [9] found a significantly higher median IL 18 gene expression among AML patients with high initial TLC (*p*=0.02), we could not confirm this finding in our work though the median IL 18 gene expression was higher among patients with elevated TLC group (*p*=0.081) which may be related to ethnic variation.

In this work, the median expression of IL18 was higher among the non responders (refractory & early death) and those with short overall survival (*p*=0.021 & 0.019 respectively). To our knowledge the relation between IL18 expression and response to treatment and survival has not been studied before. From our results, IL 18 may be considered as a poor prognostic factor in AML as evidenced by its correlation with poor response to treatment and short OS. Additionally IL 18 may be considered a positive regulatory factor for proliferation of leukemic cells as seen by its tendency to be higher among patients with high initial TLC and its correlation to FAB-M4 & 5 subtypes. However, further studies are needed to understand the full significance of IL 18 in leukemogenesis.

To our knowledge, this study is the first to address the relation between combined marker expression (Gelatinases A, B & IL18) and their clinical relevance regarding response to induction and OS, where only 7/19 (36.8%) patients that showed double or triple marker expression achieved CR compared to 14/20 (70%) in patients who failed to express any of the studied markers or expressed only one marker (*p*=0.038) suggesting an additive or cumulative effect of these markers on response to chemotherapy. However, we could not reach such results with OS although the median survival was 7 weeks with combined marker expression compared to 32 weeks in the other group.

Conclusion:

The expression of gelatinase A (MMP-2) by AML blasts supports the hypothesis that in AML, MMP-2 may be a marker of the invasive phenotype and may suggests a novel therapeutic approach in AML. Gelatinase B (MMP-9) expression might carry a poor prognosis in AML. IL18 may be considered as a poor prognostic factor acting as a positive regulator for proliferation. Combined expression of gelatinases and IL18 gene overexpression could have cumulative effect on response of AML patients to chemotherapy. In summary; these data suggests that angiogenic factors may have a role in the leukemic process and that anti-angiogenic therapy could be a new therapeutic strategy in AML. However, to validate these assumptions, a study on a larger number of patients is required.

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