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The possible role of Matrix Metalloproteinase-9 in children with acute leukemia

[Akut Lösemili Çocuklarda Matriks Metalloproteinaz-9'un Olası Rolü]

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ABSTRACT

Rationale and objectives: The main role of matrix metalloproteinase-9 in angiogenesis, tumor growth and metastasis is degradation of extra cellular matrix. The objective of this study was to evaluate the serum levels of matrix metalloproteinase-9 and different enzymes such as lactate dehydrogenase, creatine kinase and acid phosphatase in children with acute leukemia to determine the potential use of such parameters as predictive markers for acute leukemia.

Methods: The study was carried out on 30 children with acute leukemia (18 acute lymphoblastic leukemia and 12 acute myeloid leukemia, and 20 healthy children as a control group. They were subjected complete blood count, lactate dehydrogenase, creatine kinase, acid phosphatase and serum matrix metaloproteinase-9 level.

Results: The serum matrix metalloproteinase-9 level and other enzymes were significantly higher in patients with acute lymphoblastic leukemia and acute myeloid leukemia as compared to the control group ($P < 0.05$). The serum matrix metalloproteinase-9 levels significantly increased in acute lymphoblastic leukemia and acute myeloid leukemia patients. By spearman correlation serum matrix metalloproteinase-9 level was correlated positively with lactate dehydrogenase activity and number of blast cells in peripheral blood.

Conclusions: These data suggest that matrix metalloproteinase-9, lactate dehydrogenase and blast cells in peripheral blood may be useful diagnostic marker that help patient's stratification and adjust the disciplines of therapy.

Key Words: leukemia, matrix metalloproteinase-9, lactate dehydrogenase, creatine kinase, acid phosphatase

ÖZET

Amaç: Matriks metalloproteinaz-9'un anjiyogenez, tümor gelişim ve metastazında ana rolü ekstrasellüler matriks yıkımıdır. Bu çalışmanın amacı akut lösemili çocuklarda matriks metalloproteinaz-9, laktat dehidrogenaz, kreatin kinaz, ve asit fosfataz gibi farklı enzimlerin serum seviyelerini belirleyip bu parametrelerin akut lösemide olası saptayıcı belirteçler olarak kullanılıp kullanılamayacağını belirlenmekti.

Yöntemler: Çalışma akut lösemili 30 çocuk (18 akut lenfoblastik lösemi ve 12 akut myeloid lösemi) ve kontrol grubu olarak 20 sağlıklı çocuk ile yapıldı. Tam kan analizi, laktat dehidrogenaz, kreatin kinaz, asit fosfataz ve serum matriks metalloproteinaz-9 düzeyleri saptandı.

Bulgular: Serum matriks metalloproteinaz-9 ve diğer enzimlerin düzeyleri kontrol grubuna oranla akut lenfoblastik lösemi ve akut myeloid lösemi hastalarında belirgin olarak daha yüksek bulundu ($P < 0.05$). Serum matriks metalloproteinaz-9 düzeyleri akut lenfoblastik lösemi ve akut myeloid lösemi hastalarında belirgin olarak daha yüksek idi. Spearman korelasyon analizi ile serum matriks metalloproteinaz-9 düzeyleri, laktat dehidrogenaz aktivitesi ve periferal kandaki blast hücrelerini sayısı arasında pozitif korelasyon saptandı.

Sonuç: Bu veriler serum matriks metalloproteinaz-9, laktat dehidrogenaz aktivitesi ve periferal kandaki blast hücrelerinin hastaların sınıflandırılması ve tedavi şekline uyumlarında yararlı bir diyagnostik belirteç olabileceğini düşündürtmektedir.

Anahtar Kelimeler: Lösemi, matriks metalloproteinaz-9, laktat dehidrogenaz, kreatin kinaz, asit fosfataz

Introduction

Leukemia defined as the uncontrolled proliferation or expansion of hematopoietic cells that do not retain the capacity to differentiate normally to mature blood cells. In addition, this abnormal blood cells development may result in a breakdown of cell to stroma interactions leading to the subsequent egress of immature blood elements from the bone marrow to the peripheral blood (1).

Matrix metalloproteinases (MMPs) are a family of enzymes with the common ability to degrade various components of the extracellular matrix such as collagen, elastin and gelatin (2)**.** These enzymes are involved in all physiological processes that occur during tissue remodeling and repair. They also play a crucial role in pathological conditions such as rheumatoid arthritis, tumor invasion and metastasis (3). Both in vitro and in vivo investigations have shown that increased concentrations of MMPs are associated with the invasive and metastatic potential in several human malignant tumors (4- 6)**.** The catalytic activities of MMPs were controlled by various mechanisms including enzyme synthesis and specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) (7)**.**

The human MMP gene family consists of at least 18 structurally related members that fall into five classes according to their primary structure and substrate specificity: collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, MMP-11 and MMP-12), membrane type (MT)-MMPS (MT_1-MMP, MT_2-MMP, MT_3-MMP, and MT_4-MMP), and non classified MMPs (8,9)**.**

Matrix metalloproteinase-9 (MMP-9) is present in a wide variety of tissues**.** It is able to degrade several connective tissue macromolecules, including type IV collagen in basement membranes. These enzymes participate in tissue remodeling (10) and cell invasion through extracellular matrix barriers (11)**.** Several members of matrix metalloproteinase family, especially MMP-2 and MMP-9 have been associated with invasion of tumor cells and they play a role in formation of hematogenous metastasis in several malignancies as well as tumor angiogenesis (12)**.**

The objective of this study is to understand serum levels of MMP-9 in children with acute leukemia as prognostic marker, and knows the importance of this enzyme in cell invasion through extracellular matrix barriers in patients with acute leukemia and to investigate the link between MMP-9 level and different enzymes activities as LDH, CK and acid phosphatase. This will help the identification of some helpful prognostic parameters that assist patient's stratification and adjust the disciplines of therapy.

Materials and Methods

Subjects

This study was conducted on fifty subjects. Twenty healthy volunteers of matched age and sex recruited for participation as control group (group I) subjects (9 males and 11 females) their ages ranged from 4 to 15 years. Thirty children were selected with acute leukemia, from Pediatric Department, Tanta University Hospital. They were divided into: group IIa Included eighteen children with acute lymphoblastic leukemia (ALL) (10 males and 8 females), their ages ranged from 2 to 17 years and group IIb Included twelve children with acute myeloid leukemia (AML) (7 males and 5 females), their ages ranged from 4 to 16 years.

All cases included in this study were subjected to history taking and complete clinical examination.

Fasting blood sample was collected aseptically from all subjects. An aliquot of fasting sample was collected on EDTA and analyzed within few hours complete hemogram analysis using sysmex counter k-1000 Japan and differential count of peripheral blood by leishman stain.

The other aliquots were stored at -20 ^{o}C for measurement lactate dehydrogenase (LDH), creatine kinase (CK), total and tartarated acid phosphatase and serum MMP-9 activity by ELISA technique

Enzyme assays:

Lactate dehydrogenase (LDH):

LDH activity in serum was assayed by using commercial kit that was supplied by Standbio Laboratory, from Texas (13)**.** One unit of enzyme per liter (U/L) of LDH activity is that amount of enzyme, which produces one µmol/L of NADH per minute at 37 ^{o}C.

Creatine kinase (CK):

Creatine kinase activity in serum was assayed by using commercial kit that was supplied by Standbio Laboratory, from Texas (14)**.** One unit per liter (U/L) of CK activity is the amount of enzyme, which oxidizes one µmol/L of NADH per minute at 37 ^{o}C.

Acid phosphatase:

Acid phosphatase activity in serum was assayed by commercial kit that was supplied by Quimica Clinica Aplicada S.A, from Spain (15)**.**

MMP-9:

MMP-9 activity in serum was assayed by using commercial kit that was supplied by New Test Co, from Egypt (16)**.** This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MMP-9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and the immobilized antibody binds MMP-9. After washing away unbound substances, an enzyme-linked polyclonal antibody specific for MMP-9 is added to the wells. Following a wash to remove unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of total MMP-9 (pro and/ or active) bound in the initial step. The color development is stopped and the intensity of the color is measured.

Statistical analysis

Results were expressed as mean ± SE. Comparisons between groups were made using Student's t-test for continuous variables. Correlation between the two parameters was determined by Spearman correlation coefficient (r). A p value less than 0.05 was considered statistically significant.

Results

This study was carried out on twenty volunteers as a control group and thirty children with acute leukemia, the patients were classified into two groups (AML and ALL). Different parameters were studied on these groups.

The clinical data of the studied patients with acute leukemia was shown in Table 1. The fatigue and pallor symptoms was encountered in all patients (100 % in ALL and 100 % in AML), Bleeding and fever were observed 22.2 % and 16.6 %; 88.8 and 88.7, in ALL and AML, respectively. Hepatosplenomegally, lymphadenopathy and bone pain were detected in 77.8 % and 77.8 %; 50 and 66.7; 83.3 and 8.3 % in ALL and AML, respectively.

There was significant decrease in RBCs count in ALL and AML patients when compared to the control group ($P< 0.005$). In addition, there was significant decrease in hemoglobin level, HCT and platelet count in ALL and AML patients when compared to the control group ($P< 0.001$) (Table 2).

WBCs count and blast cell in peripheral blood was significant increase in ALL and AML patients when compared to the control group ($P< 0.001$), respectively. Moreover, the mean value of WBCs count in ALL significantly increased than of AML. There was significant decrease in neutrophil percentage in ALL and AML patients when compared to control group ($P< 0.001$). On the other hand, no significant difference was detected in monocyte in ALL patients when compared to the control group ($P>0.05$), while there was significant increase in AML patients when compared to control group ($P < 0.001$) (Table 3).

Table 1. Clinical data of AML and ALL patients

	Group IIa ALL n=18		Group IIb AML n=12	
	n	%	n	%
Fatigue/Pallor	18	100	12	100
Bleeding	4	22.2	2	16.6
Fever	16	88.8	8	66.6
Hepatosplenomegally	14	77.8	6	50
Lympadenopathy	9	50	8	66.6
Bone pain	15	83.3	1	8.3

Table 2. RBCs count (x 10^6 cell/ Cmm), Hb (g/dl), HCT (%) and Platelet count (x 10^3 cell/ Cmm) in control, ALL and AML patients

Groups / Parameter	Control n=20	Leukemia patients	
		ALL n=18	AML n=12
RBcs (C.mm) X10^6 Range Mean ± S.E. P value	 3.8 - 6 5 ± 1.1	 1.1- 3 2.3 ± 0.14 P* value< 0.005	 1.3-2.8 2.2 ± 0.14 P**value< 0.005
Hb (g%) Range Mean ± S.E. P value	 11.3 -14 12.65 ± 0.172	 3 - 8.5 6.1 ± 0.399 P*value< 0.001	 5.2-7.5 6.5 ± 0.23 P**value< 0.001
HCT (%) Range Mean ± S.E. P value	 37 – 47 42.7 ± 0.65	 10 – 28 20.1 ± 1.3 P*value< 0.001	 18-32 22.67 ± 1.2 P**value< 0.001
Platelet Range Mean ± S.E. P value	 186 – 260 222.9 ± 5.6	 19– 197 45.2 ± 11.5 P*value< 0.001	 13- 280 75.8 ± 25.4 P**value< 0.001

P significant < 0.05
P* ALL vs control
P** AML vs control

Table 3. Total and differential leukocytic count (x 10^3 cell / Cmm) in control, ALL and AML patients

Groups / Parameter	Control n=20	Leukemia patients	
		ALL n=18	AML n=12
Leukocyte (C.mm) x10^3 Range Mean ± S.E. P value	 4- 9 6.5 ± 0.43	 22- 395 201.7 ± 23.36 < 0.001	 11-220 152.8 ± 18.9 < 0.001
Blasts (%) Range Mean ± S.E. P value	 0 0	 0 - 83 28.7 ± 5.124 < 0.001	 0 - 20 13.67 ± 1.75 < 0.001
Stab (%) Range Mean ± S.E. P value	 2 – 3 2.3 ± 0.51	 0 – 16 3.17 ± 1.04 >0.05	 0-14 4.4 ± 1.459 < 0.05
Neutrophils (%) Range Mean ± S.E. P value	 55 – 63 59.6 ± 0.47	 3 – 72 30.44 ± 3.98 < 0.001	 30-40 35.17 ± 1.127 < 0.001
Lymphocyte (%) Range Mean ± S.E. P value	 29 - 33 31.2 ± 0.31	 10 - 88 28.11 ± 4.5 >0.05	 21-44 30.7 ± 2.1 >0.05
Monocyte (%) Range Mean ± S.E. P value	 2 - 9 4.7 ± 0.49	 0 -14 4.5 ± 1.221 >0.05	 0 - 20 11.833 ± 1.7 < 0.001

P significant < 0.05 **P*** vs control

There was no blast in bone marrow in control group, while the amount of blast cell in ALL group and AML group ranged from 8 to 85 and from 24 to 80 with a mean value of 59.1 ± 4.95, and 48.92 ± 4.9 respectively. There was significant increase of blasts in bone marrow ALL and AML patients when compared to the control group (P< 0.001) (Table 4 and Figures (1, 2).

LDH activity was significant increase in ALL and AML patients when compared to the control group (P< 0.001). Also, there was significant increase in CK activity in ALL and AML patients when compared to the control group (P< 0.025) and (P< 0.001), respectively (Table 5). Also, There was significant increase in CK activity in ALL compared to AML patients (P<0.001). There was significant increase in acid phosphatase level in ALL and AML patients when compared to control group (P< 0.001) and (P< 0.01), respectively (Table 5).

MMP-9 activity in control group ranged from 0.9 to 2.5 ng/ml with a mean value of 1.64 ± 0.119; while in ALL group and AML group it ranged from 2.5 to 11.9 ng/ml and from 3.4 to 12.3 ng/ml with a mean value of 6.9 ± 0.69 and 7.23 ± 0.88, respectively. There was significant increase in MMP-9 level in ALL and AML patients when compared to the control group (P< 0.001) (Table 6 and Figure 3).

The spearman correlation between MMP-9 and the different studied parameters are shown in Table 7. In ALL group, MMP-9 showed a significant positive correlation of MMP-9 with leukocyte count (r= 0.500, P <0.05) and blast cells in peripheral blood (r= 0.535, P<0.05). In AML group, MMP-9 showed a significant positive correlation with leukocyte count (r= 0.691, P<0.05).

There was a significant positive correlation of MMP-9 with LDH in ALL and AML with r=0.493 (P< 0.05) and r= 0.704 (p<0.05), respectively, and There was no significant correlation of MMM-9 in ALL and AML with CK and acid phosphatase.

Discussion

The acute lymph blastic leukemia is a disorder characterized by the un- controlled growth and proliferation of immature lymphoid cells. The presenting signs and symptoms reflect the degree of bone marrow infiltration with leukemia cells and the extent of extramurally disease spread (17).

The spleen and liver usually are moderately enlarged, while enlarged lymph nodes are seen mainly in the pediatric lymph blastic leukemia's (18). Massive hepatosplenomegally is uncommon except in infants with AML (19).

In this study, extramurally involvement in acute leukemia as organomegally was observed in 77.8 % in ALL. Lymphadenopathy was detected in 50 % in ALL and 66.7 % in AML and bone ache 83.3 % in ALL and 8.3

Table 4. Blasts in bone marrow in control, ALL and AML patients

Groups / Parameter	Control n=20	Leukemia patients	
		ALL n=18	AML n=12
Blasts in BM (%)			
Range	0	8 - 85	24 - 80
Mean ± S.E.	0	59.1 ± 4.95	48.92 ± 4.9
P * value		< 0.001	< 0.001

P significant < 0.05 P* vs control

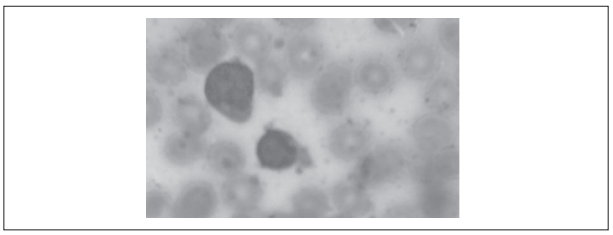

Figure 1. Photograph of bone marrow aspirated film from a case with acute lymphocytic leukemia

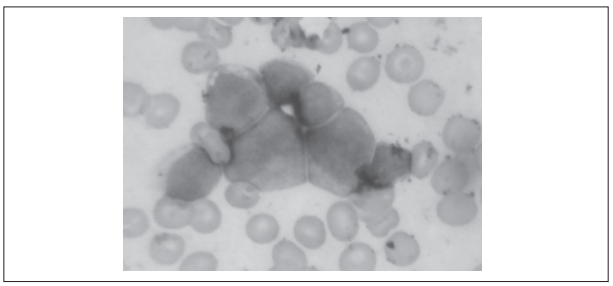

Figure 2. Photograph of bone marrow aspirated film from a case with acute myeloid leukemia

in AML these results were in agreement with Jonsson *et al.* (20).

The clinical picture of acute leukemia is marked by the effects of anemia, which is usually sever (fatigue, malaise), an absence of functional granulocytes (proneness to infection and inflammation), and thrombocytopenia (hemorrhagic diathesis) (18,21).

In this study, fatigue and pallor were the main presenting symptoms encountered in both types of acute leukemia (100 %) while fever was observed in 88.9 % in ALL and in 66.7 % in AML. Fever in acute leukemia patients caused by pyrogenic cytokines released from leukemia cells, including interleukin-1, tumor necrosis factor and interleukin-6, but in 30 % of patients is caused by infection. So CBC must be done for every patient with unexplained fatigue, pallor and fever these results were in agreement with Dinarello and Bunn (22).

In this study, a significant decrease in the mean of hemoglobin level and platelets counts was detected in two types of acute leukemia. This is consistent with Colby-Graham and Chordas (23) who stated that hemoglobin level below 10g/dl is present in the majority of acute leukemia and thrombocytopenia is present at diagnosis. Remitted acute leukemia is associated with increase in hemoglobin level and platelets count.

Leukocyte counts vary greatly in the acute leukemia. About one-fourth to one third of cases begin with a low white blood count (sub or a leukemia), while about half show some degree of leukocytosis. Mature granulocytes may still be found in the peripheral blood in addition to abnormal forms. The leukocytopenic forms are the most difficult to differentiate from a plastic anemia's, pancy-

Table 5. LDH, CK and total and tartarated acid phosphatase in control, ALL and AML patients

Groups / Parameter	Control n=20	Leukemia patients	
		ALL n=18	AML n=12
LDH (U/L)			
Range	185-325	158 - 1845	134 – 1892
Mean ± S.E.	254 ± 10.5	757.7 ± 136.96	757.83 ± 192.6
P value		P* value< 0.001	P**value< 0.001
Creatine kinase (U/L)			
Range	27 - 78	13.2 – 109.9	68.2 -279
Mean ± S.E.	47.15 ± 3.9	61.63 ± 7.13	143.9 ± 18.8
P value		P*value< 0.025	P**value< 0.001
Total acid phosphatase (U/L)			
Range	2.9 – 9.9	9.5 – 23.3	7.3 -21.5
Mean ± S.E.	6.67 ± 0.505	14.83 ± 0.95	10.28 ± 1.68
P value		P*value < 0.001	P**value< 0.01

P significant < 0.05
P* ALL vs control
P** AML vs control

Table 6. MMP-9 in control, ALL and AML patients

Groups / Parameter	Control n=20	Leukemia patients	
		ALL n=18	AML n=12
MMP-9 (ng/ml) Range Mean ± S.E. P value	0.9 - 2.5 1.64 ± 0.119	2.5 – 11.9 6.9 ± 0.69 P* value< 0.001	3.4 – 12.3 7.23 ± 0.88 P**value< 0.001

P significant < 0.05

P* ALL vs control

P** AML vs control

Table 7. Spearman correlation between MMP-9 and different parameters of ALL and AML patients

MMP-9 in studied groups				groups / Studied parameters
AML		ALL		
P	r	p	r	
0.013*	0.691	0.005*	0.500	Leukocyte
0.055	0.566	0.022*	0.535	Blasts in peripheral blood
0.185	0.411	0.143	0.360	Blasts in bone marrow
0.001*	0.704	0.038*	0.493	LDH
0.296	0.329	0.084	0.418	CK
0.060	0.557	0.143	0.360	Total acid phosphatase

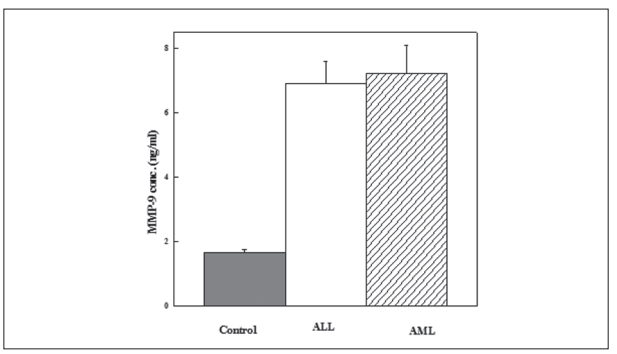

Figure 3. MMP-9 in control, ALL and AML patients

topenias, and the myelodysplastic syndromes (18).

As regard total leukocyte count (WBCs), there was a significant increase in WBCs in children with acute leukemia when compared to control group. This finding is in agreement with Devine, and Larson (1994) (24) who stated that patients with high leukocyte count without normal function result in reduction in circulating neutrophils essential to fight bacterial infection.

High levels of blast cells in ALL and AML patients were observed in this study indicating a bad prognosis. This results were in agreement with the results reported by Jabbour et al. (25) Who stated that the acute leukemia are characterized by aberrant differentiation and proliferation of malignantly transformed hematopoietic stem cells. These cells accumulate in the bone marrow and lead to suppression of growth and differentiation of normal blood cells.

LDH can show a moderate increase only in some cases of acute non lymphoblastic leukemia, whereas LDH activity in ALL patients almost always increases, an event related to the number of white cells during remission or relapse of the disease (26)**.**

In the present study, serum total LDH level were elevated in both acute lymphoblastic leukemia and acute myeloid leukemia and when these levels compared with the level in control group, were found to be statistically significant.

Our results are similar to those obtained by Stuart et al. (27) and Sactor and Dick (28), who have shown increased LDH activity in isolated lymphoblasts and in the serum of patients with ALL and in animals with transplantable lymphatic leukemia. Increased cellular LDH activity reflects a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of malignant cells accompanied by a high turnover rate (27,29)**.**

Bierman et al. (30) reported elevated serum LDH levels in 47 of 54 patients with lymphatic leukemia and in all 36 patients with AML; they recorded elevated levels in 86 % of patients with malignant lymphoma.

Fang et al. (31) the leukemic cellular CK activity was 2.2 times higher than the normal value. Only two of five

normal leukocyte samples showed positive CK isoenzyme MM. In this study, serum creatine kinase activity levels show a significant difference in ALL and AML children when compared to control group and AML children had higher serum creatine kinase activity than ALL patients.

Acid phosphatase activity is moderately to strongly positive in the blast cells of acute granulocytic and monocytic leukemia and low in acute lymphoblastic leukemia (32)**.** In this study, there were a significant difference between ALL, AML and control group of acid phosphatase activity. These results are in agreement with Catovsky et al. (33) who found that there is a clear association between T lymphocytic membrane markers and strong acid phosphatase activity in the blast cells of patients with ALL.

MMP-9 measurements in blood have provided more encouraging results in cancer than MMP-2 assays. MMP-9 was significantly increased in the plasma of patients with breast cancer and gastrointestinal tract cancer as compared with that of healthy subjects (34)**.** MMP-9 was also increased in the plasma of patients with hepatocellular carcinoma (35)**.** In addition, clinical follow up of patients with metastatic gastrointestinal cancer indicated that the survival time of patients with increased plasma levels of MMP-9 was significantly shorter than of patients with normal plasma levels (5)**.**

In this study, it was shown that the concentration of MMP-9 in the patients with either ALL and AML were higher than those in the normal control. Our result in agreement with Ringshausen et al. (36) who reported that serum levels of angiogenic factors like MMP-9 were elevated in patients with chronic lymphocytic leukemia and these levels correlated with un favorable prognosis.

In this study, there were a significant positive correlation between MMP-9 and total leukocyte count and blast cells in peripheral blood. Our result in agreement with Abd-Elhalim et al. (37) who reported that there was a significant correlation between MMP-9 levels in the plasma of leukemic patients and both total leukocytic count and peripheral blood blasts. This is supported by the assumption that MMP-9 appears to be an important regulator of the growth of tumors both at the primary site and at metastasis (38)**.**

In this study, there were a significant positive correlation between MMP-9 and LDH in children with acute leukemia (ALL and AML). This was supported by Jong et al. (39) who reported that in patients with acute leukemia there are an elevation of MMP-9 and LDH. Moreover, Park et al**.** (40) reported that MMP-9 positively correlated with LDH levels in pleural fluid in patients with lung cancer. Also, Ito et al. (41) found a significant correlation between MMP-9 and acid phosphatase in patients with bone tumors. In conclusion, serum is helpful diagnostic markers that correlate with LDH activity and number of blast cells in peripheral blood blast.

References

[1] Freireich EJ. (1984). Acute leukemia. A prototype of disseminated cancer. Cancer. 53: 2026-2033.

[2] Liotta LA and Stetler Stevenson. WG. (1991). Tumor invasion and metastasis: an inbalance of positive and negative regulation. Cancer Research. 51:5054-5059.

[3] Crawford HC and Matrisian, LM. (1994). Tumor and stromal expression of matrix metalloproteinases and their role in tumor progression. Invasion and Metastasis. 14: 234-245.

[4] Anderson IC, Sugarbaker DJ, Ganju RK, Tsarwhas DG Richards WG, Sunday M, Kobzik L and Shipp MA. (1995). Stromelysin-3 is over expressed by stromal elements in primary non-small cell lung cancers and regulated by retinoic acid in pulmonary fibroblasts. Cancer Res. 55:4120- 4126.

[5] Zucker S, Lysik RM, DiMassimo BI, Zarrabi HM, Moll UM, Grimson R, Tickle SP and Docherty AJ. (1995). Plasma assay of gelatinase B: tissue inhibitor of metalloproteinase complexes in cancer. Cancer. 76: 700-708.

[6] Murray GI, Duncan M., O´Neil P, Melvin WT and Fothergill JE. (1996). Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nature Medicine. 2:461-462.

[7] Lin LI, Lin DT, Chang CJ, Lee CY, Tang JL and Tien HF. (2002). Marrow matrix metalloproteinases (MMPs) and tissue inhibitors of MMP in acute leukemia: Potential role of MMP-9 as a surrogate marker to monitor leukemic status in patients with acute myelogenous leukemia. Br J Haematol. 117: 835- 841.

[8] Kleiner DE and Stetler-Stevenson WG. (1999). Matrix metalloproteinases and metastasis. Cancer Chemother. Pharmacol. 43: S42-51.

[9] Westermark J and Kahari VM. (1999). Regulation of matrix metalloproteinase expression in tumor invasion: FASEB J. 13: 781-792.

[10] Birkedal-Hansen H. (1995). Proteolytic remodling of extracellular matrix. Curr Opin Cell Biol, 7: 728-735.

[11] Leppert D, Waubant E, Galardy R, Bunnett NW. and Hauser SL. (1996). T cell gelatinases mediate basement membrane transmigration in vitro. J Immunol. 154: 4379-4389.

[12] Eccles SA, Box GM, Court WG, Bone EA, Thomas W and Brown PD. (1996). Control of lymphatic and hematogenous metastasis of a rat mammary carcinoma by matrix metalloproteinase inhibitor Batimastat (BB-94). Cancer Res. 56: 2815.

[13] Buhl SN and Jackson KY. (1978). Optimal conditions and comparison of lactate dehydrogenase analysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25,30, and 37 degree C. Clin Chem. 24:828-831.

[14] Kachmar JF and Moss DW (1976). In fundamentals of clinical chemistry, 2nd ed. NW Tietz, Editor. WB Saunders, Phildelphia. P682.

[15] Moss DW. (1984). In methods of enzymatic analysis, Ed. H. U. Bergmeyer, verbiage-chemie, 3d edition. V4: 92-106.

[16] Cullen B, Silcock D, Brown LJ, Gosiewska A, Geesin JC. (1997). The differential regulation and secretion of proteinases from fetal and neonatal fibroblasts by growth factors. Int J Biochem Cell Biol. 29:241-250.

[17] Pizzo PA and Poplack DG. (1993). Principles and Practice of Pediatric Oncology. 2nd. Philadelphia, Penn: JB Lippincott Co.1444-1445.

[18] Brunning RD. and McKenna RW. (1994). Acute leukemias. In: Tumor of the bone marrow, 3rd Series, fascicle 9. Washington: Armed forces institute of pathology (AFIP). 22-142.

[19] Weinstein HJ. (1999). Acute myeloid leukemia. In Pui, C (ed.), Childhood Leukemias. New York: Cambridge University Press.

[20] Jonsson O, Sartain P, Ducore J and Buchanan G. (1990). Bone pain as an initial symptom of acute lymphocytic leukemia. J Pediatrics. 117:233-237.

[21] Escalon EA. (1999). Acute lymphoblastic leukemia in childhood. Int Pediatr. 14: 83-89.

[22] Dinarello CA and Bunn PA. (1997). Fever as presenting symptom in acute lympocytic leukemia. Semin Oncol. 24: 288-298.

[23] Colby-Graham MF and Chordas C. (2003). The Childhood Leukemia. J Pediatr Nurs. 18: 87-95.

[24] Devine SM and Larson RA. (1994). Acute leukemia in adults: Recent developments in diagnosis and treatment:Ca. Cancer J Clin. 44: 326-352.

[25] Jabbour EJ, Estey E and Kantarjian HM. (2006). Adult Acute Myeloid Leukemia: Mayo Clin Proc. 81: 247-260.

[26] D'Angelo G, Giardini C and Calvano D. (1989). Clinical significance of the determination of lactate dehydrogenase in acute leukemia and non-Hodgkin's lymphoma. Minerva Med. 80:549-552.

[27] Stuart J, Simpson JS and Mann JR. (1970). Intracellular hydrogen transport system in acute leukemia. Br J Haematol. 19: 739-748.

[28] Sactor B. and Dick AR. (1960). Alpha-glycerophosphate and lactate dehydrogenase of hematopoietic cells from leukemic mice. Cancer Res. 20:1408-1412.

[29] Field M, Block JB, Levin R and Rall DP. (1966). Significance of blood lactate dehydrogenase elevations among patients with acute leukemia and other neoplastic proliferative disorders. Am J Med. 40:528-547.

[30] Bierman HR, Hill BR, Reinhardt L and Emory E. (1957). Correlation of serum lactate dehydrogenase activity with the clinical status of patients with cancer, lymphomas and leukemias. Cancer Res. 12:660-667.

[31] Fang SR, Yao EG, Wei SZ, Fan H and Dong ZR. (1989). Leukemic cell creatine kinase and its isoenzymes. Chin Med J (Engl). 102: 420-424.

[32] Li CY, Yam LT and Lam KW. (1970). Acid phosphatase isoenzyme in human leukocytes in normal and pathologic conditions. J Histochem Cytochem. 18: 473-481.

[33] Catovsky D, Cherchi M, Greaves MF, Janossy , Pain C and Kay HEM. (1978). Acid phosphatase reaction in acute lymphoblastic leukemia. Lancet. 1:749-751.

[34] Zucker S, Lysik RM, Zarrabi HM and Moll UM. (1993). M(r) 92000 typeIV collagenase is increased in plasma of patients with colon cancer and breast cancer. Cancer Research. 53:140-146.

[35] Hayasaka A, Suzuki N, Fujimoto N, Iwama S, Fukuyama E, Kanada Y and Saisho H. (1996). Elevated plasma levels of matrix metalloproteinase-9 (92-kd type IV collagenase/gelatinase B) in hepatocellular carcinoma. Hepatology. 24:1058-1062.

[36] Ringshausen I, Dechow T, Schneller F, Weick K, Oelsner M, Peschel C and Decker T. (2004). Constitutive activation of the MAPkinase p^{3}8 is critical for MMP-9 production and survival of B-CLL cells on bone marrow stomal cells. Leukemia. 18:1964-1970.

[37] Abd-Elhalim AZ, Bakr SIS, Osman MM, Abd-AlAziz MM and Hamed H. (1999). Plasma matrix metalloproteinase-9 as a prognostic marker in acute leukemia. Hematol. 24:1-19

[38] Chambers AF and Matrisian LM. (1997). Changing views of the role of matrix metallproteinases in metastasis. J Ntal Cancer Inst. 89: 1260-1270.

[39] Jong GK, Sang KS, Dong HK, Jin HB and Kyu BL. (2005). Clinical implications of angiogenic factors in patients with acute or chronic leukemia: Hepatocyte growth factor levels have prognostic impact, especially with acute myeloid leukemia. 46:885-891.

[40] Park KJ, Hwang SC, Sheen SS, Oh YJ, Han JH and Lee KB. (2005). Expression of matrix metalloproteinase-9 in pleural effusion of tuberculosis and lung cancer. Respiration. 72: 166-175.

[41] Ito M, Amizuka N, Tanaka S, Funatsu-Ozawa Y, Kenmotsu S, Oda K, Nakajima T and Ozawa H. (2003). Ultrastructural and cytobiological studies on possible interactions between PTHrP-secreating tumor cells, stromal cells, and bone cells. J Bone Miner Metab. 21: 353- 362.