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Estimation of plasma growth differentiation factor 15 level in *de novo* acute myeloid leukemia patients

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Abstract:

BACKGROUND: Acute myeloid leukemia (AML) is a diversified disorder, characterized by clonal proliferation of myeloid precursors in peripheral blood (PB) and bone marrow (BM). Growth differentiation factor 15 (GDF15) is a member of transforming growth factor-β superfamily that has an important role in cancer prognosis and pathophysiology and it can induce apoptosis and inhibit growth and invasion of tumor.

OBJECTIVES: The aim of this study was to estimate the GDF15 plasma levels in patients with *de novo* AML and their association with patients' survival.

MATERIALS AND METHODS: A cross-section samples from 60 adult patients who were newly diagnosed with *de novo* AML from September 2022 to September 2023 were included. Other 30 healthy adult individuals were involved as controls. The measurement of plasma GDF15 level was established by the ELISA technique using the human GDF15 ELISA kit.

RESULTS: Plasma (GDF15) was higher in AML patients, and it was associated with inferior overall survival (OS). Plasma (GDF15) level shows positive correlation with age, hemoglobin level, and insignificant correlation with the BM and PB blast percentages, total white blood cell count, sex, and platelets.

CONCLUSIONS: Plasma GDF15 levels in AML patients were high at the diagnosis and were associated with inferior OS.

Keywords:

Acute myeloid leukemia, enzyme-linked immunosorbent assay, growth differentiation factor 15

Introduction

Acute myeloid leukemia (AML) results from the expansion of a malignant pluripotent or multipotent clone of myeloid series, with defective multiplication and maturation. Substitutions of the normal marrow cells by AML cells subsequently lead to pancytopenia which is a common feature.[1]

Growth differentiation factor 15 (GDF15) is a pleiotropic cytokine belongs to bone morphogenetic protein subfamily of the transforming growth factor-β, GDF15 is expressed in the low concentrations in most organs. However, it can be upregulated in response to organ injury, such as in the liver, kidney, heart, and lung, elevated levels of GDF15 have been associated with different diseases, including heart diseases and cancer so it has shown to be a strong prognostic protein in these conditions.[2-4] GDF15 gene in human is located in chromosome 19.[5] GDF15 may exist in different structures within the cell: Pro-GDF15 monomer (~40 kDa), pro-GDF15 dimer (~80 kDa), and mature

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dimer (~30 kDa).[6] GDF15 has important role in the regulation of some processes in cells such as regulating inflammatory pathways, apoptosis, angiogenesis, cell repair, and cell growth.[7,8] Serum GDF15 concentrations increase during pregnancy because of high expression in the placenta.[9] Role of GDF15 in cancer: Tumor growth and metastasis, angiogenesis, and immune modulations (GDF15 may contribute to immune evasion by suppressing anti-tumor immune responses and promoting an immunosuppressive environment).[10-12]

GDF15 in hematology can regulate the proliferation and differentiation of hematopoietic stem cells.[13]

GDF15 in hematological diseases is elevated in myelodysplastic syndromes, dyserythropoietic disorders (such as β-thalassemia, congenital dyserythropoietic anemia type I, and refractory anemia with ring sideroblasts),[14-17] and some myeloproliferative neoplasm.[18] GDF15 has an important role in the growth and survival of leukemic cells in AML. It is produced by cancer-associated fibroblasts (CAFs) in marrow microenvironment and contributes to the chemo-resistance of leukemic cells,[19] also, GDF15 secreted by leukemic cells is involved in the morphological remodeling of marrow adipocytes This remodeling process promotes leukemic cell growth and survival.[13] The aim of this study: Estimation GDF 15 plasma levels of in newly diagnosed patients with *de nova* AML and their association with patients' survival and correlate the level of GDF 15 with other clinical and hematological parameters.

Materials and Methods

A cross-sectional samples from 60 adult patients who were newly diagnosed with *de novo* AML from September 2022 to September 2023 were included. Other 30 healthy adult individuals were involved as controls. The measurement of plasma GDF15 level established by the ELISA technique was measured using the human GDF15 ELISA kit.

The patients were diagnosed according to morphology, immunophenotype and genetic tests on bone marrow (BM) aspirate, and/or peripheral blood (PB) samples in the Medical City/National Center of Teaching Laboratories in Baghdad.

Sixty adult patients with AML were selected randomly regarding sex, they were sequentially selected (according to inclusion and exclusion criteria), were classified according to the FAB classification (FAB classification according to flowcytometry [M0-M1-M2-M4-M5-M7]).

All AML patients had "3+7" protocol of (Daunorubicin 30–90 mg/m^2/day continuous intravenous [IV] infusion for 3 days plus cytosine arabinoside 100–200 mg/m^2 continuous IV infusion for 7 days).

The AML patients were followed up for 6 months after the diagnosis to assess the disease outcome (whether the patient is dead or still alive).

This study was approved by the review ethics committee of the Iraqi Council for Medical Specialization.

Inclusion criteria

1. Adults
2. All AML patients were newly diagnosed
3. All were not receiving any chemotherapy before the time of collecting blood.

Exclusion criteria

1. Patients with a diagnosis of secondary or relapsed AML
2. Other types of hematological malignancies
3. Solid cancers
4. Active inflammatory disease, for example, rheumatoid arthritis
5. Pregnancy
6. Patients with neurodegenerative disease.

Control group was 30 seem healthy individuals, there sex and age were matched to that of patients, complete blood count (CBC), and blood film were done for control group and they were normal.

Data of AML patients were collected from all the participants, including their age, current place of residence, drug intake, personal, and family history of any chronic inflammatory diseases, neurodegenerative diseases, and drug intake. CBC and blood smear were done, other data including (BM study, Immunophenotyping / Flowcytometry (IPT), genetics, Lymphadenopathy (LAP), and ultrasound (U/S) for hepatosplenomagally) were taken from patients' files.

Sampling

Written informed consent was obtained from each patient and control before sample collection, a venous blood sample done for blood smear and CBC, within 30 min of collection the remaining anticoagulated blood was centrifuged to obtain plasma, at – 80°C the plasma was stored in the Medical City at the National Center of Teaching Laboratories and then measurement of plasma GDF15 level was done by using Human GDF15 ELISA kit (china).[20]

Results

The mean age of patients was 47.48 ± 19.62 years, there was male predominance in newly diagnosed AML

patient was 33/60 (55%). Age and sexology control group were matched to that of patients. The common signs and symptoms of AML patients included in this study: Pallor, fever then extramedullary disease including splenomegaly (37%), hepatomegaly (43%), and lymphadenopathy (32%).

The mean hemoglobin (Hb) concentration was 8.13 ± 2.08 g/dl (range: 4.3–16.4 g/dl) and the mean total leukocyte count was 44.2 ± 57.28 × 10^9/L (1–220 × 10^9/L). The mean absolute neutrophilic count (3.61 ± 5.73) range was 0.0433. The mean absolute lymphocytic count (4.43 ± 6.18) range was 0.2–44.4. Mean platelets count was 53.49 ± 34.02 × 10^9/L (range: 15.00–186.00 × 10^9/L). The mean blast percentage in PB (46.08% ± 26.161%), (range: 5–96) and BM were (69.62 ± 22.959), (range: 20–98). The patients' number who was alive after the follow-up for 6 months was 30/60 (50%), while 30 patients were dead (50%).

There was a statistically significant difference in the median of GDF15 level between patients and control level with a $P < 0.001$. GDF15 showed a significant positive correlation with patient age ($r = 0.534$ and $P = 0.001$) and significant positive correlation with patient Hb ($r = 0.253$ and $P = 0.05$). While no correlations were found with patients' gender ($P = 0.953$) and other hematological parameters [Table 1].

Table 1: Correlations of growth/differentiation factor-15 at presentation with hematological parameters in acute myeloid leukemia patient's group

Parameters	GDF-15	
	Patients	Control
Age (years)		
r	0.534	0.567
P	<0.001	0.001
Hb (g/dL)		
r	0.253	0.136
P	0.05	0.474
WBC (×10^3/μL)		
r	−0.005	−0.272
P	0.969	0.146
Platelets (×10^3/μL)		
r	0.183	−0.093
P	0.161	0.626
RDW CV		
r	−0.006	−0.074
P	0.961	0.698
ANC (×10^3/μL)		
r	0.121	−0.283
P	0.356	0.130
ALC (×10^3/μL)		
r	0.024	0.066
P	0.857	0.730
Blast% in PB		
r	−0.124	-
P	0.345	-
Blast% in BM		
r	0.199	-
P	0.534	-

Hb=Hemoglobin, WBC=White blood cell, RDW=Red cell distribution width, ANC=Absolute neutrophilic count, ALC=Absolute lymphocyte count, PB=Peripheral blood, BM=Bone marrow, GDF-15=Growth/differentiation factor-15

Distribution of plasma growth differentiation factor 15 levels at diagnosis in acute myeloid leukemia patients group according to the FAB classification

Distribution of AML patients group according to FAB classification (M0–M7) at the time of diagnosis in relation to plasma GDF15 levels the median plasma GDF15 level was higher in (M7) (870 pg/ml) and lowest in M0 (443 pg/ml) [Table 2].

Table 2: Distribution of plasma growth/differentiation factor-15 levels at the diagnosis in acute myeloid leukemia patients group according to French-American-British classification

Flow cytometry	*n*	GDF-15 (pg/mL)	
		Median (range)	Mean±SD
M0	3	443 (391–715)	516.33±174
M1	9	573 (426–690)	558.22±99.53
M2	19	599 (328–895)	579.37±139.84
M4	10	634 (393–972)	686.6±221.51
M5	17	690 (386–1552)	737.18±280.43
M7	2	870 (812–928)	870±82.02

SD=Standard deviation, GDF-15=Growth/differentiation factor-15

Relation of plasma growth differentiation factor 15 levels and patient prognosis

The AML patients were followed up for 6 months after the diagnosis to assess the disease outcome and there are statistically significant positive correlations that were found between plasma GDF level and death in AML patient (inferior overall survival [OS]). Mean in live and dead patients was 543.77 ± 131.37 and 746.87 ± 230.55, respectively. Median (range) (505 [328–895]) and (702.5 [386–1552]) and $P < 0.001$.

Discussion

The mean age of patients in this study was comparable with studies reported from Iraq done by Tawfiq *et al*.,[21] Muhsin and Al-Mudallal[22] and Mohammad *et al*.[23] In this study, among the sixty patients, the most common subtype of AML was the M2 subtype (31%) that is comparable with study done in Iraq by Muhsin and Al-Mudallal[22] and Pouls *et al*.[24] Presentation, the mean white blood cells (WBCs) count in our study was comparable to Hussein[25] and Pouls *et al*.[24] and more than what was described in other studies in Iraq and in Egypt.[13,21] However, the mean Hb in our study was close

to that reported in other studies in Iraq[21,24] and Egypt[13] which results from the leukemic cells accumulation in the BM suppressing the normal hematopoietic cells production.[26]

Thrombocytopenia is a significant manifestation of acute leukemia, which results from the leukemic cells accumulation in the BM suppressing the normal hematopoietic cells production including platelets.[26] The mean platelet count was comparable to another study in Iraq.[21,24]

In this study, by the ELISA, we evaluated plasma GDF15 in AML patients. A significantly higher plasma GDF15 level in patients than controls ($P < 0.001$), is in agreement with two studies.[13,27] After 6 months of follow-up, the patients for the assessment of OS and the median GDF15 plasma level were significantly higher in AML patients who died within 6 months (702.5 pg/ml) while in those who still alive was (505 pg/ml) and ($P < 0.001$). These findings were in agreement with the result of study on AML patients[28] and study reported on myeloma patients.[29] The possible reason is that GDF15 plays a crucial role in the growth and survival of leukemic cells in AML. It is produced by CAFs in the BM microenvironment and contributes to the chemo-protection of leukemic cells. In addition, GDF15 secreted by leukemic cells is involved in the morphological remodeling of marrow adipocytes, promoting leukemic cell growth.[13,19] In this study, the GDF15 level and patient age were significantly correlated $P < 0.001$ and $r = 0.534$, for its role in the different processes involved with aging.[28,30] In this study, the GDF15 plasma level and Hb levels had significant positive correlation $P = 0.05$ and $r = 0.253$. which agree with that study.[28] It had been revealed that slight inhibition effect of the recombinant human GDF15 on hematopoiesis.[31]

Plasma GDF15 level not correlated with patient WBC count, PB and BM blast percent and sex; similar result was reported in another study.[28] In this study, there was a description of the differences in median plasma levels of GDF15 and FAB classification according to flowcytometry (M0-M1-M2-M4-M5-M7) and the highest level was in M7; this could be explained by the excretion of GDF15 by megakaryocytes in the BM[27] The lowest level was in (M0).[19,28]

Conclusions

Plasma GDF15 is higher in AML patients than control. High-plasma GDF15 levels at the time of diagnosis were associated with inferior OS in AML patients. Plasma GDF15 level in patients is correlated with patient's age and hemoglobin and plasma GDF15 level is not correlated with sex, percentage of blasts in the PB and BM, WBCs count, and platelet at the time of diagnosis.

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Conflicts of interest

There are no conflicts of interest.

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