Evaluation of Hepatocyte Growth Factor in Iraqi Patients with Acute Myeloid Leukemia: Its Correlation with Clinical Parameters and Response to Induction Therapy

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Abstract

BACKGROUND: Hepatocyte growth factor (HGF) is a proangiogenic factor that exerts different effects over stem cell survival growth, apoptosis, and adhesion. Its impact on leukemogenesis has been established by many studies.

AIM: This study aimed to determine the effect of plasma HGF activity on acute myeloid leukemia (AML) patients at presentation and after remission.

PATIENTS AND METHODS: This was a cross-sectional prospective study of 30 newly-diagnosed, adult, and AML patients. All patients received the 7+3 treatment protocol. Patients' clinical data were taken at presentation, and patients were followed up for 6 months to evaluate the clinical status. Plasma HGF levels were estimated by ELISA based methods in the patients at two intervals first at diagnosis and the 2nd time after remission of disease. Plasma HGF levels were only measured once in twenty healthy control individuals.

RESULTS: A statistically significant lowering in the median levels of HGF after remission induction therapy has been found when compared with those before treatment (p = 0.006) and in the group of patients who achieved complete remission in comparison with those before treatment (p = 0.005). Furthermore, a significant reduction was also demonstrated after treatment in patients presenting with extramedullary involvement (p = 0.003) and in the alive group (p = 0.001).

CONCLUSION: Plasma HGF estimation can be a useful parameter in predicting treatment outcomes in patients with AML, and it can add potentially useful information to risk stratification parameters.

Introduction

Leukemia is an aggressive malignant neoplasm of hemopoietic tissue characterized by progressive uncontrollable proliferation and accumulation of blasts in the bone marrow and peripheral blood [1]. In Iraq, leukemia ranks number three in the top ten types of cancer, accounting for about 6.48% of total cancers with an incidence rate of 4.3/100,000 [2].

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults and becomes increasingly common with age. In children, AML accounts for only 10–15% of childhood leukemia, with slightly more male preponderance [3], [4]. Most cases arise *de novo* and only 10–20% of cases are therapy-related. The etiology of AML is multifactorial with acquired diseases, environmental factors, and inherited conditions predisposing to its development [5].

Despite advances in understanding the pathogenesis and the impact of molecular defects in the progression of AML, there is variability in remission rates ranging from 50% to 90% in adults. This variation reflects the heterogeneity of the disease, the underlying molecular abnormality, and the possibility of other factors that could influence disease progression and treatment response [6], [7].

There is growing evidence that suggests the impact of angiogenesis on leukemia development and treatment response [8]. Many studies have revealed that evaluation of the microvessel density of the bone marrow might have potential prognostic implications in patients with AML [9], [10].

Hepatocyte growth factor (HGF) is a polypeptide proangiogenic factor synthesized by mesenchymal cells, which acts to regulate diverse biological processes that are involved in hemopoietic stem cell survival such as cell growth, apoptosis, and cell adhesion [11]. The cellular responses to HGF are mediated through Met, a cell surface receptor encoded by the c-met proto-oncogene. The effect of HGF in the regulation of angiogenesis maybe by both autocrine and paracrine mechanisms, as both the HGF and its receptor are expressed by vascular tissue [12], [13].

Most studies that address the clinical significance of HGF in AML include all AML subtypes, with different modalities of the treatment regimen. In this study, our aim was to measure HGF levels in adult,

newly diagnosed, AML patients that received the "7+3" treatment regimen. In our opinion, this would lead to more consistent results and accurate evaluation.

Patients and Methods

This was a cross-sectional prospective study that included 30 adult newly diagnosed *de novo* AML patients (14 males and 16 females) who were admitted to the hematology ward of the Medical City Complex. The diagnosis of AML was established by cytomorphology, cytochemistry, and immunophenotyping. Patients' clinical data were all taken at presentation. Patients with secondary AML or acute promyelocytic leukemia were excluded from the study because of the different disease characteristics. Patients who received different treatment options other than 3+7 treatment protocol (3 days of anthracycline "doxorubicin" 30 mg/m^2 or daunorubicine 45 mg/m^2 and 7 days of cytarabine arabinoside 100 mg/m^2 continuous IV) were also excluded from the study. After 21–35 days from the start of chemotherapy, all patients were considered in complete remission (CR) status when: The bone marrow blast cells <5%, there was absence of Auer rods with recovery of white blood cells (WBC) and platelet counts, and absence of evidence of extramedullary leukemia [14]. Patients were followed up for 6 months to evaluate their clinical outcomes.

The study also included 20 healthy age- and sex-matched medical staff and family members as a control group. They were 11 males and nine females, all of them showed negative reaction to C reactive protein test and normal complete blood counts.

The study was approved by the research ethics committee in College of Medicine, University of Baghdad and was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all participants.

Blood samples were withdrawn from AML patients twice (at diagnosis and post-induction) and once in control individuals. Venous blood, 2.5 ml, was collected from each patient and control in K3-EDTA tubes and used for complete blood counts and blood film smear preparation. Plasma was separated by centrifugation at 1000 g for 15 min, poured into small aliquots and kept in deep freezer below −40°C until plasma HGF assay was done by sandwich enzyme-linked immunosorbent assay following the manufacturer's instruction [15].

Statistical analysis

Statistical analyses were done using SPSS version 20, data are represented as mean ± standard deviation (SD), median, and interquartile range for continuous data. Non-parametric Mann–Whitney U-test and Wilcoxon Rank tests were used to compare continuous data. p < 0.05 was considered significant.

Results

Clinical parameters

The mean age for patients' group was 34.67 ± 12.65 years and the mean age for the control group was 35.15 ± 11.3 years. AML was observed in 16 (53%) females and 14 (47%) males with a male:female ratio of 1:1.14. According to subtypes of French-American-British classification, of the 30 AML cases studied, three were in M1 subtype, 15 were in M2, three were in M4, five were in M5a, and four were in M5b (Figure 1).
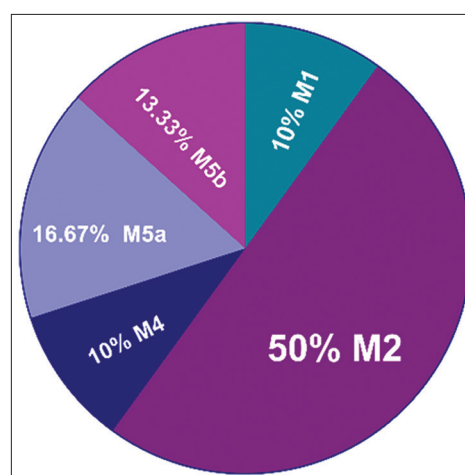

Figure 1: The distribution of patients group among French-American-British classification

Fever was the most common presentation of patients included in the study (63%), followed by pallor (60%), extramedullary disease (hepatosplenomegaly and lymphadenopathy) and bleeding in 50% and 32%, respectively (Figure 2).
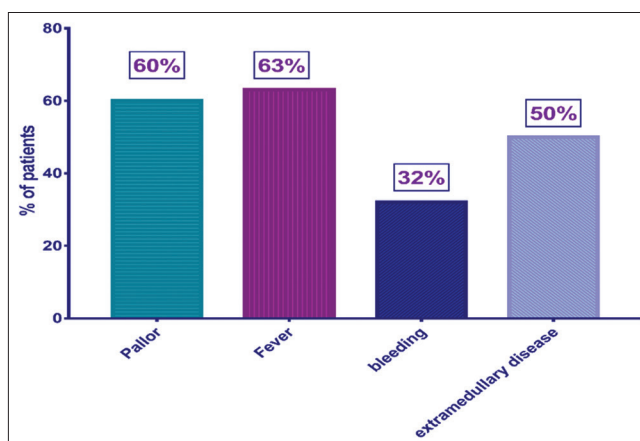

Figure 2: Common signs and symptoms of the patient's group

Various hematological parameters concerning peripheral blood and bone marrow are demonstrated in Table 1.

Table 1: The median (IQR) of hematological laboratory parameters of the study groups

Parameters	AML (n=30)	Control (n=20)	p value*
Hb g/dL	7.85 (2.41)	14.05 (1.9)	0.000
WBC (×10⁹/L)	22.3 (78.73)	6.4 (1.83)	0.09
ANC (×10⁹/L)	1.78 (4.38)	4.7 (1.77)	0.002
Platelet count (×10⁹/L)	45.5 (75.8)	284 (124)	0.000
PB blasts %	47 (38.0)	-	
BM blasts %	64 (33.5)	-	

*Mann–Whitney U-test. Hb: Hemoglobin, WBC: White blood cells, ANC: Absolute neutrophil count, PB: Peripheral blood, BM: Bone marrow, IQR: Interquartile range, AML: Acute myeloid leukemia.

HGF level in AML patients before and after remission induction therapy and in the control group

The mean (±SD) levels of HGF were 3098 ± 3214 pg/mL, 1664 ± 1810 pg/mL, and 702 ± 188 pg/mL for AML patients before remission induction therapy, after therapy, and in the control group, respectively.

There was a statistically significant increase in the median HGF level of patients when compared with control (p < 0.001). Furthermore, there was significant reduction in the median HGF level of patients after treatment with p = 0.006 (Table 2).

Comparison of HGF levels before and after remission induction therapy in AML Patients' groups according to their response to treatment

AML patients who achieved CR had a significant reduction in median levels of HGF after

Table 2: Comparison of HGF in AML patients group before and after treatment

Parameter	AML at presentation	AML After treatment	p value*
HGF (pg/mL)	1592.5 (2409.7)	1094.5 (863.3)	0.006
Median (IQR)	680.7–13838.9	512.2–8664.5	
Range		Control	
		683.3 (305.9)	<0.001
		(471.3–1094.5)	

*Mann–Whitney U-test. IQR: Interquartile range, AML: Acute myeloid leukemia, HGF: Hepatocyte growth factor.

treatment. This was statistically significant (p = 0.003), whereas patients with no response had insignificant lowering in median HGF levels after treatment, with p = 0.334 (Table 3).

Comparison of HGF levels before and after remission induction therapy in AML patients according to extra-medullary involvement (lymphadenopathy and hepatosplenomegaly)

In AML patients without extramedullary involvement, there was a statistically insignificant decrease in the median level of HGF after treatment, whereas the median level of HGF in patients with

Table 3: Comparison between HGF levels before and after remission induction therapy in AML patients groups according to their response to treatment and to extramedullary involvement

Variables	HGF (pg/mL) Median (IQR) Range		p value*
	Before	After	
Response to treatment			
No response (n=19)	1472.5 (985.8)	1319.2 (1026.7)	0.334
	680.7–8470.4	512.2–8664.5	
Yes, complete remission (n=11)	3173.4 (4699.3)	885.1 (505.7)	0.003
	1012.8–13838.9	573.5–1625.7	
Extramedullary involvement			
Absent (n=15)	1600.2 (2068.7)	1140.5 (878.6)	0.191
	680.7–13838.9	512.2–8664.5	
Present (n=15)	1584.9 (4260.0)	1048.5 (975.6)	0.005
	956.6–10406.3	573.5–3730.2	

*Wilcoxon Signed-Ranks test. IQR: Interquartile range. AML: Acute myeloid leukemia, HGF: Hepatocyte growth factor.

extramedullary involvement showed a significant statistical reduction after treatment, p = 0.005 (Table 3).

Comparison of HGF levels in AML patients according to disease outcome

Six months after remission induction therapy, the total number of AML patients died was 16. The mean (±SD) level of HGF after treatment in patients who remained alive and those deceased was 930 ± 342 and 2307 ± 2300 pg/mL, respectively.

Moreover, the median level of HGF after treatment in deceased patients was remarkably higher than those who remained alive. This observation shows statistically significant difference (p = 0.003), whereas the median HGF level before treatment between alive and deceased patients was statistically insignificant with p = 0.377. A significant decrease in the HGF level was demonstrated in the group of patients who remained alive (p = 0.001) unlike the deceased patients in whom the difference was insignificant with a p = 0.501 (Table 4).

Table 4: Comparison of HGF level in AML patients groups according to disease outcome

Parameter	Alive (n=14) Median (IQR) Range	Deceased (n=16) Median (IQR) Range	p value*
HGF (pg/ml)			
Before	2783 (5196)	1575 (756)	0.377
	681–13839	798–7740	
After	910.6 (533.8)	1480 (1218)	0.003
	512–1779	619–8665	
p value**	0.001	0.501	

*Mann–Whitney U-test, **Wilcoxon Signed-Ranks test. IQR: Interquartile range, AML: Acute myeloid leukemia, HGF: Hepatocyte growth factor.

Discussion

The impact of angiogenesis over the progression of hematological malignancies has been extensively studied with growing evidence over its implication in the progression of multiple myeloma, acute lymphoid leukemia, and AML [16], [17], [18].

HGF is one of the proangiogenic multifunctional cytokines that act through regulating cell adhesion, cell growth, and survival of hemopoietic cells through MET pathway [11].

High levels of HGF in AML patients at diagnosis which declines following induction remission, in particular those who achieved CR support the hypothesis that blasts are potential sources of HGF in patients with AML, besides other sources such as stromal cells and endothelial cells. The eradication of blasts from bone marrow and peripheral blood was associated with a decrease in HGF levels toward normalization and these findings were in agreement with the previous studies [19], [20]. The significant reduction in HGF levels in patients with extramedullary involvement following induction therapy disagree with other studies where a significant differences were demonstrated in both patients with and without extramedullary involvement [19], [21]. No such a difference was found in our study, possibly due to different sample characteristic. For example, in the study of Ahmed _et al_. [19], patients with all were included in the study, whereas our study only included _de novo_ AML patients, and excluded patients with acute promyelocytic leukemia. The significant reduction in HGF levels we found in patients with extramedullary infiltration could be due to the higher tumor mass in those patients, and the subsequent decrease in levels after therapy may be an indicator of regression in tumor mass.

After 6 months of follow-up, a significant decline in HGF was seen in patients who remained alive. This is consistent with the study of Kim _et al_. [22], who found that serum HGF was the only parameter that was strongly predictive for CR, and that AML patients with low HGF tend to have better leukemia free survival. Similarly, Verstovsek _et al_. [23] demonstrated an association between higher levels of HGF and inferior survival in AML patients, and suggested that the enhancement of angiogenesis triggered by this factor and by other proangiogenic factors maybe an explanation for poor response to treatment and inferior outcomes. On the other hand, Hjorth-Hansen _et al_. [20] used serum samples unlike the other studies that mainly relied on plasma samples, and did not show a significant association between survival and HGF levels. Plasma samples have proven to be more valid and precise when compared to serum samples, as the clotting of blood can induce the release of angiogenic factors from platelets [24].

High HGF levels at presentation may not be significant, as marked reduction of HGF levels after remission induction did not reflect the actual response to treatment. This is because the median level of HGF in alive patients at presentation was much greater than that of the deceased group. Furthermore, there was a significant reduction in HGF levels in the living group and a limited decrease in levels in the deceased group.

The presence of a significant positive correlation between WBC count and serum HGF was also seen in other studies [19], [21], [22] and this may reflect the association between tumour mass and HGF levels.

Conclusion

Plasma HGF estimation can be a useful parameter in predicting treatment outcomes in patients with AML and it can add potentially useful information to the risk stratification parameters.

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