Evaluation of the Level of Tumor Necrosis Factor -α in Acute Myeloid Leukemia Patients in Mosul

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ABSTRACT

BACKGROUND: Acute myeloid leukemia (AML) is an abnormal clonal proliferation of early hematopoietic precursor cells leading to maturation arrest and accumulation of immature myeloid cells in the bone marrow, blood, and other tissues. Bone marrow microenvironment plays a crucial role in supporting hematopoiesis, illness progression, and response to chemotherapy. Tumor necrosis factor -α is one of these microenvironments with a pleiotropic action.

OBJECTIVE: Evaluate the level of tumor necrosis factor-α in patients with AML and study its relation to other clinical prognostic factors and laboratory parameters.

METHODS: This study is a prospective case series study conducted on 40 recently diagnosed AML patients from May 2023 to October 2023. The patients were collected from the hematology units of Ibn Sina and Ibn Al-Atheer Teaching Hospitals in Mosul City, Iraq. Verbal consent was obtained for each patient, and a questionnaire was administered; blood samples and laboratory investigations were also conducted.

RESULTS: Out of 40 patients, 24 males and 16 females were between 5 and 82 years old. The serum TNF-α level of patients at presentation was much higher than that in the control group (p-value 0.000), with a significant positive relation with the marrow blast percent (p-value 0.002). The relation between TNF- α and age, neutrophils, and LDH (Lactate Dehydrogenase) level are negative and non-significant; the relation between TNF- α mean and the disease outcome is significant, with a higher level in the dead group, near the normal control level in patients went to complete remission and in between in relapse group.

CONCLUSION: A high level of TNF- α is a bad prognostic factor as it is associated with high blast count and high mortality rate secondary to high disease-related complications.

Keywords: AML, Leukemia, TNF- α, DIC, TLS.

تقييم مستوى عامل نخر الورم ألفا في مرضى ابيضاض الدم النخاعي الحاد في الموصل

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الخلاصة

خلفية البحث: ابيضاض الدم النخاعي الحاد (AML) هو تكاثر نسيلي غير طبيعي للخلايا السليفة المكونة للدم في وقت مبكر مما يؤدي إلى توقف النضج وتراكم الخلايا النخاعية غير الناضجة في نخاع العظم والدم والأنسجة الأخرى. تلعب البيئة الدقيقة لنخاع العظم دورًا حاسمًا في دعم تكون الدم وتطور المرض والاستجابة للعلاج الكيميائي. عامل نخر الورم -الفا هو أحد هذه البيئات الدقيقة ذات التأثير متعدد المظاهر.

الاهداف: تقييم مستوى عامل نخر الورم (ألفا) في مرضى ابيضاض الدم النخاعي الحاد و دراسة العلاقة مع عوامل التكهن السريرية والمختبرية.

طُرائق البحث: هذه الدراسة عبارة عن سلسلة حالات تشمل ٤٠ مريضًا تم تشخيصهم حديثًا بسرطان الدم النخاعي الحاد (AML) في الفترة من ايار ٢٠٢٣ إلى تشرين الاول ٢٠٢٣. تم جمع المرضى من قسم أمراض الدم في مستشفى ابن سينا وابن الأثير التعليمي في مدينة الموصل،العراق. تم أخذ الموافقة اللفظية لكل مريض وإجراء استبيان، كما تم إجراء عينات الدم والفحوصات المخبرية.

النتائج: من بين ٤٠ مريضًا، ٢٤ ذكرًا و١٦ أنثى، تتراوح أعمارهم بين ٥-٨٢ عامًا. كان مستوى TNF-α في الدم لدى المرضى عند العرض أعلى بكثير من ذلك الموجود في المجموعة الضابطة (قيمة p 0.000)، وهي علاقة إيجابية مهمة مع نسبة خلايا البلاست في نخاع العظم (قيمة p 0.002). العلاقة بين TNF-α والعمر والخلايا العدلة ومستوى LDH (لاكتات ديهيدروجينيز) سلبية وغير مهمة، والعلاقة بين متوسط TNF-α ونتيجة المرض مهمة، مع ارتفاع المستوى في المجموعة الميتة، وقريب من مستوى التحكم الطبيعي في المرضى الذين وصلوا إلى مرحلة الشفاء التام وبين مجموعة الانتكاس.
الاستنتاج: يعد المستوى المرتفع لعامل نخر الورم (ألفا) من عوامل التكهن السيئة لأنه يرتبط بارتفاع عدد خلايا البلاست وارتفاع معدل الوفيات الناتج عن المضاعفات العالية المرتبطة بالمرض.

الكلمات المفتاحية: ابيضاض الدم النخاعي الحاد، ابيضاض الدم، عامل نخر الورم-الفا، تخثر منتشر داخل الاوعية الدموية، متلازمة انحلال الورم.

INTRODUCTION

Acute myeloid leukemia (AML) is a form of cancer caused by infiltration of the bone marrow, blood, and other tissues by abnormal clonal proliferation of poorly differentiated hematopoietic system; it is characterized by an increase in the number of progenitor myeloid cells in the marrow and an arrest in their maturation, frequently resulting in hematopoietic insufficiency, including (granulocytopenia, thrombocytopenia, and/ or anemia) [1], with cure rate about 40% of adult patients who are 60 years of age or younger and about 15% of patients who are older than 60 years of age [2]. AML forms about 1% of all cancers, with an incidence of 4.1 per 100000 per year, 1.9% of all cancer deaths [3]. In the United States, the annual incidence of AML is approximately 4.3 per 100,000 [4] and is slightly more common in men compared to females, with a male-to-female ratio of 1.3:1 [1,5]. The incidence increases with age, and it accounts for about 90% of all acute leukemia in adults but forms a very rare fraction in children [6]. The median age at diagnosis is 63 years. The rate of therapy-related AML (that is, AML caused by previous chemotherapy) is rising; it accounts for about 10–20% of all cases of AML. The incidence of AML also altered according to the geographical location; for instance, in adults, the highest rate is seen in North America, Europe, Oceania, and Africa, while the incidence of adult AML is less in Asia and Latin America. In contrast, childhood AML is less common in North America and India. These may be due to differences in population genetics and/or environmental factors [7].

Tumor necrosis factor-α is a soluble bone marrow microenvironmental factor belonging to the pro-inflammatory cytokines factors. It can be produced by monocytes, macrophages, activated NK (Natural Killer) cells, neutrophils, CD4+ T helper 1 (Th1) cells, CD8+ T cells, and even AML blasts [8]. The effect of TNF in AML is providing a tumor-supportive environment and playing an important role in the establishment and progression of malignant cells; it is produced ectopically by malignant cells, and immune cells of malignant microenvironment create a suitable environment to :

i. Activating NF-KB and c-Jun N-terminal kinase/activator protein -1 establishes and promotes malignant cell proliferation and apoptosis inhibition.
ii. Heme oxygenase-1 upregulation promotes malignant cell survival and resistance to treatment.
iii. Increased TNFR2 on the regulatory T cell in the microenvironment suppresses immune response by preventing cytotoxic T cell activation. [9].

This research aims to evaluate the level of tumor necrosis factor-α and study the relation with other clinical prognostic factors and laboratory parameters.

PATIENT MATERIALS AND METHODS

A total of 40 AML patients were sequentially selected (according to inclusion and exclusion criteria as mentioned below). The sample size is calculated as follows. [10].

$$N = \frac{Z^2 * (p*q)}{e^2}$$

Where:
n=required sample size
z=1.96 at alpha 5% level of significance
P=prevalence in Mosul city[11]. = 0.08; q (compliment of prevalence) =0.72
e=allowable error, 0.0741
So the sample size calculated = 40

Ethical Consideration

The approval was obtained from the Ministry of Health / Nineveh Health Directorate. In addition, the agreement was obtained from all patients before the interview.

Inclusion Criteria

A.Newly diagnosed patients with AML of all age group
B.Patients were not receiving any chemotherapy before the time of blood collection.

Exclusion Criteria

A.Patient with AML already receiving chemotherapy.
B.Patients with a diagnosis of secondary or relapsed AML.

Control Group

Twenty-six healthy individuals were included in this study as a control group for TNF-α level. These subjects matched the sex age distribution of the patient sample, and CBC, blood film, and C-RP tests were done for all controls, and their results were negative.

This prospective case series study was conducted on 40 recently diagnosed AML patients from May 2023 to October 2023. The patients were collected from the hematology units of Ibn Sina and Ibn Al-Atheer Teaching Hospitals in Mosul City, Iraq. For each patient, verbal consent was obtained, a questionnaire was administered, and blood samples and laboratory investigations were conducted. The diagnosis was based on the morphology of the peripheral blood and/or bone marrow aspirate samples in teaching laboratories of Ibn-Sina and Ibn Al-Atheer teaching hospitals in Mosul City, and flow cytometry was done by FACS-CantoII (4-color, Becton Dickinson, USA) in the nursing home hospital of the Medical City, Baghdad, Iraq, and flow cytometry unit in Ibn Al-Atheer hospital in Mosul City, Iraq. Cytogenetic studies were performed for selected patients.

Estimation of Serum TNF- α

Serum TNF- α assay done by a sandwich enzyme immunoassay performed using a 40-microliter serum sample, using the immunoassay analyzer and the human tumor necrosis factor-alpha ELISA kit by YLbiont made in China, No: YLA1337HU, the test done according to instructions of kit by semiautomated device chromate.

Test Principle

This kit used enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the human (TNF- α). (TNF- α) The wells were added to pre-coated wells with (TNF- α) monoclonal antibody and then incubated. After that, anti-TNF-α antibodies labeled with biotin were added to unite with streptavidin-HRP, which forms an immune complex.

Unbound enzymes were removed after incubation and washing. Then, substrates A and B were added to the solution, changing the blue color into yellow with the effect of acid. The shades of solution and human tumor necrosis factor-alpha (TNF- α) concentration are positively correlated.

TNF has no global reference, and each lab should have its own reference value.

Biochemical Tests

All investigations are performed using the standard procedure recommended by the manufacturer of each kit.

Biochemical tests assay by Cobas c111. The normal levels according to the kits.

LDH by fully automated Abbot C4000 bioch. Instrument. Normal range (125-220 U/L) [12]

Statistical Analysis

All statistical analyses were performed using SPSS software. Chi-square was used to compare categorical variables. Spearman correlation was done to show the relation between S.TNF- α and different hematological parameters. P-values < 0.05 were taken as statistically significant. Microsoft Excel 2016 and SPSS (Statistical Package for the Social Sciences) version 23 were used as software to perform the statistics.

RESULTS

Of the 40 patients in this study, 24 were male, and 16 were female, with a male-to-female ratio of 3:2.

The ages ranged from 5 to 82, with a median of 45 years, as presented in Table (1). The distribution of the control sample according to age is presented in Table (2).

Table(1): Distribution of the study sample according to age.

Range	No. of patients	Percent %
Less 10 years	3	7.5
10-19 years	3	7.5
20-29 years	5	12.5
30-39 years	6	15
40-49 years	6	15
> 50 years	17	42.5

Table (2): Distribution of the control sample according to age

Range	No. of patients	Percent %
Less 10 years	3	11.5
10-19 years	4	15.4
20-29 years	6	23.1
30-39 years	6	23.1
40-49 years	5	19.2
> 50 years	2	7.7

Hematological Parameters

The mean, mode, minimum, and maximum of Hemoglobin (Hb), WBC, neutrophil, platelets, and ESR (Erythrocyte Sedimentation Rate) [13] are shown in Table (3).

Table (3): Hematological parameter in patients with AML

Hematological parameter	Minimum	maximum	median	mean	mode	SD	Normal range
Hb(g/dl)	4.3	14	7.6500	8.1	6.80	2.129	13-17g/dl (men) 12-15g/dl (women) 10-14 g/dl (children)
WBC ($\times10^9$/l)	0.55	457.7	24	66.2	1.17	106.4	4-10$\times10^9$/L
Neutrophil ($\times10^9$/l)	0.05	55	1.45	7.1	.12	11.95	2 -7$\times10^9$/L
Platelets ($\times10^9$/l)	5	641	55	86.3	165	105.6	150-410$\times10^9$/L
ESR (mm/hr)	45	172	92.5	92.7	82	24.11	<14(men) <20 (women)

Tumor Necrosis Factor- α

Table 4 shows a significant variation between the mean level TNF- α in patients and control with a p-value of 0.00

Table (4): TNF- α level variation between patient and control.

variable	Mean	SD	median	minimum	maximum	p-value
Patients (TNF)	53.15	33.52	49	6	162	0.00
Control (TNF)	15.07	3.67	14	10	26	

Level of the Tumor Necrosis Factor- α in Different Age Groups and AML Morphological Subtypes

Evaluation of TNF-α in different ages shows no significant variation. Also, when evaluated according to AML subtypes(divided according to FAB on morphological bases and supported by immunophenotyping), there is no significant variation; the mean TNF- α and P- value for each group was illustrated in Table (5):

Table (5): variation of TNF- α level according to different age groups and AML subtype

Variable		TNF- α mean	P-value
Age/ years	<20	71.8	0.998
	20-50	52.7	
	>50	46.8	
AML subtypes	M0	64	0.237
	M1	53	
	M2	44.4	
	M3	44.6	
	M4	64.25	
	M5	44	

Complications and Outcomes

There is significant variation in the level of TNF- α in cases of clinical complication and disease outcome.

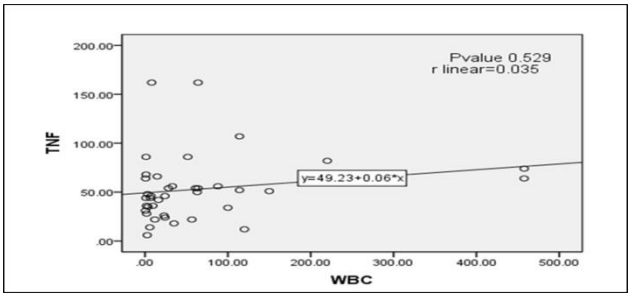
Tumor lysis syndrome occurs in 5 cases (12.5%), and DIC (Disseminated Intravascular Coagulation) occurs in 4 cases (10%), these represent the nine deaths (22.5%), remission occurs in 14 cases (35%), relapse in 17 cases (42.5%) as shown in table (6).

Table (6): Complication and outcome

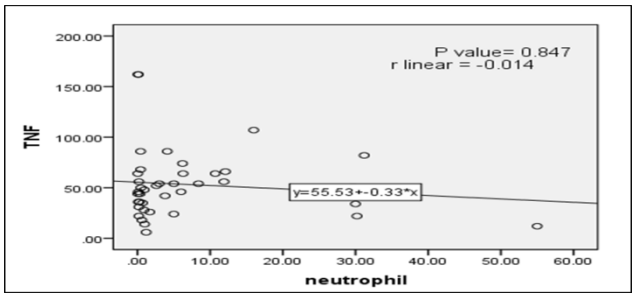
Variable		No	%	Mean of TNF- α	p-value
Tumor lysis	Yes	5	12.5	110.4	0.015
	No	35	87.5	44.97	
Total		40	100		
DIC	Yes	4	10.0	84.4	0.05
	No	36	90.0	49.7	
Total		40	100		
Outcome	Remission	14	35.0	24.5	0.00
	Relapse	17	42.5	52.4	
	Dead	9	22.5	99	
Total		40	100		

Relation between the Level of TNF-α with Hematological and Biochemical Parameters

The relation between levels of TNF- α and different hematological parameters and LDH is an important prognostic factor. The relation between white blood cell count and TNF- α was positive and non-significant, with a p-value of 0.529. At the same time, with neutrophils, it was negative non-significant with a p-value of 0.847; it was positive non-significant, with a p-value of 0.292 with platelet count. Regarding the relation with blast percent, it was positively significant with bone marrow blast with a p-value of 0.002, and the relation with LDH was negative non-significant, with a p-value of 0.875. all these relations are illustrated in the following figure (1):



a-



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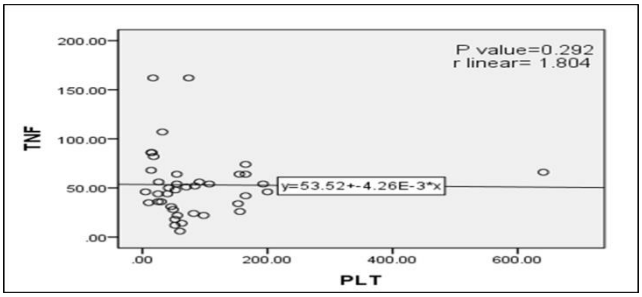
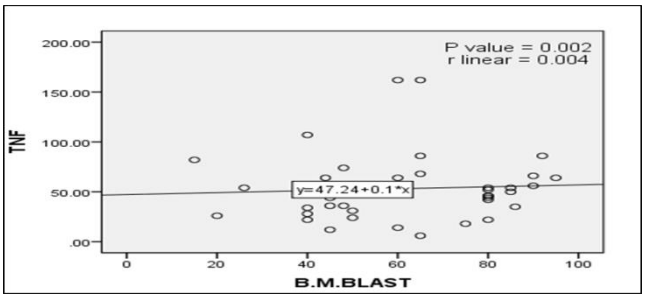
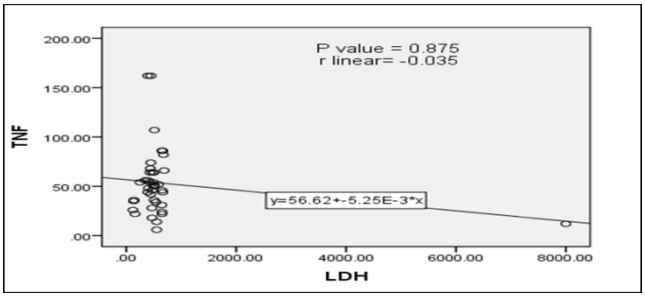


C-



d-



e-

Figure(1): Relation between TNF- α and laboratory parameters; a relationship with white blood cell count, b- relation with neutrophils, c- relation with platelet count, d- relation with B.M. blast, e- relation with LDH.

DISCUSSION

Early mortality in acute myeloid leukemia is significantly influenced by disease characterization and clinical conditions driven by genetic abnormalities, and advancements in classification and management are based on understanding these factors. [14]. In the last decades, research has focused on understanding the correlation between genetic changes and disease cure rates and updating anti-cancer therapies to target these altered genes. [15,16].

Still, there is a wide variation in the severity of the disease, high rate of relapse, and high death rate; these variations are modified by other factors [17]. The bone marrow microenvironment (BMM) is a crucial factor in hematopoietic differentiation [18]; the evaluation of factors like tumor necrosis factor- α (as part of BMM) is a broad field that may modify the clinical presentation and gives a chance for adding new therapeutic agents for managing critical diseases [18-20].

The age and sex distribution, leukemic subtypes, and laboratory parameters at the time of diagnosis of this study are within what is reported in Iraq and neighboring countries [21-25].

The mean level of hemoglobin (8.1 g/dl) in this study is similar to that of the group of studies [26-28], but it is a little bit higher than the mean of other groups of studies [29,30]. The mean leucocyte count (66.2x 10^9/L) in this study is higher than what was reported by others [27,31,32.] There is a variation between studies regarding platelet counts [26,27,30,31,] which may be due to the sample size of this study.

In the current study, 35% (14/40) of patients developed remission, and 42.5% (17/40) of patients developed relapse, which is in disagreement with other studies [33,34]. In comparison, death occurs in 22.5% (9/40) of them, which is higher than what was reported [34]. The higher mortality rate probably may be due to the higher risk group of the current studies (older age groups, leukocytosis). Disease-related complications in the current study, which are still considered as one of the prognostic clinical factors, are represented by DIC and tumor lysis

syndrome. DIC occurs in 10% (4/40) patients, 2/4 belong to promylocytic (M3), and tumor lysis syndrome in 12.5% (5/40) patients, which is consistent with others [35].

Regarding TNF-α, the exact mechanism of its effect on malignancy in general and acute myeloid leukemias is complex and under investigation; characterized by its pleotropic role, it can maintain survival and proliferation of precursor malignant cells and apoptosis of mature cells, the specific and critical level of TNF-α in AML has adverse effects according to many studies [9,19,36,37].

The Mean of TNF-α level in the current study in patients with AML was (53.1ng/L) more than in healthy volunteers (mean: 15.07 ng/L), with a p-value

of(0.000), which is in agreement with other studies in which the mean of TNF-α level was substantially greater in patients with AML than in healthy volunteers (P-0.003), [30,38-40], it means that this characteristic relation is not limited in our locality, the age group and disease subgroup has no significant effect on the level of TNF-α(table-5).

In the current study, statistically non-significant relations between the level of TNF α and WBC counts, neutrophil count, and platelet count (figure 1-a,b,c) this was similar to the other study [39] but were in disagreement with a study which showed a significant relation between the concentration of TNF-α and WBC counts [40] this may be due to difference in the sample size.

The other serious finding in the present study is the positive significant association between the level of TNF-α and bone marrow blast percentage (p-value less than 0.005), which is in agreement with others [30,41,42].

This may be because TNF-α expression levels were linked to a greater percentage of blasts in AML. Results of the relation between WBC, neutrophils, and blast TNF- α may support the idea reported by Waters JP TNF- α was ectopically secreted by blast cells, and this effect may overcome the immune apoptotic effects [43].

Regarding the relation with Lactate dehydrogenase, which is one of the bad prognostic factors in AML and statistically increased in AML [44], in this study, there is no significant relation between TNF- α and LDH; this may belong to the difference in LDH activity and FAB classification which reflect that each type of leukemic cells have its pathway in aerobic glycolysis according to their morphological features

The important finding in the current study is the association between high TNF-α levels and poor outcomes; all patients who died had high TNF- α levels with a P value (< 0.005), which is consistent with other studies [39,40.] Also, there was a significant relation between TNF- α level (mean 99 ng/l) and complication of the disease and poor outcome, the number of died patients in the current study is nine patients representing both groups of DIC 4 patients, and TLS (Tumor Lysis Syndrome) 5 patients, the most possible explanation is that TNF- α is one of the pro-inflammatory factors and the role of pro-inflammatory factors in the pathogenesis of DIC is well-studied and clarified since the last century [45-47].

Lower TNF-α level (mean 24.5 ng/l) was noticed in patients who went into remission; the level between the two groups (remission and dead),

which is still high (mean 52.4ng/l), represents the group of relapse. These findings are consistent with what was reported by Zhou et al. and Verma et al. [30]. The significant relation of TNF- α with marrow blast and disease outcome, while non-significant relation, whether positive or negative, with other parameters, means that the TNF- α is an independent prognostic in cases of AML.

CONCLUSION

- AML is associated with a higher TNF – α level than normal control.
- TNF – α is an independent clinical bad prognostic factor.
- The results have demonstrated that TNF- α serum concentrations could constitute a useful biomarker of AML disease activities and progression.
- A higher level of TNF – α level is associated with high blast count and high mortality rate, most probably secondary to disease-related complications.
- Serum TNF-α levels may potentially be of clinical significance in the future, given follow-up and the role of anti-TNF-α therapy in acute leukemia.

Acknowledgments

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Conflicts of Interest

The authors declare that there are no conflicts of interest to report regarding the present study.

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