

Assessment of Serum P53 Protein Level in Adult Patients with Acute Myeloid Leukemia in Correlation with Response to Treatment

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Abstract:

Background: Acute myeloid leukemia (AML) is an adult leukemia characterized by rapid proliferation of undifferentiated myeloid precursors, leading to bone marrow (BM) failure and impaired erythropoiesis. The p53 tumor suppressor protein regulates cell division and inhibits tumor development by preventing cell proliferation of altered or damaged DNA. It orchestrates various cellular reactions, including cell cycle arrest, DNA repair, and antioxidant properties.

Objectives: To investigate the relationship of P53 serum level with hematological findings, remission, and survival status in de novo AML patients.

Methods: This is a cross-sectional study that enrolled 63 newly diagnosed de novo AML patients, and 15 sex- and age-matched healthy persons as a control group. Serum P53 levels were assessed using the enzyme-linked immunosorbent assay (ELISA) technique before initiating induction chemotherapy. The study was performed between November 2022 and May 2023 at the Hematology and Bone Marrow Transplant Center of the Medical City Complex in Baghdad.

Results: There were significantly lower P53 serum levels in AML patients before starting chemotherapy compared to the control group. However, no substantial difference in P53 levels was identified between AML patients achieving complete remission and those exhibiting no response, nor between alive and deceased individuals. Furthermore, there was a positive yet statistically non-significant correlation between serum P53 levels and age, and no significant relationship between P53 levels and sex or various hematological parameters.

Conclusion: P53 levels are low in AML patients. They are not associated with remission status or survival after six months and are not correlated with hematological values.

Keywords: AML; ELISA; Remission; Survival status; 53.

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Introduction:

Acute myeloid leukemia (AML) is the predominant form of leukemia in adults. It is recognized by rapid proliferation of undifferentiated myeloid precursors (blasts) in both the bone marrow (BM) and peripheral blood (PB). This leads to BM failure and impaired erythropoiesis (1). The TP53 gene encodes the p53 tumor suppressor protein and is often called the "Guardian of the Genome." (2). The p53 protein functions as a tumor suppressor and transcription factor, governing cell division and inhibiting tumor development by preventing the proliferation of cells harboring altered or damaged deoxyribonucleic acid (DNA). It accomplishes this through orchestrating transcriptional control to induce apoptosis. In response to cellular stress or DNA damage, it elicits the activation of several transcriptional targets. The p53 protein orchestrates a diverse array of cellular reactions,

metabolic changes, antioxidant properties, anti-angiogenic effects, autophagy, senescence, and apoptosis (3). As P53 plays a crucial role in hematopoietic stem cell activities, its abnormalities significantly impact the development, characteristics, and responsiveness to treatment of AML, often indicating a poor prognosis. Understanding the precise pathways responsible for p53 malfunction will provide valuable insights into the development of targeted treatments for AML (4). This study aimed to investigate the serum level of P53 in de novo AML patients to demonstrate its prognostic value and its relation to laboratory findings at diagnosis.

Patients, Materials, and Methods:

This cross-sectional study was performed between November 2022 and May 2023 at the Hematology and Bone Marrow Transplant Center of the Medical City Complex in Baghdad on 63 patients who had just been diagnosed with de novo AML. They were selected using

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a non-probability sampling method (sequential selection), and 15 healthy individuals, who were matched in terms of age and sex, as a control group. These individuals were conveniently selected from healthcare workers, friends, and relatives of the cases. They had normal complete blood counts and normal C-reactive protein, and did not complain from any illness. Patients under 18, those with secondary AML, AML-M3, other malignancies, pregnant or lactating women, and those with comorbid diseases were excluded from the study. A comprehensive assessment of the patient's medical records was conducted, focusing on the diagnosis, therapy, and any other relevant clinical data. Complete patient history and clinical examination were performed. The results of the patient's complete blood count (CBC), blood films, bone marrow aspirate (BMA), and flow cytometric immunophenotypic analysis were collected from patients' data records. Patients were assessed for response to remission induction therapy after one month of starting chemotherapy by assessment of CBC, PB, and BMA blast percentage. Patients < 60 years of age, received the 3+7 protocol, which consists of daunorubicin from days 1–3 and Cytarabine from days 1–7. Patients ≥ 60 years of age were treated with decitabine and oral venetoclax. Patients were split into two categories: The complete remission (CR) category, which included those who achieved CR [i.e., BM blast count <5%, no circulating blast cells, no extramedullary disease, absolute neutrophil count (ANC) $\geq 1.0 \times 10^9/L$, platelet count (PLT) $\geq 100 \times 10^9/L$], or CR with incomplete hematological recovery (CRi) (meeting all CR criteria except neutropenia $< 1.0 \times 10^9/L$ or PLT $< 100 \times 10^9/L$), and the no response (NR) category (5). Patients were followed for up to six months to document their survival status and accordingly were divided into two groups based on whether they were alive or deceased. This study has obtained the approval of the Pathology Research Ethics Committee that of the College of Medicine, University of Baghdad (approval number: 144 on 4 October 2022). All participants involved in this study were informed, and their verbal consent was obtained before sample collection. Serum P53 level was determined by quantitative sandwich enzyme immunoassay ELISA technique using Human p53 tumor protein ELISA Kit, Catalog no. E1711Hu (BT LAB, China).

Statistical analysis

On version 26 of the Statistical Package for the Social Sciences (SPSS), a full explanation of each variable was made. The mean, standard deviation, and frequency (percent) were used to show the data, depending on the type of variable. The chi-square test was used to test the association between two variables. Both an independent sample t-test (for normally distributed data) and a Mann-Whitney U test (for non-normally distributed

data) were used to find the difference between the two means of continuous variables. The Pearson correlation was used for data that was normally distributed and the Spearman correlation for data that was not normally distributed. A confidence level of 95% with a P-value equal to or less than 0.05 was considered significant.

Results

The mean age of the 63 AML patients was 50.0 ± 19.01 (Mean \pm SD) years, ranging from 18 – 80 years. The male-to-female ratio was 1: 2.15 (20/43). The most prevalent clinical presentations were pallor (71%), and fever (68%) followed by bleeding (22%), splenomegaly (14%), hepatomegaly (11%), bone pain (9.5%), weight loss (8%), and lymphadenopathy (5%). There was a statistically significant lower mean P53 serum levels in AML patients when compared with the control group with a P-value of 0.001. The mean values for males and females were not statistically significant (Table 1).

Table 1: P53 levels in AML patients and controls and male and female patients

Characteristics	P53 (ng/L)(Mean \pm SD)	P value [†]
Control	1324.7 \pm 1304.64	0.001
AML patients	586.0 \pm 651.95	
Male (n=20)	450.7 \pm 77.71	0.478
Female (n=43)	648.9 \pm 782.25	

[†]Mann-Whitney U test

The response to treatment after 28 days of starting chemotherapy was CR in 20 patients (31.7%), and NR in 32 patients (50.8%). Eight (12.7%) patients died during treatment and three patients (4.8%) died before treatment. The follow-up after six months to document the survival status revealed that 36 patients (57.1%) were still alive and 27 (42.9%) died. No statistically significant association was found between CR and NR categories with patients' sex (P= 0.213), and no significant difference in mean age (P= 0.609), Table 2. At the time when the cases were diagnosed with AML there was a statistically significant difference between the means of BM blast percentage in CR patients (38.5 ± 25.43) and NR patients (56.8 ± 28.62) with a P-value of 0.031. No statistically significant difference was observed between remission status and white blood cell count (WBC), hemoglobin (Hb), PLT, ANC, and PB blasts. No statistically significant difference was found in the mean serum level of P53 in AML patients between those who achieved CR and those with NR (P = 0.430).

Table 2: Distribution of CR and NR surviving AML patients by sex and difference between their mean hematological values

Variable	Remission				P value	
	Complete remission		No response			
Sex	Male	9	45%	9	28.1%	0.213 [‡]
	Female	11	55%	23	71.9%	
Age (years)	47.6 ± 21.60		50.6 ± 19.31		0.609*	
WBC (×10 ⁹ /L)	38.0 ± 48.31		39.7 ± 54.72		0.605 [‡]	
Hb (g/dL)	8.5 ± 2.71		8.0 ± 1.93		0.371*	
Platelets (×10 ⁹ /L)	58.0 ± 41.29		91.6 ± 82.71		0.058*	
ANC (×10 ⁹ /L)	7.0 ± 9.12		4.0 ± 6.14		0.585 [‡]	
Peripheral blood blast (%)	39.8 ± 26.02		40.8 ± 30.40		0.992 [‡]	
Bone marrow blast (%)	38.5 ± 25.43		56.8 ± 28.62		0.031 [‡]	
P53 level (ng/L)	510.7 ± 176.95		688.7 ± 898.05		0.430 [‡]	

[‡] Chi-square test *Independent t-test [‡] Mann-Whitney U test
 Note: Eleven patients who passed away before or during treatment are not included because they did not receive or finish the induction chemotherapy.

After six months of follow-up, there was no statistically significant association between sex and or difference in mean age with survival status in AML patients (P-values = 0.815 and 0.200 respectively (Table 3). There were no statistically significant differences between the means of hematological parameters and P53 levels between alive and deceased patients (P-values > 0.05).

Table 3: The association of survival status with sex, and the differences in mean age, hematological parameters, and P53 levels between alive and deceased AML patients

Variables	Survival status after 6 months				P value	
	Alive		Deceased			
	N=36	%	N=27	%		
Sex	Male	11	30.6	9	33.3	0.815 [‡]
	Female	25	69.4	18	66.7	
Age (years)	47.4 ± 20.23		53.6 ± 16.96		0.200*	
WBC (×10 ⁹ /L)	33.3 ± 46.84		43.5 ± 54.82		0.917 [‡]	
Hb (g/dL)	8.2 ± 2.47		7.9 ± 1.63		0.573*	
PLT (×10 ⁹ /L)	68.3 ± 62.84		80.1 ± 72.23		0.739 [‡]	
ANC (×10 ⁹ /L)	5.2 ± 7.52		3.5 ± 6.22		0.151 [‡]	
PB blast (%)	38.9 ± 25.73		46.3 ± 34.88		0.453 [‡]	
BM blast (%)	49.1 ± 27.39		58.4 ± 31.26		0.199 [‡]	
P53 level (ng/L)	579.3 ± 555.43		594.8 ± 773.37		0.117 [‡]	

[‡] Chi-square test *Independent t-test [‡] Mann-Whitney U test

Table 4 shows positive but non-significant correlations between serum levels of P53 and age, Hb, PLT, and ANC in AML patients. There was a negative but non-significant correlation between serum levels of P53 and WBC, PB blast percentage, and BM blast percentage (P > 0.05).

Table 4: The correlations of P53 with age, and hematological parameters in 63 AML patients

Variable	P53 level	
	r	P
Age	0.116	0.366*
WBC	-0.007	0.959**
Hb	0.128	0.316*
PLT	0.146	0.255*
ANC	0.092	0.474**
PB blast %	-0.184	0.148*
BM blast %	-0.034	0.797*

*Pearson correlation

**Spearman correlation

Discussion

The mean age of AML patients was comparable to other Iraqi studies (6-8), and with British, Iranian, and Egyptian studies (9-11), respectively. The sex distribution of the patients in the current study revealed a female majority, which is consistent with another local study that reported a higher prevalence of AML in females (80%) compared to males (12). Other recent Iraqi studies have also revealed a modest female preponderance (13, 14), although AML is reported to be more frequent in males. The small sample size of the current study may have contributed to the differences in sex predominance. Regarding clinical characteristics at presentation, the current study found that the most common symptoms at the time of presentation were pallor, fever, and bleeding tendency, in addition to other complaints like bone pain and weight loss that were manifested to a lesser extent, which are in agreement with previous Iraqi studies (13, 15), and in studies from other countries (16, 17), which reported that pallor and fever were among the most common presenting symptoms in adult AML. The most frequent signs at presentation were splenomegaly, hepatomegaly, and lymphadenopathy, respectively, these results were consistent with an Iraqi (18), and an Indian (16) study. The CR in the current study is lower than that reported by Zayed, et al. in Egypt (19) (CR rate of 40%), Moulod, et al. in Iraq (20) (CR rate of 40%), Alwan, et al. in Iraq (CR rate of 69.5% in 115 patients diagnosed with de novo AML) (18), and Udupa, et al. in India (CR rate of 65.6%) (21). The discrepancies in the findings may be attributed to the different sample size, the risk stratification of these patients, and the efficiency of supportive care during the myeloablative period. In the present study, there was a significant decrease in the serum level of P53 in AML patients as compared to the control group, in agreement with an Egyptian study (22), which reported a significantly lower serum level of P53 in AML patients when compared with the control group, which was significantly increased after treatment when compared to its level before treatment. Unfortunately, we did not assess serum p53 after induction remission to compare the level before treatment in AML patients. In contrast to other Egyptian studies; El-Toukhy et al. in 2019 (11), and Abdel-Aziz in 2013 (23) reported a significant increase in the serum level of P53 at presentation by ELISA in AML patients. Suppression of the p53 level may occur as a result of other genes, such as P63, P73, among others, which may cause the inhibition, preventing an increase in P53 levels (24). There were no significant correlations between the serum level of P53 and the patients' age, or hematological parameters, and a non-significant association with sex. Similarly, El-Toukhy, et al. reported no statistically significant association between the age and sex of AML patients and P53 levels by ELISA. However, they found a strong relationship

between high P53 and Hb, BM blast percentage, and PB blast percentage (11).

Study limitation

The limitations of this study include a restricted sample size and a short duration of patients' follow-up.

Conclusions

P53 levels are low in AML patients. They are not associated with remission status or survival after six months or correlated with hematological values.

Authors' Declaration

We confirm that all the tables in the manuscript are ours. The project was approved by the Research Ethics Committee in the College of Medicine, University of Baghdad (issue number 144 dated 4 Oct 2022).

Conflict of interest: None

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Authors' contributions

Study conception & design: (Laith A. Jebur & Haithem A. Al-Rubaie). Literature search: (Laith A. Jebur). Data acquisition: (Laith A. Jebur). Data analysis & interpretation: (Laith A. Jebur). Manuscript preparation: (Laith A. Jebur & Haithem A. Al-Rubaie). Manuscript editing & review: (Haithem A. Al-Rubaie).

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تقييم مستوى بروتين (p53) في المصل لدى المرضى البالغين المصابين بابيضاض الدم النخاعي الحاد وارتباطه بالاستجابة للعلاج

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الخلاصة:

الخلفية: يبرز سرطان الدم النخاعي الحاد (AML) كشكل بارز من أشكال سرطان الدم لدى البالغين، والذي يتميز بالانتشار السريع لسلائف نخاع الشوك غير المتميزة، والذي يبلغ ذروته في فشل نخاع العظم (BM) وتكوين الكريات الحمر المعرضة للخطر. يعتبر البروتين الكابت للورم p53 هو البروتين الأساسي في علم الأمراض، والذي يلعب دورًا محوريًا في التحكم في انقسام الخلايا وإحباط تكوين الورم عن طريق وقف تكاثر الخلايا ذات الحمض النووي التالف أو المتحور. فهو ينسق مجموعة من الاستجابات الخلوية، بما في ذلك إيقاف دورة الخلية، وإصلاح الحمض النووي، ووظائف مضادات الأكسدة. فهو ينظم التفاعلات الخلوية المختلفة، بما في ذلك إيقاف دورة الخلية، وإصلاح الحمض النووي، وخصائص مضادة للأكسدة.

الهدف من الدراسة: استكشاف العلاقة بين مستويات مصل P53 والنتائج المختبرية لدى المرضى الذين تم تشخيص إصابتهم بسرطان الدم النخاعي الحاد (AML).

المنهجية: سجلت هذه الدراسة المقطعية 63 مريضًا تم تشخيصهم حديثًا بمرض سرطان الدم النخاعي الحاد (AML) إلى جانب 15 فردًا يتمتعون بصحة جيدة من حيث العمر والجنس والذين يعملون كمجموعة مراقبة. تم تقييم مستويات P53 في الدم باستخدام تقنية مقايضة المتميز المناعي المرتبط بالإنزيم (ELISA)، سواء في مرضى سرطان الدم النخاعي الحاد قبل بدء العلاج الكيميائي التعريفي أو في المجموعة الضابطة.

النتائج: لوحظ انخفاض ملحوظ في مستويات مصل P53 لدى المرضى مقارنة بالمجموعة الضابطة، مما يشير إلى وجود فرق كبير ومع ذلك، لم يتم تحديد أي تباين كبير في مستويات P53 بين مرضى سرطان الدم النخاعي الحاد الذين حققوا استجابة كاملة وأولئك الذين لم يظهروا أية استجابة، ولا بين الناجين والأفراد المتوفين. علاوة على ذلك، كان هناك ارتباط إيجابي ولكن غير مهم إحصائيًا بين مستويات P53 في الدم والعمر، في حين لم يتم اكتشاف علاقة معنوية بين مستويات P53 والجنس أو البارامترات الدموية المختلفة.

الاستنتاج: مستويات P53 بين مرضى سرطان الدم النخاعي الحاد منخفضة. وهي لا ترتبط بحالة سكون المرض أو البقاء على قيد الحياة بعد ستة أشهر، ولا ترتبط بقيم الدم.

الكلمات الدالة: ابيضاض الدم القوي الحاد، مقايضة الامتصاص المناعي المرتبط بالإنزيم، بروتين P53، حالة الشفاء، البقاء على قيد الحياة.