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Evaluation of human transforming growth factor -β and some biochemical markers in leukemia patients in Kirkuk city

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Abstract: *Acute myeloid leukemia is defined by the invasion of the bone marrow, blood, and other organs by rapidly growing, genetically identical, immature cells that come from the hematopoietic system.* ***The aim of the present study*** *was to assess the levels of human transforming growth factor-β and several biochemical markers in individuals diagnosed with leukemia, and to determine their significance in the context of these cases.* ***Materials and study****: The present research was carried out in the Kirkuk oncology center. The study was conducted from January to May 2023. The research groups were examined to identify the presence of TGF-β using ELISA technology. Moreover, a variety of biochemical tests were conducted.* ***Results****: The study had a total of 90 individuals, including sixty persons in the patient's group and thirty healthy persons as control group. The study included participants aged 18 to 70 years. The current study has revealed a statistically positive connection between the presence of TGF-β in patients diagnosed with leukemia. Moreover, a notable relation was seen between the serum creatinine levels in the patients and the control group. Furthermore, the present investigation demonstrates a statistically significant correlation between white blood cell (WBC) count, hemoglobin concentration, and platelet count (PLTs) in patients diagnosed with leukemia and individuals in a healthy control group.* ***Conclusion****: The current investigation determined that patients with leukemia had a significant increase in TGF-β levels.*

.**Keywords:** *Leukemia; TGF-β; AML.*

1.Introduction

Acute myeloid leukemia (AML) is a fast-growing malignancy characterized by the infiltration of immature leukemic cells into the bone marrow. Despite being the most prevalent form of acute leukemia (AML) (Brenner and Bruserud, 2019). The term leukemia may be turn back to its Greek origins, namely the words "leukos" & "heima," which denote an abundance of white blood cells (WBCs) within the human body. Leukemia, formerly regarded as a singular ailment, was initially identified around the 4th century (Blackburn *et al.*, 2019). The development of leukemia is attributed to a sequence of genetic alterations occurring in

hematopoietic stem and progenitor cells, resulting in their malignant conversion (Zinngrebe *et al.*, 2020). Leukemia is a prevalent malignancy that has a significant impact on the global population. In the year 2018, leukemia was identified as the fifteenth most prevalent form of cancer worldwide, with a total of 437,033 reported cases and 309,006 recorded deaths. Consequently, it stood as the eleventh leading cause of mortality attributed to malignant conditions (Tebbi, 2021). Leukemia is the fourth most common type of cancer in Iraq, affecting both males and females. According to study by (Juliusson *et al.*, 2016), between 1991 and 2009, the Iraqi Cancer Board documented 13,951 cases of leukemia in Iraq, accounting for 6.59% of all newly diagnosed cases. Also, study reported by (Chennamadhavuni *et al.,* 2023), in Karbala province in central Iraq, has leukemia as the 6th most prevalent form of cancer. Hematopoietic cells undergo a malignant transformation, resulting in the appearance of abnormal leukemic cells. This, in turn, impairs the production of normal blood cells. Only a small percentage of leukemia cases may be traced to known causal factors, such as prior chemotherapy treatment or particular chemical exposures.   However, most instances are mainly caused by genetic alterations, such as chromosomal abnormalities or single gene mutations, without clearly identified causative factors (Pelcovits *et al.*, 2020).   The significance of transforming growth factor-β (TGF-β) in the development of immune system cannot be overstated. Moreover, it plays a pivotal role in preserving immune tolerance and homeostasis, while also governing other facets of immune responses. TGF-β plays a crucial function in regulating the healing process after acute tissue damage. It exerts its impact on several cell types involved in this process (Zhang *et al.,* 2019).

2.Methodology

The current study was carried out at Kirkuk Oncology Center. The inquiry started in the period of (January to May) 2023.   The study utilized a sample size of 90 participants, which was separated into two distinct groups: a group of 60 persons who were patients, and a group of 30 individuals who served as the control group.   The research included individuals ranging in age from 18 to 70 years.   The researcher conducted a structured interview with the participants, employing a well-crafted and customized questionnaire specifically created for this study.   The research groups were examined to identify the presence of TGF-β using the ELISA technique. Furthermore, a range of biochemical tests were performed, including the measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as hematological examinations involving the assessment of hemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), and platelet count. All enzyme-linked immunosorbent test (ELISA) kits utilize the sandwich-ELISA technique, adhering to the instructions supplied by the manufacturer, namely (Sunlong/ China). The data were recorded utilizing a questionnaire that was particularly designed for this purpose. Subsequently, the data were collected and entered into a computer system. The data was evaluated using version 22 of the Statistical Package for Social Sciences (SPSS). The acquired results were subsequently compared across patients demonstrating different variables, employing a predetermined threshold of statistical significance established at P < 0.05. The results are presented in tabular and graphical formats, depicting rates, ratios, frequencies, and percentages. Subsequently, the obtained findings are subjected to statistical analysis by the use of t-tests and Chi-square tests.

3.RESULTS

According to the gender distribution data presented in Table 1, it is apparent that the present study included 41 participants (68.3%) who identified as male, while 19 participants (31.7%) identified as female. The present investigation revealed that the age group

exhibiting the highest frequency of patients was >51, accounting for 32% of the sample. Similarly, the control group exhibited the highest prevalence in the same age group, constituting 23% of the sample. Conversely, the age group with the lowest percentage of patients was <10, representing 8% of the sample. Likewise, the control group displayed the lowest percentage in the age group (41-50), amounting to 7% of the sample. These findings are presented in Table-2. The present study's results suggest that a significant proportion of individuals diagnosed with leukemia demonstrated the presence of many concurrent medical illnesses, in accordance with the idea of comorbidity. The study findings revealed that splenomegaly was seen in 15 individuals, accounting for 25% of the patient population. Additionally, fever was detected in 12 patients, representing 20% of the sample. Hematuria was reported in 10 patients, corresponding to 17% of the participants. Lastly, pallor was identified in 6 individuals, making up 10% of the study cohort, as evidenced by the data reported in Table 3. The current investigation revealed a significant correlation ($P= 0.0086$) in the concentrations of TGF-β in individuals with leukemia and those healthy group, as seen in Figure 1. The present study, as depicted in Figure 2, did not reveal any correlation ($P= 0.5467$) between the calcium concentration in individuals with leukemia and control group. Figure 3 illustrates a clear correlation between serum creatinine concentration in patients and the control group, indicating a favorable connection. In contrast, there was no correlation was seen in blood urea levels in study groups. The present study found a significant association between WBC count ($P= 0.0142$), hemoglobin level ($P= 0.0034$), and platelet count ($P= 0.0033$) in study groups, as shown in Figure 4 during the analysis of hematological parameters. The average ± variation value for WBC, Hb, and PLTs count were ($7.605 ± 7.61$, $10.99 ± 2.83$, and $199.7 ± 103.1$) respectively.

Table (1): Sex distribution in patients' group.

Gender distribution	Patients		Control	
	No.	%	No.	%
Males	41	68.3	15	50
Females	19	31.7	15	50
Total	60	100	30	100

Table (2) Distribution of patients according to age.

Age group	Patients		Control	
	No.	%	No.	%
<10	5	8	4	13
11-20	9	15	7	23
21-30	11	18	5	17
31-40	10	17	5	17
41-50	6	10	2	7
>51	19	32	7	23
Total	60	100	30	100

Table (3): Comorbidity factors in patients' and control group.

Comorbidity factors	Patients (60)		Control (30)	
	No.	%	No.	%
Splenomegaly	15	25	0	0
Fever	12	20	0	0
Pallor	6	10	0	0
Hematuria	10	17	0	0
Total	43	72	0	0
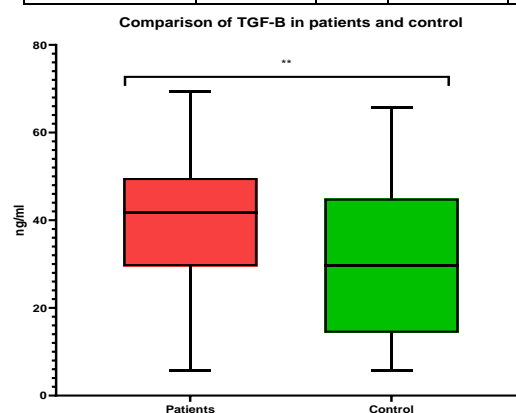
Figure (1) Comparisons of TGF- β in patients and control (P. value 0.0086).
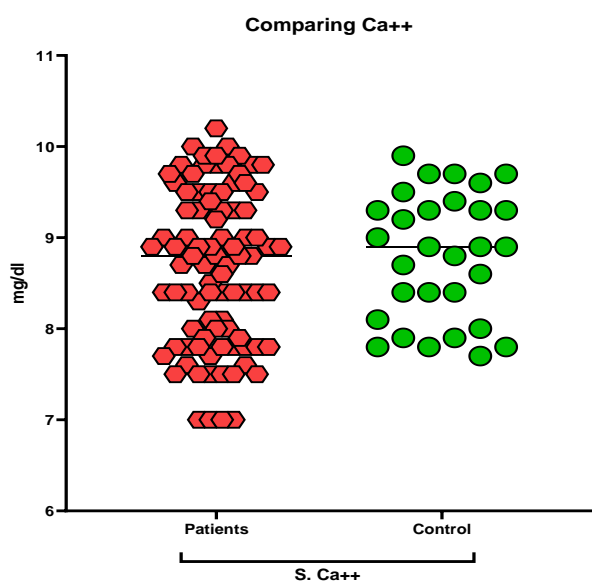
Comparing Ca++

mg/dl

Figure (2) Comparisons of calcium concentration in patients and control (*P.* value 0.5467).

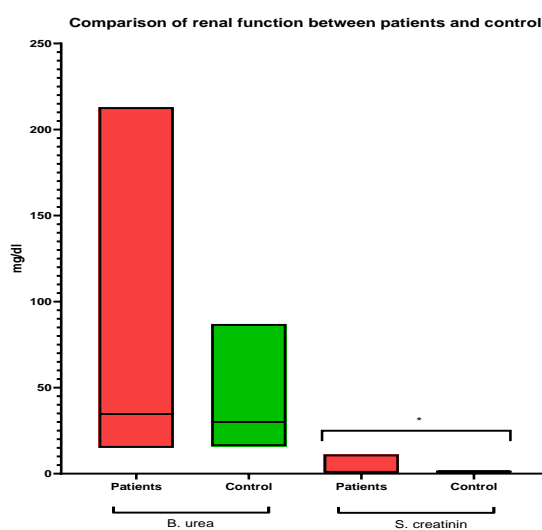**Comparison of renal function between patients and control**

mg/dl

Figure (3): Comparison of renal function between patients and control, B. urea with non-significant correlation (P > 0.05), While, S. creatinine with significant correlation (P < 0.05).
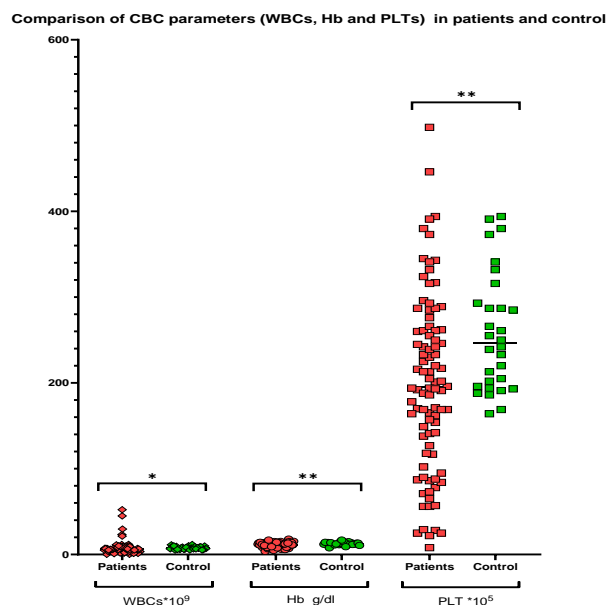
Comparison of CBC parameters (WBCs, Hb and PLTs) in patients and control

WBCs*10⁹   Hb g/dl   PLT *10⁵

Figure (4): Comparison of hematological parameter in patients and control group.

Discussion

Leukemia is a disease that exhibits a wide range of clinical signs and is known for its aggressive nature (Maktoof et al., 2020). The result of our study is consistent with Tawfiq *et al*., findings who revealed that 53% of study group were categorized as male, whereas 47% were categorized as female (Tawfiq *et al*., 2019). The survey found that there was a ratio of 1.1 males for every 1 female. Our study presents a different perspective on the findings of (Sleurs *et al.,* 2021) regarding the utilization of the abbreviated health survey, SF-12, within the context of juvenile acute lymphoblastic leukemia. Our study challenges the authors' claim there is a lack of substantial disparities in scores between females and males. According to study done by (Tawfiq *et al.,* 2019), the majority of the 98 patients diagnosed with acute myeloid leukemia (AML) were younger than 60 years old (81%), while the remaining patients (19%) were 60 years of age or over. Research conducted by Creutzig et al. (2018) indicates a greater prevalence of acute myeloblastic leukemia in individuals between the ages of (14 – 60 years), compared to older people and geriatric patients. This discovery is consistent with the results of a distinct study

conducted by (Mohammed *et al*., 2020). Also, study done by (Tawfiq et al., 2019) found that males had a little greater vulnerability to the previously mentioned illness, a finding consistent with both local and international research. According to a previous investigation conducted by (Döhner, et al., 2015), it was shown that the remission rates for AML were around the prevalence of this condition is around (35-40%) in adult patients aged ≤60 years, and (5-15%) in persons > 60 years. The preliminary inquiry encompassed a group of 58 individuals who were diagnosed with AML and underwent therapy at the Al-Nasiriyah Center/Thiqar, the duration of this investigation extended over a period of 6 years. Furthermore, according to comorbidity factor this finding presents a contradiction to a prior investigation carried out in Iraq, which suggested that the predominant symptom among leukemia patients was fever (90.9%), followed by pallor (70.5%) (Dhahir *et al*., 2012). According to the research done by (Mohammed *et al*. 2020), the results revealed that across the population of individuals diagnosed with acute leukemia, the most commonly reported complaint associated with comorbidity was pallor. Specifically, 11 patients, accounting for 19.3% of the sample, reported experiencing this symptom. Subsequently, a total of 10 patients (17.54%) reported experiencing epistaxis, while 7 instances (12.28%) experienced fever. Inflammatory disorders and tumor development are attributed to disruptions in the signaling of human TGF-β, which plays a crucial role in suppressing the immune response inside the microenvironment of tumor. Recent inquiries have shown its role in tumor evasion of immune system and the less-than-ideal results seen in cancer immunotherapy. Based on a latest study carried out by (Batlle *et al*., 2019), who demonstrated that the reactions of TGF-β are affected by specific transcriptional processes. The coordination of these programs occurs through the interaction of SMADs with distinct transcription factors specific to diverse cells and tissues. The genetic alterations in cancer cells result in the rewiring of transcriptional circuits, which in turn alters the functioning of TGF-β signaling. This metamorphosis undergoes a shift in its function, transitioning from inhibiting the formation of tumors to promoting other tumor processes that facilitate growth, invasion, and spread to other parts of the body. Low levels of TGF-β in mononuclear cell cultures have a stimulating effect, whereas large amounts of TGF-β have an inhibiting effect. The duration of TGF-β injection is also a crucial element to take into account (Wang, Yu, & He, 2019). Study by (Tai *et al.,* 2015) reported that homeostatic systems regulate blood calcium levels, and maintain normal range to calcium level, and bone integrity may be impaired. The current findings contradict prior research that indicated a low frequency of hypercalcemia and osteolytic bone lesions as consequences of adult acute B-ALL. Moreover, the coexistence of these diseases might indicate a more unfavorable outlook in patients with B-ALL. As per the findings of (Yao., 2023), that found the patient who had a low level of calcium (4.09 mmol/L), Indicating a lack of response to treatment and a negative outlook for recovery. The observed anomalies consist of reduced levels of calcium in both blood and urine, as well as decreased levels of serum phosphate and 25-hydroxy vitamin-D. Additionally, there are higher levels of alkaline phosphatase and parathyroid hormone (Crandall *et al.,* 2016). Leukemia is a multifaceted and highly aggressive disease that presents with a diverse array of clinical symptoms, as evidenced by the study conducted by (Sun *et al*., 2019). The findings documented in this research are consistent with the research done by (Abdul *et al*., 2016) about the concentrations of creatinine and urea in the patient's blood diagnosed with different forms of leukemia. These results also support the results published by (Mohammed, 2007), which demonstrated that certain patients exhibited elevated levels of

urea and creatinine after receiving chemotherapy treatment. Leukemia, a type of cancer that impacts the lymphatic system, bone marrow and blood cells occur due to abnormal cellular growth, leading to a reduced ability for RBCs and platelets to inhabit this area (Maktoof *et al*., 2020). The disorder can have several consequences for patients, including the occurrence of anemia, a reduction in platelet count, and reduced immunological function due to the abnormal properties of white blood cells (Maktoof *et al*., 2020). While, (Tawfiq *et al.* 2019) observed a mean count of WBCs at (17,850 × 106/L), that exceeded the numbers recorded in other study in Iraq (Al-dulaimi *et al.*, 2023). The present observations regarding Hb levels do not align with Tawfiq *et al.* data, who found that among 98 patients, the lowest Hb level was (4.1 g/dl), while the highest level was (15.8 g/dl).   A previous study done by Tawfiq et al. in 2019 reported a median PLTs count of (44,000 × 106/L).   Other results in Egypt by (Abuhelwa et al., 2017) found that the median PLTs count was measured at (62 × 106/L).

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