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OPEN ACCESS ORIGINAL ARTICLE

Evaluation of the Caspase-3, Calpain-2, GSH, and MDA in AML and ALL Patients Undergoing Chemotherapy

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ABSTRACT

Background: leukemia is one of the most common and dangerous types of cancer around the world. The exact mechanisms that lead to leukemia development are still unknown. However, this study evaluates the biochemical dynamics of caspase-3, calpain-2, malondialdehyde (MDA), and reduced glutathione (GSH) in patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) during three treatment stages: pre-chemotherapy, during chemotherapy, and post-chemotherapy.
Materials and methods: A total of 270 blood samples were analyzed, divided equally among AML, ALL, and healthy controls. Sample collection during the period continued from July 2022 to June 2024 from Medical City and Al-Kadhimain Medical City and Al-Amal National Hospital for Cancer Management in Baghdad province.
Results: We observed significant alterations in apoptotic markers (Caspase-3 and Calpain-2), oxidative stress (MDA), and antioxidant defenses (GSH). Both AML and ALL patients exhibited elevated Caspase-3 and Calpain-2 levels pre-treatment, which declined progressively during and post-treatment. MDA levels were elevated across all stages, peaking pre-treatment, while GSH levels were significantly reduced. Comparative analysis revealed distinct biochemical profiles between AML and ALL patients, with AML exhibiting higher apoptotic activity. Correlation analyses highlighted complex interplays between apoptosis, oxidative stress, and antioxidant responses.
Conclusion: These findings confirm the potential of Caspase-3, Calpain-2, MDA, and GSH as biomarkers for monitoring therapeutic efficacy and managing the leukemia treatment protocols.

Keywords: Leukemia, ALL, AML, Apoptosis, Caspases, Calpain 2, Chemotherapy

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INTRODUCTION

Cancer is a significant challenge to global health and social development, with a rapidly increasing incidence. In 2012 alone, the world saw 14.1 million new cancer cases and 8.2 million cancer-related deaths, underscoring the urgent need for more effective anti-cancer therapies. Among various malignancies, leukemia represents a group of cancers that affect blood-forming tissues, including the bone marrow and lymphatic system (1, 2).

The abnormal buildup of undifferentiated blasts that can proliferate unchecked in the bone marrow and disrupt the generation of healthy blood cells is a hallmark of hematopoietic stem cell (HSC) cancers. Acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphoblastic leukemia (CLL) are the four primary subgroups of leukemia. One of the most prevalent and deadly malignancies is leukemia, particularly acute leukemia (AL) (3).

People of different ages, sexes, and races have varying incidences of leukemia. These differences are mostly linked to exposure levels to genetic and environmental risk factors. For example, ionizing radiation is a known causative exposure for pediatric ALL, as demonstrated by the slight but noticeably increased risk brought on by X-ray pelvimetry during pregnancy, and approximately 10% of people who acquire CLL have a family history of the disease (4).

Acute Myeloid Leukemia (AML) is an uncommon but deadly kind of leukemia that accounts for 1.2% of all new cancer diagnoses in the US each year. It is a malignancy of the stem cell progenitors in the myeloid lineage. Despite its rarity, AML constitutes nearly one-third of all leukemia cases diagnosed. It results from genetic variants that fuel clonal growth and neoplastic alterations, leading to impaired blood cell production (5).

AML is a hematologic malignancy with a high rate of treatment failure due in part to high relapse of the disease following initial or subsequent therapy (6). Early diagnosis and rapid cytogenetic and molecular analysis are now crucial in tailoring therapies and enhancing survival rates (7).

Acute lymphocytic leukemia (ALL) is defined as a cancer of B or T lymphoblasts that is typified by the unchecked growth of aberrant, immature lymphocytes and their progenitors. This ultimately results in the replacement of bone marrow components and other lymphoid organs, giving rise to the typical disease pattern associated with ALL. The percentage of lymphoid neoplasms diagnosed in the US that are ALL is about 2%. Males are somewhat more likely than females to have acute lymphocytic leukemia, and White people are three times more probable than Black people to develop it (8). However, the major challenge associated with leukemia is treatment. However, all efforts that attempt to understand the mechanism of leukemia development and response to chemotherapy are valuable and may aid in improving efficiency and reducing the side effects of chemotherapy treatment protocols. Therefore, the current study is designed to monitor the changes that occur in caspases 3 and calpain 2 in patients with AML and ALL before, during, and after the treatment.

MATERIALS AND METHODS

Study Design and Participants

The current study included a total of 270 blood samples that were collected and analyzed. These samples were divided into three groups: AML patients (n=90), 30 samples collected before treatment, 30 during treatment, and 30 after treatment. ALL patients (n=90) comprise 30 samples collected before treatment, 30 during treatment, and 30 after treatment.in addition to 90 samples as a control group. The mean age for AML patients was 41.5±2.6 years, while for ALL patients, it was 28±2.4 years.

Ethical Approval

The study was approved by the ethics committee in the College of Medical and Health Techniques, University of Bilad Alrafidain (No. E-232/2022-5-24), and informed consent was obtained from all participants.

Sample Collection

Sample collection during the period continued from July 2022 to June 2024 from Medical City and Al-Kadhimain Medical City and Al-Amal National Hospital for Cancer Management in Baghdad province. The patients were selected in the current study based on treatment protocol. This means the patients who were selected during and after treatment were undergoing the same chemical treatment protocol (drugs set including Doxorubicin, Vincristine, Cyclophosphamide, Topotecan, and Paclitaxel). The serum was separated from whole blood via centrifugation at 3000 rpm for 15 minutes and

stored at −80°C until analysis. Part of blood storage in an EDTA tube for blood film tests to confirm the cases with either AML or ALL.

Inclusion and Exclusion

Only Iraqi patients who were newly diagnosed with AML or ALL and who had written informed consent were included in the current study. While any patients with other hematological disorders, such as other forms of leukemia or lymphoma, were excluded from the study to maintain specificity for ALL and AML, chronic diseases like autoimmune diseases, liver dysfunction, or chronic kidney disease, which could affect serum caspase and calpain-2 levels, previous treatment, and pregnancy were excluded from the study.

Biochemical Assays

Caspase-3 Levels: Quantified using an ELISA kit. The manufacturer's instructions were followed for doing the measurements, and the results were reported in ng/ml. The Human Caspase-3 ELISA Kit is specifically designed for the precise quantification of Caspase-3 in various biological fluids, including serum. Prior to the assay, reagents and samples are equilibrated to room temperature. Standards and samples are then added to the designated wells and incubated at 37°C. Following this, the detection antibody and HRP-streptavidin conjugate are sequentially applied, with multiple washing steps performed between additions to ensure specificity. The 3,3',5,5'-Tetramethylbenzidine (TMB) substrate is subsequently added to initiate the enzymatic reaction, which is terminated using a stop solution. Absorbance is measured at 450 nm, and the results are interpreted using optical density (OD) values. A standard curve is generated to accurately determine the concentration of Caspase-3 in the test samples.

Calpain-2 Levels: Determined using an ELISA kit, with measurements in ng/ml. The manufacturer's instructions and procedure summarized as: The assay is designed to detect calpain 2 concentrations within a range of 0.156 ng/mL to 10 ng/mL, with a sensitivity of <0.06 ng/mL, ensuring precise and reliable measurements. Prior to the procedure, reagents and samples are equilibrated to room temperature. Standards and samples are then added to the microplate wells and incubated at 37°C. Sequential additions of Detection Reagent A and Detection Reagent B are performed, with washing steps in between to ensure assay specificity. Following the addition of the 3,3',5,5'-Tetramethylbenzidine (TMB) substrate, the enzymatic reaction proceeds until it is halted by a stop solution. The absorbance is measured at 450 nm, and sample concentrations are determined by comparison to a standard curve.

Oxidative Stress Marker (MDA): Malondialdehyde levels were measured spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) method. Serum was separated from whole blood. After a series of solutions is added, measure the absorbance of the supernatant at 532 nm using a spectrophotometer or microplate reader. Results were expressed in mmol/ml.

Antioxidant Marker (GSH): Reduced glutathione (GSH) levels were quantified enzymatically, expressed in U/ml. Add serum to a reaction mixture containing phosphate buffer, nicotinamide adenine dinucleotide phosphate (NADPH), glutathione reductase, and Ellman's reagent. Incubate at room temperature, allowing DTNB to react with GSH to produce a yellow TNB product. Measure the absorbance at 412 nm and calculate GSH concentration using a standard curve.

Blood film staining

The samples were diagnosed as ALL and AML via blood film staining. The slides were read by a specialist hematology doctor. The diagnosis was based on the previous criteria as mentioned in (9, 10). According to the French-American-British (FAB) Cooperative Group classification system, acute lymphocytic leukemia (ALL) is an aggressive neoplasm that is identified by the presence of more than 30% lymphoblasts in the bone marrow or peripheral blood (11).

Statistical Analysis

Data were analyzed using SPSS software (version 8.0). Results are expressed as mean ± standard deviation. The significance of differences between groups was determined using one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

RESULTS

One hundred eighty samples were collected from study patients, divided into groups as follows: 90 samples from AML patients (30 before treatment, 30 during treatment, and 30 after treatment) and 90 samples for ALL patients (30 before treatment, 30 during treatment, and 30 after treatments). And 90

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cases as a control group. The mean age of patients in the current study for AML patients was 41.5 ± 2.6 years, and for ALL patients, it was 28 ± 2.4 years.
According to the current study's findings, the level of caspase-3 increased significantly (p value 0.01). in AML patients before treatment (9.278 ± 0.1062), during treatment (6.077 ± 0.1423), and after treatment (3.915 ± 0.2644), as well as in ALL before treatment (6.707 ± 0.1165), during treatment (5.210 ± 0.1468), and after treatment (2.824 ± 0.04932) compared to the apparently healthy control (2.176 ± 0.02546). Also, the results showed a significant increase (p-value 0.01) in caspase-3 levels in AML patients before treatment compared to patients during treatment and after treatment, as well as in ALL patients before treatment, during treatment, and after treatment. Furthermore, the current study's findings demonstrated that ALL patients had significantly higher (p value 0.01) caspase-3 levels. before treatment compared to AML patients and ALL patients during and after treatment. Also, the results showed a significant increase (p value 0.05) in caspase-3 in AML patients under treatment compared to AML patients after treatment and ALL patients during and after treatment, and also a significant increase (p value 0.01) in caspase-3 in ALL patients during treatment compared to AML patients and ALL patients after treatment. Finally, there was also an increase in significant (p value 0.05) caspase-3 in AML patients after treatment compared to ALL patients after treatment, as shown in Figure 1.

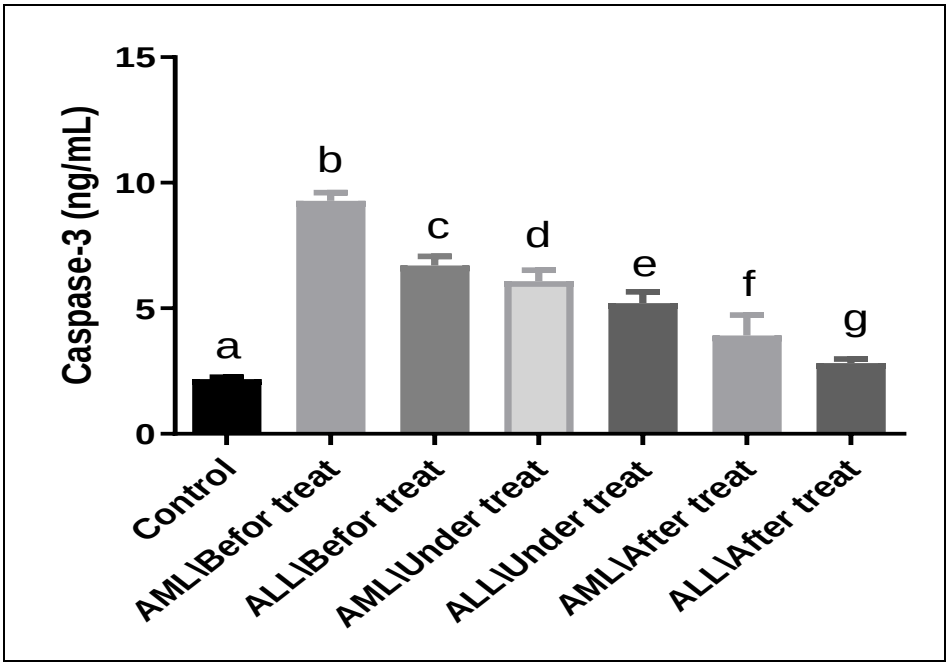

Fig 1: The comparison of Caspase-3 concentration in the serum between AML and ALL before treatment, under treat and after treat with healthy control, a, b, c, d, e, f, g: different letter mean ($p<0.05$)

From Figure No. 2, the results of the current study showed that there is a significant increase in the levels of Calpain-2 in all stages of the disease and treatment compared to the control group. To clarify this, the results showed a significant increase (p value 0.01) in the levels of Calpain-2 in AML patients before treatment (4.198 ± 0.1242), during treatment (3.549 ± 0.1415) and after treatment (3.074 ± 0.07634), as well as in ALL before treatment (4.064 ± 0.09482), during treatment (3.306 ± 0.05060) and after treatment (2.821 ± 0.01364) compared to the apparently health control (2.155 ± 0.02436). On the other hand, the results showed a significant increase (p-value 0.01) in calpain-2 levels in AML patients before treatment compared to patients during treatment and after treatment, as well as in ALL patients; during treatment and after treatment, there was no significant difference between ALL and AML patients before treatment. In addition to that, the results of the present study showed a significant

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increase (p-value 0.01) in calpain-2 levels in ALL patients before treatment compared to AML patients and ALL patients during and after treatment; there was no significant difference between AML and ALL patients during treatment and AML patients after treatment. Also, the results of the present study showed that there was no significant difference between AML and ALL patients after treatment, while there was a significant decrease (p-value 0.09) in ALL patients after treatment compared to all other study groups.

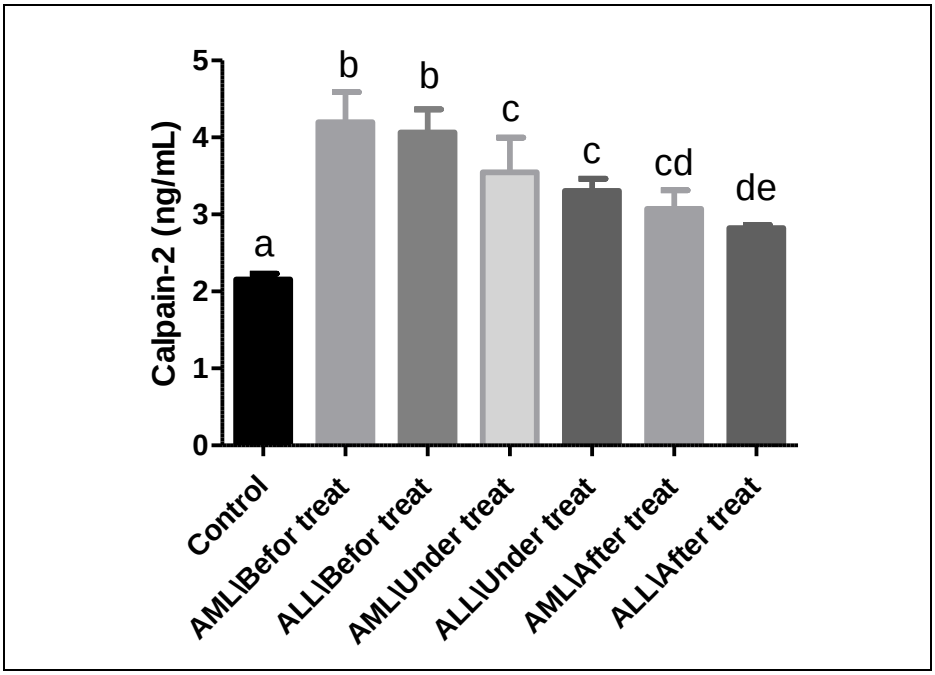

Fig 2: The comparison of Calpain-2 concentration in the serum between AML and ALL before treatment, under treat and after treat with healthy control, a, b, c, d, e: different letter mean ($p<0.05$)

As for the results of the current study regarding oxidative stress, the results showed that there was a significant increase (p value 0.01) in the levels of MDA in AML patients before treatment (192.7 ± 2.501), during treatment (148.6 ± 2.756) and after treatment (119.2 ± 0.8625), as well as in ALL before treatment (155.1 ± 1.680), during treatment (118.0 ± 3.464) and after treatment (106.5 ± 0.9379) compared to the apparently health control (91.88 ± 0.2415). On the other side, the results showed a significant increase (p value 0.01) in MDA levels in AML patients before treatment compared to all other study groups, while there was no significant difference between ALL before treatment and ALL during treatment and also between ALL during treatment, AML and ALL after treatment. Finally, there was a significant decrease (p value 0.08) in ALL patients after treatment compared to all others study groups, as shown in Figure 3. By measuring the levels of antioxidants in the serum, specifically GSH, the results showed that there was a significant decrease in the levels of GSH in all patients in the study group, which is: AML patients before treatment (8.466 ± 0.2558), during treatment (8.574 ± 0.4507) and after treatment (18.52 ± 0.4158), as well as in ALL before treatment (7.437 ± 0.3713), during treatment (17.01 ± 0.5980) and after treatment (16.20 ± 0.1134) compared to the apparently health control (21.20 ± 0.2542). On the other hand, there was no significant difference in GSH between AML patients before treatment and during treatment and ALL before treatment, and also, there was no significant difference in GSH between ALL during treatment and after treatment and AML after treatment, while there was a significant decrease (p value 0.06) in ALL patients after treatment compared to all others study groups except with AML after treatment and ALL during treatment, Figure 4. By studying the

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correlation between the markers of the study's disease groups, the results showed that there is a positive correlation between each of the following: Caspase-3 and Calpain-2, Caspase-3 and GSH, Calpain-2 and MDA, and also between MDA and GSH, while there was a negative correlation between Caspase-3 and MDA and Calpain-2 and GSH in AML patients before treatment, as shown in Table 1.

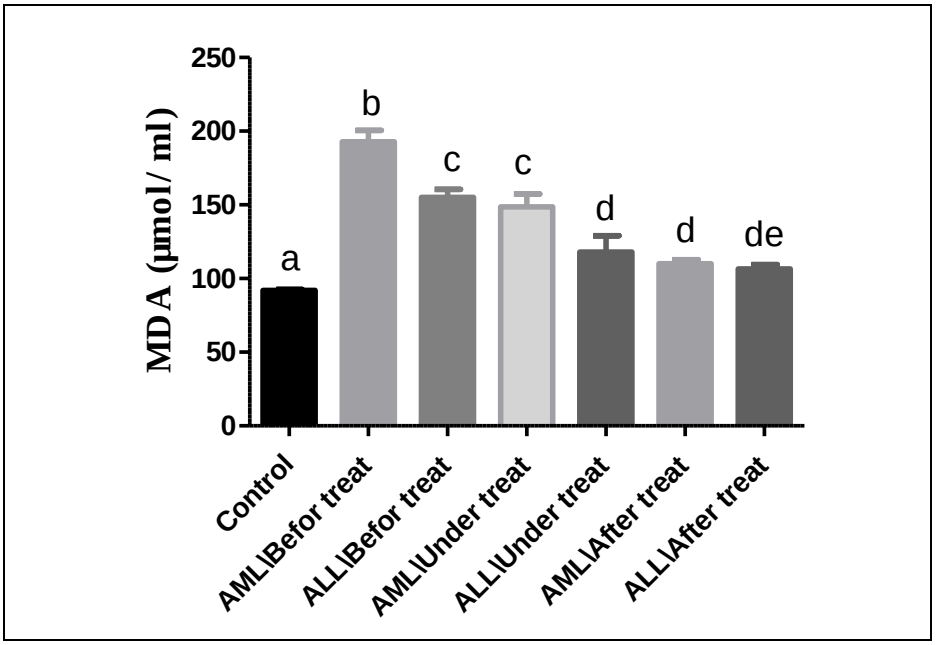

Fig 3: The comparison of MDA concentration in the serum between AML and ALL before treatment, under treat and after treat with healthy control, a, b, c, d, e: different letter mean ($p<0.05$)

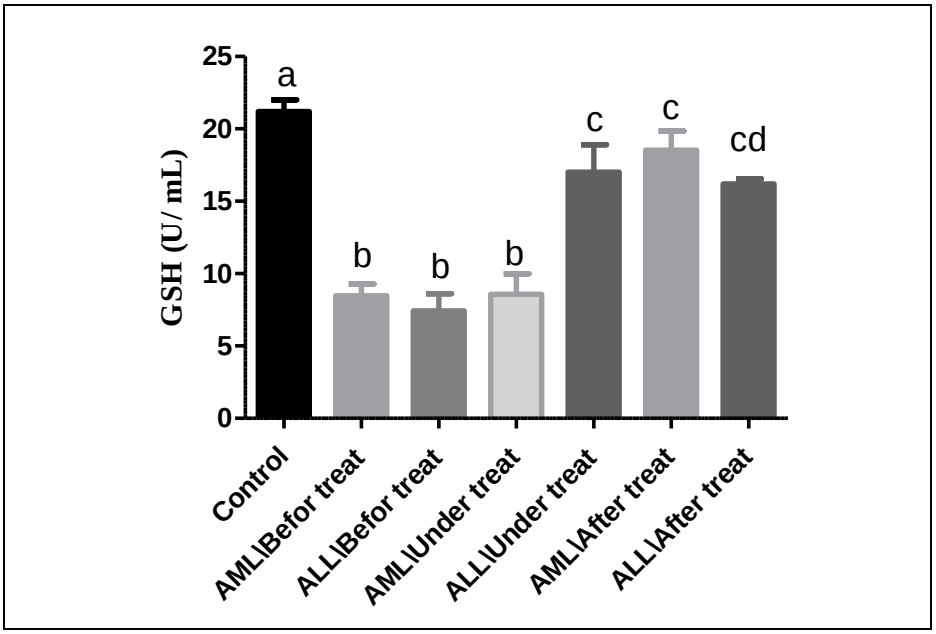

Fig 4: The comparison of GSH concentration in the serum between AML and ALL before treatment, under treat and after treat with healthy control, a, b, c, d: different letter mean ($p>0.05$)

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On the other hand, the results showed a positive correlation between each of Caspase-3 and Calpain-2, Caspase-3 and MDA, and between MDA and GSH, while there was a negative correlation between Caspase-3 and GSH, Calpain-2 and MDA, and between Calpain-2 and GSH in ALL patients before treatment, as shown in Table 2. As shown in Table 3, the results of the current study showed that there is a positive correlation between Calpain-2 and MDA, Calpain-2 and GSH, and between MDA and GSH, while there was a negative correlation between Caspase-3 and Calpain-2, Caspase-3 and MDA, and between Caspase-3 and GSH in AML patients during treatment. In ALL patients during treatment, as shown in Table 4, the results of the present study showed that there was a positive correlation between each of the following: Caspase-3 and MDA, Caspase-3 and GSH, Calpain-2 and GSH, and between MDA and GSH. In addition to that, the results showed a negative correlation between Caspase-3 and Calpain-2 and between Calpain-2 and MDA. On the other hand, as shown in Table 5, the results showed a positive correlation between each of Caspase-3 and Calpain-2, Calpain-2 and MDA, Calpain-2 and GSH, and between MDA and GSH, while there was a negative correlation between Caspase-3 and MDA and between Caspase-3 and GSH in AML patients after treatment. In addition to that, in ALL patients after treatment, the results showed a negative correlation between each of the following: Caspase-3 and Calpain-2, Caspase-3 and MDA, Calpain-2 and GSH, and between MDA and GSH, while there was a positive correlation between Caspase-3 and GSH and between Calpain-2 and MDA Table 6.

Table 1: The Correlation Between Caspase-3 Concentration (Ng/Ml), Calpain-2 (Ng/Ml), MDA (Mmol/Ml) and GSH Concentrations (U/Ml) in AML Patients Before Treatment

		Caspase3	**Calpain2**	**MDA**	**GSH**
Caspase3	Pearson Correlation		0.069	-0.030	0.565
	Sig. (2-tailed)		0.850	0.934	0.089
Calpain2	Pearson Correlation	0.069		0.028	-0.136
	Sig. (2-tailed)	0.850		0.939	0.709
MDA	Pearson Correlation	-0.030	0.028		0.200
	Sig. (2-tailed)	0.934	0.939		0.581
GSH	Pearson Correlation	0.565	-0.136	0.200	
	Sig. (2-tailed)	0.089	0.709	0.581	

Table 2: The correlation between Caspase-3 concentration (ng/ml), Calpain-2 (ng/ml), MDA (mmol/ml) and GSH concentrations (U/ml) in ALL patients before treatment

		Caspase3	Calpain2	MDA	GSH
Caspase3	Pearson Correlation		0.299	0.711*	-0.279
	Sig. (2-tailed)		0.401	0.021	0.435
Calpain2	Pearson Correlation	0.299		-0.001	-0.212
	Sig. (2-tailed)	0.401		0.997	0.556
MDA	Pearson Correlation	0.711*	-0.001		0.028
	Sig. (2-tailed)	0.021	0.997		0.939

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GSH	Pearson Correlation	-0.279	-0.212	0.028	
	Sig. (2-tailed)	0.435	0.556	0.939	
*. Correlation is significant at the 0.05 level (2-tailed).					

Table 3: The Correlation Between Caspase-3 Concentration (Ng/Ml), Calpain-2 (Ng/Ml), MDA (Mmol/Ml) and GSH Concentrations (U/Ml) in AML Patients During Treatment

		Caspase3	Calpain2	MDA	GSH
Caspase3	Pearson Correlation		-0.074	-0.364	-0.322
	Sig. (2-tailed)		0.839	0.301	0.364
Calpain2	Pearson Correlation	-0.074		0.567	0.523
	Sig. (2-tailed)	0.839		0.087	0.121
MDA	Pearson Correlation	-0.364	0.567		0.482
	Sig. (2-tailed)	0.301	0.087		0.159
GSH	Pearson Correlation	-0.322	0.523	0.482	
	Sig. (2-tailed)	0.364	0.121	0.159	

Table 4: The correlation Between Caspase-3 Concentration (ng/ml), Calpain-2 (ng/ml), MDA (mmol/ml) and GSH Concentrations (U/ml) in ALL Patients During Treatment

		Caspase3	Calpain2	MDA	GSH
Caspase3	Pearson Correlation		-0.554	0.278	0.138
	Sig. (2-tailed)		0.097	0.437	0.705
Calpain2	Pearson Correlation	-0.554		-0.745*	0.373
	Sig. (2-tailed)	0.097		0.013	0.288
MDA	Pearson Correlation	0.278	-0.745*		-0.575
	Sig. (2-tailed)	0.437	0.013		0.082
GSH	Pearson Correlation	0.138	0.373	-0.575	
	Sig. (2-tailed)	0.705	0.288	0.082	
*. Correlation is significant at the 0.05 level (2-tailed).					

Table 5: The Correlation Between Caspase-3 Concentration (ng/ml), Calpain-2 (ng/ml), MDA (mmol/ml) and GSH Concentrations (U/ml) in AML Patients After Treatment

		Caspase3	Calpain2	MDA	GSH
Caspase3	Pearson Correlation		0.427	-0.006	-0.044
	Sig. (2-tailed)		0.218	0.987	0.905

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Calpain2	Pearson Correlation	0.427		0.790**	0.434
	Sig. (2-tailed)	0.218		0.006	0.210
MDA	Pearson Correlation	-0.006	0.790**		0.700*
	Sig. (2-tailed)	0.987	0.006		0.024
GSH	Pearson Correlation	-0.044	0.434	0.700*	
	Sig. (2-tailed)	0.905	0.210	0.024	
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Table 6: The Correlation between Caspase-3 Concentration (ng/ml), Calpain-2 (ng/ml), MDA (mmol/ml) and GSH Concentrations (U/ml) in AML Patients After Treatment

		Caspase3	Calpain2	MDA	GSH
Caspase3	Pearson Correlation		-0.182	-0.148	0.273
	Sig. (2-tailed)		0.615	0.683	0.446
Calpain2	Pearson Correlation	-0.182		0.159	-0.714*
	Sig. (2-tailed)	0.615		0.661	0.020
MDA	Pearson Correlation	-0.148	0.159		-0.425
	Sig. (2-tailed)	0.683	0.661		0.221
GSH	Pearson Correlation	0.273	-0.714*	-0.425	
	Sig. (2-tailed)	0.446	0.020	0.221	
*. Correlation is significant at the 0.05 level (2-tailed).					

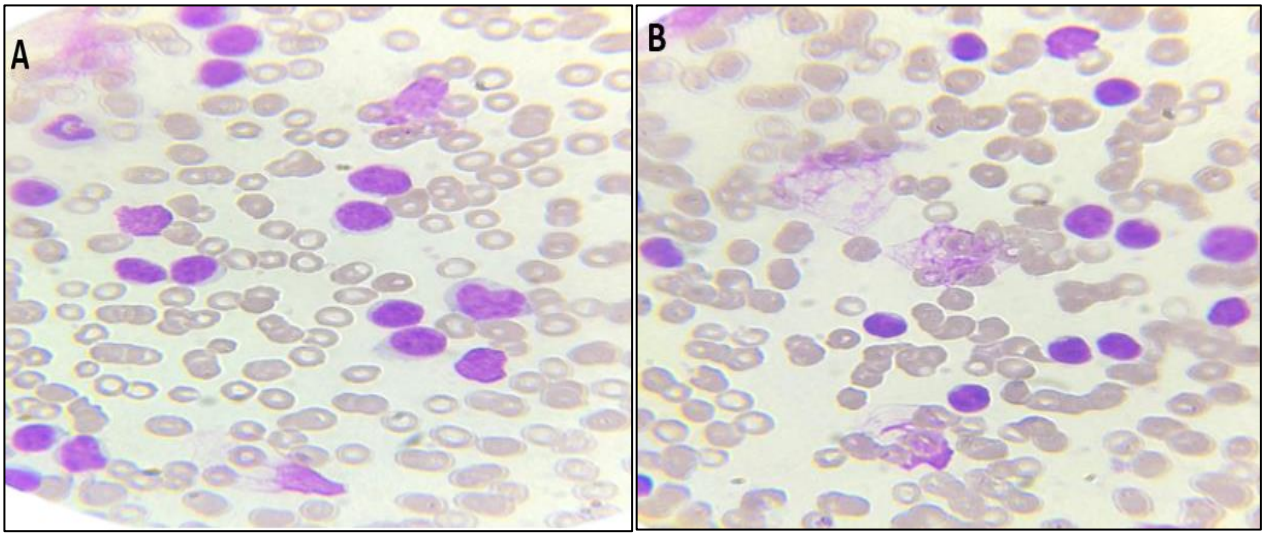

Fig 5 (A, B): shown blood smears stained with Giemsa stain to patients with ALL

Figures 5 (A, B) shown blood smear to cases of patients diagnosed with ALL. The diagnosis consistent with previous study found Acute lymphocytic leukemia (ALL) is an aggressive neoplasm that has been defined by the presence of more than 30% lymphoblasts in the bone marrow or peripheral blood in the French-American-British (FAB) Cooperative Group classification system. (Lai et al., 2000), while Figure 6 (A, B) shown the cases of AML.

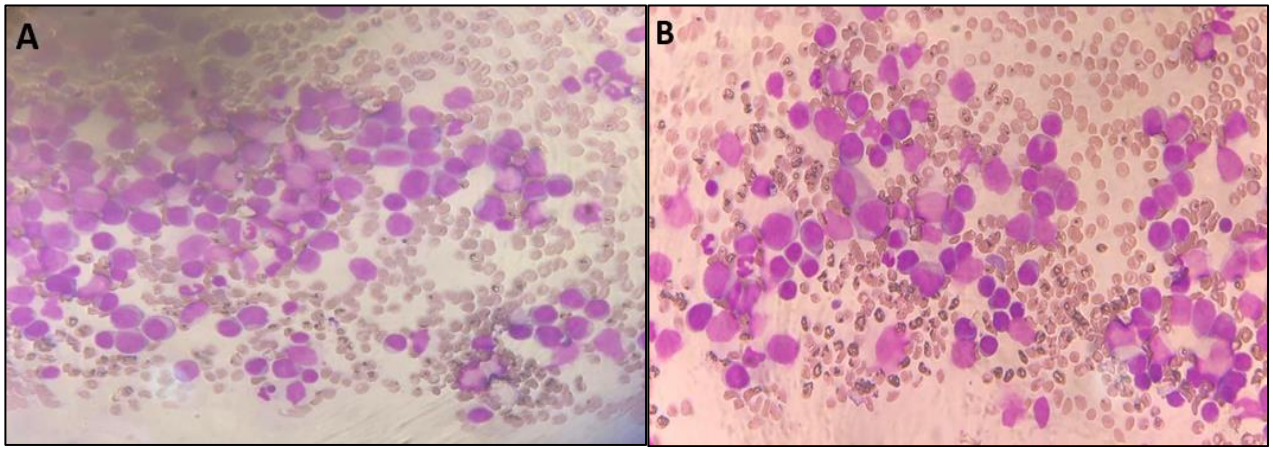

Fig 6 (A, B): Shown Blood Smears Stained with Giemsa Stain to Patients With AML

DISCUSSION

Cancer is undoubtedly a major obstacle to social and national development, and its prevalence is rising quickly. According to data, there were 14.1 million new instances of cancer worldwide in 2012, compared to 8.2 million cancer-related deaths. After heart disease, cancer is the second most common cause of mortality worldwide. In the United States, one-third of women and half of men will get cancer at some point in their lives. Radiation therapy, chemotherapy, and surgery are some of the current cancer treatment options; chemotherapy remains the main cancer management strategy (1, 12, 13).

Due to their high proliferative capacity, the majority of chemotherapy drugs, such as doxorubicin, vincristine, cyclophosphamide, topotecan, and paclitaxel, select cancer cells. This can increase toxicity in normal tissues, including bone marrow, the gastrointestinal tract, and highly proliferative hair follicles. As a result, low doses are frequently prescribed in anti-cancer treatment strategies, which ultimately fail to treat the tumor. Chemotherapy can also cause cancer cells to become resistant, which can lead to tumor regrowth and higher mortality rates. The complex problem of drug resistance in cancer cells is probably influenced by both the microenvironment and cell tissue (14, 15).

The introduction of less toxic treatment options and evolving therapies brings new hope to leukemia patients. Continued research is a critical issue (5). Therefore, the current study is designed as an attempt to understand the mechanism of ALL and AML and finally may aid in developing the treatment protocol.

The current study found the mean age of patients in the current study for AML patients was 41.5 ± 2.6 years, and for ALL patients it was 28 ± 2.4 years. These findings are consistent with a prior study that found that ALL is the most common cancer in younger people, accounting for about 25% of all cancer diagnoses around the world. Approximately 60% of all cases occur in children and adolescents under the age of 20, with an annual incidence of 36.2 cases per million and a peak incidence of >90 cases per million between the ages of two and five years. With a ratio of roughly 1.3:1, boys are diagnosed with ALL more often than girls (16).

According to a previous study (17), acute lymphocytic leukemia is the most prevalent kind of juvenile leukemia and most commonly occurs in youngsters. roughly account for fewer than one-third of acute

leukemia cases in adults with an ALL diagnosis and more than two-thirds of acute leukemia cases in children. According to another study, acute myeloid leukemia (AML) is the most common acute type in adults, making up 1.3% of new cancer cases in the USA, while acute lymphoblastic leukemia (ALL), one of the acute leukemia types, is most commonly diagnosed in children and young adults, with incidence peaks between the ages of 2 and 5(4) All these previous studies are consistent with current results, which found the mean age of AML patients was 41.5 ± 2.6 years, and for ALL patients, it was 28 ± 2.4 years.

One of the most popular methods for aiming for tumor cell death is by activating apoptosis, the process by which a multicellular organism eliminates damaged cells without damaging nearby cells, also known as programmed cell death (PCD). Either the mitochondrial outer membrane permeabilization (MOMP), which is brought on by a variety of physical or chemical stressors, or the interaction between death receptors and their ligands (the extrinsic pathway) can cause apoptosis. In both methods, initiator caspases are drawn to a multiprotein signaling platform (the apoptosome in the internal system or the death-inducing signaling complex in the extrinsic pathway), where they dimerize to become active (18).

Cell shrinkage, chromatin condensation and fragmentation, and blebbing of the cell membrane are all features of apoptotic cells, which are followed by the production of apoptotic bodies. Apoptosis, a self-destructive procedure, may also occur in healthy cells to maintain the regular operations of tissues and organs, according to shards of evidence (19).

The key of apoptosis processes is the caspase-3 enzyme. The cysteine-aspartic acid protease Caspase-3 has garnered a lot of interest lately due to its amazing functions in neuronal development, tissue differentiation, and regeneration. A crucial zymogen in cell death, this enzyme is not activated until initiator caspases cleave it during apoptotic flux (20). This previous study is consistent with the results of the current study, which found significantly elevated caspase-3 levels ($p < 0.05$) in both AML and ALL patients compared to healthy controls. Also, caspase-3 levels were highest before treatment, decreased during treatment, and further reduced after treatment for both AML and ALL. Caspase-3 levels were significantly higher in AML patients compared to ALL patients before, during, and after treatment. And the ALL patients showed higher caspase-3 levels before treatment compared to AML patients and during/after treatment phases. Furthermore, AML patients undergoing treatment had higher caspase-3 levels than AML patients after treatment or ALL patients during/after treatment. Finally, post-treatment caspase-3 levels remained higher in AML patients compared to ALL patients.

The current study demonstrated significantly elevated calpain-2 levels ($p < 0.05$) in both AML and ALL patients at all stages of disease and treatment compared to healthy controls. Calpain-2 levels were highest before treatment, decreased during treatment, and further reduced after treatment in both AML and ALL patients. While no significant difference was observed between AML and ALL patients before treatment. And the ALL patients showed higher calpain-2 levels before treatment compared to AML patients and during/after treatment stages. No significant differences were noted between AML and ALL patients during treatment or post-treatment. Finally, all the ALL patients after treatment exhibited significantly lower calpain-2 levels compared to all other groups.

Calpains, non-lysosomal cysteine proteases, play a key role in apoptosis regulation before, during, and after treatment. These calcium-dependent enzymes, requiring varying calcium levels for activation, are regulated by calpastatin, which inhibits up to four calpain heterodimers simultaneously. Unlike traditional proteases, calpains modulate protein activity, influencing cell motility, adhesion, autophagy, and apoptosis. Their involvement spans tissue-specific functions and disorders, including acute myeloid leukemia (21). The same study found a

strong overexpression of calpain-2 in glioblastoma on both protein and mRNA levels. These findings are completely consistent with the results of the current study.

Numerous critical physiological processes, such as the cell cycle, cytoskeleton remodeling, cellular proliferation, migration, invasion, metastasis, survival, autophagy, apoptosis, and signaling, as well as the pathophysiology of numerous disorders, potentially involving the promotion of tumorigenesis, depend on the calpain system. By catalyzing and controlling the proteolysis of their particular substrates—significant signaling molecules along the course of cancer—calcains, intracellular conserved calcium-activated neutral cysteine proteinases, contribute to the development of cancer (22). TP53, a known calpain substrate, undergoes calpain-dependent cleavage crucial for the G1/S-phase transition upon DNA damage. Calpain inhibition stabilizes TP53, as shown by increased nuclear levels in Mcf-7 cells, while truncated TP53 is quickly degraded in vivo. While calpain-2 expression aids in DNA repair and cell survival, its silencing improves the detection of DNA damage and encourages apoptosis. According to these results, (temozolomide) TMZ in conjunction with anti-calpain-2 medication may be beneficial for glioblastoma (GBM) patients with elevated calpain-2 levels. This approach holds promise for improving clinical outcomes (21). These results agree with the results of the current study.

To monitor the oxidative and antioxidative stress in the patients with ALL and AML, GSH and MDA were included in the current study. Normal cells maintain tightly regulated pathways in response to external stimuli, while cancer cells exhibit altered physiology, including unchecked growth and reduced apoptosis. These changes are often linked to mutations in oncogenes and tumor suppressor genes, as well as imbalanced anti-death and pro-death protein expression (23).

Glutathione (GSH), a tripeptide composed of γ-glutamate, cysteine, and glycine, is the most abundant non-protein thiol in eukaryotic cells. Its synthesis involves two key cytosolic enzymes: γ-glutamate-cysteine ligase (rate-limiting step) and GSH synthetase. High intracellular GSH concentrations play crucial roles in protecting cells from free radicals, regulating carcinogenesis, supporting DNA synthesis, and supporting cell proliferation (24).

Cancer cells often exhibit deregulated GSH metabolism, which contributes to tumor survival by protecting against oxidative stress, evading apoptosis, enhancing colonization, and developing resistance to drugs and radiation. Elevated GSH levels are linked to resistance against chemotherapeutic agents (25). Another study found overexpression of glutathione (GSH) has been observed in many cancer cells (26). The findings of this study are completely consistent with the results of the current study. Which found significantly reduced serum GSH levels in all patient groups compared to healthy controls. GSH levels were lowest before and during treatment for both AML and ALL patients, with partial recovery after treatment. Then no significant differences were observed in GSH levels between AML patients before and during treatment or between ALL patients before treatment. GSH levels in ALL patients during and after treatment were comparable to AML patients after treatment but remained significantly lower than healthy controls. Finally, the ALL patients after treatment exhibited lower GSH levels compared to most groups, except AML after treatment and ALL during treatment.

The current study revealed significantly elevated levels of MDA, a marker of oxidative stress, in AML and ALL patients at all stages compared to healthy controls. MDA levels were highest before treatment and progressively decreased during and after treatment for both AML and ALL patients. And the AML patients before treatment had significantly higher MDA levels compared to all other groups. No significant differences were shown between ALL patients before treatment and during treatment or between ALL during treatment and AML/ALL after treatment. Among the ALL patients, after treatment,

they showed the lowest MDA levels among all patient groups. these results in line with a study found Compared to controls, patients' serum MDA levels were noticeably higher. Level was considerably lower following treatment. Patients with bigger tumors and lymph node metastases had significantly higher pre-chemotherapy serum MDA levels than patients without lymph node metastases. These results indicated the level of MDA increase with tumor development is progressive and may aid in tumor cell resistance to chemotherapy (27). According to correlation studies, the current study revealed key correlations among Caspase-3, Calpain-2, MDA, and GSH in AML and ALL patients. Before treatment, groups showed positive correlations between the markers, except for negative correlations involving caspase-3 with MDA and calpain-2 with GSH. Positive correlations among most markers indicate heightened oxidative stress and apoptosis activity. Negative correlations suggest regulatory imbalances in oxidative stress and antioxidant defenses. These results are consistent with findings in previous studies (28, 29).

While in patients during treatment, AML showed positive correlations between Calpain-2, MDA, and GSH, while ALL had positive correlations among all markers except negative correlations involving Caspase-3 with Calpain-2 and Calpain-2 with MDA. These findings agree with the findings of these two studies (28, 30). Correlations shift, with markers like Calpain-2 and MDA showing sustained activity, while apoptosis pathways (Caspase-3) appear modulated. A study achieved with breast cancer subgroup-specific settings found significant correlations between caspase-3 and calpain-2 expression in the basal-like subgroup (31). This finding is inconsistent with current results.

Finally, in patients after treatment groups, AML exhibited positive correlations among Caspase-3, Calpain-2, MDA, and GSH, with negative links between Caspase-3 and MDA/GSH. In ALL, most correlations reversed, showing negative relationships between key markers. Positive correlations between recovery-related markers (e.g., Calpain-2 with MDA/GSH) indicate normalization, while negative correlations suggest restored balance in stress-response pathways. These findings are in line with results in line with two previous studies (21, 32). In summary, the difference between ALL and AML may belong to the difference in the mechanism of these types of leukemia development. These biomarkers, which are included in the current study, may have interfered with the response to chemotherapy; therefore, we suggest more studies about, if possible, using medication(s) or plant extract to reduce the level of these markers. and if possible, considered as a route test with chemotherapy treatment.

CONCLUSION

The current study highlights the significant role of Caspase-3, Calpain-2, MDA, and GSH in understanding the biochemical and apoptotic changes in AML and ALL during chemotherapy. Elevated levels of apoptotic markers and oxidative stress pre-treatment, along with reduced antioxidant capacity, emphasize the aggressive nature of leukemia. The progressive normalization of these markers post-treatment reflects the therapeutic effect of chemotherapy. These findings refer to the potential of these biomarkers for evaluating treatment efficacy, therapeutic strategies, and improving prognosis in leukemia management.

Conflict of interest

The authors declare that no conflict of interest.

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