ORIGINAL A R T I C L E

Targeting of CD38 and other NAD-dependent Enzymes in Leukemia Patients

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Abstract

Background   Leukemia is a type of cancer that affects the blood and bone marrow. This paper highlights the role of nicotinamide adenine dinucleotide (NAD) consuming enzymes such as CD38, PARP1, and SIRT1 in patients with acute lymphocytic leukemia, acute myeloid leukemia, and patients suffering from chronic myeloid leukemia.

Materials and methods   Based on recent data. 40 patients with acute lymphocytic leukemia (ALL), 40 patients with acute myeloid leukemia (AML), 30 patients with chronic myeloid leukemia (CML), and 40 healthy persons served as controls. The enzyme-linked immunosorbent assay (ELISA) sandwich technique was used to detect the serum levels of CD38, PARP1, and SIRT1.

Results   In patient, groups compared to the control group, CD38 concentrations were discovered to be considerably higher ($p < 0.05$), especially in the CML group, also Comparing patient groups to the control group, it was discovered that the amounts of PARP1 were considerably greater ($p < 0.05$), especially CML group, and When compared to the control group, the patient groups' SIRT1 concentrations were discovered to be considerably greater, especially AML group ($p < 0.05$).

Conclusion   The results obtained show that there is a reliable correlation between the NAD-dependent enzymes and groups of patients suffering from leukemia and are considered predictive indicators of the pathological condition that can be used in the future in treatments.

1   Introduction

Leukemia is a form of blood tissue cancer. The delicate inside of the body is called bone marrow, and hematopoietic stem cells are composed of bone marrow. The latter develops into various blood components, each with a different purpose, including platelets, red blood cells, and white blood cells [1]. Notably, the contribution of NAD-dependent enzymes in the pathogenesis of leukemia has remained largely ignored.

NAD^+ participates in cellular redox reactions and is integral to fundamental energy metabolisms such as glycolysis, citric acid cycle, and mitochondrial electron transport [2]. In addition, NAD^+ is a substrate for many NAD-dependent enzymes such as poly ADP ribose polymerase (PARP), sirtuins and ADP ribosyltransferases, mono ADP ribosyltransferase (ART), and the CD38/CD157 system [3]. These NAD^+-consuming enzymes have been known to mediate many fundamental cellular processes [4]. NAD^+ is created in cells by three pathways: the traditional Preiss-Handler method, the NAD^+ salvage pathway, and de novo synthesis beginning with tryptophan. The latter path recycles nicotinamide, a byproduct of NAD^+-consuming enzymes [5]. A balance of NAD^+ production and consumption is necessary for NAD^+ homeostasis. The three major classes of enzymes that use NAD^+ as a

co-substrate are cyclic ADP-ribose synthases. In the heart, mainly CD38 [6], sirtuins (SIRTs), and poly ADP-ribose (PAR) polymerases (PARPs), is a group of enzymes that transmit ADP ribose (ADPR) to target proteins. There are 17 members of the PARP family in humans [7]. A possible therapeutic target is likely the role of NAD^+ biosynthesis pathways in cancer. Involved in both DNA repair and genomic stability, PARPs are significant mediators of cellular responses. Since PARP uses a lot of NAD^+ during the DNA repair process, activating PARP in cells that have DNA damage or are under external stress causes NAD depletion. BRCA mutations result in DNA double-strand breakdowns that can't be effectively fixed, which causes cancer cells to die. PARP1 is a therapeutic drug that is effective against BRCA1- and BRCA2-associated malignancies. NAD^+ metabolism is strongly influenced by the control of PARP1 activity [8]. The endogenous PARP1 inhibitor macroH2A1.1 improves the availability of mitochondrial NAD^+ and lowers the consumption of NAD^+ in developing cells, allowing for an increase in OXPHOS [8]. However, PARP1 only works against some types of cancer.

On the other side, SIRTs are a session of NAD^+-dependent proteins and have deacetylase activity. Sir2 is the family's first member that comes up with the term sirtuin. There are 7 (Sir2) homologs found in mammals. SIRT1, SIRT6, and SIRT7 are mostly nuclear, whereas SIRT2, SIRT3 and SIRT5 are mitochondrial [9]. Sirtuins control a wide range of cells involved in several processes, including tumor genesis energy metabolism, genomic stability, inflammation, and apoptosis [1][10]. Sirtuins control the effectiveness of DNA repair. The selective suppression of NAD^+ production showed tumor cells' activation of apoptosis [11]. Sirtuin inhibitors' activity as anticancer medications has been attributed to suppressing SIRT1, which deacetylates p^{53} to support cell survival. Cancer cells' proliferation is halted by SIRT1 inhibition, and their level of treatment resistance is decreased *in vitro* [12].

Finally, a cluster of differentiation 38 (CD38) is a glycoprotein known as cyclic ADP ribose hydrolase [13]. CD38 functions in cell adhesion, signal transduction, and calcium signaling [14]. CD38 can also play a role as a receptor or an enzyme [15]. As a receptor, CD38 can attach to CD31 on the surface of T cells, activating those cells to produce a variety of cytokines [16]. According to cellular localization, CD38 is a transmembrane protein in four distinct forms. Numerous immune cell types, including bone marrow progenitors, natural killer cells, monocytes, and activated T- and B-lymphocytes, express CD38, as do non-hematopoietic cells [17].

This current investigation aims to ascertain how NAD-consuming enzymes like CD38, PARP1, and SIRT1 contribute to the emergence of various types of leukemia kinds.

2 Experimental part

2.1 Subjects and Methods

This research had 150 individuals who were separated into four groups: individuals who have acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), patients who have chronic myeloid leukemia (CML), and healthy controls. All patient data, including sex, age, and length of illness, were recorded. The control group was carefully chosen to make sure that none of the participants had any other diseases or disorders. The mean age of the population ranged from 17 to 70 years, selected from (October 2022 to February 2023). All laboratory test analysis was performed at Al-Kindi laboratory/ Al-Qadisiyah.

From each study group, five milliliters of blood were drawn. The blood was centrifuged for 15 minutes at (4000 rpm) after being allowed to clot for 15 minutes. The separated serum was split and distributed in Eppendorf tubes (kept at -20 °C). The levels of CD38, PARP1, and SIRT1 were measured by using the sandwich enzyme-linked immunosorbent assay method. Human Cluster of differentiation 38 (CD38) Elisa Kit, BT-lab China, Human Poly [ADP-ribose] polymerase 1 (PARP1) Elisa Kit, BT-lab China, and human Sirtuin 1 (SIRT1) Elisa Kit, (BT-lab China), were all used in the study.

2.2 Statistical analysis

GraphPad Prism 9.2.0 collected, examined and showed the data. The mean and standard error were used to express numerical data. One way, in the case of regularly distributed variables, the ANOVA test was done to competition the mean values between the several groups. The P-value was seen to be significant at p-value ≤ 0.05.

3 Results

The CD38 level was more significant in patients when compared to the control as shown in Figure 1, with high CD38 levels in CML (32.89 ± 1.853) compared to AML (30.36 ± 0.4703) ng/mL, ALL (26.86 ± 1.009) ng/mL, and control (21.4 ± 0.6173) ng/mL as shown in Figure 2. This study presented a significant difference (p-value < 0.0001) in the concentrations of CD38 between patients compared to the control, also showed a significant difference in mean values between ALL with AML, and between ALL with CML, while showed non-significant difference between AML with CML.
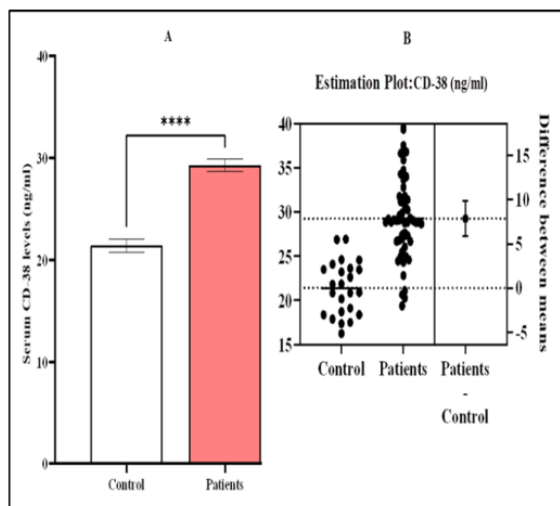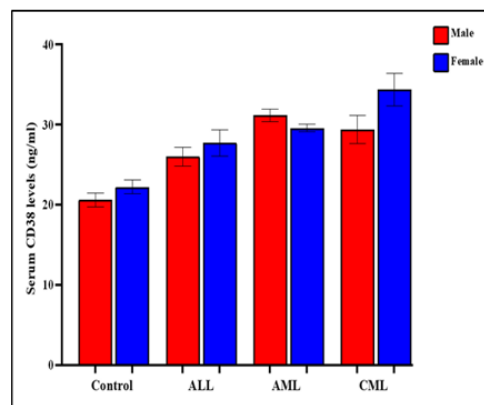
Figure 1: Estimation of concentrations of CD38 (ng/mL)], a contrast between control and patients with Leukemia (LKA). (A) a contrast between the patient's group and control, (B) an estimated plot that explains the presence of a significant increase in the level of CD38 in the patient's groups as compared to the control, the significant difference (p-value <0.0001). Data are presented as means with SEM. * Shows statistically different results between the patient groups and the control (P < 0.05)
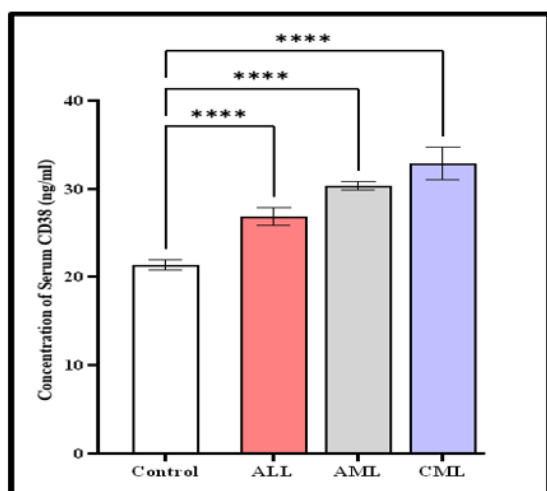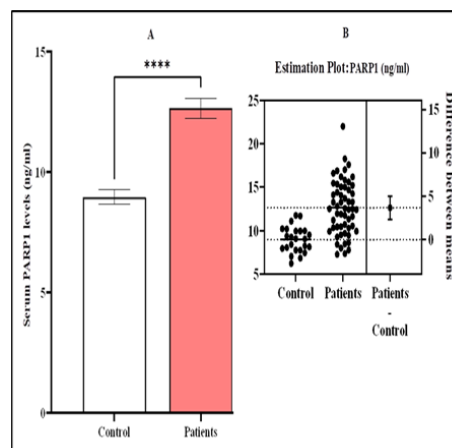

Figure 2: Comparison of CD38 levels in different studied groups controls ALL, AML, and CML. Data are presented as means with SEM. * Shows statistically different results between the patient groups and the control (P < 0.05).

Comparing the difference between males and females, the cluster of differentiation 38 [CD38 (ng/mL)] level was high in males than in females in patients with AML, while was level was high in females than in males in patients with ALL and CML. At the same time, there was a non-significant difference between males and females in the control as shown in Figure 3.

Figure 3: Estimation of a cluster of differentiation 38 [CD38 (ng/mL)] serum concentration in males and females in all patients with (ALL, AML, and CML) as compared with control. Compared to the control, a non-significant difference was found between males and females in all patients with (ALL, AML, and CML).

The serum PARP1 level was higher in patients compared to the control as shown in Figure 4 with CML (15.22 ± 0.9409) ng/mL compared to AML (13.05 ± 0.6538), ALL (11.38 ± 0.5291), and control (8.958 ± 0.3033) ng/mL as shown in Figure 5. This study exhibited a significant difference (p-value < 0.0001) in the concentrations of PARP1 between patients compared to the control, also showed a significant difference in mean values between ALL and CML, while showed non-significant difference between ALL with AML, and AML with CML.
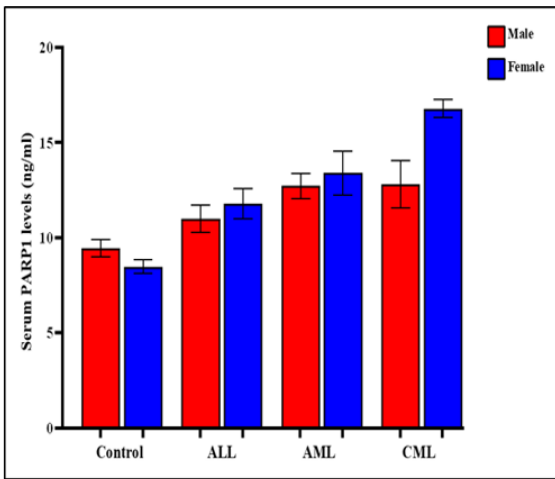

Figure 4: Estimation of concentrations of PARP1 (ng/mL). A contrast between control and patients with Leukemia (LKA). (A) a contrast between the patient's groups and control, (B) an estimated plot that shows the presence of a significant rise in the level of PARP1 in the patient's group as compared to the control, the significant difference (p-value <0.0001). Data are presented as means with SEM. * Shows statistically different results between the patient groups and the control P≤0.05.
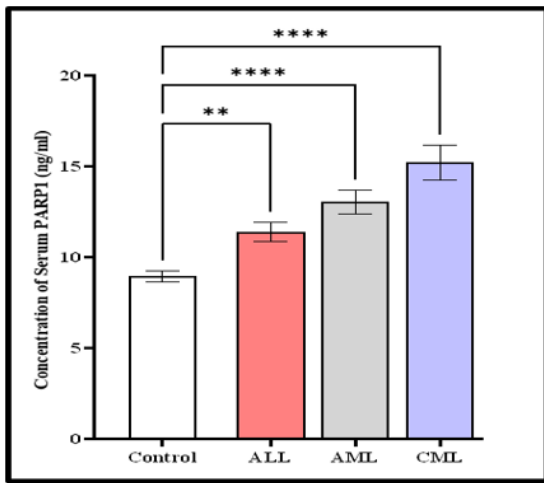
Figure 5: Comparison of PARP1 level in different studied groups, control ALL, AML, and CML. Data are presented as means with SEM. * Shows statistically different results between the patient groups and the control (P < 0.05).

Comparing the difference between males and females, the Poly [ADP-ribose] polymerase 1 [PARP1 (ng/mL)] level was high in females than in females in patients with ALL, AML, and CML. At the same time, there was a non-significant difference between males and females in the control as shown in Figure 6.


Figure 6: Estimation of Poly [ADP-ribose] polymerase 1 [PARP1 (ng/mL)] serum concentration in males and females in all patients with (ALL, AML, and CML) as compared with control. As compared with the control, a non-significant difference was found between males and females in all patients with (ALL, AML, and CML).

The serum SIRT1 levels were higher in patients compared to the control as shown in Figure 7 with AML (19.60 ± 1.148) ng/mL compared to CML (17.83± 2.309), ALL (14.97± 0.6651), and control (11.75± 0.3213) ng/mL as shown in Figure 8. Our

finding presented a significant difference (p-value < 0.0001) in the concentrations of SIRT1 between patients compared to the control, also showed a significant difference in mean values between ALL and AML, while showed non-significant difference between ALL with CML AML, and with CML.
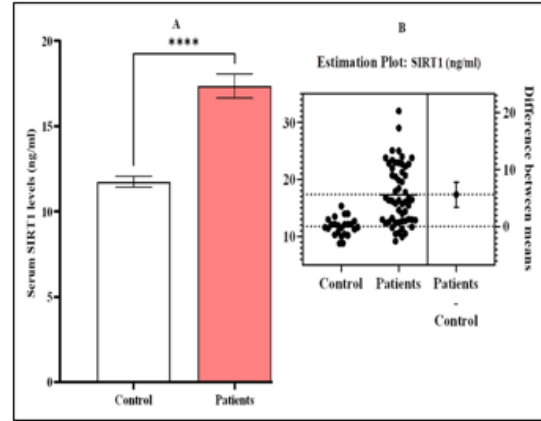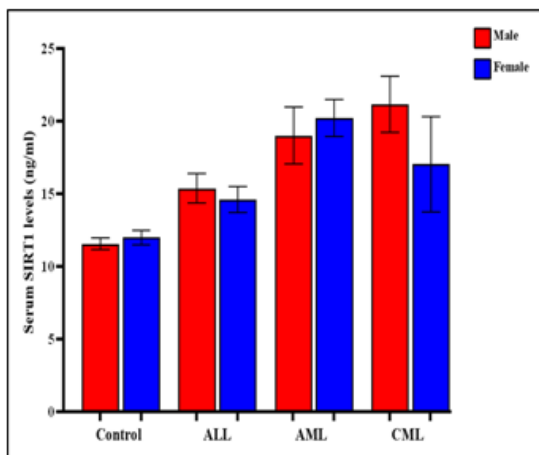

Figure 7: Estimation of concentrations of sirtuin 1 [SIRT1 (ng/mL)] A contrast between control and patients with Leukemia (LKA). (A) a contrast between the patients groups and control, (B) an estimate plot that proves the presence of a significant rise in the level of SIRT1 in the patients group as compared to the control, the significant difference (p-value <0.0001). Data are presented as means with SEM. * Shows statistically different results between the patients groups and the control P≤0.05.
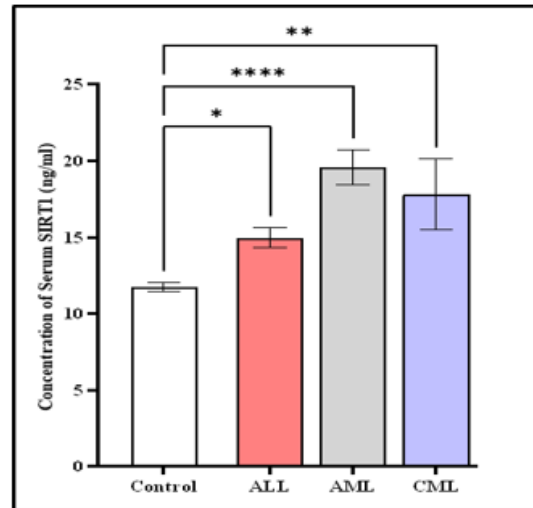
Figure 8: Comparison of SIRT1 level in different studied groups control ALL, AML, and CML. Data are presented as means with SEM. * Shows statistically different results between the patient groups and the control (P < 0.05).

Comparing the difference between males and females, the sirtuin 1 [SIRT1 (ng/mL)] level was high in

males than in females in patients with ALL and CML, while was level was high in females than in males in patients with AML. At the same time, there was a non-significant difference between males and females in the control, as shown in Figure 9.

Figure 9: Estimation of sirtuin 1 [SIRT1 (ng/mL)] serum concentration in males and females in all patients with (ALL, AML, and CML) as compared with control. Non-significant difference was found between males and females in all patients with (ALL, AML, and CML) compared to the control.

4 Discussion

Compared to the control group, the findings revealed a rise in CD38 concentration in the patient groups, especially the CML group. Our research showed that CD38 is a potentially valuable independent marker that enables illness outcome prediction. A better prognosis was formerly thought to be predicted by rising CD38 expression, which is a differentiation marker [18, 19].

NAD$^+$ is hydrolyzed by CD38, a potent cellular NAD$^+$ regulator, to create cyclic ADP ribose. It is known that CD38 is a multifunctional enzyme that uses NAD$^+$ as a substrate to make a second messenger and participates in signal transduction pathways that regulate cell proliferation and differentiation in addition to acting as a cell surface marker [16]. In particular, it controls the levels of NAD$^+$ in many different tissues and cells as the primary cellular NADase in mammals [20]. According to various stimuli, CD38, which also functions as an adhesion molecule, has been linked to cellular proliferation processes and to the inhibition and prevention of programmed cell death [21]. Although well-defined at the molecular and biochemical levels, little is known about the importance of CD38 in AML and ALL. The data above concurred with Keyhani and others [18].

In addition, when compared to the control group, the patient groups, particularly the CML group, had higher PARP1 concentrations, according to the results. The second essential NAD-consuming enzyme, PARP1, the best extensively studied and characterized by the players involved in single-strand break (SSB) repair, is activated when DNA is damaged. Poly-ADP-ribosyl (PAR) chains are added by PARP1 to other proteins and themselves to catalyze actions [22]. Along with DNA repair, PARP1 PARylation activities also have a role in transcription, cell death, and protein stability and activity control. Recent research has demonstrated that STAT3, another critical signaling protein in malignancies, is controlled by PARP1-dependent PARylation by inhibiting STAT3's transcriptional activity and causing STAT3 dephosphorylation [23]. Given the overexpression of PARP1 in most AML patients, this raises the possibility that PARP1 plays a role in the genesis of AML. This finding is particularly noteworthy because several PARP inhibitors have shown promise as treatments for AML and have shown good tolerability in clinical trials for breast and ovarian cancer. Our results concur with those Wang et al. published [24], by establishing that PARP-1 inhibition inhibits the growth of AML cells, promotes apoptosis in vitro, and improves the prognosis for AML in mice, in addition to demonstrating PARP-1 up-regulation in AML patients.

Finally, SIRT1 data revealed that patient groups, particularly the AML group, had higher concentrations of SIRT1 than the control group. SIRTs control various cellular processes, such as energy metabolism, stress response, tumorigenesis, and aging. Nuclear protein SIRT1 regulates various cellular processes, including aging, genome stability, DNA repair, metabolism, and oxidative stress responses. SIRT1 is expressed in almost all cell types and is involved in the deacetylation of histones, transcription factors, and signaling proteins that control many metabolic and stress-response pathways [25]. The most researched gene in AML is SIRT1. Patients with high- and intermediate-risk conditions exhibit high levels of SIRT1 expression, which is regarded as a great prognostic predictor. The significance of this gene in therapeutic situations is further supported by its participation in destroying cancerous cells by caspases and inducing cellular cytotoxicity [26]. Interestingly, the results agreed with the study of Chen and Bhatia [27] and Abraham et al. [28].

Further research was done to study the impact of patients' sex on prognosis by comparing the levels of NAD-consuming enzymes between males and females, investigating NAD-dependent enzyme levels, and analyzing their roles in illness progression, as indicated above. Interestingly, CD38 and PARP1 expression levels were higher in females with CML but not in

men, and there were no apparent differences in these levels between males and females in the other study groups. Contrarily, SIRT1 levels were higher in males with CML, but in the other groups under investigation, there were no apparent differences between males and females in the activity of the NAD-consuming enzymes. The findings demonstrated that gender impacted NAD-consuming enzyme levels and disease development, particularly in CML patients. Our results could shed new light on the biology of the three different leukemia types. It's interesting to note that Rai et al.'s 1975 study of 125 CLL patients included the first examination of the gender issue [29]. Though the difference was not statistically significant after adjusting for age and stage, it was noticed that women had a greater survival rate. However, additional research on the CLL experiment revealed that women did have a more remarkable overall survival, regardless of age and disease stage [30]. According to earlier studies, the biology of leukemia patients (CLL) may differ significantly between the sexes, although this hasn't been widely acknowledged. In CLL and other cancers, such as cutaneous head and neck melanoma, the female sex has become a predictor of improved outcomes [31].

5   Conclusions

Recent study provide further evidence for the hypothesis that CD38 and other NAD-dependent enzymes are a reliable markers that are physiologically involved in patients with acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) during disease progression

Authors contribution: Zainab Mohammed Hillel and Dr. Zainab Nejim Al-Abady were engaged in the study's conception, analysis of the findings, and report writing. The authors confirmed the final version before it was submitted.
Conflict of Interest: No conflicts of interest exist between the authors and the publication of this work.
Ethical consideration: The ethical committee approved the study at University of Al-Qadisiyah, Diwaniyah, Iraq.

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