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The relationship of p53, BCL-2, sphingosine and sphingosine-1-phosphate in leukemia patients

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Abstract:

Leukemia is a malignancy of the blood that occurs when a certain part of the life span of a blood cell, its division, or its precursor is improperly regulated. A large group of cells that develop from a single cell are formed once the cell begins to multiply uncontrollably. Cancers affecting the bone marrow, lymph nodes, and blood are referred to as "hematological malignancies. Chronic Myeloid Leukemia (CML), Chronic Lymphocytic Leukemia (CLL), Acute Myeloid Leukemia (AML), and Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), and lymphoma are all included. Lymphoma and myeloma In this classification, targeting different signaling pathways is a potential newer treatment for leukemia. The main naturally occurring base found in sphingolipids is sphingosine (SPH). It is a key component of sphingolipids, a group of lipids found in cell membranes that also includes the significant phospholipid sphingomyelin. Sphingosine-1-Phosphate (S1P) is a signaling sphingolipids, also known as lysosphingolipid. Sphingosine-1-phosphate and sphingosine are highly expressed by leukemia cells. The tumor suppressor protein p53 is a protein that is made using the TP53 gene. This protein controls cell division by acting as a tumor suppressor, which means it prevents cells from proliferating and growing too quickly or in an uncontrolled manner. It has a positive interaction with the BCL2 protein, which controls whether a cell dies or survives by preventing an apoptotic kind of cell death. The BCL2 gene is located on chromosome 18, and numerous B-cell leukemias and lymphomas exhibit the transfer of the BCL-2 gene to another chromosome. The study was conducted on 80 individuals (40 participants and 40 controls). Result showed a highly significant difference at p≤0.05 of values of SPH (7.6), p53 (16), and BCL-2 (6.4) in patients when compared with controls values (3.9, 1.4, and 2.4) respectively except value of S1P increased in patients but not significant. Therefore can concluded the SPH increased with leukemia and led to development of leukemia because it associated as signaling for cell proliferation and increasing p53and BCL-2.

Key words: Leukemia, sphingosine, sphingosine-1-phosphate, p53, and BCL2

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INTRODUCTION

Leukemia is known as an abnormal clonal growth generated by hematopoietic stem cells [1]. Currently, it is generally believed that most leukemias are caused by genetic material in cells and a combination of environmental factors [2]. Leukemia has been classified into two types based on the kind of cell that is improperly multiplying: lymphoid and myeloid leukemia, and there are 4 subtypes of leukemia: chronic lymphocytic leukemia , chronic myeloid leukemia, acute myeloid leukemia, and acute lymphocytic leukemia [3, 4]. Numerous phospholipid metabolites serve as crucial signal transduction mediators in cells. One of the essential elements of the lipid bilayer that makes up the cell membrane is phospholipid. Sphingolipids such as sphingosine and Sphingosine-1phosphate are crucial in the growth of cancer. While S1P encourages cell proliferation, migration, and invasion, sphingosine mediates cell cycle arrest and triggers death. Lipid kinases called sphingosine kinases phosphorylate sphingosine to create S1P. There are actually two SPHK isoenzymes: SPHK2 and SPHK1. The synthesis of S1P is aided by the enzyme SPHK1, which also encourages the formation of S1P from sphingosine [5]. Sphingolipids have been linked to a number of disorders, including atherosclerosis, cancer, obesity, and sphingolipidoses. However, the investigation of blood Sphingolipids SPL levels as illness biomarkers is still in its early stages [6]. S1P is produced after the phosphorylation of sphingosine (SPH) by sphingosine kinase. Serum S1P levels are regulated by the actions of Sphingosine-1phosphate phosphatase and Sphingosine-1phosphate lyase, which dephosphorylate or degrade Sphingosine-1phosphate (S1P), respectively. Sphingosine-1-phosphate is produced

by activated platelets, white blood cells, and RBCs cells. Although Endothelial Cells (EC) are the major source of plasma S1P under physiological conditions [7- 9], S1P levels are typically high in blood and lymph and low in lymphoid tissues; this gradient is crucial for lymphocyte egress. Erythrocytes, platelets, and lymphatic endothelial cells are cellular sources of high S1P levels [10]. The p53 gene exists on chromosome 17p13.1, known as the "protector of the genome," and plays a critical role in the cellular protection against the transformation of cells in the presence of genotoxic stress and oncogenic conditions [11]. The p53 transcription factor's capacity to activate target genes and cause biological reactions including DNA damage repair, apoptosis, senescence, and cell cycle arrest is how it accomplishes this [12]. Higher p53 expression was associated with TP53 mutation and higher rates of leukemic transformation [13]. The BCL-2 family of proteins regulates mitochondrial outer membrane permeabilization, which leads to the release of intermembrane space proteins after apoptosis and caspase activation, which is what kills cells, through direct binding interactions [14, 15]. BCL-2 proteins regulate the mitochondrial apoptotic response. BCL-2 expression that is constitutionally high causes resistance to apoptosis. Many different kinds of cancer, including CLL, acute myeloid leukemia, and CML, have been linked to aberrant BCL-2 activity [16, 17].

MATERIALS AND METHODS

Study plan: This study was ratified by the AL-Karama Teaching Hospital and External Laboratories after determining their infection in Wasit Province, Iraq. Patients (n= 40) with leukemia Participants in this study were diagnosed with leukemia infections between March 2021 and February 2022. The patients were 25 males and 15 females. The control group (27 males and 13 females) they had no pathological conditions at the time of the study, and no history of systemic disorders.The control group in this study consisted of 40 people who appeared to be in good health (27 males and 13 females), had no pathological conditions at the time of the study, and had no history of systemic disorders.

Laboratory screening of blood samples

Almost 3–4 ml of peripheral blood were acquired with a collection tube from the subjects in all groups.1.250 ul of blood was inserted in a Trizol tube for RNA extraction and maintained in deep freeze until required. Two milliliters of blood in an anticoagulant (EDTA) tube for molecular investigation and keep immediately at -20°C till use. The gel tube received three milliliters of blood, and after a few minutes at room temperature, the sample began to clot. Serum samples were separated by 3000 rpm/15 min centrifugation used to separate serum for ELISA tests preservation at -20°C. The serum was placed in a cool box and was then transferred to the laboratory. Detection of human SPH, S1P by an ELISA kit.

Statistical analysis

SPSS has been used to manage data. Qualitative data are expressed in frequency, median, and percent, and quantitative data in average. The statistical analysis used determines the frequency with a significant p-value of $p \leq 0.05$. ANOVA test revealed correlations between more than two groups. A t-test was used for correlations between two groups.

RESULTS AND DISCUSSION

In this study, the sample includes forty (40) patients with leukemia (the test group). There were 15 (37.5%) females and 25 (62.5%) males. Chronic Myeloid Leukemia (CML) was the most common form of leukemia in this study, accounting for 14 (52.5%) of all cases; ALL was the second most common type, encompassing 13 (32.5%) of cases, followed by CLL in 3 patients (7.5%) and AML in 7 patients (7.5%). and forty (40) healthy individuals (the control group). 13 (32.5%) females and 27 (67.5%) males as a control group.

The study was conducted on 80 people. There is a difference between the values of SPH (0.0001) and SIP (0.08) in patients when compared with controls, with the mean ± SD of the total indicating a significant increase ($p \leq 0.05$). As shown in (Table 1). The results showed an increase in the levels of all parameters except SIP. It seems a little loud, but there isn't a lot of difference.

Tab. 1. Association between SPH and SIP for patients and control

Item	participant	No	Mean	SD	p-value
SPH	Patients	40	7.6	2.08	0.0001
	Control	40	3.9	0.83	
SIP	Patients	40	49.1	4.1	0.08
	Control	40	37.7	1.1	

SD= Standard Deviation; No= Number

Apoptosis signaling is endogenously regulated by sphingosine. DNA fragmentation in the nucleus and morphological alterations in DNA fragmentation within the nucleus indicative of apoptosis were noticed when leukemia cells were treated to sphingosine or its methylated derivative "N,N-dimethylsphingosine" [18]. SPH level showed a significant increase in leukemia patients compared to the control group, and this is consistent with [19]. SPH is produced by the transformation of sphingomyelin to ceramide, which is later converted to sphingosine by ceramidase and phosphorylated to S1P by two sphingosine kinases, SPHK2 and SPKH1. S1P is the end result of sphingolipid SPLs metabolism [20]. The relationship between carcinogenesis and sphingolipid metabolism has attracted interest in light of recent reports of abnormal sphingolipid metabolism in a number of cancers [21]. According to studies, sphingosine and ceramide promote apoptosis and inhibit cell growth [22]. And the metabolite S1P inhibits apoptosis and promotes cell proliferation [23]. Hence, whether cancer cells undergo apoptosis or proliferation depends on the dynamic balance of ceramide/sphingosine-1phosphate. Sphingosine kinase-1 is a crucial enzyme for S1P synthesis and the control of ceramide/sphingosine-1phosphate balance because it produces S1P by phosphorylating SPH [24].

The levels of P53 and BCL-2 were evaluated in 40 patients and 40 controls. The mean ± SDs for P53 and BCL-2 levels in patients and controls are 16 ± 2.8, 6.11, and and 6.4 ± 9.9, respectively. The findings in patients show a significant increase in P53 and BCL-2 ($p \leq 0.05$, 0.002, and 0.02, respectively) as shown in the (Table 2).

Tab. 2. Association between gene expression P53 and BCL-2 for patient and control

Item	Participant	No	Mean	SD	P value
P53	Patients	40	16	2.8	0.002
	Control	40	1.4	3.1	
BCL-2	Patients	40	6.4	9	0.02
				9	
	Control	40	2.4	4.7	

SD= Standard Deviation; No= Number

Changes in the mRNA expression of genes associated with cell cycle apoptosis, such as P53 and BCL-2, shown in (Figure 1, 2) respectively, were assessed using quantitative RT-PCR. The level of P53 demonstrated a highly significant increase in patients with leukemia compared with the control group, which the results agree with [25]. Phosphorylation, protein ubiquitylation, protein folding, molecular chaperones, and other processes can all affect the accumulation of p53 protein, which is a defining characteristic of cells with p53 mutations [26]. About 50% of cancer cases are associated with P53 inactivation [27]. Apoptosis, senescence, angiogenesis, cell cycle regulation, cellular differentiation, and DNA metabolism are some of the major biological roles of p53 [28].

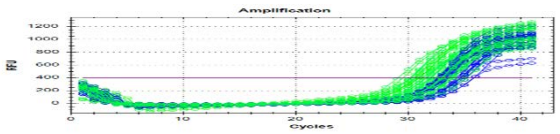

Fig. 1. The Real Time PCR amplification plots of P53 gene expression in patients (Green plots) and healthy control (Blue plots).

BCL-2 levels were significantly elevated in leukemia patients compared to controls, with consistent results agreeing with [29]. which confirmed what was reported in previous studies: that overexpression of BCL-2 did not, as expected, cause cell growth but rather inhibit cell death. The BCL-2 protein, a chromosomal A translocation oncogene that results in malignant lymphomagenesis, is a member of the BCL-2 family. BCL-2 was discovered as an anti-apoptotic protein that delays cell death. However, the BCL-2 Associated X (Bax) protein (binding and inducing BCL-2 family proteins, acting on MOM permeabilization, and altering cellular mitochondria to induce cell death), which forms heterodimers with BCL-2 and shares sequence and structural similarities with it, triggers apoptosis [30]. The BCL-2 family of proteins is consequently categorized as either pro- or anti-apoptotic. These proteins' effects on the permeabilization of the mitochondrial outer membrane enable the release of cytochrome C and DIABLO (SMAC, or the second mitochondrial-derived activator of caspases) from the intermembrane mitochondrial space into the cytosol. The released cytochrome C is bound by apoptosis-protease-activating factor 1 (Apaf-1) and caspases [30].

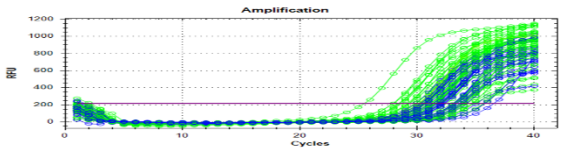

Fig. 2. The Real Time PCR amplification plots of BCL-2 gene expression in patients (Green plots) and healthy control (Blue plots).

Correlation between P53 level and other parameters are shown in (Table 3). Showed non-significant correlations (P≤ 0.05) in leukemia patients between the gene expression of P53, SPH, and S1P.

Tab. 3. Association between gene expression P53 and BCL-2 for patient and control

Variable	p-value	Pearson correlation
SPH	0	-0.05
S1P	0.5	0.08

Significant differences (P≤ 0.05).

The relationship between sphingolipid metabolism and carcinogenesis has attracted attention in light of recent reports of abnormal sphingolipid metabolism in a number of malignancies [31]. According to studies, Ceramide and Sphingosine promote apoptosis and inhibit cell growth [32]. And the metabolite S1P promotes cell proliferation and inhibits apoptosis [33]. Hence, whether tumor cells undergo apoptosis or proliferation depends on the dynamic balance of Ceramide and Sphingosine-1 phosphate. Sphingosine kinase-1 is a crucial enzyme for Sphingosine-1 phosphate synthesis and the control of Ceramide/Sphingosine-1 phosphate CER/S1P balance because it produces Sphingosine-1 phosphate by phosphorylating Sphingosine (34). Our findings demonstrated that there is no connection between p53 and sphingosine, and this is consistent with the fact that sphingosine's pro-apoptotic and anti-proliferative effects are via down-regulation of MYCN, which acts independently of TP53 mutation [34,35]. Reported that there is no positive relationship between SPHK1 expression and p53, and since SPHK1 is responsible for the formation of SIP, we believe that this is in line with our results and is the lack of a relationship between S1p and P53.

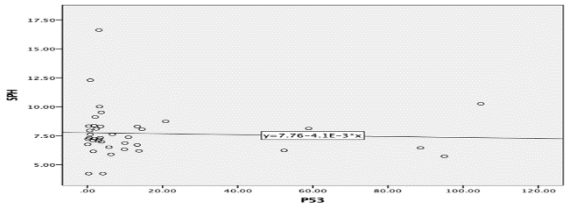

Fig. 3. Correlation between P53 level and PSH level

The Correlation between BCL-22 level and other parameters as illustrated in (Table 4). Showed non-significant correlations (P≤ 0.05) in leukemia patients between the genes BCL-2 and SPH (-0.1) and S1P (0.1).

Tab. 4. Correlation between BCL-2 level and other parameters

Variable	P value	Pearson correlation
SPH	0.2	-0.1
S1P	0.2	0.1

Significant differences (P<0.05).

Our results showed no correlation between the BCL-2 level and SPH, which agrees with, who pointed out that BCL-2 is a major regulator of the ceramide-mediated crosstalk between autophagy and apoptosis [36,37]. In their paper, BCL-2L13 in mitochondria, which is different from BCL-2 and related proteins and inhibits apoptosis differently than other BCL-2-related proteins, indicated an association between BCL-2L13 and sphingosine metabolism in leukemic patients.

According to the results we obtained, we found that there was no relationship between BCL-2 and SPH. Our findings demonstrated that there is no correlation between BCL-2 and S1p, and in this, we disagree with who reported that S1P produced by Spk-1 can increase BCL-2 and MCL-1 in cells [38]. I think that there is no correlation between the SphLs, BCL-2, and P53 because of their different pathways inside the cell.

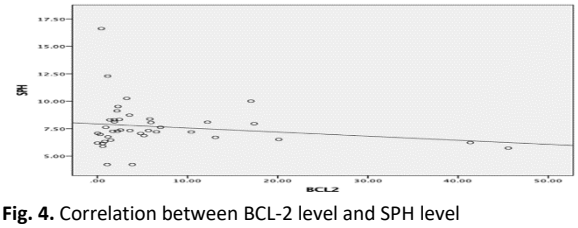

Fig. 4. Correlation between BCL-2 level and SPH level

REFERENCES

1. Li Y, Zhou F. Efficacy of bone marrow transplantation in treating acute myeloid leukemia: a systematic review and meta-analysis. Am J Transl Res. 2023;15(1):1-12.
2. Kayser S, Levis MJ. Clinical implications of molecular markers in acute myeloid leukemia. Eur J Haematol. 2019;102:20-35.
3. Leeuw DC, Ancker W, Denkers F, Menezes RX, Westers TM et al. MicroRNA profiling can classify acute leukemia of ambiguous lineage as either acute myeloid leukemia or acute lymphoid leukemia. Clin Cancer Res. 2013;19:2187-2196.
4. Aftab MO, Awan MJ, Khalid S. Executing Spark BigDL for Leukemia Detection from Microscopic Images Using Transfer Learning. 1st Int Conf Artif Intell Data Anal. 2021;978(1):6654-1511-8.
5. Yi X, Tang X, Li T, Chen L, He H, et al. Therapeutic potential of the sphingosine kinase 1 inhibitor, PF-543. Biomed Pharmacother. 2023;163:114401.
6. Hammad SM, Pierce JS, Soodavar F, Smith KJ, Al Gadban MM et al. Blood sphingolipidomics in healthy humans: impact of sample collection methodology. J Lipid Res. 2010;51(10):3074-3087.
7. Kyle RA, Rajkumar SV. An overview of the progress in the treatment of multiple myeloma. Expert Rev Hematol. 2014;7:5-7.
8. Garcia JG, Liu F, Verin AD, Birukova A, Dechert MA et al. Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. Clin Invest. 2001;108(5):689-701.
9. Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y et al. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. Circ Res. 2008;102(6):669-6760.
10. Liu M, Seo J, Allegood J, Bi X, Zhu X et al. Hepatic apolipoprotein overexpression stimulates formation of larger apoM/sphingosine 1-phosphate-enriched plasma high-density lipoproteins. J Biol Chem. 2014;289(5):2801-2814.
11. Neilsen PM, Pishas KI, Callen DF, Thomas DM. Targeting the p53 pathway in Ewing sarcoma. Sarcoma. 2011;2011:746939.
12. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004;10(8):789-799.
13. Saft L, Karimi M, Ghaderi M. p53 protein expression independently predicts outcome in patients with lower-risk myelodysplastic syndromes with del (5q). Haematologica. 2014;99:1041-1049.
14. Zhang LK, McGraw DL, Sallman AF. The role of p53 in myelodysplastic syndromes and acute myeloid leukemia: molecular aspects and clinical implications. Leuk Lymphoma. 2017;58(8):1777-1790.
15. Kale J, Osterlund EJ, Andrews EW. BCL-2 family proteins: changing partners in the dance towards death. Cell Death Differ. 2018;25:65-80.
16. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science. 1984;226:1097-1099.
17. Przespolewski ER, Baron J, Kashef F, Fu K, Sait SNJ et al. Concomitant Venetoclax and Imatinib for Comanaging Chronic Lymphocytic Leukemia and Chronic Myeloid Leukemia: A Case Report. J Natl Compr Canc Netw. 2023;21(2):102-107.
18. Wątek M, Durnas B, Wollny T, Pasiarski M, Gozdz S et al. Unexpected profile of sphingolipid contents in blood and bone marrow plasma collected from patients diagnosed with acute myeloid leukemia. Lipids Health Dis. 2017;16:235.
19. Raza Y, Atallah J, Luberto C. Advancements on the Multifaceted Roles of Sphingolipids in Hematological Malignancies. Int J Mol Sci. 2022;23(21):12745.
20. Spampinato SF, Sortino MA, Salomone. Chapter Four: Sphingosine-1-phosphate and Sphingosine-1-phosphate Receptors in the Cardiovascular System: Pharmacology and Clinical Implications. Adv Pharmacol. 2022;94:95-139.
21. Watson C, Long JS, Orange C, Tannahill CL, Mallon E,et al. High expression of sphingosine 1-phosphate receptors (S1P1 and S1P3), sphingosine kinase 1, and extracellular signal-regulated kinase-1/2 is associated with the development of tamoxifen resistance in estrogen receptor-positive breast cancer patients. Am J Pathol. 2010;177:2205-2215.
22. Xu R, Antwi Boasiako P, Mao C. Alkaline ceramidase family: the first two decades. Cell Signal. 2021;78:109860.
23. Roy S, Khan S, Jairajpuri DS, Hussain A, Alajmi MF et al. Investigation of sphingosine kinase 1 inhibitory potential of cinchonine and colcemid targeting anticancer therapy. J Biomol Struct Dyn. 2021;10:1-13.
24. Hii LW, Chung FF, Mai CW, Yee ZY, Chan HH et al. Sphingosine kinase 1 regulates the survival of breast cancer stem cells and non-stem breast cancer cells by suppressing STAT1. Cells. 2020;9:886.
25. Ghasemi H, Jamshidi A, Ghatee MA, Mazhab-Jafari K, Khorasani M, et al. PPARγ activation by pioglitazone enhances the anti-proliferative effects of doxorubicin on promonocytic THP-1 leukemia cells via inducing apoptosis and G2/M cell cycle arrest. J Recept Signal Transduct. 2021;42(5).
26. Tashakori M, Kadia T, Loghavi S, Daver N, Kanagal-Shamanna R, et al. TP53 copy number and protein expression inform mutation status across risk categories in acute myeloid leukemia. Blood. 2022;140(1):58-72.
27. Suzuki K, Matsubara H. Recent advances in p53 research and cancer treatment. J Biomed Biotechnol. 2011;2011:978312.
28. Rahman N, Khan H, Zia A, Khan A, Fakhri S et al. BCL-2 Modulation in p53 Signaling Pathway by Flavonoids: A Potential Strategy towards the Treatment of Cancer. Int J Mol Sci. 2021;22(21):11315.
29. Pollyea DA, Pei S, Stevens BM, Smith CA, Jordan CT. The Intriguing Clinical Success of BCL-2 Inhibition in Acute Myeloid Leukemia. Ann Rev Cancer Biol. 2021;5:277-289.
30. Rahman N, Khan H, Zia A, Khan A, Fakhri S et al. BCL-2 Modulation in p53 Signaling Pathway by Flavonoids: A Potential Strategy towards the Treatment of Cancer. Int J Mol Sci. 2021;22(21):11315.
31. Watson C, Long JS, Orange C, Tannahill CL, Mallon E et al. High expression of sphingosine 1-phosphate receptors, (S1P1 and S1P3), sphingosine kinase 1, and extracellular signal-regulated kinase-1/2 is associated with development of tamoxifen resistance in estrogen receptor-positive breast cancer patients. Am J Pathol. 2010;177:2205-2215.
32. Xu R, Antwi Boasiako P, Mao C. Alkaline ceramidase family: the first two decades. Cell Signal. 2021;78:109860.
33. Roy S, Khan S, Jairajpuri DS, Hussain A, Alajmi MF et al. Investigation of sphingosine kinase 1 inhibitory potential of cinchonine and colcemid targeting anticancer therapy. J Biomol Struct Dyn. 2021;10:1-13.
34. Hii LW, Chung FF, Mai CW, Yee ZY, Chan HH, et al. Sphingosine kinase 1 regulates the survival of breast cancer stem cells and non-stem breast cancer cells by suppressing STAT1. Cells. 2020;9:886.
35. Ahn EH, Lee MB, Seo DJ, Lee M J, Kim Y, et al. Sphingosine Induces Apoptosis and Down-regulation of MYCN in PAX3-FOXO1-positive Alveolar Rhabdomyosarcoma Cells Irrespective of TP53 Mutation. Anticancer Res. 2018;38:71-76.
36. Young MM, Kester M, Wang H. Sphingolipids: regulators of crosstalk between apoptosis and autophagy. J Lipid Res. 2013;54:5-19.
37. Kim J, Kim H, Lee S. BCL2L13 protein prevents apoptosis in acute myeloid leukemia cells. Oncologie. 2023;25:0112.

38. Bonnaud S, Niaudet C, Pottier G, Gaugler MH, Millour J et al. Sphingosine-1-phosphate protects proliferating endothelial cells from ceramide-induced apoptosis but not from DNA damage-induced mitotic death. Cancer Res. 2007; 67:1803–1811.