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Evaluation levels of sphingosine, sphingosine-1-phosphate, and interleukin 2 and 3 in leukemia patients

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ABSTRACT

Sphingosine is the major naturally occurring base present in sphingolipids. It forms a primary part of sphingolipids, a class of cell membrane lipids that include sphingomyelin, an important phospholipid. Sphingosine-1-Phosphate (S1P) is a signaling sphingolipids, also known as glycosphingolipid. It is also referred to as a bioactive lipid mediator. The expression and localization of S1P receptors is dynamically regulated and controls immune cell trafficking. In vertebrates, S1P is found in the extracellular milieu and interacts with cell-surface receptors to regulate an array of cellular responses, including cell migration, differentiation and survival. Targeting various signaling pathways is a potential neoteric therapeutic for the treatment of leukemia. "Sphingosine and sphingosine 1 phosphate" are expressed in large amounts by leukemia cells. Leukemia is a group of blood malignancies that frequently start in the bone marrow and produce a lot of aberrant blood cells. Blasts, or leukemia cells, are the term for these immature blood cells. The study was participating 80 people (40 leukemia patients and 40 as healthy control group).

Result showed highly significant mean values at $p \leq 0.05$ of SPH (7.6), IL2 (283.2), and IL3 (196.1) in patients when compared with controls (3.9, 95.2, 145.5) respectively and S1P increased in patients but not significant. The association values of SPH, SIP, IL2 and IL3 in types of leukemia showed highly significant only in IL2 only. Therefore, can concluded the SPH increased with leukemia and led to development of leukemia because it is associated as signaling for cell proliferation with increasing levels of IL2-3.

Keywords: leukemia, sphingosine, sphingosine-1-phosphate, interleukin 2, interleukin 3

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INTRODUCTION

Leukemia is the most prevalent type of cancer across all age groups, particularly among kids. It happens because of the excessive proliferation and immature development of blood cells, which can harm red blood cells, bone marrow, and the immune system [1]. Leukemia has been classified into two types based on the kind of cell that is improperly multiplying: lymphoid and myeloid leukemia, and there are four subtypes of leukemia: Acute Lymphocytic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), and Chronic Myeloid Leukemia (CML) [2, 3]. The Sphingosine (SPH) class of natural chemicals is known to include compounds with long aliphatic and polar chains with 2-amino-1,3-diol-termini (2-amino-4-trans-octadecene-1,3-diol), an 18-carbon unsaturated alkyl chain amino alcohol that serves as the foundation for other sphingolipids. It is present in the membranes of all animal cells and many plant cells and is essential for an array of complex biological processes, including autophagy activity, cell proliferation, differentiation, and development [4]. One bioactive lipid known as Sphingosine-1-Phosphate (S1P) has been connected to the regulation of several physiological cell processes, including apoptosis, cell division, and angiogenesis [5]. Sphingosine-1-phosphate is produced by activated white blood cells, red blood cells, and platelets. Although endothelial cells are the major source of plasma S1P under many physiological conditions [5-7]. Sphingosine is created by two enzymes, Sphingosine Kinase 1 (SK1) and Sphingosine Kinase 2 (SK2), and a molecule of S1P has a ceramide backbone (SK2). By interacting with a G protein-coupled S1P Receptor (S1PR) on the cell membrane, S1P exerts its activity both inside and outside the cell membrane. Numerous investigations have revealed that S1P affects the development of cancer [8, 9]. S1P levels are typically high in blood and lymph and low in lymphoid tissues [10]. The formation of memory and effector cells, as well as the proliferation of T cells, depends on the T-cell growth factor IL2. Based on this function, the initial therapeutic application of IL-2 was to boost cancer patients' immune systems. It was so surprising that, in addition to the anticipated immunological deficit, systemic autoimmunity and lymphoproliferation also arose from the genetic deletion of the cytokine or its receptor. Later studies demonstrated that IL-2 is essential for survival and encourages development and functional activity [11]. Inter Leukin-3 (IL-3) is a multipotent hematopoietic growth factor generated by antigen-activated T lymphocytes, NK cells, monocytes, and endothelial cells. This

cytokine promotes hematopoietic progenitor cell growth and differentiation into granulocytes, megakaryocytes, erythrocytes, and mast cells. Interleukin 3 plays a significant role in diseases that are related to inflammation as well as autoimmunity. This is mostly generated by activated T-cells in response to stimuli. It acts as a link between the hematopoietic system, which produces cellular components for cellular defense in response to outside stimuli, and the immune system (T-lymphocytes) [12, 13].

MATERIALS AND METHODS

Study design

This study involved 80 individuals (40 leukemia patients and 40 healthy as control group). The patients were divided according to type of leukemia was previously diagnosed by their doctors responsible for their treatment. Acute Lymphoid Leukemia (ALL) included 13 patients, Acute Myeloid Leukemia (AML) included 3 patients, Chronic Lymphocytic Leukemia (CLL) included 3 patients and Chronic Myeloid Leukemia (CML), which included 21 patients. The study was conducted at the postgraduate research center of the College of Medicine and Al-Karamah Teaching Hospital in Wasit Province, Iraq during the period from February 2021 to October 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 whole blood (3 ml-5 ml) was collected from patient and control group for assay.

Examination of blood samples

Approximately 3 ml-5 ml of peripheral blood was obtained from all individual that participate in this study. Serum samples were separated by 3000 rpm/15 min centrifugation to separate serum for ELISA tests and preservation at -20°C. The serum was placed in a cool box and then transferred to the laboratory. All assays for (IL2, IL3, SPH, and S1P) all down according special its kit and all kits were bought from Foresight USA they designed for accurate quantitative measurement.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) has been used to manage data. Qualitative data are expressed in frequency and percent, and quantitative data in average and median. The statistical analysis used determines the frequency with a significant when p-value is $p \leq 0.05$. A one-way 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, as shown in table 1.

Tab. 1. Patients and healthy persons demographic data

Item	No.	Frequency
Patients	40	100
Male	25	62.5
Female	15	37.5
Disease		
ALL	13	32.5
AML	3	7.5
CLL	3	7.5
CML	21	52.5
Healthy persons No.	**40**	**100**
Male	27	67.5
Female	13	32.5

No.= Number

The study was conducted on 80 people. There is a difference between the values of SPH (0.0001), SIP (0.08), IL2 (0.0001), and IL3 (0.0001) in patients when compared with controls, with a mean ± SD of Overall, the findings indicate a significant increase ($p \leq 0.05$). As shown in table 2, statistical analysis revealed an increase in the levels of all parameters except SIP. It seems a little loud, but there isn't much difference.

The endogenous mediator of apoptotic cell death signaling is sphingosine. Upon exposure to sphingosine or its N-methylated derivative, N-dimethyl sphingosine causes morphological changes and DNA fragmentation within the nucleus in leukemic cells, indicating apoptosis [14].

Tab. 2. Association between SPH, SIP, IL2, and IL3 for patients and controls

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	
IL2	Patients	40	283.2	5.4	0.0001
	Control	40	95.2	1.1	
IL3	Patients	40	196.1	5	0.0001
	Control	40	145.5	1.8	

SD= Standard Deviation; No.= Number

The level of sphingosine demonstrated a highly significant increase in patients with leukemia compared with the control, a result that agrees with the literature [15, 16]. An increase in intracellular sphingosine is also accompanied by an increase in ceramide, and this increase results from the degradation of ceramide. Notably, the rise of these two sphingolipids metabolites occurs before the start of apoptosis [6]. The ground breaking discovery that sphingosine regulates apoptosis in HL-60 pro-myelocytic leukemic cells served as the catalyst for the discovery of the bioactive activities of Sphingo Lipids (SLs) in the regulation of vital cellular functions, such as apoptosis [17]. In addition to the cellular structural significance of some of its components, this relationship not only established a new function for sphingolipids metabolism but also firmly established SL metabolism association with hematological cancers [16]. This has served as the foundation for a growing corpus of research and sparked numerous fresh insights into the role of Sphingo Lipids (SLs) in hematological malignancies. Multiple normal and pathological disorders, including numerous hematological malignancies including leukemias, lymphomas, and myelomas, have been found to include SLs in cellular differentiation, senescence, proliferation, and other processes [16]. S1P a bioactive lipid that cells can release can activate a family of G protein receptors, can also connect to intracellular target proteins like HDAC (sister proteins that control access to DNA by modulating chromatin) to activate cellular responses. S1P receptors, such as S1P4 and SK1, are increasingly being shown to have a role in cancer [13]. In chronic myeloid leukemia (S1PR2), S1P promotes the anti-apoptotic protein Mcl-1 (Myeloid Cell Leukemia-1) and its binding to S1P receptor type 2. In acute myeloid leukemia, S1P promotes mutagenic signaling by activating NF-kB (the transcription factor plays a crucial role in mediating inflammatory reactions and encourages the expression of a number of pro-inflammatory genes, including those that code for cytokines), which prevents apoptosis in U937 and HL-60 cells. Moreover, T Acute Lymphoblastic Leukemia S1P suppresses classical apoptosis (T-ALL). Moreover, the amount of SPHK1 is elevated in B-ALL, which aids in the growth of murine BCR/ABL1 ALL. Low S1P and ceramide levels are essential regulators of leukemic cells' resistance to drug-induced apoptosis [18]. In the current study, the level of S1P was slightly elevated but not significant, which is in agreement with [18]. In CML, S1P enhances Mcl-1 (An Anti-Apoptotic Protein) and its binding to S1P receptor-2 [19]. S1P induces mutagenic signaling in acute myeloid leukemia through activation of NF-kB [20]. Furthermore, sphingosine-1-phosphate inhibits apoptosis in T-cell acute lymphocytic leukemia [20]. Reduced levels of S1P and/or ceramide are important regulators of leukemia cells' resistance to drug-induced apoptosis, according to the results of most studies [21]. There is a dual role for cytokines in the biology of cancer. They may play a role in the immune system's ability to regulate cancer, but they may also have an impact on how quickly cancer spreads and develops [22]. It has been shown in several studies that the levels of IL2 and IL3 are elevated in patients with leukemia, and this is consistent with the current study. The level of IL-3 expression was investigated by Testa, and they showed a higher level of IL-3 in leukemic stem cells and lower expression in normal hematopoietic stem cells, making it a marker of leukemic stem cells and a target for treatment [23]. Individuals with most types of lymphoid malignancies also have higher serum levels of soluble interleukin-2 receptors, as do individuals with reactive diseases or solid tumors, such as severe inflammation. To assess the diagnostic value of soluble interleukin-2 receptor levels for lymphoma screening and differential diagnosis [24].

The result showed revealed a link between SPH and AML-detected leukemia in 3 patients with a mean ± SD of 9.8 ± 5.8, while all including ALL, 13 patients, showed a mean ± SD of 7.4 ± 1.0, and CML included 21 patients. Finally, 7.6 ± 1.8 in three CLL patients means ± SD, with 6.8 ± 1.1. We noticed a high level of sphingosine in all types of leukemia at varying rates, with no significant difference in the p-value (0.2). As shown in table 3, types of leukemia and S1P include: AML (3) patients mean ± SD, 49.9 ± 1.4. ALL (13) patients mean ± SD, 58.9 ± 5.9; CML (21) patients mean ± SD, 42.1 ± 2.7 and CLL (3) patients mean ± SD, 54.2 ± 3.9. We observe a high level of sphingosine in all types of leukemia at varying rates with no significant difference ($p \leq 0.05$) (0.7). The relationship between IL-2 and the four types of leukemia is as follows: AML (3) patients mean ± SD, 270.8 ± 1.8; ALL (13) patients mean ± SD, 280.6 ± 3.5; CML (21) patients mean ± SD, 301.3 ± 4.3; and CLL (3) patients mean ± SD, 179.6 ± 1.1. We observe a high level of sphingosine in all types of leukemia at varying rates, with a highly significant difference of 0.002 ($p \leq 0.05$), Finally, the following is the link between four types of leukemia and IL3: AML (3) patients mean ± SD, 189.4 ± 2.4. ALL (13) patients mean ± SD, 179.0 ± 1.6; CML (21) patients mean ± SD, 212.6 ± 6.4 and CLL (3) patients mean ± SD, 161.7 ± 1.1. We observe a high level of sphingosine in all types of leukemia at varying rates, with no significant difference ($p \leq 0.05$) (0.1).

Tab. 3. Association between SPH, SIP, IL2 and IL3 for patients and types of leukemia

Item		No.	Mean	SD	p-value
SPH	ALL	13	7.4	1	0.2
	AML	3	9.8	5.8	
	CLL	3	6.8	1.1	
	CML	21	7.6	1.8	
SIP	ALL	13	58.9	5.9	0.7
	AML	3	49.9	1.4	
	CLL	3	54.2	3.9	
	CML	21	42.1	2.7	
IL2	ALL	13	280.6	3.5	0.002
	AML	3	270.8	1.8	
	CLL	3	179.6	1.1	
	CML	21	301.3	4.3	
IL3	ALL	13	179	1.6	0.1
	AML	3	189.4	2.4	
	CLL	3	161.7	1.1	
	CML	21	212.6	6.4	

SD= Standard Deviation; No.= Number

In HL-60 leukemic cells (the cell line for human promyelocytic leukemia), sphingolipids and apoptosis were first linked historically, which led to the discovery that deregulation of sphingolipids metabolism plays a complicated role in hematological cancer [25]. Less complicated sphingolipids and metabolic enzymes are also essential components for cell activity. A perturbed balance between lipid species may cause a wide range of diseases, including neurodegenerative diseases and cancer [26]. In our results, we noticed an increase in the level of SPH but not a significant difference in all types of leukemia, with similar proportions. High SPH may come from a high level of SPHK2 in the blood. SPHK catalyzes the phosphorylation of SPH to form S1P, considerably altering its function and changing sphingosine's charge. Also, SPHK2 promotes apoptosis and cell cycle arrest, and in addition to its "pro-apoptotic" functions, it may also play a cytoprotective role. [27]. Most studies confirm a high percentage of SIP in leukemia patients, and our results showed a high but not significant difference, and this is agreed upon [28]. In the past, it has been demonstrated that sephengolipid metabolism helps Chronic Lymphoblastic Leukemia (CLL) cells survive, at least in part because of known CLL survival cues [29]. In contrast to sphingosine and ceramide, which cause apoptosis and cell growth arrest, S1P encourages cell survival and proliferation [30]. The delicate balance between these sphingolipids, which have competing roles and are interconvertible inside cells, can determine the fate of the cell. One of the two known SPHKs, sphingosine kinase 1, controls the progression of cancer, making it an important component of the sphingosine-S1P balance. S1P interacts with the S1PR1–S1PR5 family of G protein-coupled receptors. The five G protein-coupled S1P Receptors (S1PR1–S1PR5) that S1P binds to are distributed differently in different cell types. Through the heterodimerization of these receptors via different G-alpha subunits, S1P is able to accurately exert its impact on a number of pathways to stimulate growth, differentiation, cell migration, and cell trafficking [31]. Increased S1P-driven signaling can exacerbate pathological conditions and put people at risk for developing cancer [30]. Actually, ceramide derivatives, sphingosine, and agents that modulate their endogenous levels are being considered as potential anti-cancer agents. However, understanding the molecular mechanisms and biological activity of these lipids may serve as targets for therapeutic development and lead to drugs with better specificity and efficacy [32]. The main growth agent for T lymphocytes, Inter Leukin-2 (IL-2), also promotes the activity of natural killer cells. IL-2 has now been demonstrated to be an effective treatment for a small number of leukemias that are often refractory. Patients with chronic myelogenous leukemia who have relapsed after receiving an allogeneic transplant can achieve long-term remission by receiving T-cell re-infusions. Therefore, IL-2 levels are generally elevated in leukemic patients, which agrees with our results [33].

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