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Diagnostic Value of ^{1}H NMR-Based Metabolomics in Acute Lymphoblastic Leukemia, Acute Myeloid Leukemia, and Breast Cancer

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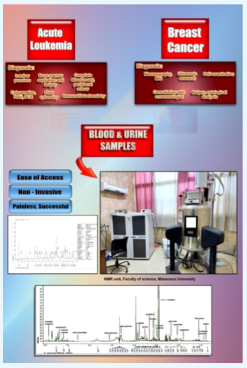
ABSTRACT: Cancer refers to a massive number of diseases distinguished by the development of abnormal cells that divide uncontrollably and have the capability of infiltration and destroying the normal body tissue. It is critical to detect biomarkers that are early detectable and noninvasive to save millions of lives. The aim of the present work is to use NMR as a noninvasive diagnostic tool for cancer diseases. This study included 30 plasma and 21 urine samples of patients diagnosed with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), 25 plasma and 17 urine samples of patients diagnosed with breast cancer (BC), and 9 plasma and urine samples obtained from healthy individuals as controls. They were prepared for NMR measurements; then, the metabolites were identified and the data were analyzed using multivariate statistical procedures. The OPLS-DA score plots clearly discriminated ALL, AML, and BC from healthy controls. Plots of the PLS-DA loadings and S-line plots showed that all metabolites in plasma were greater in BC than in the healthy controls, whereas lactate, *O*-acetylcarnitine, pyruvate, trimethylamine-*N*-oxide (TMAO), and glucose were higher in healthy controls than in ALL and AML. On the other hand, urine samples showed lower amounts of lactate, melatonin, pyruvate, and succinate in all of the studied types of cancer when compared to those of healthy controls. ^{1}H NMR can be a successful and noninvasive tool for the diagnosis of different types of cancer.



1. INTRODUCTION

There is no doubt that cancer is an unusual growth of cells. It is produced by numerous modifications in gene expression affecting the stability of cell proliferation and death. It finally develops into a population of cells that have the ability to attack tissues and metastasize to remote sites, leading to significant disease and, if untreated, death.[1]

It is known that leukemia is one of the main causes of death worldwide.[2] The leading etiology of acute leukemia is the malignant alternate of myeloid or lymphoid cells into unique and homogeneous cells.[3]

It could be divided into two chief categories: chronic and acute leukemia; both chronic and acute leukemias are considered myeloid or lymphocytic.[4] Among the reasons that cause the progress of leukemia are gene mutations and translocations, deregulation of the immune system, and adjustments within the bone marrow surroundings.[5]

Acute myeloid leukemia (AML) is common in adults than in youngsters,[4] while acute lymphoblastic leukemia (ALL) is the regular form of youth leukemia and the second most common in adults.[6]

For analysis of leukemia, immunohistochemical and immunologic techniques in addition to the examination of smears of bone marrow aspirates are used.[7] Regardless of the use of these strategies, numerous patients are not recognized early enough as the symptoms are vague and unspecified.[2] Without suitable treatment, patients with acute leukemia live only for a few weeks.[8] Consequently, it is crucial to detect biomarkers that are early detectable and noninvasive to save the lives of a countless number of patients through timely intervention.[2] It is noted that breast cancer (BC) develops when cells in the breast tissue divide and proliferate without control. It is the most common cancer among women and affects approximately 10% of all women at some stages of their life.[9]

Blood tests for tumor markers, periodic mammography, self- or physician-performed examination, and carcinoembryonic antigen (CEA), tissue polypeptide specific antigen, and human epidermal growth factor receptor 2 identification are all common methods of routine monitoring for BC.[10] The fact that the diagnosis is often delayed due to limitations in screening tests is an additional factor that contributes to the poor prognosis of BC patients.[11] There is a growing significance of superior magnetic resonance (MR) techniques in most cancer diagnostics.[12]

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Table 1. Patients' Characteristics (WBC = White Blood Cells, HGB = Hemoglobin, PLT = Platelets)

no	sex	age	diagnosis	grade/stage	WBC (K/μL)	HGB (g/dL)	PLT (K/μL)
1	F	60	infiltrating ductal carcinoma	II	9.1	10.4	215.3
2	F	33	invasive ductal carcinoma	II	7.027	13.48	507.2
3	F	34	invasive ductal carcinoma	III	8.751	12.13	442.2
4	F	38	invasive ductal carcinoma	II	4	12.2	415
5	F	36	invasive ductal carcinoma	II	7.836	11.34	402.7
6	F	58	invasive ductal carcinoma	III	8.349	13.23	139.7
7	F	38	infiltrating ductal carcinoma	II	4.714.	11.94	240.3
8	F	37	invasive ductal carcinoma	III	2.579	12.06	255.8
9	F	61	invasive ductal carcinoma	II	10.1	12	243
10	F	59	invasive ductal carcinoma	III	5.5	12.9	229
11	F	45	invasive ductal carcinoma	III	9.516	11.33	299.6
12	F	44	infiltrating ductal carcinoma	III	3.8	12.3	180
13	F	48	metastatic poorly differentiated carcinoma	IV	5.1	13.5	324
14	F	66	invasive mammary carcinoma	II	6.9	11.8	373
15	F	78	infiltrating ductal carcinoma	II	8.955	9.396	413.6
16	F	51	infiltrating ductal carcinoma	II	9.884	11.97	310.6
17	F	59	ductal carcinoma	I	7.7	11.1	223
18	F	38	infiltrating ductal carcinoma	II	10	12.86	390.7
19	F	35	invasive ductal carcinoma	II	9.078	8.61	283.9
20	F	53	ductal carcinoma	III	5.987	11.81	427.3
21	F	74	infiltrating ductal carcinoma	II	6.392	11.98	217
22	F	41	infiltrating ductal carcinoma	II	5.81	13.5	328
23	F	54	invasive ductal carcinoma	II	7.9	12.1	315
24	F	22	invasive ductal carcinoma	II	7.183	13.39	298.8
25	F	49	invasive ductal carcinoma	III	8.514	12.19	232.1
26	F	51	AML	M(1−2)	26.75	7.43	18.21
27	F	62	AML	M(4−5)	9.64	7.22	67.55
28	F	28	AML	M(4−5)	38.52	9.7	38.6
29	F	50	AML	M4	41.4	6.2	159
30	M	47	AML	M6	1.842	7.249	18.91
31	F	52	AML	M2	3.8	6.8	47
32	F	37	AML	M4	22.7	7.6	125
33	M	61	AML	M4	34.6	7.84	38.6
34	F	38	AML	M(1−2)	28.22	10.29	72.17
35	F	31	AML	M(1−2)	2.1	8.1	169
36	M	42	AML	M(1−2)	2.1	7.1	241
37	F	63	AML	M2	61.1	5.81	80.13
38	F	23	AML	M(4−5)	41.6	6.9	56
39	F	31	AML	M3	2.55	6.9	16.1
40	F	42	AML	M(1−2)	14.41	11.54	11.96
41	M	59	AML	M(1−2)	153.8	7.58	9.6
42	M	21	ALL	T-ALL	116	9.1	35
43	M	34	ALL	T-ALL	142	10.9	104
44	F	44	ALL	B-ALL	12.5	6.4	5
45	M	22	ALL	T-ALL	205	7.6	40
46	M	58	ALL	T-ALL	93.9	6.9	9
47	M	18	ALL	B-ALL	0.9	6.9	28
48	M	26	ALL	B-ALL	35.2	11	33
49	M	41	ALL	T-ALL	151.2	12.77	30.56
50	M	68	ALL	B-ALL	119.4	7.41	148.2
51	F	42	ALL	T-ALL	4.16	11.71	188.8
52	M	37	ALL	T-ALL	8	12.1	53.7
53	M	26	ALL	B-ALL	3.1	3.8	35
54	F	54	ALL	T-ALL	89.37	11.29	22.86
55	F	53	ALL	B-ALL	36.2	7.5	35

It has been shown that the assessments of bone marrow and blood are essential, in particular for prognosis. Studies of immunology and cytogenesis are beneficial in the statistics of prognosis. There is no doubt that the early stages of leukemia during illness or remission can establish the presence of leukemia through blood tests.[2]

Variations in the concentrations of metabolites have been connected to the biochemical status of organisms and reflect

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alterations in metabolism due to biologic conditions, including disease and response to chemical treatment. Recent studies demonstrate the applicability of NMR-based metabolomics using samples of serum for the diagnosis and prognosis of disease.[13,14]

Urine samples also offer some advantages for carrying out metabolomics studies as they can be collected noninvasively and have a less-complex composition compared to other biofluids, therefore simplifying the novel biomarker discovery.[15] Many new techniques in most cancers' metabolism have been utilized to discover metabolites and metabolic activities.[16]

Metabolites are considered to be the end products of gene expression and a direct result of enzymatic and protein activity. Thus, metabolites are more closely related to a phenotype or illness than genetic or proteomic data.[17,18] Quantifying metabolites (metabolomics) is a more complex system of metabolic evaluation than evaluating the events of metabolic pathways, according to one popular theory.[19]

Metabolomics is regularly being employed as a biomarker discovery technique. Tissues and biofluids have been used for the detection of early diagnostic metabolite biomarkers of most cancers in current years.[20,21]

Nuclear magnetic resonance (NMR) has been utilized in biofluid analysis that include plasma,[22] serum,[23] cerebrospinal fluid,[24] pus,[25] saliva,[26] feces,[27] cervicovaginal secretions,[28] and urine.[29] NMR has also been applied for intact tissue sample analysis.[30,31]

Blood promptly revealed the internal state of the body, including metabolic, immune, and nutritional states.[32] The withdrawal of the sample is invasive, and the high-abundance molecules affect the identification of low-abundance proteins.[33]

It is known that urine has been checked for a long time as a base of assistive information for the determination of several disorders. Urine carries waste materials from numerous metabolic pathways.[32] Information of the inside organs can be provided by urine and urinary tracts instead of plasma by kidney glomerular filtration.[33,34]

NMR permits smooth quantitative analysis of metabolite concentrations and offers numerous resources of metabolite identification. One of the main benefits of using NMR as a tool for metabolomics is its ease in dealing with sample.[35]

Primarily NMR-based metabolomics was integrated with the corresponding statistical strategies and completed with independent samples to discover disease-precise variations of metabolites and, in addition, validate disorder biomarkers.[36] This is because metabolomics provides more accurate information about the biological system,[37] which can be used for disorder diagnosis[38,39] and cure,[40,41] drug toxicological mechanism analysis,[42,43] and precision medicine.[44]

The aim of the present study is to use NMR as a noninvasive diagnostic tool for different cancer diseases.

2. MATERIALS AND METHODS

2.1. Collection of Blood Plasma and Urine Samples. A total of (14 ALL + 16 AML + 25 BC) patients participated from the Oncology Center of Mansoura University (between 2018 and 2020). They were first diagnosed and had not taken any previous therapy. Consents have been obtained from all patients prior to the study according to the Helsinki declaration. The study has been approved by the Mansoura University, Faculty of Medicine IRB.

The diagnosis of acute leukemia was based on the morphological examination of bone marrow smear (blast cells are equal to or more 20%) and the peripheral blood smear and was confirmed by immunophenotyping using flow cytometry.

The panels used for the diagnosis of acute leukemia include flow cytometry determination of CD25/CD123 cell antigen expression, while the diagnosis of BC was based on the histopathological examination of surgical biopsy obtained from breast tumors. The samples were collected after overnight fasting; the blood was collected in standard green top glass vacutainers for clotted blood, and the urine was collected in cups of 25 mL volume. The characteristics of patients are shown in Table 1.

The samples were centrifuged for 5 min in a centrifuge type MPW 300, and the supernatant was taken for NMR sample preparation.

2.2. Preparation of the NMR Sample. A total of 120 μL of phosphate buffer 0.5 M (pH was adjusted to 7) containing 0.75% w/v sodium azide was added to plasma (420 μL). In total, 180 μL of 0.4 M phosphate buffer (pH 7; containing 0.75% w/v sodium azide) was added to urine (540 μL) present at room temperature. These samples were allowed to stand for 20 min and allowed to centrifuge at 13 000 rpm for 3 min in a centrifuge type MPW 300. The filtrate was moved to a dry-cleaned tube, and the pH was measured and adjusted to 7. After 20 min, the samples were centrifuged again. After that, precipitation was no longer observed following centrifugation, and the supernatant was gathered. A total of 540 μL of the resulting sample was mixed with 200 μL of D_2O.[45]

2.2.1. Nuclear Magnetic Resonance (NMR). A JEOL JNM.ECA II 500 MHZ (JAPAN) high-performance Fourier transform (FT) NMR-500 MHZ spectrometer (Faculty of Science, Mansoura University), supported with a broad band probe fully automatic tune matching with a pulse field gradient (Liquid Royal Probe), (instead of using TMS as a reference, FT-NMR automatically adjusts the zero point of the chemical shifts using a deuterium as a looking agent), was used for one-dimensional (1D), proton NMR data acquisition of both blood plasma and urine samples. ECA standard software Delta 5.0 is used.

Both blood plasma and urine samples were measured using D_2O as a solvent. Water suppression uses presaturation at 4.662 ppm; the resulting spectra can be mostly free of the solvent signal causing the improvement.

1D spectra were acquired with a relaxation delay of 5 s. With a flip of 45° to guarantee near-complete longitudinal relaxation, spectral width of 14.5 kHz, and acquired time of 1.74587904 s, the NMR receiver gain was 54, temperature was 21 Celsius degree, and the number of scans was 180.

2.3. Metabolite Identification and Statistical Analyses. Metabolites were identified using Chenomx NMR Suite 8.5 Professional (Spectral Database, Edmonton, Alberta, Canada). All the ^{1}H NMR signals were fit into the Chenomx database. All processed data were binned to a 0.04 ppm as bin size, except for the water signal region (4.68−4.88 ppm) and numerically transformed. Analyses of transformed data by multivariate statistical procedures were performed with statistical software SIMCA 16.0 (Umetrics, Umeå, Sweden). Data were analyzed using principal component analysis (PCA). To maximize the separation between samples, partial least-squares discriminant analysis (PLS-DA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) methods were utilized.

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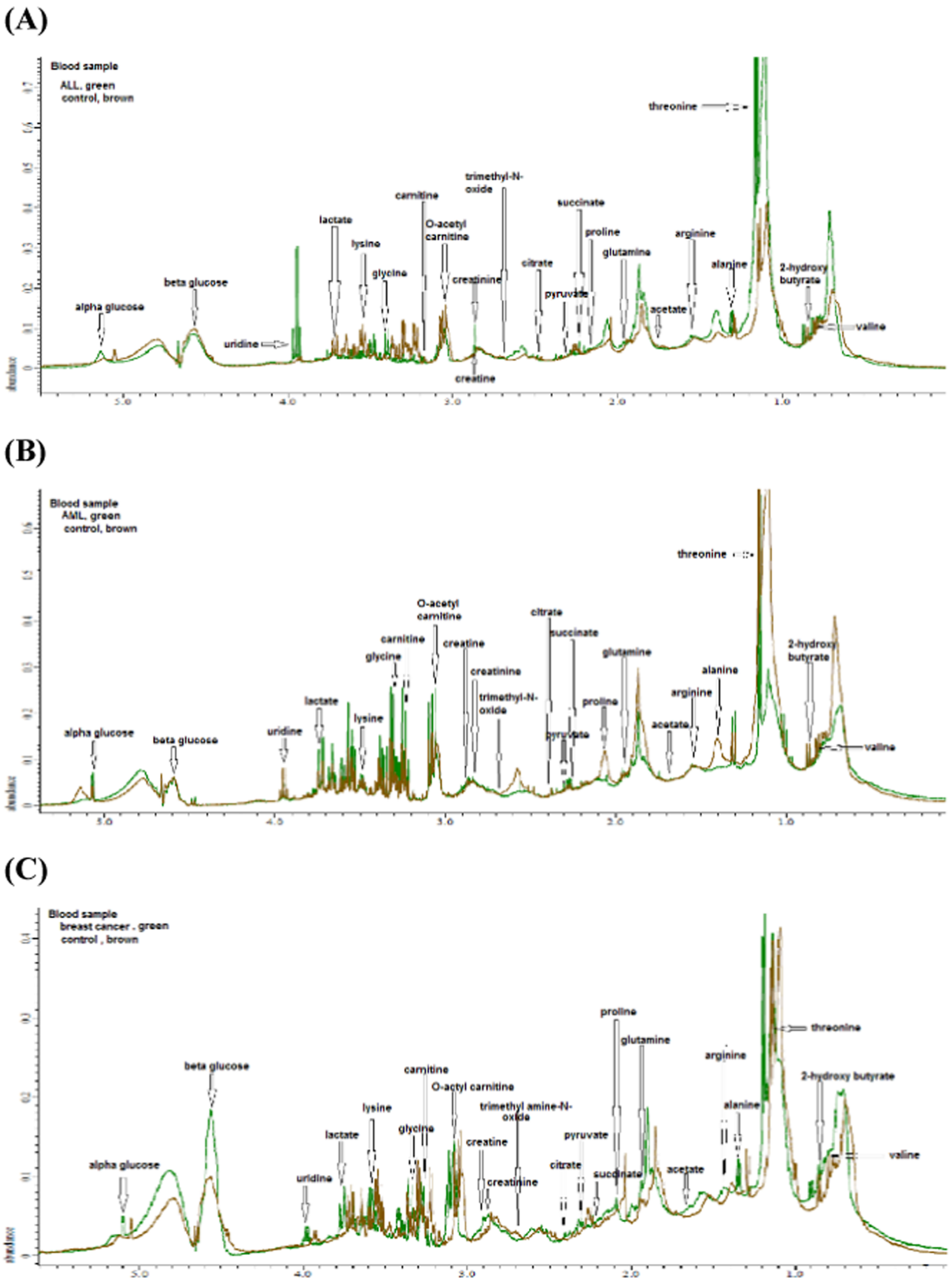

Figure 1. Comparison of blood plasma samples; (A) ALL vs healthy control, (B) AML vs healthy control, and (C); BC vs healthy control. The green color indicates the disease type, and the brown color indicates the control.

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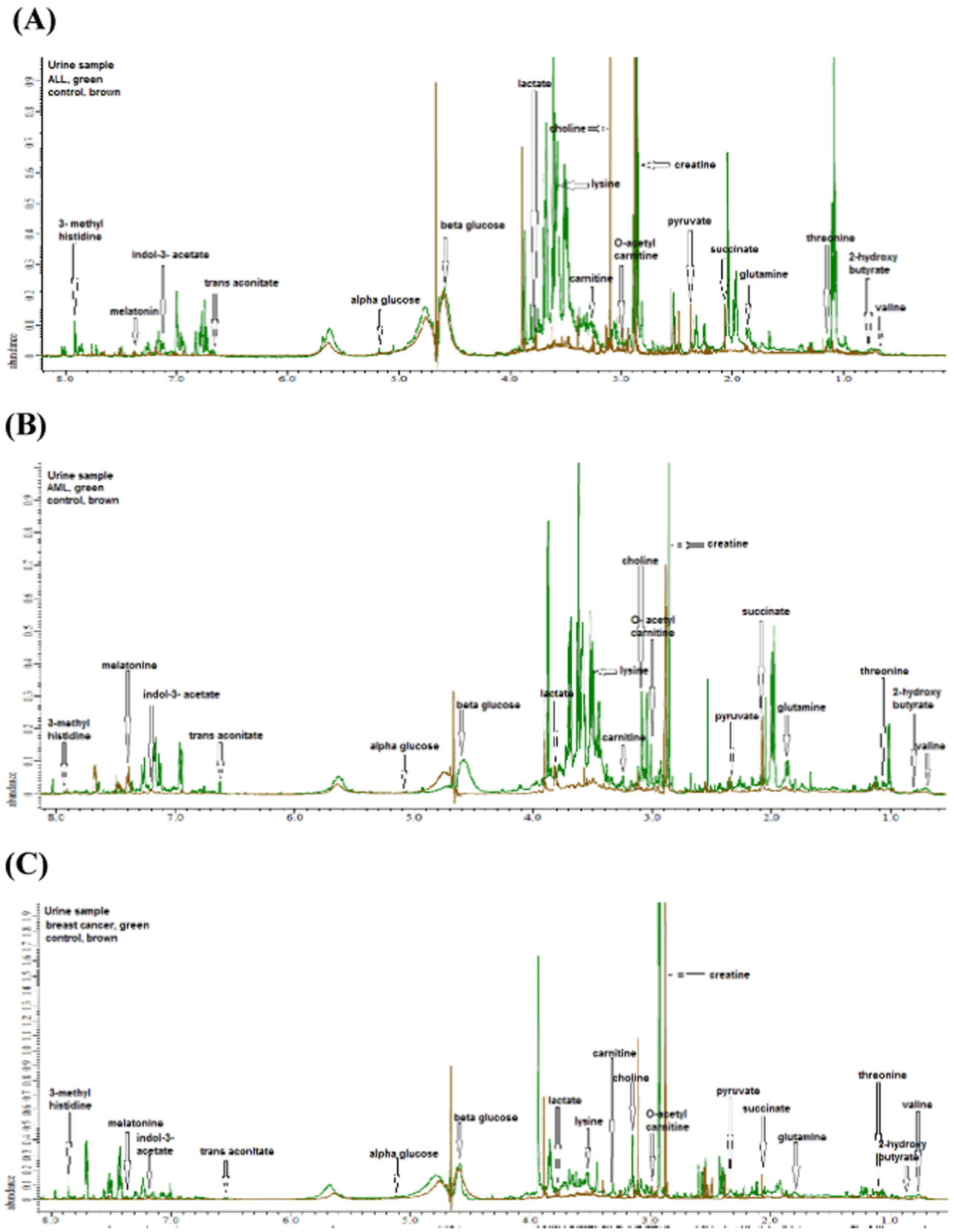

Figure 2. Comparison of urine samples; (A) ALL vs healthy control, (B) AML vs healthy control, and (C) BC vs healthy control. The green color indicates the disease type, and the brown color indicates the control.

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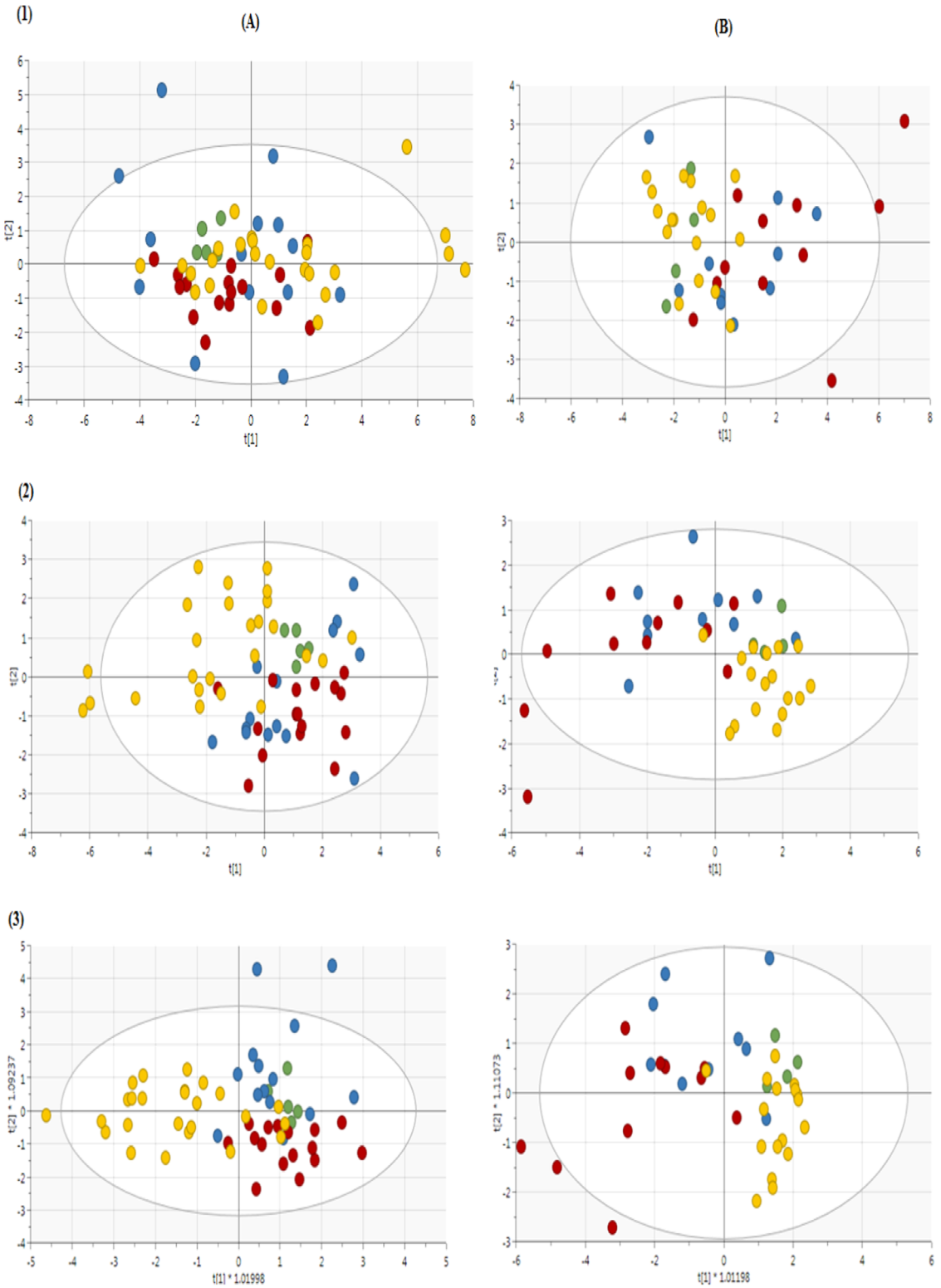

Figure 3. (1) PCA, (2) PLS-DA, and (3) OPLS-DA score plots for healthy controls (green), ALL (blue), AML (red), and BC (yellow) in blood (A) and urine (B) samples.

3. RESULTS

One-dimensional ^{1}H NMR spectra were acquired on a total of 60 blood plasma and 42 urine samples from ALL, AML, and BC patients, together with healthy controls. Sections of blood plasma and urine NMR spectra of one AML patient, one ALL patient, one BC patient, and a healthy control are represented in Figures 1A–C and 2A–C.

To confirm these visual observations, multivariate analysis on the data was implemented. The unsupervised principle component analysis (PCA) was initially done to attain a tendency of separation of samples according to groups. Values

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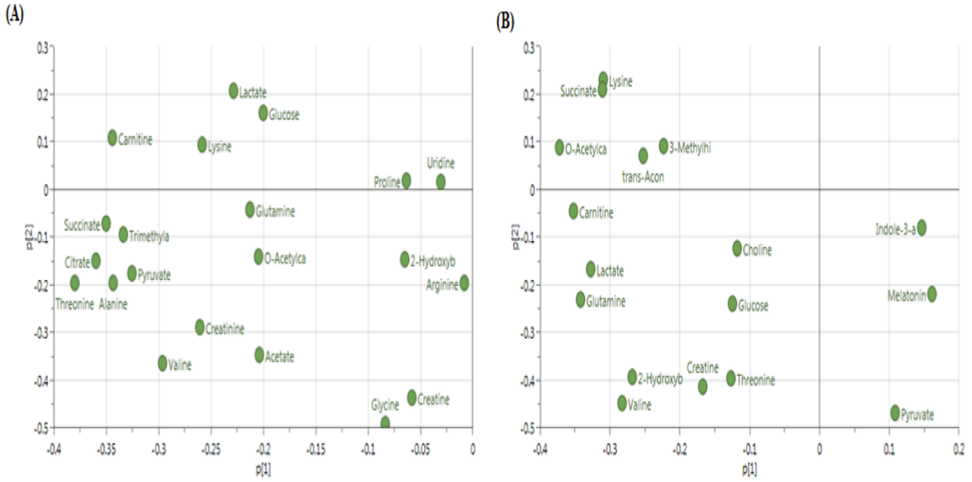

Figure 4. PLS-DA Loading plots of (A) blood and (B) urine samples.

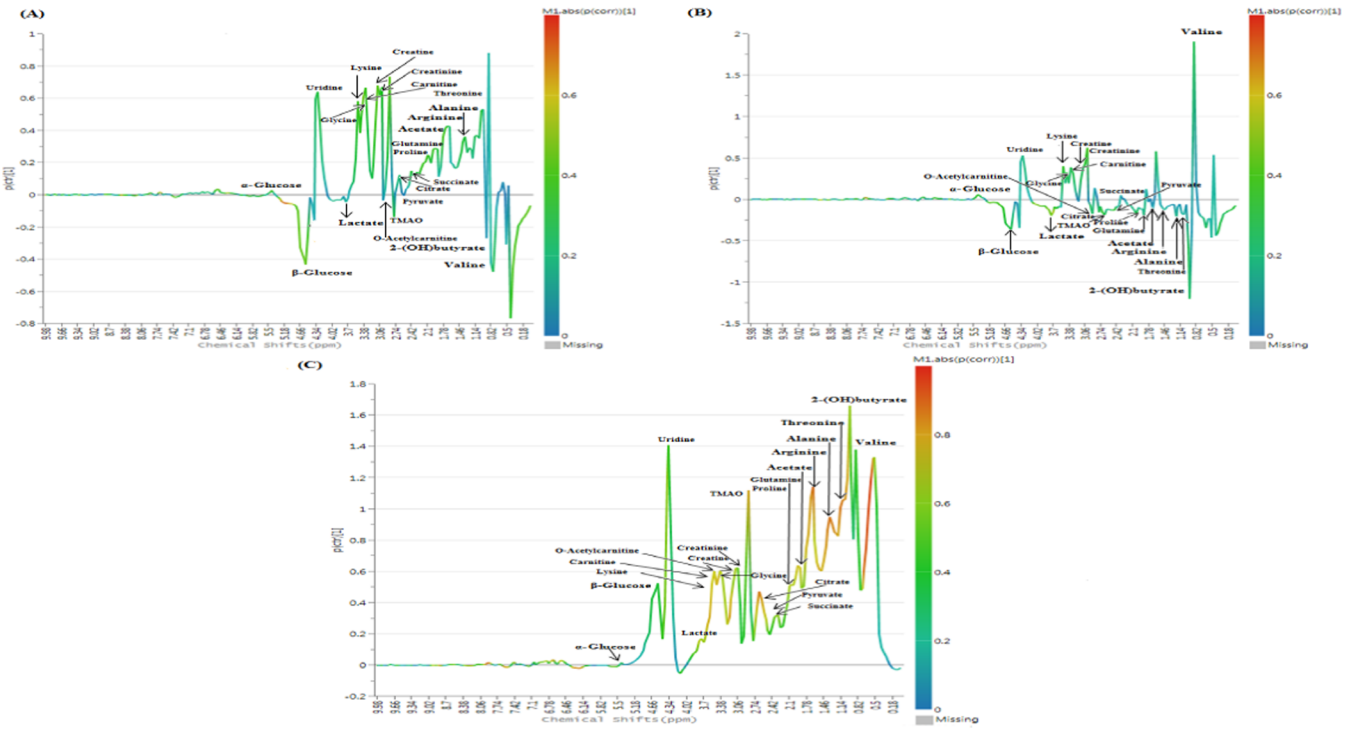

Figure 5. OPLS-DA S-line plots of the healthy controls versus (A) ALL, (B) AML, and (C) BC blood plasma samples.

falling outside Hotelling's T2 with a confidence limit of 95% are considered outliers. For further separation of groups, PLS-DA and OPLS-DA models were also generated (Figure 3A,B).

Plots of the PLS-DA loadings (Figure 4) showed that all metabolites are significant in characterizing BC samples from other groups in blood. However, in urine, lysine, succinate, *O*-acetylcarnitine, 3-methylhistidine, and *trans*-aconitate separated ALL patients from the other groups. On the other hand, carnitine, lactate, choline, glutamine, glucose, creatine, 2-hydroxybutrate, threonine, and valine were somehow significant in AML urine samples. BC urine samples were distinguished with indole-3-acetate, melatonin, and pyruvate. Interestingly, none of the metabolites have separated the healthy controls from all patients.

Supervised analyses using OPLS-DA were employed to identify the spectral features that discriminate each group form the others. S-line plots were performed to show differentiating features in NMR metabolomics (Figures 5 and 6). Representative metabolites appeared slightly different according to each cancer type.

In blood, all metabolites were higher in BC than in healthy controls, whereas relative amounts of lactate, O-acetylcarnitine, pyruvate, trimethylamine-*N*-oxide (TMAO), and β-glucose were higher in healthy controls than in ALL and AML. On the other hand, urine samples showed lower amounts of lactate, melatonin, pyruvate, and succinate in all types of cancer (ALL, AML, and BC) when compared to those of healthy controls.

4. DISCUSSION

The metabolism of cancer is one of the early trends of research in the biology of cancer. It depends on the fact that metabolic activities are changed in cancer cells compared to those in

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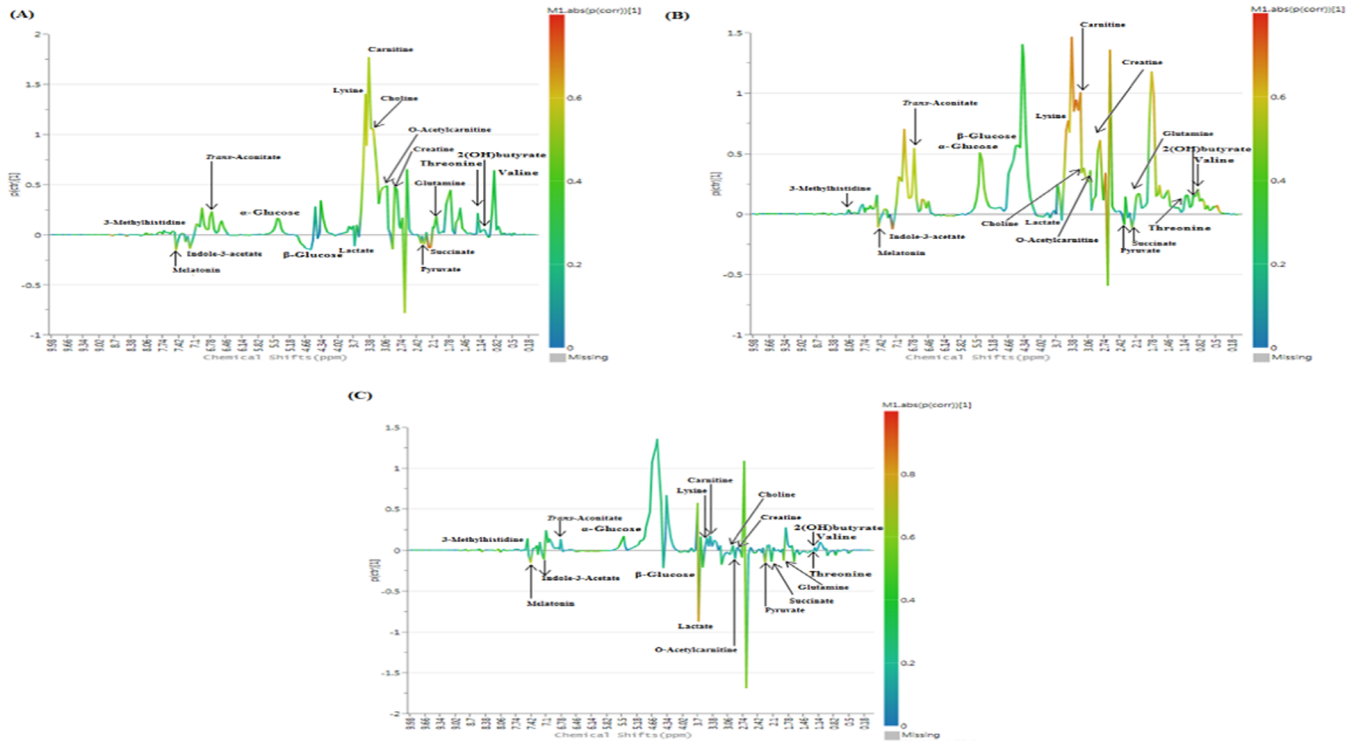

Figure 6. OPLS-DA S-line plots of the healthy controls versus (A) ALL, (B) AML, and (C) BC urine samples.

normal ones.[46] As a heterogeneous disease, every type of cancer has its own metabolic characteristics.[47]

4.1. Metabolites in Blood Plasma. The metabolic conditions of patients can be affected by the alterations of the structure and concentration of amino acids.[48] In the patients of cancer, the amino acid metabolism properties can be that (1) the amino acids decrease in the body of cancer patients as tumor cells have the ability to take amino acids faster than the normal ones and (2) tumor tissue can compete with the host for nitrogen compounds and consume a different group of essential and nonessential amino acids to meet the metabolism and growth needs and also for the proliferation of cells.[49,50]

Normal cells compared to tumors posses less amino acid demand from extracellular fluids.[51] Valine, isoleucine, and leucine, i.e., branched-chain amino acids (BCAAs), are essential amino acids, and energy manufacturing in cancer cells depends on them. Hattori et al. said that changes in the metabolism of BCAAs affect the cancer progress in myeloid leukemia.[52] Similarly, the present study showed that the plasma level of valine in AML patients was higher than in healthy controls.

Miyagi et al. reported that there was a higher plasma level of threonine, proline, glycine, alanine, and lysine in breast cancer than in the control group.[53] This was in line with the results obtained from ALL and BC patients.

Recently, it has been reported that glycine is related to the cancer cell proliferation; Taherizadeh and his co-workers found high plasma glycine levels in patients with esophageal cancer.[54] The high level of glycine in the plasma of patients with ALL, AML, and BC in our results was confirmed by other reports.[54,55]

Glutamine, the most abundant amino acid found in the body, is released from the skeletal muscles and is an essential interorgan transporter of nitrogen and carbon.[56] Our results showed that the circulating glutamine level was low in the patients with AML as its consumption was increased, in agreement with another report.[57]

The metabolism of creatine/creatinine plays an important role in the energy production of muscles, carcinogenesis, and progression of cancer. Creatine and phosphocreatine are the primary origins of energy-producing ATP. The level of serum creatinine was high, and this shows the kidney function impairment. A higher risk of cancer is connected to higher serum creatinine concentrations.[58] These findings were in agreement with the increased levels of creatine and creatinine in ALL, AML, and BC found in our results.

Tumor biology and carcinogenesis are affected by a number of biosynthetic pathways involving arginine. Nitric oxide (NO), which is a signal transduction molecule, has been found to be derived from the metabolism of arginine. It can participate in different events that result in cancer.[59] The decreased levels of arginine in patients with AML in this study confirmed these findings.

It is known that lactate is the outcome of anaerobic glycolysis, and its level is high in hypoxia, ischemia, and poorly vascularized cancer. The level of lactate significantly increased in several cancers in humans.[60,61] High levels of lactate have been found in breast tumors due to metabolic alterations.[62] However, in some other types of cancer such as pancreatic cancer, lactate levels were lower in the serum.[63] This confirmed the results of the patients suffering from ALL and AML.

An increased glycolysis rate can be found in cancer cells for their needs of energy, and hence, they produce a high level of pyruvic acid, which is an end product of glycolysis.[64] This was also found in the plasma of BC patients.

The rate of metabolism and use of glutamine are significantly altered in the case of hypoxia or in the cells of cancer with mitochondrial dysfunctions. α-ketoglutarate (α-KG) derived from glutamine can be converted into isocitrate, which is eventually converted into citrate.[65,66] This explained the high levels of citrate obtained in the case of ALL and BC.

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Some evidence proposes that citrate has a function in the biology of cancer and the aggressiveness of tumor may be connected to the low concentration of citrate in cancer cells.[67] We also found reduced levels of citrate in patients with AML.

The satisfaction of energy demands in cancer cells depends on the majority of blood glucose used in glycolysis. When glycogen is not broken down into an adequate amount of glucose, another pathway for glucose production takes place. This secondary metabolic pathway uses glucogenic amino acids in the form of glycerol, lactate, and pyruvate.[3] It is said that the cells of solid tumors have a different glucose metabolism that leads to high levels of lactate production.[68] Our results showed that BC patients have higher lactate levels than acute leukemia patients. They also showed high blood glucose levels, which agrees with what has been reported by Raza and co-workers.[69]

It is known that uridine is an endogenous nucleoside and its modification by different antimetabolites can affect the synthetic process of *de novo* pyrimidine.[70,71] Protecting the preformed pyrimidines from their environment is a way by which tumor cells can protect themselves from the cytotoxic effects of inhibitors of the *de novo* pyrimidine synthesis,[72,73] and hence, uridine levels should be high as occurred in the results of ALL, AML, and BC.

Butyric acid is considered as a fatty acid found in the animal fat ester form and plant oils.[74] The fermentation of starch by colonic bacteria can also produce butyrate, which has a key role in promoting cell differentiation and apoptosis and preventing cell growth in colorectal and other cells of cancers.[75]

2-Hydroxybutyric acid is produced from the α-ketobutyrate formation by a reaction that is catalyzed by lactate dehydrogenase (LDH) or α-hydroxybutyrate dehydrogenase.[76] It is found in the serum of multiple myeloma patients whose 2-hydroxybutyrate levels gradually decreased.[45] Leukemia, lymphoma, and myeloma are types of blood cancer where leukemia affects the leukocyte cells, lymphoma affects lymphocytes, and myeloma affects plasma cells.[77] Acute leukemia (ALL and AML) in the present study showed reduced levels of 2-hydroxybutyrate similar to the findings of multiple myeloma in another report.[45]

Acetic acid is required by cancer cells to live under the nutrient-limiting conditions.[78] These reasons confirmed the high level of acetate in our results in ALL and BC patients.

Organic acids are considered end products of metabolic pathways, and their levels are essential indicators of physiological conditions and can be related to metabolic alterations in cancer.[79] The results of patients with ALL, AML, and BC showed high levels of succinic acid, which was in agreement with the findings reported by Hur et al.[79]

The immune response, antibodies, can be encouraged by carnitine by increasing cell differentiation of plasma or/and by promoting the synthesis and secretion of immunoglobulin (Ig) by the cells of plasma.[80,81] The important function of carnitine is represented in the metabolism of fatty acids, where it can stimulate the transfer of acyl groups through the inner membrane of the mitochondria for fatty acid β-oxidation.[82,83] It is observed that there was an accumulation of carnitine and/or acetylcarnitine in the serum of patients with multiple myeloma at diagnosis and after relapse.[45] This confirmed the results obtained from of ALL, AML, and BC patients regarding the carnitine and *o*-acetylcarnitine plasma levels.

Trimethylamine-*N*-oxide (TMAO) is obtained mostly from carnitine and choline of the diet by the work of microbiota of the gut that produces trimethylamine (TMA) from these ingredients. TMA is oxidized in the liver by flavin-containing monooxygenases to TMAO.[84]

It has been found that TMAO stimulates the accumulation of cholesterol in macrophages and foam cells in the walls of artery, causing atherosclerosis, leading to cardiovascular diseases.[85] It can immediately lead to advanced fibrosis of organs and dysfunction in animal models.[86,87] In addition, higher levels of TMAO have been reported in the serum of patients with colorectal cancer than in the healthy controls.[88] Similarly, TMAO showed higher plasma levels in patients with BC, which is a solid tumor, when compared to controls.

4.2. Metabolites in Urine. Compared to different biological fluids, urine has been found to be low cost, rich in metabolites, simple for handling and collection, and available in large quantities.[33]

Nuclear magnetic resonance or mass spectroscopy is primarily employed in the studies of urinary metabolomic biomarkers. Urinary NMR measurements are most commonly conducted using proton NMR spectroscopy, which identify molecules by detecting the unique electrochemical environment of its constituent protons.[89]

Investigation of urinary metabolite alterations has shown low levels of several metabolites such as succinate present in patients' urine with epithelial ovarian cancer and BC when compared to the normal cases. There were significantly high levels of several metabolites such as creatine and glucose in the urine of BC patients, and other metabolites, which increased in cancer tissue (such as some of the amino acids), were found to be in reduced levels in the urine of BC patients.[90] Higher levels of creatine and α-glucose were observed in the urine of BC patients and also in ALL and AML patients. Also, our urinary succinate levels were reduced in BC patients with respect to controls in agreement with another report.[91]

Glutamine that has been reported to show a high concentration in breast tissue[90] lowered in the urine of BC patients as also found by other researchers.[91] Moreover, threonine levels were found to be reduced in the urine of BC patients when compared to controls, which was similar to what has been reported in another study.[92]

Kidney damage can be caused by the proteins secreted by the malignant plasma cells and can sometimes lead to total renal failure. Some multiple myeloma patients have severe renal failure, needing dialysis.[93] Proteinuria is connected to leukemia. With the high frequency of hematologic malignancy and new medical care that extends the survival in patients suffering from leukemia and lymphoma, kidney injury and its problems will be more common.[94] This might have led to increased creatinine levels in the urine of patients with ALL and AML as compared to those in controls.

Our results showed that the urinary levels of 2-hydroxybutyrate and choline were higher in the patients with BC, ALL, and AML than in healthy controls. These findings were confirmed by the studies of other researchers.[95,96]

Amino acids are used to generate more energy in most cancer cells through glycolysis rather than oxidative phosphorylation *via* tricarboxylic acid (TCA).[97,98] Valine is a glucogenic amino acid, and its urinary level in cancer patients is higher as it is important for gluconeogenesis.[99] Similarly, the levels of valine in our results increased in the urine of patients with ALL, AML, and BC than in healthy individuals.

It is known that amino acids have a significant function in biological metabolism and regulation of physiological activity of

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organisms. The alterations of endogenous amino acids can be related to the type of diseases.[100]

Urinary lysine levels were observed to be higher in patients with ALL, AML, and BC than in healthy individuals as reported in another study.[101]

It is known that lipid metabolism provides the necessary building units for the proliferation of cells containing phospholipids and cholesterol for the formation of the cell membrane. Several cells of cancer have high rates of *de novo* lipid synthesis. Fatty acids are catabolized through β-oxidation by some types of breast cancer and prostate cancer. These types of cancer might use fatty acids from the environment.[102] Choline shows a lipotropic part in the metabolism of lipid as a primary matter.[103] Carnitine and acetylcarnitine work as transporters to carry long-chain fatty acids into the mitochondria for β-oxidation to supply energy for different aspects of cell activity.[104]

In a study of another type of cancer, the increases in pyruvate and lactate levels in urine were not statistically significant. The increased levels of *o*-acetylcarnitine, carnitine, and methylhistidine were observed.[105] In our results, it is detected that urinary levels of lactate and pyruvate were lower in patients with ALL, AML, and BC than in the normal cases. Urinary levels of carnitine and methylhistidine were higher in all patients, and the levels of *o*-acetylcarnitine in ALL and AML increased when compared to those in healthy controls.

Aconitic acid is considered as an organic acid and has two isomers which are *cis*-aconitic acid and *trans*-aconitic acid.[106] The urinary level of *trans*-aconitate in colorectal cancer has been reported to be higher than in normal cases.[107] This confirmed our results where increased levels of *trans*-aconitate in all patients were obtained when compared to those in controls.

In various microorganisms, indole-3-acetate (IAA) is found to be a signaling molecule.[108,109] It has been reported that the cell death of human tumor cells in bladder carcinoma is due to IAA.[109,110] This report confirmed the lower level of urinary indole-3-acetate in ALL, AML, and BC than in healthy subjects.

Melatonin is secreted in the body by the pineal gland and can be synthesized in the bone marrow, the retina, bile, and the gastrointestinal tract. It is released into the bloodstream and then into the cerebral spinal fluid, saliva, and bile. A total of 50−75% of melatonin is bound reversibly to albumin and glycoproteins in the blood. Melatonin metabolism takes place in the liver and results in a 90% clearance rate with small amounts excreted in the urine unmetabolized.[111]

The urine of patients with ALL, AML, and BC showed low levels of melatonin when compared to the normal cases, as reported by other researchers.[112,113]

5. CONCLUSIONS

This study gives a summary about the analytical parts connected to the use of quantitative ^{1}H NMR for metabolic profiling of different types of cancer such as ALL, AML, and BC based on the analysis of blood and urine because of their ease of access and noninvasiveness. It is suggested that the blood plasma and urinary metabolic profiles of ALL, AML, and BC are different. They are also unlike when compared to the normal individuals. We recommend that studies on diagnosing cancer should be linked to metabolomics with the use of nuclear magnetic resonance as a rapid and noninvasive diagnostic tool.

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