Metabolomics in Lipoid Proteinosis

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Abstract: Background: Lipoid proteinosis (LP) is an autosomal recessive transfer lysosomal storage disease that is characterized by the accumulation of hyalin substance in the mucous membranes, skin, internal organs, and brain. Thus far, no biochemical diagnostic method has been identified.

Objective: The aim of this study was to determine the carnitine and acylcarnitine metabolic profiles of LP patients and to examine the potential of LC-MS/MS as a new biochemical method for the identification of biochemical markers.

Methods: In this study, 27 carnitine and acylcarnitine esters were measured with LC-MS/MS in serum samples taken from 14 healthy control subjects and 14 patients. The patients, who presented at the Skin and Venereal Diseases Polyclinic, were diagnosed with LP on the basis of clinical, radiological, and histopathological examinations.

Results: The results of the study showed that the C0 (free carnitine) C3, C4, C4:DC, C5DC, C6, C8, C14:1, C14:2, C16, and C18 acylcarnitines were statistically significantly reduced in the LP patients (p < 0.05, p < 0.01).

Conclusion: It was concluded that the application of carnitine profile screening, an inexpensive, rapid, and reliable method, as a supporting laboratory test could make a contribution to the differential diagnosis for individuals with suspected LP.

Keywords: LC-MS/MS, lipoid proteinosis, carnitine, acylcarnitine, suspected LP, metabolomics.

1. INTRODUCTION

Lipoid proteinosis (LP), also known as hyalino-osis cutis et mucosae or Urbach-Wiethe disease, is an infrequently seen lysosomal storage disease with a chronic course that is characterized by hyalin substance accumulation in the mucous membranes, skin, internal organs, and brain. A genetic disorder causes a mutation in the extracellular matrix protein-1 gene, and this results in hyalin accumulation [1-3]. The clinical findings for LP show variations and generally start with voice restriction in the neonatal period. Various skin symptoms, such as papillary, acneiform scars at the eyelash roots, and wart-like papillae and plaque, emerge during childhood. When there is no airway obstruction or occurrence of life-threatening epileptic seizures, this disease is consistent with a normal life course. Mortality generally occurs because of laryngeal obstruction. Because the incidence is low and the etiology is associated with a genetic mutation, there is no recommended treatment strategy for intervention on the systemic effects associated with the prognosis. LP has a stable or slowly progressive course [4]. A wide clinical heterogeneity may be seen even in a family or an isolated patient population [5]. In a 2002 study using genome link analysis, the pathogen mutations of the ECM1 gene, with an accompanying location on chromosome 1q21, were identified in LP [6]. A total of 52 different pathogen mutations have been reported. They include missense and small and large deletions and insertions. Approximately 50% of the mutations have been

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shown to be in exons 6 and 7 [5]. Although some data about the genetic mutations in LP have been obtained, there is insufficient information about the biochemical pathophysiology. The determination of the metabolic profile of systemic diseases has been useful for discerning the biochemical clues for the accumulated metabolites discussed in previous studies about the disease [4]. With metabolomic and proteomic studies, the determination of new biochemical markers with LC-MS/MS, which is often used for congenital metabolic diseases, can contribute to the development of protective and therapeutic strategies [7]. The normal acylcarnitine balance in healthy individuals is altered because of the metabolic changes. The determination of the free carnitine (FC) and acylcarnitine (AC) profiles through the use of mass spectrometry provides a significant contribution to the understanding of the pathogenesis of diseases [8, 9]. The determination of FC and AC levels from dry blood test was used to evaluate the metabolic status of some systemic and genetic diseases (paroxysmal atrial fibrillation, autism, stroke, ventricular diastolic dysfunction, breast and colorectal cancer) [10-15]. A review of the literature suggests that this is the first study to use LC-MS/MS to determine the levels of FC and AC concentrations with AC/FC ratio.

The aim of the current study was to determine the carnitine and acylcarnitine metabolic profiles of LP patients. In addition, the study aimed to establish the efficacy of LC-MS/MS as a new method for the objective identification of biochemical markers in LP patients.

2. MATERIALS AND METHODS

2.1. Clinical Samples

The study included 14 patients who, after presenting at the skin and venereal diseases polyclinic, were diagnosed with LP diagnosis on the basis of clinical, radiological, and histopathological examinations. The control group consisted of 14 age- and gender-matched healthy individuals. Patients were excluded from the study if they were pregnant or breastfeeding, smoked cigarettes or drank alcohol, were un-cooperative, or had a systemic disease. Approval for the study was granted by the Local Ethics Committee (Harran University, Medical Faculty, Number: 05-07-23; Date: 01.02.2018). The study was designed and conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all the study participants. Blood samples were taken in the morning after 8 hours of fasting. The samples were withdrawn into gel tubes and centrifuged at 5,000 rpm for 10 min. The separated sera were then placed in Eppendorf tubes and stored at -80°C until assay. A total of 27 parameters of the FC and AC esters were measured (C0, C2, C3, C4, C4DC, C5, C5:1, C5OH, C5DC, C6, C6DC, C8, C8:1, C8DC, C10, C10:1, C10DC, C12, C14, C14:1, C14:2, C16, C16:1, C18, C18:1, C18:2, and C18:1 OH). The acyl-carnitine esters were measured with a Shimadzu Nexera X2 ultra-high-performance liquid chromatograph (UHPLC) coupled with a Shimadzu LCMS-8040 triple quadrupole liquid chromatograph mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA).

2.2. Materials and Reactives

HPLC-graded formic acid, methanol, Acenonitrile Sigma (Loughborough, UK) acylcarnitine internal standard (Cambridge Isotope Laboratories) were purchased.

2.3. Sample Preparation

The samples for the routine screening tests were prepared in accordance with the 2008 study by La Marca et al. except for the butylation procedure, which was not performed. The carnitine profile was examined with a modification of the neonatal screening method developed by LaMarca and Azzari [16, 17]. A 5 µl plasma sample was then placed in each well and left to dry at room temperature overnight. Filter paper (Whatman filter paper 10538018) was cut into discs of 3.2 mm diameter and placed in 96-well plates.

2.4. Tandem Mass Spectrometry

The sample (5 µl plasma) was extracted by dispensing 300 µL of extraction solution consisting of methanol and aqueous mixture of 3 mmol/L hydrazine hydrate at approximate relative volume/volume ratios of 66.6% and 33.3%. The internal standards and the stable heavy isotope analogues of the carnitine and acylcarnitines were also present in the extract solution. The extracted sample was injected into the Shimadzu LCMS-8040. The mass spectral data for the acylcarnitines
were acquired through a precursor ion scan of 85 m/z in a positive mode (CE-25V). The percentage of each analyte that was recovered was determined through a comparison with an internal standard for each analyte. The standard concentrations were 7.6-152 µmol/L for the acylcarnitines. Spiked samples with different concentrations of analytes were used as the daily control quality tests. Examples of chromatograms and spectra are shown in Figs. (1 and 2).

Fig. (1). Chromatograms of free carnitine (C0) and isovaleryl carnitine (C5OH).
2.5. Analysis Condition

A run of 2.2 minutes was applied on an FIA flow of 0.070 µL/min (A: water + 0.05% formic acid; B: acetonitrile; A/B: 30%/70%). Next, 40 µL of the sample was injected in a column oven at 30°C, desolvation line at 300°C, heat 500°C, nebulizing gas at 3 L/min, and drying gas at 20 L/min. All of the data were reprocessed with Shimadzu neonatal mass processing software, which automatically calculated the concentration of each compound.

2.6. Statistical Analysis

All of the data analyses were performed with IBM SPSS Statistics for Windows, Version 20.0 software (IBM Corp., Armonk, NY, USA). The data were statistically analyzed with Levene’s test and the Shapiro–Wilks test of equality of variances and the normality assumption. Next, the data (C0, C2, C3, C4, C4DC, C5, C5:1, C5OH, C5DC, C6, C6DC, C8, C8:1, C8DC, C10, C10:1, C10DC, C12, C14, C14:1, C14:2, C16, C16:1, C18, C18:1, C18:2, and C18:OH) were analyzed through the Mann–Whitney U test to determine any further differences among the groups. The results were presented as mean and standard deviation values. A value of $p < 0.05$ was accepted as statistically significant for all of the tests.

3. RESULTS

No significant difference was found between the LP patient group and the control group regarding the demographic data (Table 1). The results of the FC and AC amounts determined with the LC-MS/MS of the serum samples from the LP patients (n:14) and the healthy control group (n:14) are presented in Table 2. A comparison of the groups showed an increase in C5 and C5:OH in the LP group. A statistically significant increase was found in C12 ($p < 0.05$). C0, C2, C3, C4, C4:DC, C5DC, C6, C8, C8DC, C10, C10:1, C10DC, C14, C14:1, C14:2, C16:1, and C18:1 were determined to be significantly reduced lower in the LP patients ($p < 0.05$, Table 1). The AC/FC ratios, a marker of mitochondrial dys-function, were compared. The C2/C0, C5OH/C0, C5DC/C0, C6/C0, C10/C0, C10:1/C0, C12/C0, C14:1/C0, and C14:2/C0 ratios were found to be statistically significantly lower in the LP patients than in the control group ($p < 0.05$, $p < 0.01$ respectively; Table 3).

4. DISCUSSION

In recent years, metabolomics analysis has been widely used in the etiopathogenesis research of diseases [18-21]. The most commonly measured
Table 1. The demographic variables of the patient and control groups. The p values for the comparison of the variables between two groups were calculated according to the Mann-Whitney U test.

<table>
<thead>
<tr>
<th>-</th>
<th>Group</th>
<th>N</th>
<th>Mean±Sd</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Patient</td>
<td>14</td>
<td>15.07±7.20</td>
<td>0.927</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>15.42±8.54</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Patient</td>
<td>14</td>
<td>150.07±29.82</td>
<td>0.346</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>144.07±30.77</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>Patient</td>
<td>14</td>
<td>51.35±19.70</td>
<td>0.260</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>46.0±17.95</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Patient</td>
<td>14</td>
<td>21.63±1.70</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>21.13±1.28</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The acylcarnitines in the serum samples of the two studied groups quantified using the LC-MS/MS method. * p<0.05 and **p<0.001 values for the comparison of the variables between two groups were calculated according to the Mann-Whitney U test. (m/z: mass of main ion and product ion).

<table>
<thead>
<tr>
<th>Acylcarnitines</th>
<th>m/z</th>
<th>Formula</th>
<th>Status</th>
<th>Patient Group (n:14) Mean ± SD</th>
<th>Control Group (n:14) Mean ± SD</th>
<th>Fold Change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0 (free carnitine)</td>
<td>218.20&gt;103.00</td>
<td>C7H15NO3</td>
<td>↓</td>
<td>98.3±21.77</td>
<td>120.87±28.72</td>
<td>0.81</td>
<td>0.05*</td>
</tr>
<tr>
<td>C2 (acetyl carnitine)</td>
<td>260.20&gt;85.00</td>
<td>C9H17NO4</td>
<td>↓</td>
<td>33.57±9.06</td>
<td>57.17±9.72</td>
<td>0.59</td>
<td>0.001*</td>
</tr>
<tr>
<td>C3 (propionyl carnitine)</td>
<td>274.20&gt;85.00</td>
<td>C10H19NO4</td>
<td>↓</td>
<td>0.93±0.3</td>
<td>1.25±0.49</td>
<td>0.74</td>
<td>0.05*</td>
</tr>
<tr>
<td>C4 (butyryl carnitine)</td>
<td>288.20&gt;85.00</td>
<td>C11H21NO4</td>
<td>↓</td>
<td>0.4±0.21</td>
<td>0.43±0.25</td>
<td>0.93</td>
<td>0.945</td>
</tr>
<tr>
<td>C4DC (methylmalonyl carnitine)</td>
<td>374.30&gt;85.00</td>
<td>C11H19NO6</td>
<td>↓</td>
<td>0.06±0.02</td>
<td>0.09±0.02</td>
<td>0.67</td>
<td>0.003*</td>
</tr>
<tr>
<td>C5 (isovaleryl carnitine)</td>
<td>302.20&gt;85.00</td>
<td>C12H23NO4</td>
<td>↑</td>
<td>0.23±0.1</td>
<td>0.21±0.08</td>
<td>1.10</td>
<td>0.645</td>
</tr>
<tr>
<td>C5:1 (tiglyl carnitine)</td>
<td>300.20&gt;85.00</td>
<td>C12H21NO4</td>
<td>↓</td>
<td>0.18±0.06</td>
<td>0.22±0.11</td>
<td>0.82</td>
<td>0.475</td>
</tr>
<tr>
<td>C5OH (isovaleryl carnitine)</td>
<td>318.20&gt;85.00</td>
<td>C12H23NO4</td>
<td>↑</td>
<td>0.16±0.14</td>
<td>0.11±0.05</td>
<td>1.45</td>
<td>0.145</td>
</tr>
<tr>
<td>C5DC (glutaryl carnitine)</td>
<td>388.30&gt;85.00</td>
<td>C12H21NO6</td>
<td>↓</td>
<td>0.24±0.1</td>
<td>0.51±0.11</td>
<td>0.47</td>
<td>0.001*</td>
</tr>
<tr>
<td>C6 (hexanoyl carnitine)</td>
<td>316.20&gt;85.00</td>
<td>C13H25NO4</td>
<td>↓</td>
<td>0.06±0.02</td>
<td>0.11±0.03</td>
<td>0.55</td>
<td>0.001*</td>
</tr>
<tr>
<td>C6DC (adipoyl carnitine)</td>
<td>344.20&gt;85.00</td>
<td>C13H23NO6</td>
<td>↔</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>1.00</td>
<td>0.698</td>
</tr>
<tr>
<td>C8 (octanoyl carnitine)</td>
<td>342.20&gt;85.00</td>
<td>C15H29NO4</td>
<td>↓</td>
<td>0.07±0.09</td>
<td>0.31±0.21</td>
<td>0.23</td>
<td>0.001*</td>
</tr>
<tr>
<td>C8:1 (octenoil carnitine)</td>
<td>302.20&gt;85.00</td>
<td>C15H27NO4</td>
<td>↔</td>
<td>0.12±0.06</td>
<td>0.12±0.07</td>
<td>1.00</td>
<td>0.695</td>
</tr>
<tr>
<td>C8DC (suberyl carnitine)</td>
<td>430.40&gt;85.00</td>
<td>C15H27NO6</td>
<td>↓</td>
<td>0.03±0.01</td>
<td>0.05±0.04</td>
<td>0.60</td>
<td>0.037*</td>
</tr>
<tr>
<td>C10 (decanoyl carnitine)</td>
<td>372.30&gt;85.00</td>
<td>C17H33NO4</td>
<td>↓</td>
<td>0.21±0.14</td>
<td>0.75±0.33</td>
<td>0.28</td>
<td>0.001*</td>
</tr>
<tr>
<td>C10:1 (decenoil carnitine)</td>
<td>370.30&gt;85.00</td>
<td>C17H31NO4</td>
<td>↓</td>
<td>0.39±0.20</td>
<td>0.98±0.38</td>
<td>0.40</td>
<td>0.001*</td>
</tr>
<tr>
<td>C10DC (sebacoyl carnitine)</td>
<td>458.40&gt;85.00</td>
<td>C17H31NO6</td>
<td>↓</td>
<td>0.019±0.01</td>
<td>0.02±0.001</td>
<td>0.95</td>
<td>0.015*</td>
</tr>
<tr>
<td>C12 (dodecanoyl carnitine)</td>
<td>400.30&gt;85.00</td>
<td>C19H37NO4</td>
<td>↑</td>
<td>0.1±0.05</td>
<td>0.029±0.01</td>
<td>3.45</td>
<td>0.001*</td>
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<tr>
<td>C14 (myristoyl carnitine)</td>
<td>428.40&gt;85.00</td>
<td>C21H41NO4</td>
<td>↓</td>
<td>0.04±0.02</td>
<td>0.07±0.02</td>
<td>0.57</td>
<td>0.001*</td>
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<tr>
<td>C14:1(tetradecenoyl carnitine)</td>
<td>426.40&gt;85.00</td>
<td>C21H39NO4</td>
<td>↓</td>
<td>0.1±0.06</td>
<td>0.27±0.12</td>
<td>0.37</td>
<td>0.001*</td>
</tr>
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(Table 2) Contd…
<table>
<thead>
<tr>
<th>Acylcarnitines</th>
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<th>Formula</th>
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<th>Control Group (n:14) Mean ± SD</th>
<th>Fold Change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:2(tetradecadienoyl carnitine)</td>
<td>424.40&gt;85.00</td>
<td>C21H37NO4</td>
<td>↓</td>
<td>0.26±0.15</td>
<td>0.71±0.3</td>
<td>0.37</td>
<td>0.001*</td>
</tr>
<tr>
<td>C16 (palmitoyl carnitine)</td>
<td>456.40&gt;85.00</td>
<td>C23H45NO4</td>
<td>↓</td>
<td>0.19±0.05</td>
<td>0.21±0.05</td>
<td>0.90</td>
<td>0.333</td>
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<tr>
<td>C16:1 (hexadecenoyl carnitine)</td>
<td>454.40&gt;85.00</td>
<td>C23H45NO4</td>
<td>↓</td>
<td>0.09±0.04</td>
<td>0.16±0.05</td>
<td>0.56</td>
<td>0.001*</td>
</tr>
<tr>
<td>C18 (stearoyl carnitine)</td>
<td>484.40&gt;85.00</td>
<td>C25H49NO4</td>
<td>↔</td>
<td>0.07±0.02</td>
<td>0.07±0.01</td>
<td>1.00</td>
<td>0.814</td>
</tr>
<tr>
<td>C18:1 (oleoyl carnitine)</td>
<td>482.40&gt;85.00</td>
<td>C23H45NO4</td>
<td>↓</td>
<td>0.04±0.01</td>
<td>0.05±0.01</td>
<td>0.80</td>
<td>0.014*</td>
</tr>
<tr>
<td>C18:2 (linoleyl carnitine)</td>
<td>480.40&gt;85.00</td>
<td>C23H45NO4</td>
<td>↓</td>
<td>0.1±0.04</td>
<td>0.11±0.03</td>
<td>0.91</td>
<td>0.105</td>
</tr>
<tr>
<td>C18:1OH (hydroxoleoyl carnitine)</td>
<td>498.40&gt;85.00</td>
<td>C25H49NO4</td>
<td>↓</td>
<td>0.008±0.005</td>
<td>0.011±0.003</td>
<td>0.73</td>
<td>0.109</td>
</tr>
</tbody>
</table>

Table 3. The AC/FC ratios. As a marker of mitochondrial dysfunction, were compared. * p<0.05 and **p<0.001 values for the comparison of the variables between two groups were calculated according to the Mann-Whitney U test.
parameters are acylcarnitines, organic acids and amino acids.

L-carnitine is an amino acid-like molecule that has an important mission for the transportation of long-chain fatty acids in the mitochondrial membrane [22]. Acylcarnitines, the esterized form, are the reaction products that are transferred from the primary CoA of the main parts to carnitine. Carnitine can be synthesized endogenously in the kidneys, liver, and brain [23]. The theory that systemic involvement in LP, especially brain involvement, can lead to defects in carnitine metabolism explains the low levels of acylcarnitine that were observed in the patient group [23].

C0, C2, C3, C4, C4:DC, C5DC, C6, C8, C8DC, C10, C10:1, C10DC, C14, C14:1, C14:2, C16:1, and C18:1 were significantly lower in the LP patients. Decrease in levels of Acylcarnitine may be indicative of a defect in the transport of fatty acids. Variation of C16:1 and C18:1 blood level is an indicator of Carnitine palmitoyltransferase II (CPTII) deficiency, and change of C14 level is indicative of Very Long-Chain Acyl-CoA Dehydrogenase (VLCAD) deficiency. Although carnitine has transporter properties, some intermediate metabolic pathways in the binding of fatty acid molecules to carnitine have not yet been elucidated. It is not clear which metabolic pathways affect the genetic etiology of rare LP patients. However, the determination of the change in acylcarnitines reinforces the possibility of carnitine metabolism deficiency in LP patients. Especially in other diseases with carnitine deficiency, clinical benefit has been obtained with supplementary therapies [24]. The data in this study may provide clinicians with a new perspective for LP patients.

Ochoa et al., measured the levels of acylcarnitine by HPLC-ESI-QTOF-MS in Systemic Sclerosis, which led to similar clinical findings like LP. The levels of some acylcarnitines (2-octenoylcarnitine, decatrienoylcarnitine, 2-nonenoylcarnitine, 2,6-dimethylheptanoylcarnitine, 9-decenoylcarnitine, hydroxydodecenoylcarnitine, undecenoyl carnitine) were found to be lower than healthy individuals [25].

In a study by Hamada et al. the impairment that plays a role in LP pathogenesis was shown to originate from the long arm of chromosome-1 [6]. Chromosome-1 has been related to collagen tissue disease and lipid metabolism disorders [26]. Finocchiaro et al. demonstrated that there was a relationship between carnitine palmitoyltransferase (CPTase) activity and chromosome 1q12. More than 52 mutations have been identified in LP, together with ECM1, which encodes extracellular matrix proteins in chromosome 1q12 [5, 27]. In histological studies, the disease is characterized by resistance to diastase in the dermoepidermal component, thickening of the basal membrane, and the accumulation of hyalin material in the blood vessels, adnexal epithelium, and dermis. Abnormal lysosomes similar to those associated with Farber disease have been observed in LP patients. They are thought to reflect the abnormality in the impaired pathway of the glycolipids or sphingolipids. In some studies, the pathology of LP has been evaluated through the biochemical analyses of skin and cultured fibroblasts [28, 29]. In particular, Bauer et al. suggested that because of the complexity of lysosomal material accumulation, there could be multiple enzyme defects, together with mucopolysaccharide lipid and glycolipid metabolism disorders [30].

Because of the lipid accumulation with this disease, the blood acylcarnitine levels are also affected. Carnitines are known to have a significant role in the transport of active fatty acids along the internal mitochondrial membrane, and they are important to produce energy from fatty acids.
These transport molecules have a place in the elimination of stored toxic fatty acyl-CoA metabolites, and they assist in the protection of the equilibrium between FC (C0) and AC, the esterized forms of carnitine. The L-carnitine deficiency that leads to functional carnitine deficiency can increase the AC/FC ratio, a measurement of the carnitines that have acquired acyl compared to free carnitines. Enzymatic disorders or changes in carnitine metabolism may cause higher acylcarnitine levels and, therefore, a high AC/FC ratio. Consequently, while a low AC/FC ratio indicates normal mitochondria, a high ratio indicates reduced mitochondrial function [31]. The C2/C0, C5OH/C0, C5DC/C0, C6/C0, C10/C0, C10:1/C0, C12/C0, C14:1/C0, and C14:2/C0 ratios were determined statistically significantly lower in the LP patients. These data may be a clue in terms of mitochondrial dysfunction.

Because LP cases are rarely seen and few patients can reach advanced diagnostic centers, the laboratory data for providing ideas about the etiopathogenesis of the disease and prognosis are insufficient. In the current study, C0, C2, C3, C4, C4:DC, C5DC, C6, C8, C8DC, C10, C10:1, C10DC, C14, C14:1, C14:2, C16:1, and C18:1 acylcarnitine levels, which were considered to be potential biomarkers in LP patients, were determined to be significantly lower than the values in healthy individuals. The lower levels observed in the patient group are most likely associated with a genetic defect combined with carnitine metabolism and accumulated metabolites. This change in carnitine metabolism is explained by the multiple enzyme defect theory in LP, as proposed by Bauer et al. However, the identification of lower C2/C0, C5OH/C0, C5DC/C0, C6/C0, C10/C0, C10:1/C0, C12/C0, C14:1/C0, and C14:2/C0 ratios in the LP group than in the healthy control group suggests that mitochondrial capacity was not reduced. Although there was no reduction in mitochondrial capacity, the reduction in the blood concentrations of the acylcarnitines in LP, a lysosomal metabolic storage disease, requires advanced genetic analyses of the enzyme defects.

CONCLUSION

In this study, which aimed to reveal the differences in the acylcarnitine levels of LP patients and healthy individuals, LC-MS/MS-based metabolomics was successfully applied. The application of the inexpensive, rapid, and reliable carnitine profile screening from dry blood as a supportive laboratory test can contribute to the differential diagnosis of individuals with suspected LP. The findings of this study indicate that with the identification of the carnitine metabolic pathways, the pathogenesis of LP could be clarified, and new treatment protocols could be investigated with interventions to these pathways.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Approval for the study was granted by the Local Ethics Committee (Harran University, Medical Faculty, Number: 05-07-23; Date: 01.02.2018).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The research was performed on human in accordance with the standard set forth in the Declaration of Helsinki principle of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Informed consent was obtained from all the study participants.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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