Phospholipids and L-Carnitine Screening in Children with Congenital Heart Diseases Undergoing Surgical Correction

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ABSTRACT

Objectives: Congenital heart defect (CHD) represents almost 33% of all major congenital deformities, representing a worldwide health problem. The aim of the study is to identify the value of lecithin, cephalin, sphingomyelin & other phospholipids screening in the pathogenesis and prognosis of CHDs and consequently improve their management.

Methods: A total of 89 child with CHDs were included [35 atrial septal defect (ASD), 27 ventricular septal defect (VSD) and 27 patent ductus arteriosus (PDA)] and 34 child as a control group. Biochemical analysis of the plasma levels of total and different component of phospholipids for both CHD and control group were done by colorimetry and two-dimensional thin layer chromatography. Assay of plasma L-carnitine level was done by ELISA for both patients and control group.

Results: The overall results of the present study revealed a significant reduction in the total phospholipids among CHD patients in comparison to the control group; also, a significant change in the phospholipid profile. A significant lower plasma L-carnitine levels in the CHDs group when compared with the control group (p < 0.001).

Conclusions: Disturbed total and differential types of phospholipids & plasma L-carnitine levels occurs in children with CHDs. Moreover, cell-specific targeting of L-carnitine and phospholipid biosynthetic pathways might serve as a possible strategy for helping favorable outcome in management of CHDs.

Keywords: Lecithin; Cephalin; Sphingomyelin; L-carnitine; Congenital Heart Disease.

1. INTRODUCTION

Congenital heart defect (CHD) represents almost 33% of all major congenital deformities (1). Its prevalence predominant worldwide, however its total outline is absent (2). Procedures used to investigate the whole metabolic processes in the body are known as Metabolomics. It used to identify metabolic biomarkers and in the management
of some diseases (3). Also, it is documented that phospholipid production is very essential for the cell life, natural growth and the maintenance of wellbeing as they give:

a. The cell membrane substance, allowing intracellular enzymatic and chemical reactions;
b. Energy reserve;
c. Bioactive particles that are utilized in cellular signal transmission and molecular recognition pathways; and
d. Active particles that interact with proteins and glycoproteins; that is very important for cellular membrane constitution and function (4,5,6).

The cardiac phospholipid toxicity occurs due to alterations in the phospholipid homeostasis, as it is connected to the energy metabolism (7,8). Lecithin is the most common phospholipid found in the mammalian cell. However, cephalin that is found in the surface of the mitochondrion forms about 40% (9). Alterations in the cellular Lecithin / cephalin molar ratios can affect energy metabolism in different organelles, and it is related to many diseases and metabolic disorders (9).

L-Carnitine (β-hydroxy-γ-trimethylammonium butyrate) is naturally biosynthesized from the amino acids lysine and methionine. It is very important for the intracellular transmission of long-chain fatty acids from the cytoplasm to the mitochondria, where β-oxidation of unsaturated fatty acids and thus, adenosine triphosphate (ATP) generation happen (10,11).

Acylcarnitines is formed by combination of long-chain fatty acids with carnitine. In case of carnitine deficiency, β-oxidation stops, lipid accumulates, and organ dysfunction starts. In starvation, these fatty acids provide the energy supply to the cardiac muscle. Around 70% of cardiac vitality is given by mitochondrial fatty acid oxidation. Also, by different pathways it can save the heart from oxidative stress-related risk factors and cardiac damage. Carnitine protects the heart against ischemia-reperfusion injury (12). Post cardiac surgery, some metabolic disorders that decrease carnitine level may result in weakness of the cardiac muscle (12,13). Moreover, carnitine can augment the cardiac function by prevention of fatty acid collection and lactic acid synthesis (14).

The aim of this original study was to investigate the the total and differential phospholipids profile, in addition to L-carnitine, among children with CHDs (ASD, VSD and PDA) and link the changes of the phospholipids and L-carnitine, if present, to the post-operative outcome.

2. PATIENTS AND METHODS

Participant patient population
A prospective cross-sectional case control study was conducted from January 2015 to January 2017 in the department of Cardiothoracic Surgery- Faculty of Medicine- Assiut and Qena University hospitals. Informed consents were obtained from the parents of the included children.

Inclusion criteria
This study included 89 patients with CHDs (ASD, VSD and PDA). Elective open-heart surgery was done to correct these defects. These cases weren’t receiving drugs or intravenous infusions and routine investigations were normal. This group of patients was compared to 34 age-matched, apparently healthy children forming the control group.

Exclusion criteria
Children with fever, infections, electrolyte imbalance, cancer, hepatic or kidney disease were excluded. Children with aortic stenosis, moderate or severe regurgitation of the mitral or tricuspid valves, pulmonary stenosis, Eisenmenger syndrome or increased pulmonary artery pressure (>25 mmHg) were excluded (15).

Data collections
Complete history taking, general and systemic examination, ECG, abdominal sonar and chest X-ray were done for all cases. Routine preoperative investigations were done for all cases in the form of complete blood count, INR, ESR, urine analysis, liver and kidney function tests. Echocardiography was done for all cases. All included patients had an ejection fraction (EF) more than 57%.

Anesthesia technique and type of operation done for CHD group
Standard median sternotomy with the usual cardiopulmonary bypass were used for closure of VSD and ASD. While, PDA was ligated via left posterior thoracotomy. Intravenous midazolam (0.03 mg/kg) was given to all cases half an hour preoperatively. Induction of anesthesia was done with Fentanyl (0.3 μg/kg) and Propofol (1-2 mg/kg). Propofol leads to decreased left-to-right flow with increased right-to-left flow with significant decrease in QP/QS ratio (16). Rocuronium bromide (1 mg/kg) was given to relax.
skeletal muscle before endotracheal intubation. Anesthesia was maintained by frequent administration of fentanyl (1 μg/kg/hour) and Propofol (3-8 mg/kg/hour).

**Laboratory workup**

A 3 cc of venous blood sample was obtained from the CHD and the control groups. In the CHD group, every 1.5 ml was placed into a tube. The first 1.5 ml on EDTA as anticoagulant was used for measurement of total and differential phospholipids, the remaining 1.5 ml was added on EDTA tubes, then centrifuged at 3500 rpm for 15 min at 4 ○C and the plasma was transferred into 1 ml cryotubes, and stored at -80 ○C for later measurement of L-carnitine level.

**Assay of total and differential phospholipids**

0.5 ml of plasma was enough for the analysis. Samples were spotted onto precoated silica gel using two-dimensional thin layer chromatography (TLC). To visualize the spots of phospholipids, the plate was dipped into a 0.2 per cent aqueous solution of the detection reagent. To remove excess reagent, the plate was placed face downwards on lint-free tissue paper. When inspected in ultraviolet light at 365 pm, the spots appeared yellow-green on a dark, uncolored background that was only faintly fluorescent. the spots were scraped into Pyrex tubes. To each tube 0.3ml 10N sulphuric acid and 0.1 ml hydrogen peroxide (100 volume) were added. The tubes were then heated at 180oC for 30 minutes.A further 0.1ml hydrogen peroxide (100 volume) was added and the tubes re-heated at 180oC for a further 30 minutes. The optical density was measured at 820 pm using aPye-UnicamSp500oraCecil spectrophotometer. Total phospholipids were estimated in the extract by using kits supplied by Sigma St Louis and the amount counted per ml of the extract. By using the percentage of different kinds of the phospholipids, quantitation of each parameter was also counted.

**Assay of plasma L-carnitine level**

This was done by ELISA, using the available L-Carnitine assay kit according to manufacturer protocol (Catalog No.: WH-1775 WEKA MED supplies corp, China) (using ELISA multiskan EX microplatephotomter, thermo scientific, STAT FAX-2100, USA).

**Statistical analysis**

It was performed using Statistical Package for the Social Sciences (SPSS-version 17) software. The results were expressed as mean ± standard deviation. One-way ANOVA (analysis of variance) test was used to compare more than two groups as regard quantitative variable (LSD =least significant difference). Pearson correlation analysis was used to evaluate the correlations between different parameters. P value of less than 0.05 was considered significant.

**3. RESULTS**

**Baseline characteristics**

The mean age was 6.95 ± 2.90 years for the CHD children (n =89) and 8.04 ± 3.05 years for the other group (n =35, 16 males) without significant difference between both groups. Regarding the CHD group, 27 with VSD (15 males), 35 with ASD (14 males) and 27 with PDA (9 males). In the second postoperative day, three cases with CHD were died in the ICU (two ASD & one VSD), while 86 patients were improved.

**Plasma L-carnitine level in CHD children versus control group**

The plasma L-carnitine level in CHD children versus control group was presented in Table (1). It revealed that CHD children had a significant low plasma L-carnitine level other than the control group (3.8±1.7pg/ml). Also, there was low plasma L-carnitine level among children with VSD (1.3±0.7pg/ml) other than those with ASD(1.6±0.5pg/ml) and PDA(1.4±0.6pg/ml), p value <0.001 for all.

**Table 1: Baseline characteristics, plasma lecithin, cephalin, sphingomyelin, other phospholipids and total phospholipids & L-carnitine levels in CHD group and control group**

<table>
<thead>
<tr>
<th></th>
<th>CHD group</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>ASD</td>
</tr>
<tr>
<td>Age(years)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>16</td>
<td>14</td>
</tr>
</tbody>
</table>
Plasma lecithin, cephalin, sphingomyelin and other phospholipids levels & the lecithin/cephalin in CHD children versus control group

The total and differential plasma phospholipids levels were expressed as mg/ml, Table (1). It was noticed that there is a marked decrease in the plasma lecithin, cephalin, sphingomyelin and other phospholipids levels in CHD cases in comparison to normal children (p value <0.001). Also, marked changes in the phospholipid profile were observed. There was a significant decrease in lecithin levels in CHD group in comparison to normal children (p value <0.001). Interestingly, sphingomyelin and other phospholipids levels increased markedly in CHD cases in comparison to normal children (p<0.001 for both). The lecithin/cephalin ratio for the control group was 5.62, for ASD was 2.77, for VSD was 3.19 and for PDA was 2.91.

The correlations between plasma lecithin, cephalin, sphingomyelin and other phospholipids & L-carnitine in CHD children and control group

In normal children we found a significant positive correlation between total phospholipids and each of Lecithin, sphingomyelin and other phospholipids. However, there was a significant negative correlation between L-carnitine and both lecithin and total phospholipids (table 2). In ASD cases, we noticed a significant positive correlation between total phospholipids and each of Lecithin, cephalin and sphingomyelin. Also, there was a significant positive correlation between Lecithin and cephalin, and there was a significant positive correlation between other phospholipids and both sphingomyelin and L-carnitine (table 3). In VSD cases, we noticed a significant positive correlation between other phospholipids and sphingomyelin. Also, there was a significant positive correlation between cephalin and total phospholipids. However, there was a significant negative correlation between other phospholipids and Lecithin (table 4). In PDA cases, we noticed a significant positive correlation between total phospholipids and each of Lecithin, cephalin, sphingomyelin and other phospholipids. Also, there was a significant positive correlation between cephalin and both sphingomyelin and other phospholipids (table 5).

Table 2: The correlations between plasma lecithin, cephalin, sphingomyelin, other phospholipids and total phospholipids levels & L-carnitine level in the control group

<table>
<thead>
<tr>
<th></th>
<th>Lecithin</th>
<th>Cephalin</th>
<th>Sphingomyelin</th>
<th>Other phospholipids</th>
<th>Total Phospholipids</th>
<th>L-carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>p</td>
</tr>
<tr>
<td>Lecithin</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalin</td>
<td>-0.039</td>
<td>0.881</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>0.467</td>
<td>0.059</td>
<td>0.310</td>
<td>0.226</td>
<td>0.241</td>
<td>0.352</td>
</tr>
<tr>
<td>Other phospholipids</td>
<td>0.280</td>
<td>0.276</td>
<td>0.442</td>
<td>0.075</td>
<td>0.241</td>
<td>0.352</td>
</tr>
<tr>
<td>Total Phospholipids</td>
<td>0.863</td>
<td>&lt;0.001</td>
<td>0.417</td>
<td>0.096</td>
<td>0.661</td>
<td>0.004**</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>-0.656</td>
<td>0.004</td>
<td>0.185</td>
<td>0.477</td>
<td>-0.354</td>
<td>0.163</td>
</tr>
</tbody>
</table>

Table 3: The correlations between plasma lecithin, cephalin, sphingomyelin, other phospholipids and total phospholipids levels & L-carnitine level in ASD cases

<table>
<thead>
<tr>
<th></th>
<th>Lecithin</th>
<th>Cephalin</th>
<th>Sphingomyelin</th>
<th>Other phospholipids</th>
<th>Total Phospholipids</th>
<th>L-carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Lecithin</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalin</td>
<td>0.649</td>
<td>0.005</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>0.219</td>
<td>0.399</td>
<td>0.035</td>
<td>0.894</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Other phospholipids</td>
<td>0.001</td>
<td>0.998</td>
<td>-0.221</td>
<td>0.395</td>
<td>0.570</td>
<td>0.017</td>
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<tr>
<td>Total Phospholipids</td>
<td>0.827</td>
<td>&lt;0.001</td>
<td>0.629</td>
<td>0.007</td>
<td>0.509</td>
<td>0.037</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>-0.046</td>
<td>0.861</td>
<td>-0.119</td>
<td>0.649</td>
<td>0.130</td>
<td>0.619</td>
</tr>
</tbody>
</table>

Table 4: The correlations between plasma lecithin, cephalin, sphingomyelin, other phospholipids and total phospholipids levels & L-carnitine level in VSD cases.

<table>
<thead>
<tr>
<th></th>
<th>Lecithin</th>
<th>Cephalin</th>
<th>Sphingomyelin</th>
<th>Other phospholipids</th>
<th>Total Phospholipids</th>
<th>L-carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Lecithin</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalin</td>
<td>0.521</td>
<td>0.068</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>-0.497</td>
<td>0.084</td>
<td>0.182</td>
<td>0.552</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Other phospholipids</td>
<td>-0.739</td>
<td>0.004</td>
<td>-0.32</td>
<td>0.917</td>
<td>0.872</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Phospholipids</td>
<td>.515</td>
<td>0.072</td>
<td>.656</td>
<td>0.015</td>
<td>.312</td>
<td>0.299</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>-0.268</td>
<td>0.375</td>
<td>.253</td>
<td>0.405</td>
<td>.443</td>
<td>0.130</td>
</tr>
</tbody>
</table>

Table 5: The correlations between plasma lecithin, cephalin, sphingomyelin, other phospholipids and total phospholipids levels & L-carnitine level in PDA cases.

<table>
<thead>
<tr>
<th></th>
<th>Lecithin</th>
<th>Cephalin</th>
<th>Sphingomyelin</th>
<th>Other phospholipids</th>
<th>Total Phospholipids</th>
<th>L-carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Lecithin</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cephalin</td>
<td>0.044</td>
<td>0.886</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sphingomyelin</td>
<td>0.360</td>
<td>0.226</td>
<td>.822</td>
<td>0.001</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Other phospholipids</td>
<td>0.089</td>
<td>0.772</td>
<td>.601</td>
<td>0.030</td>
<td>0.542</td>
<td>0.056</td>
</tr>
<tr>
<td>Total Phospholipids</td>
<td>.765</td>
<td>0.002</td>
<td>.627</td>
<td>0.022</td>
<td>.838</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>.054</td>
<td>0.860</td>
<td>.115</td>
<td>0.708</td>
<td>.119</td>
<td>0.698</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Phospholipids are fundamental natural parts of the cell membrane, regulating the cell membrane function, and intercellular connections. Clinically, the serum total phospholipids level is proved to be a liver and biliary disorders marker (17). Lately, it has been accounted that phospholipids and phospholipid hydrolysates assume imperative parts in bioactivity as lipid mediators and in atherosclerosis progression from dyslipidemia (18,19). Moreover, phospholipid metabolism disorders can affect the body energy metabolism, that lead to some metabolic diseases like atherosclerosis and obesity (9). Lecithin, cephalin, sphingomyelin and other phospholipids Form the plasma phospholipids. It was found that lecithin is the main constituent. L-carnitine is a natural amino acid which It assumes a critical value in the intracellular transmission of long-chain fatty acids from the cytoplasm to the mitochondria for energy production (10,11).

To the best of our knowledge, the present study is the first trial to prove any correlation between the different phospholipids components and the CHDs and their complications. VSD or PDA cases are associated with the presence of left ventricular overload, while, those with ASD are associated with the presence of right ventricular overload (15). The findings of the present study revealed significant lower plasma L-carnitine levels.
Among CHD patients especially those having ASD or VSD which indicate that L-carnitine could reflect significant load over the ventricles when they are under pressure or volume stress. The significant decrease in L-carnitine plasma level in our patients may result in improper cardiac metabolism, that may lead to cardiomyopathy later. In our study, the most significant early finding was the significant decrease in the total phospholipids in CHD cases in comparison to normal children. Significant changes in the phospholipid profile were observed in CHD cases with a significant decrease in lecithin and a significant increase compared to normal children. These compositional changes in the phospholipid profile are similar to that reported by Hamplová et al. They noticed that reduction in the total phospholipids (PL), phosphatidylcholine and phosphatidylethanolamine in children with CHD. Interestingly, they found that increase in the total phospholipid level in cyanotic heart disease cases. So, they suggested that this is may be a prophylactic mechanism in these children (20).

However, Ohkawaa et al. found that marked increase in Sphingomyelin level with acute myocardial infarction in comparison to normal population (21). Also, Lu J and colleagues, noticed that marked increase in plasma lipids level in acute coronary syndrome patients in comparison to normal population (22).

The present study showed, statistically significant lower serum L-carnitine levels in CHD when compared with the normal children. Sharma et al. they studied carnitine metabolism disorders. They found that its level is significantly decreased in their models. So, they announced that disorders in carnitine metabolism lead to mitochondrial dysfunction, that result in occurrence of endothelial dysfunction, increase pulmonary artery pressure and flooding of pulmonary circulation (23).

So that, Black al. 2017, reported that carnitine intake may prevent endothelial breakdown associated with increased pulmonary blood flow (24).

The present study revealed that the three cases who died within two days post-operatively in the I.C.U were had the least level of both L-carnitine and phospholipids in all group studied so, these findings might indicate that L-carnitine and phospholipids might help in prediction of the outcome following the congenital heart surgery.

5. CONCLUSION

The findings of this study prove that the phospholipids and the L-carnitine levels have a valuable and important role in the pathogenesis and prognosis of congenital heart disease with right or left ventricular volume or pressure overload as in ASD, VSD or PDA. Cell-specific targeting of L-carnitine and phospholipid biosynthetic pathways could serve as a potential strategy for helping in management of congenital heart diseases.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors report no conflict of interest.

FUNDING

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ETHICAL APPROVAL

The Research Committee at Faculty of Medicine, Assiut University approved this study.

REFERENCES
