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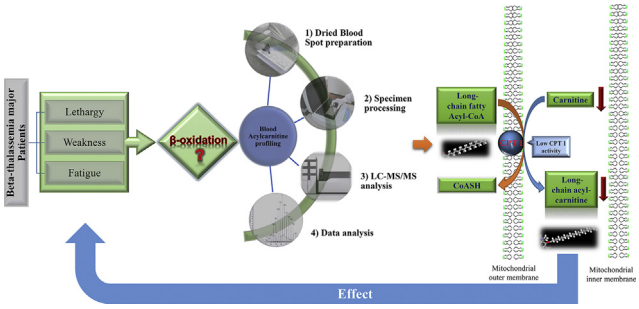
Original Article

Impaired acylcarnitine profile in transfusion-dependent beta-thalassemia major patients in Bangladesh

Suprovath Kumar Sarker [a,i,1], Md Tarikul Islam [a,1], Golam Sarower Bhuyan [b], Nusrat Sultana [a], Mst. Noorjahan Begum [a], Mohammad Al Mahmud-Un-Nabi [b], Md. Abdulla Al Noman Howladar [c], Tashmim Farhana Dipta [d], A.K.M. Muraduzzaman [e], Syeda Kashfi Qadri [f], Tahmina Shirin [e], Salma Sadiya [g], Manzoor Hussain [h], Waqar Ahmed Khan [g], Sharif Akhteruzzaman [i], Syed Saleheen Qadri [a], Firdausi Qadri [a,j], Kaiissar Mannoor [a,*]

[a] Laboratory of Genetics and Genomics, Institute for Developing Science and Health Initiatives, Mohakhali, Dhaka 1212, Bangladesh
[b] Infectious Diseases Laboratory, Institute for Developing Science and Health Initiatives, Mohakhali, Dhaka 1212, Bangladesh
[c] Research and Development, Incepta Chemicals Ltd., Barabaria, Saturia, Dhankora, Manikganj, Dhaka 1810, Bangladesh
[d] Transfusion Medicine and Clinical Haematology, BIRDEM General Hospital and Ibrahim Medical College, Shahbag, Dhaka 1000, Bangladesh
[e] Department of Virology, Institute of Epidemiology, Disease Control and Research, Mohakhali, Dhaka 1212, Bangladesh
[f] Department of Paediatric Medicine, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899, Singapore
[g] Department of Biochemistry and Molecular Biology, Dhaka Shishu Hospital, Dhaka 1207, Bangladesh
[h] Department of Pediatric Medicine and Cardiology, Dhaka Shishu Hospital, Dhaka 1207, Bangladesh
[i] Department of Genetic Engineering & Biotechnology, University of Dhaka, Dhaka 1000, Bangladesh
[j] Department of Enteric and Respiratory Infectious Diseases, Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka 1212, Bangladesh

GRAPHICAL ABSTRACT



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ABSTRACT

Patients with beta-thalassemia major (BTM) suffer from fatigue, poor physical fitness, muscle weakness, lethargy, and cardiac complications which are related to an energy crisis. Carnitine and acylcarnitine derivatives play important roles in fatty acid oxidation, and deregulation of carnitine and acylcarnitine metabolism may lead to an energy crisis. The present study aimed to investigate carnitine and acylcarnitine metabolites to gain an insight into the pathophysiology of BTM. Dried blood spots of 45 patients with BTM and 96 age-matched healthy controls were analyzed for free carnitine and 24 acylcarnitines by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Although medium chain acylcarnitine levels were similar in the patients with BTM and healthy controls, free carnitine, short chain

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* Corresponding author.
E-mail address: kaiissar@ideshi.org (K. Mannoor).

[1] These authors contributed equally to this work

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Carnitine-acylcarnitine levels
Impairment in fatty acid oxidation
Carnitine Palmitoyltransferase-1 activity

acylcarnitines, long chain acylcarnitines, and total acylcarnitine levels were significantly lower in patients with BTM than in the healthy controls ($P < 0.05$). Moreover, an impaired fatty acid oxidation rate was observed in the patients with BTM, as manifested by decreased fatty acid oxidation indicator ratios, namely C2/C0 and (C2 + C3)/C0. Furthermore, an increase in the C0/(C16 + C18) ratio indicated reduced carnitine palmitoyltransferase-1 (CPT-1) activity in the patients with BTM compared with that in the healthy controls. Thus, a low level of free carnitine and acylcarnitines together with impaired CPT-1 activity contribute to energy crisis-related complications in the patients with BTM.

Introduction

Beta-thalassemia, an inherited hemoglobinopathy caused by beta-globin gene mutations and deregulation of the synthesis of the β-globin chain, is one of the most common autosomal recessive disorders worldwide. Approximately 65,000–70,000 babies are born each year with different types of thalassemia, most of whom are affected by beta-thalassemia major (BTM), the most severe form of the disorder [1]. In beta-thalassemia, pathophysiology caused by an imbalance in the ratio of alpha globin chains to beta globin chains, and the presence of excess alpha-globin chain in red blood cells (RBCs), results in oxidative damage to RBCs [2]. Consequently, patients with beta-thalassemia major suffer from hemolytic anemia, which triggers ineffective erythropoiesis through the action of erythropoietin, which is produced in excess by the kidneys [3]. These sequential phenomena lead to an expansion of bone marrow, ultimately causing bone deformities, hepatosplenomegaly, growth retardation, iron-induced dysfunctions of various organs, including the kidney and liver; and above all, muscular and cardiac complications [4]. The pathophysiology of these complications is associated with deregulation of metabolite levels in patients with BTM [5]. Therefore, metabolite profiling, in terms of free carnitine and acylcarnitines, amino acids, and other untargeted metabolites, might be a useful approach to manage BTM and to devise treatment strategies to ameliorate its complications.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) is a high-throughput technique that has shown analytical superiority for low molecular weight biomolecules or metabolites in biological specimens. For example, LC-MS/MS is now used as a dedicated high-throughput platform to screen and diagnose inborn errors of metabolism, such as aminoacidopathies, fatty acid oxidation disorders, and organic acidemia, in many countries worldwide [6]. An alteration in the metabolite profile of a biological specimen can provide valuable phenotypic information and mechanistic insights into the biochemistry of disease processes and their related pathophysiology. It is believed that multi-organ involvement in beta-thalassemia pathophysiology might be associated with an altered metabolite profile. However, very few studies have been conducted on metabolite profiling of BTM specimens [5,7,8].

L-Carnitine plays a significant role in fatty acid transportation across the mitochondrial membrane. In addition, L-carnitine participates in the fatty acid oxidation cycle by releasing Coenzyme-A (CoASH) molecules from shortened products of fatty acid oxidation [9]. Thus, L-carnitine and its acylcarnitine derivatives are important for energy production from fatty acids. Deregulation of carnitine and acylcarnitine levels inflicts damaging effects on organs that depend on fatty acid oxidation for energy, such as the heart and muscle [10]. Although previous studies reported data on free carnitine and total acylcarnitines, those studies did not focus on individual acylcarnitines [8,11]. The present study aimed to perform an LC-MS/MS-based analysis of dried blood spot (DBS) specimens of patients with BTM and age-matched healthy controls to gain an insight into the metabolite profiles of patients with BTM in terms of free carnitine and 24 individual fatty acylcarnitines.

Patients and methods

Ethical approval, study participants and specimen collection

Ethical approval for the present study was obtained from the Bangladesh Medical Research Council (BMRC) of National Ethics Review Committee (NERC), Dhaka, Bangladesh. The study enrolled 45 patients with transfusion-dependent BTM in the age range of 1–15 years. The patients were attending the Dhaka Shishu hospital for follow-up examination and chronic blood transfusions. All the patients continued to receive Desferoxamine (20–40 mg/kg/day) as an iron chelator. Patients with BTM with other comorbid conditions affecting the liver and kidney, and the respiratory, gastrointestinal, and cardiovascular systems, were excluded from the study. In addition to the patients with BTM, 96 age-matched healthy controls were included in the study. The healthy participants had no history of hemoglobinopathy or related disorders.

Before sample collection, written informed consent was obtained from the guardians of both the patients with BTM and the healthy controls. Blood specimens (2 mL) were collected from overnight-fasted patients with BTM and the healthy controls using the standard venipuncture method. Blood collection from the patients with BTM was performed before their regular blood transfusion. Collected blood specimens were immediately spotted on Whatman™ 903 Generic Multipart filter paper (GE Healthcare, Westborough, MA, USA) (~75 μL per spot) to prepare DBS specimens. The rest of the blood was transferred to a BD vacutainer containing dipotassium EDTA (Becton Dickinson, Franklin Lakes, NJ, USA) for DNA isolation and subsequent genetic analysis.

Genotyping of study participants

Genomic DNA (gDNA) was extracted from the EDTA-treated blood using a QIAGEN flexigene® DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted DNA templates were amplified using polymerase chain reaction (PCR) with the forward primer 5′-GGCAGAGCCATCTATTGCTTAC-3′ and the reverse primer 5′-CAGGCCATCACTAAAGGCACC-3′, which together flanked a mutational hot-spot region of beta-globin gene (*HBB*) of the Bangladeshi population [12,13]. The following thermal cycling conditions were applied for PCR amplification: initial denaturation at 94 °C for 15 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 40 s and extension at 72 °C for 40 s; and a final extension at 72 °C for 5 min. The PCR composition was as follows: 4.0 μL of 10 × PCR buffer (with 1.5 mM MgCl2), 1.2 μL of MgCl2 (25 mM), 8.0 μL of Q-solution (Qiagen), 6.4 μL of dNTP mixture (2.5 mM), 0.8 μL of forward primer (10 mM) and 0.8 μL of reverse primer (10 mM), 0.4 μL of HotStart Taq DNA polymerase (Qiagen). Finally, a total reaction volume of 40.0 μL was made with nuclease-free water. PCR product purification was accomplished using a MinElute® PCR purification kit (Qiagen) following the manufacturer's instructions.

Using a BigDye® v3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK), the column-purified PCR products were subjected to cycle sequencing to obtain the chain termination