Karolina Dąbrowska

Uszkodzenie wątroby związane z żywieniem pozajelitowym i wpływ żywienia pozajelitowego na poziom stresu oksydacyjnego przewlekle żywionych pacjentów

Parenteral nutrition associated liver disease and the effect of parenteral nutrition on oxidative stress levels of chronically fed patients

Rozprawa doktorska na stopień doktora
w dziedzinie nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki o zdrowiu
przedkładana Radzie Dyscypliny Nauk o Zdrowiu
Warszawskiego Uniwersytetu Medycznego

Promotor: **Dr hab. n med. Jacek Sobocki, prof. CMKP**

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Abstract

Introduction

Parenteral nutrition (PN) is a type of life-saving nutritional therapy that involves intravenous administration of nutrients such as amino acids, glucose, lipids, electrolytes, vitamins, trace elements, and water to patients who are unable to meet their nutritional needs orally and/or enterally. The most common clinical conditions that directly indicate the use of PN include short bowel syndrome, high intestinal fistulas, Crohn's disease, and cancer with accompanying gastrointestinal obstruction. One of the most common metabolic complications of PN is liver dysfunction. Prolonged use of PN often leads to an increase in liver enzymes such as alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). One of the main complications in adult patients is slowly progressing fatty liver inflammation leading to fibrosis and cirrhosis.

The primary energy substrate in the nutritional mixture for parenteral nutrition is lipid emulsions. They provide the patient with essential fatty acids, but they can also lead to the development of parenteral nutrition-associated liver disease (PNALD). Lipid peroxidation is a complex free-radical reaction initiated by reactive oxygen species (ROS), which results in the formation of lipid hydroperoxides and secondary products of their breakdown, including toxic aldehydes. Oxidative stress is caused by an imbalance between ROS production and the body's ability to detoxify reactive intermediates or repair the resulting damage.

Activation of liver macrophages and the generation of IL1-β, TNF-α, as well as other pro-inflammatory cytokines, ROS, and inflammatory mediators contribute to changes in gut microbiota, increased absorption of microorganisms, and episodes of PN-associated bacteremia. As a result, damage to membranes, proteins, DNA, and RNA may occur, leading to increased permeability of the intestinal mucosa and a toxic increase in bile acid concentration for hepatocytes. The worsening of liver tissue damage is a consequence of these cellular and molecular pathways.

The intrinsic pathway of cell death can be induced by the interaction of ROS with the endoplasmic reticulum, mitochondrial and lysosomal membranes, as well as by the process of lipid peroxidation causing an increase in ROS in serum. Predictive factors for liver and other organ damage due to parenteral nutrition have not been identified.

Oxidative damage may be one of the main mechanisms of liver dysfunction associated with PN. We hypothesized that patients receiving parenteral nutrition would have an increased level of oxidative stress. Differentiation of factors that would be associated with changes in liver enzyme levels in the blood could improve the planning of nutritional treatment and avoid complications.

This doctoral thesis consists of three papers: a review article summarizing the state of knowledge on PNALD and two original publications. The total number of ministerial points is 200, and the combined Impact Factor is 7.584.

Aim of the study

The aim of the doctoral thesis was to discuss the mechanisms contributing to liver dysfunction during PN use and potential methods of prevention, as well as to evaluate the activity of aminotransferases as a marker of liver dysfunction. The correlation between the concentrations of liver aminotransferases: AST and ALT, depending on the rate of oxygen consumption in platelet mitochondria with the degree of oxidative stress induced by lipid emulsions in patients chronically receiving PN under the care of a reference center was also analyzed.

Materials and methods

This doctoral dissertation consists of a series of three papers - one review paper discussing issues related to liver dysfunction associated with parenteral nutrition (publication 2) and two original papers: a retrospective study evaluating the use of liver aminotransferase activity levels as a marker of liver dysfunction (publication 1) and a cohort study (publication 3) examining the correlation between AST and ALT levels, oxygen consumption rate in blood platelet mitochondria, and oxidative stress levels in patients on long-term parenteral nutrition under the care of a reference center.

Publication 1.

The study group consisted of all patients (N=251, 140 women and 111 men) undergoing home parenteral nutrition (HPN) at a reference center in Poland as of December 31, 2012, according to the National Health Fund report. A comprehensive analysis of patients' medical records from a 9-year period (December 2012 – December 2021) was conducted. The following parameters were evaluated:

composition of the nutritional mixture and blood test results, including total bilirubin level in serum, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, standardized time, international normalized ratio (INR), and serum albumin.
Patients were divided into three groups according to their clinical status as of December 31, 2021: group 0 – patients who died, group 1 – patients who were taken off HPN due to recovery of intestinal autonomy, group 2 – patients still receiving home parenteral nutrition. Group 2 was further divided into subgroups: 2A – patients without PNALD features, 2B – patients with PNALD features.
Statistical analysis was performed using the SPSS program. Basic descriptive analyses were obtained. Differences between the groups were calculated using the Mann-Whitney U test and Kruskal-Wallis test. The null hypothesis for each analysis was verified by considering a statistically significant level of $p < 0.05$.

Publication 2.

In this review paper, literature on Parenteral Nutrition Associated Liver Disease was reviewed, and based on that, the mechanisms of PNALD development and therapeutic strategies were presented.

Publication 3.

The study group consisted of 86 patients (43 women and 43 men) who were receiving home parenteral nutrition. During a routine visit to the parenteral nutrition center, blood serum was collected for control tests, including liver enzyme activity (AST, ALT) and morphology. Mitochondria were isolated from blood platelets, and the extent of their damage was determined using the Oxygen Consumption Test. The results were compared with a control group of 86 healthy people who were receiving oral nutrition. The study was conducted using two methods: a traditional method using a Sysmex reagent kit and Sysmex XN-200 machine according to the manufacturer's instructions, and a method that isolated mitochondria from blood platelets and determined their damage using the MitoXpresIntra kit from Agilent (USA), which uses porphyrin-based fluorescent probes to measure mitochondrial molecular oxygen consumption in real-time. Genomic DNA was isolated from whole blood and digested with Fpg (formamidopyrimidine-DNA glycosylase) according to the manufacturer's instructions (New England Biolabs, Ipswich, MA, USA). The obtained results were subjected

to statistical analysis using the STATISTICA™ ver. 13.3 software package (TIBCO Software Inc. Palo Alto, California, USA). Basic descriptive analyses were performed, and the Student's t-test was used to determine the statistically significant difference between means in two independent samples. The null hypothesis was rejected and the alternative hypothesis was accepted when the p-value was less than 0.05, indicating a statistically significant difference between means in two independent samples.

Results

Publication 1.

Eleven patients who received parenteral nutrition were diagnosed with an elevated level of total bilirubin (=> 1.5 mg/dl) in serum, resulting in an incidence rate of 8.3% over 9 years. Five out of eleven patients in this group showed moderate liver enlargement and features of steatosis on liver ultrasound. None of the analyzed patients experienced end-stage liver failure. During the study, 63 patients died. In 61 patients, the cause of death was unrelated to liver disease. Two deaths were classified as liver disease-related, but not related to PNALD.

Publication 2.

PNALD is associated with a spectrum of liver diseases, including disturbances in liver enzyme activity, steatosis, fibrosis, and ultimately liver cirrhosis. The pathophysiology of PNALD is believed to be multifactorial. The paper describes the mechanisms of PNALD development and potential preventive measures.

Publication 3.

The study observed a significant increase in AST and ALT levels (13.9%) in the group. The oxygen uptake test detected an increase in the oxidation levels of platelet mitochondria in 67.04% of cases, with hyperoxia observed in 26.13% of cases. The level of DNA damage of 3.5% or higher was observed. The degree of platelet mitochondrial oxidation in the studied group was 18%, while in the control group, it was 33%, indicating hyperoxia. Over time, there was a tendency to decrease the percentage of genetic DNA damage and increase the percentage of oxygen content in cells during parenteral nutrition. The olive oil-based lipid emulsion caused the lowest

(on average 30%) molecular oxygen level but the highest (on average 2.1%) DNA damage.

Conclusions

The results suggest that a properly balanced, individualized parenteral nutrition program may reduce the risk of developing PNALD. ALT markers may serve as potential markers of the oxidative layer, which can estimate the degree of damage not only to the liver but also mitochondria-induced damage after nutritional treatment. However, the direct impact of parenteral nutrition on genomic DNA damage and levels of molecular oxygen in cells during treatment is not clear. Complex interactions between lipids and the immune system may intensify during the disease process and with oxidative stress, and are still not fully understood. It is essential to monitor patients receiving parenteral nutrition carefully for any adverse effects and manage potential risks properly.