Document:

Study protocol and statistical analysis plan

Protocol:

EFFECT OF A NUTRITIONAL SUPPORT SYSTEM (DIET, SUPPLEMENTS AND PROBIOTIC) FOR IMPROVING GROSS MOTOR FUNCTION IN CEREBRAL PALSY. AN EXPLORATORY RANDOMIZED CONTROLLED CLINICAL TRIAL

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EFFECT OF A NUTRITIONAL SUPPORT SYSTEM (DIET, SUPPLEMENTS AND PROBIOTIC) FOR IMPROVING GROSS MOTOR FUNCTION IN CEREBRAL PALSY. AN EXPLORATORY RANDOMIZED CONTROLLED CLINICAL TRIAL

Abstract

Background: Most patients with cerebral palsy (CP) are dependent on parents due to the spasticity and limitations in their gross motor function. Additionally, many of them do not respond to physical therapy due to deterioration in their nutritional status, which is secondary to gastrointestinal disorders, parasitosis, dysbiosis and the catabolic state of the disease itself. Evidence suggests that greater independence and better clinical response can be achieved by correcting the nutritional status. However, basic treatments only contemplate the calculation of energy requirements and do not consider important nutrients in particular, supplementation with glutamine, arginine, zinc, selenium, colecalciferol, nicotinic acid, spirulina, omega 3, ascorbic acid, vegetal protein or even probiotics.

Objective: To determine the effect of using a nutritional support system (NSS) diet, supplements and probiotic on the gross motor function in children with CP with spastic diparesic and Gross Motor Function Classification System III (GMFCS III).

Material and methods: In an exploratory study with controlled clinical trial design, 30 patients were randomly assigned to receive: 1) dietary surveillance and conventional therapy (FG), 2) deworming and WHO diet (CG), or 3) deworming and the NSS (IG). The patients were recruited from the CRIT Tlalnepantla Estado de México. Males and females aged 4-12 years were included with CP and spastic diparesic GMFCS III, and who had a full-time caregiver and whose parents agreed to participate. They were studied for thirteen weeks. Gross motor function was evaluated at baseline and at 7 and 13 weeks after therapy using the GMFM scale.

Results: The IG-treated group presented a significant improvement in standing and walking parameters analyzed in the GMFM compared with FG and CG groups. Fifty percent of the IG-treated patients managed to walk, while in the other groups no patients were able to walk. **Conclusion:** The NSS used in the present work, improves gross motor function and promotes walking in patients with CP.

1. Introduction

Cerebral palsy (CP) is the most common physical disability in childhood. The prevalence worldwide and in developed countries is approximate to 2-2.5 cases per 1,000 live births [1-2]. According to the Center for Disease Control (CDC) the cost per person was estimated at around 921,000 dollars per year in the United States (2003), while the costs for general medical assistance amounted to 11,500 million dollars [3]. CP is a permanent movement disorder characterized by a persistent postural tone, causing limitation of activity. This is attributed to non-progressive damage on a developing and immature brain that is originated in the fetal, perinatal period (greater percentage) or first years of life. CP is accompanied by alterations in sensation, cognition, communication, perception, spasticity, seizures, disorders in swallowing and malnutrition [4].

An important aspect affecting evolution and functional independence in CP is spasticity which is a reflection of the damage in the Pyramidal System (PS). Traditional treatment for CP-patients, generates a recovery of 2% [5] per year and is based on rehabilitation, botulinum toxin therapy, general care and in the case of malnutrition or disorders in swallowing, nutritional support which is based on the adaptation of Krick to the Schofield formula [6].

In spite of these approaches, CP continues to be the aim of numerous investigations, especially to find new therapeutic strategies.

Currently, the use of functional foods, supplements or even the administration of probiotics or prebiotics as a therapeutic strategy has become a very important research area.

In line with this, several investigations have shown that a number of nutrients are endowed with therapeutic properties including the protection and repair of the central nervous system (CNS). Arginine, for instance, participates in the formation of Nitric Oxide (NO) which is associated with neuronal regeneration and protection of the CNS [7]. Docosahexaenoic acid (DHA) and sphingosine 1 phosphate (S1P) prevent the early death of newly generated neurons [8]. Omega 3 poliunsaturated fatty acids (PUFAs n-3) participate in brain plasticity, neurogenesis, memory and brain repair. Neuromuscular alterations after cerebral ischaemia, improve after omega-3 treatment [9-10].

On the other hand, supplementation with probiotics have also been proposed as a therapeutic strategy for alleviating CNS pathologies [11]. In this case, probiotics have been used to stimulate the production of neurotransmitters, memory, neuroregeneration and also for correcting malabsorption [12].

In order to provide CP-patients with the best therapeutic strategy, it is also important to consider the use of several metabolic rescue pathways to produce energy, including lactate and alanine cycles, where glutamine and glutamic acid are the main substrates [13]. Additionally, deficiencies have also been reported in plasma concentrations of iron, folate, niacin, calcium, vitamin D and E, zinc and selenium even in children with CP who were being supplemented [14-15-16].

Finally, it is relevant to take into account that, 3,500 million people in the world are parasitized and of them, mainly are children, increasing the risk of malnutrition in this group of people [17]. This concomitant factor, makes malnutrition, an even more important problem in CP patients.

It can be said, therefore, that there are several factors that need to be addressed in order to establish the best therapeutic strategy for CP. That is why; in the present study, we tested

an integral food system that includes nutrition, metabolic support, deworming and neurological remodeling support.

2. Materials and methods

The exploratory study was designed as a controlled clinical trial which was randomized and blind, whereby sampling was achieved using the design of consecutive cases. CP-patients with spastic diparesis and GMFCS III were enrolled in this study and treated at the Children's Telethon Rehabilitation Center (CRIT) in Tlalnepantla Estado de México. The duration of the study was 13 weeks for each participant.

The trial complied with the principles of the Declaration of Helsinki and the Mexican norm NOM-012-SSA3-2012 for scientific research in humans. This clinical trial was approved by the Committee of Research of the Faculty of Health Sciences of the Universidad Anáhuac México Campus Norte with the number 2014/03001. The parent or guardian and the patient (in the event that they could sign) signed the informed consent letter voluntarily.

2.1 Participants

Fifty-three children were interviewed. Thirty met the inclusion criteria (see figure 1).

Inclusion criteria: CP-patients with spastic diparesis and GMFCS III (these patients demonstrate elevated functionality in the categories of lying/rolling, sitting and crawling), of both genders aged between 4 and 12 years old, who had support from a full-time caregiver, and who were able to feed orally.

Non-inclusion criteria: CP-patients presenting another catabolic disease which could increase the risk of malnutrition (renal, cardiovascular, pulmonary, hepatic or immunological disease), those presenting infections or receiving antibiotic treatment at least 15 days before starting the study. Patients who had received therapy with botulinum toxin or muscle

relaxants in the last 6 months. CP-patients presenting severe gastroesophageal reflux or with any type of surgery performed in the last 9 months and, patients walking by themselves.

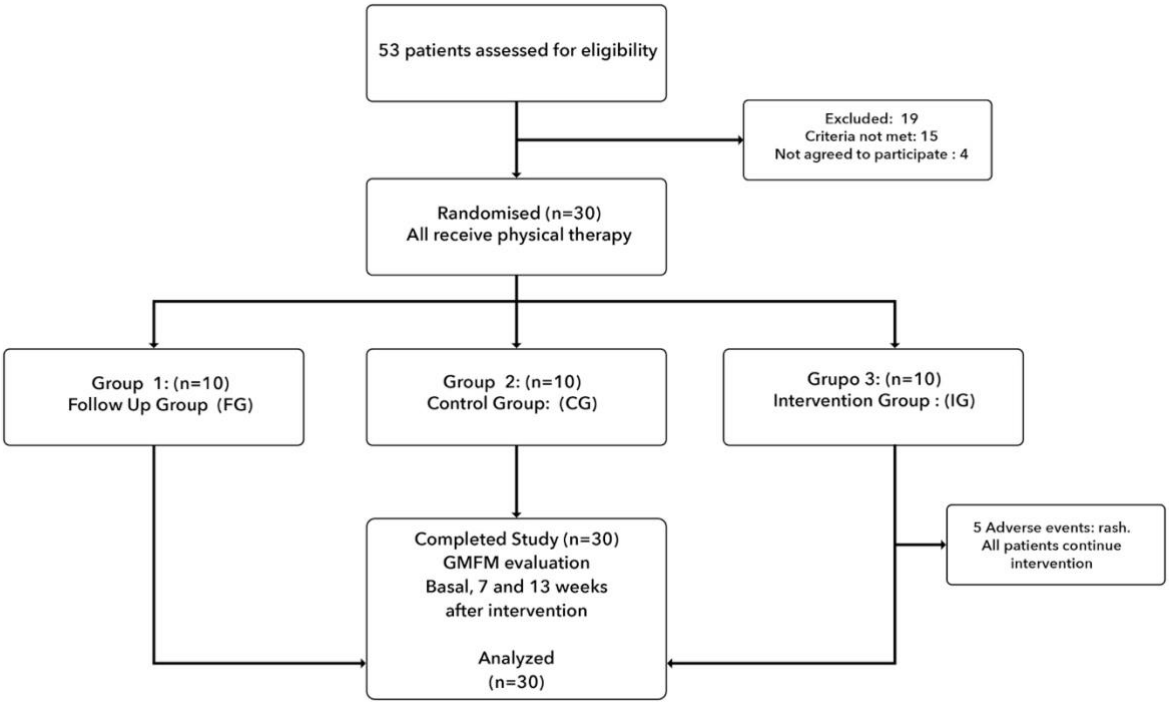

Fig. 1. CONSORT flow diagram.

2.2 Group Assignments

The thirty eligible patients were randomly assigned through a sequence of numbers. These were then divided into three groups: 1) Follow-up group (FG, n=10), only their usual diet was monitored; 2) Conventional Group (CG, n=10), were deworming and received the nutritional therapy recommended by the WHO; 3) Intervention Group (IG, n=10), were deworming and received the NSS which consists of a shake based diet with functional ingredients, high levels of vegetables, fruits, cereals, root-vegetables and fish as well as supplementation with glutamine, arginine, folic acid, nicotinic acid, zinc, selenium, cholecalciferol, ascorbic acid, spirulina, protein vegetable, PUFAs n-3 and probiotics (Saccharomyces Boulardii (200 mg every 12 hours for 3 days in the basal period and at week 7). All participants received Bobath physical therapy.

2.3 Procedures

Once the participants were selected, parents and CP-patients were called to explain the protocol and to collect their informed consent forms. Upon entering the study, the nutritional history and GMFM scale qualification was obtained and recorded from each patient. Thereafter CP-patients were randomly allocated into 3 groups (FG, CG and IG). The energy calculation for the CG and IG groups was performed with the Krick formula (50% carbohydrates, 30% lipids, and 20% proteins).

The parents and / or caregivers of the participants were trained on the project, procedures, as well as feeding and supplementation schemes if required. At the beginning of the treatment, the CG and IG groups were dewormed with nitazoxanide at a dosage of 7.5 mg / kg every 12 hours for 3 days.

2.4 Shakes and Supplementation

The IG supplements were mixed in numbered envelopes and administered in three shakes: shakes 1 and 3 contained amaranth, oats, avocado, banana, egg white, cinnamon and almond milk. Shake 1 was to be taken in the morning and shake 3 in the evening. Shake 2 contained celery, orange, pineapple, nopal, radish and ginger and was to be drunk in the morning together with shake 1.

The ingredients and administration of the envelopes were as follows: **Envelope 1** contained 4.9g of Spirulina Maximum, 100mg ascorbic acid, 5mg folic acid and 10mg of glutamine. This envelope was to be added to shake 1 during the first 10 days of the intervention. **Envelope 2** contained 1g PUFAs n-3 and was to be added to shake 2 which was given throughout the intervention. **Envelope 3** contained 4.9g of Spirulina Maximum, 100mg ascorbic acid, 5mg folic acid, 5.2g vegetable protein, 125mg nicotinic acid, 50mg zinc, 100 mcg selenium and 800 UI cholecalciferol. This envelope was to be added to shake 1 from day 11 until the end of week 6, after which it was suspended for 10 days and substituted for

envelope 5 and then to be retaken until the end of the intervention. **Envelope 4** contained 1g arginine and was to be added to shake 3 from day 8 until the end of the intervention. **Envelope 5** contained the same ingredients as envelope 3 with an additional 10mg glutamine and was to be added to shake 1 from the start of week 7 for 10 days, after which envelope 3 was restarted.

2.5 Follow up

The participants attended CRIT twice a week to receive physical therapy and once a week for a clinical and nutritional checkup where they would hand in their food diaries and their supplement envelopes to be counted to evaluate their adherence to the course of treatment. On days when the patients attended physical therapy, they were supervised to ensure that they had breakfast, mid-morning snack and lunch.

2.6 GMFM assessment

The GMFM scale was performed at baseline time and weeks 7 and 13 after intervention. It was applied by CRIT staff. The evaluators were the blind aspect of the study as they did not have access to any information about the treatment each child was receiving.

This scale assesses five general parameters: 1. Lying (decubitus) and rolling over (GMFAV), 2. Sitting (GMFB), 3. Crawling and kneeling (GMFC), 4. Standing (GMFD), 5. Walking (GMFE) and one final total item (GMFF). The scoring system consists of 88 items and each one is valued based on the following criteria: 0= No, 1= start, 2= Partially Complete, 3= Complete, NE= Not evaluated [18-19].

2.7 Statistical methods

In order to know the type of distribution of the data, the tests of Kolmogorov-Smirnov, D'Agostino-Pearson and Shapiro-Wilk were applied. Analysis of data was performed using

the continuous variables mean and standard deviation were used if the distribution was normal, and if it didn't present normal distribution the median and range were used. Percentages and proportions assessed the ordinal variables. Analysis of data with normal distributions was performed using One-way ANOVA. ANOVA of repeated measurements was used to analyze the intragroup difference. When the distribution did not show normality, Kruskal Wallis test followed by the Dunn post hoc test. and Tukey were applied respectively to post hoc tests. The level of significance was <0.05 in all cases.