J Biochem Tech (2018) Special Issue (2): 85-89
ISSN: 0974-2328

Effects of Ketogenic Diet for Breast Cancer Treatment. A Protocol for Randomised Controlled Clinical Trial

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Received: 25 December 2017 / Received in revised form: 14 May 2018, Accepted: 18 May 2018, Published online: 05 September 2018
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Abstract

Background: A protective effect of the ketogenic diet (KD) on cancer cells has been indicated by in vivo and preclinical studies. It is suggested that the ketogenic diet confer protections through reducing cellular proliferation, tumor growth, inflammation, neovascularization, angiogenesis and an increased programmed cellular death via apoptosis. However there is no concrete evidence from high quality clinical trials that shows whether cancer patients would benefit from the ketogenic diet. Aim: we aim to investigate effects of a ketogenic diet in patients with breast cancer. Methods: we conduct a randomized clinical trial to investigate the effects of Medium-Chain Triglycerides (MCT) based KD on circulating biomarkers including lipid profiles, serum ketones, fasting blood sugar (FBS), insulin, insulin-like growth factor 1(IGF1), CEA, CA15-3, ESR, CRP, IL10, TNF alpha, LDH, liver and kidney marker, electrolyte, ammonia, albumin, TSH, T3, T4), pathological and biological features of tumor, body composition and quality of life. This study will be conducted in patients with locally advanced and metastatic breast cancer that will be referred to neoadjuvant chemotherapy in a medical oncology clinic. The intervention group (n=30) will undertake the KD for 3 months and patients in control group (30) will continue with their standard diet. Discussion: The findings of current study, which investigate the effectiveness of KD as an adjuvant or alternative therapy for cancer, will provide new insights into feasibility and safety of the KD as well as the potential mechanisms contributed in antitumor activity of this diet.

Keywords: Breast Cancer, Ketogenic Diet, Chemotherapy

Introduction

The KD is a low-carbohydrate and high-fat diet that shifts the body's metabolism away from carbohydrate towards fat .The ketogenic diet has been prescribed to control seizures in epileptic patients and is reported to be safe in the long term (Freeman & Kossoff, 2010). Several animal tumor models and humans studies proposed the beneficial effects of the ketogenic diet as an adjuvant therapy in cancers

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(Abdelwahab et al., 2012; Allen et al., 2013; Fine et al., 2012; Otto et al., 2008; Klement et al., 2016). Unlike normal tissues, cancer cells are unable to use ketones for energy (Poff, et al., 2014). It is suggested that the ketogenic diet could enhance cell responses to standard-of-care radiation and chemotherapy via decreasing glucose and increasing in metabolic oxidative stress. Even at epigenetic level ketogenic diet and fatty acids inhibit, histone deacetylases and effect on methylation (Kobow et al., 2013; Kuroiwa-Trzmielina et al., 2009). Feasibility, safety and the beneficial effects of this diet have been shown by previous human studies. However, there is not enough evidence for beneficial effects of KDs in cancer patients (Huebner et al., 2014). Previous studies lacked enough power to assess the effect of the KDs on tumor characteristics due to small sample size and patient heterogeneity. To date there is also no human study investigating the effects of ketogenic diet on factors related to complete response in breast cancer patients. To address this gap in the literature, we conduct a clinical trial to investigate safety and feseability and the effects of a ketogenic diet on blood parameter, tumor characteristics such as pathological and biological features of tumor, body composition and quality of life in patients with locally advanced and metastatic breast cancer.

Project Methodology

Patients

This study was a randomised controlled open-label trial that will be conducted in patients with locally advanced and metastatic breast cancer that will be referred to neoadjuvant chemotherapy in a medical oncology clinic in Shohadae-Tajrish hospital, Tehran, Iran. The eligible participants (80) will randomized in a 1.1 ratio using Block Balanced Randomization. This protocol will computer-generated by a statistician who was not working with the patients. The intervention group (n=40) will undertake the ketogenic diet and patients in control group will continue with standard diet. Patient with locally advance and metastatic breast cancer undergoing chemotherapy for at least 3 months, age between 18 and 70 years will be included. Exclusion criteria will be as follows: history of significant cardiac, renal or neurologic comorbidities, being in active state of malnutrition, having Karnofsky index less than 70, diabetes and pregnancy, known defects in ketogenesis, ketolysis, fatty acid oxidation or gluconeogenesis enzymes.

Sample size

Sample size was calculated by using the following formula, a 5% α error and an 0.20 % β value were selected, and the 20% dropout rate during 12 weeks. With these data to obtain statistical significance the necessary number of participants was 40 patients in each group.

$$n\mathrm{Ku}/_{ml} = \left\{(z_{1-a}\sqrt{2\bar{p}(1-\bar{p}} + z_{1-B}\sqrt{(p_1(1-p_1) + p_2(1-p_2)}\right\}^2 /_{(p_1-p_2)^2}$$

Intervention

Intervention will coincide with the first 3 months of chemotherapy treatment. Participant will be prescribed the KD (6% of daily energy intake from CHO, 19% from Protein, 20% from MCT, and 55% from fat) by a trained dietician. MCT is from nutricia company (Germany). We speculate that the MCT based ketogenic diet would be more acceptable to patients which results in reduced dropout. Study participants will be instructed to consume one ketogenic meals over the course of the day on the first and second day of intervention which will gradually increase to 2 meals on day 3 and 4, followed by 90% of meals from the ketogenic foods until the end of study. Participant will be counselled by dietician, who obtain information about food preferences, determine calorie needs, provide food menu, food samplings, and recipes and discuss dietary procedures. Compliance will be assessed through weekly telephone call. Patients will be asked to avoid carbohydrate rich food containing sugars, pasta, rice and starchy vegetables such as potatoes, fruits, milk and yoghurt. Protein will be provided from high protein foods e.g. meat, fish, poultry, eggs and cheese as well as fat from MCT, olive, canola oil, butter and nut. Participants will be allowed to consume only some vegetable. Nutrient composition was calculated using the USDA Standard Reference Database

The blood glucose levels will be used to assess the therapeutic efficacy of ketogenic diet on the body metabolism. Blood ketone levels > 0.3mmol/l will be considered as stable ketosis (Klement & Sweeney, 2016).

Patients in Control group will recommended to follow a standard diet. A diet based on (55% CHO, 15% Protein, 30% fat).

Variables assessment

Dietary intake assessment: A twenty-four hour recall for 3 days including a weekend day and 2 working days will be obtained to assess dietary intakes at the beginning and end of the study.

Blood sampling: Fasting venous blood samples (10ml) in baseline, middle and last time, 3 ml in 2 and 3 nd follow up were taken from all patients. Blood sample will be immediately centrifuged at 3000 x g for 10 minutes (5702R, Eppendorf, Germany). Serum were fractioned into clean micro-tubes in aliquots and they were also kept at -80 for subsequent analysis.0.5 cc serum sent to lab for another lab test.

Monitoring: lipid profile, serum ketones, glucose, LDH,ALP,lactat, ammonia, AST, ALT, Blood Urea Nitrogen (BUN), Creatinine (Cr), electrolyte (Mg,K,P,Ca.Na) ,albumin, will be assessed at baseline and every 3 week and at the time of leaving the study. Circulating insulin, IGF1, CEA, CA15-3, IL10, TNF-alpha levels will be also measured at the beginning middle and end of the study as well as TSH, T3 and T4 at the beginning and end of the study.

Clinical and Pathologic assessment: The effect of diet on tumor characteristics will be evaluated by imagin:sonography and MRI for neoadjuvant patient as well as CT scan for metastatic patients. Background and pathologic data including estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2) expression, histologic grade, will be obtained from medical records. After completion of neoadjuvant chemotherapy patients will undergo surgery, histologic grade, tumor size, tumor stage and grade, lymph node involvement will be obtained from pathological report.

Biochemical assay: TC, LDL-C, HDL-C, TG, ALT, AST, BUN, Cr, LDH, Lactat will be measured using the enzymatic calorimetric method. All these biochemical tests will be carried out using commercial kits (Pishtaz Teb Inc., Tehran, Iran) by the auto-analyzer (Roche Hitachi 912, Basel, Switzerland).

Concentration of the study hormones was determined by enzyme-linked immunosorbent assay using IGF1 ELIZA kit(ab,100545) Abcam,USA. Insulin ELIZA kit (5825300) Monobind, USA. IL 10 ELIZA kit (SK0074501) Aviscera Bioscience, USA. TNF-alpha ELIZA kit (SK0010901)) Aviscera Bioscience, USA.

Quality of life (QOL) assessment: The patients' quality of life will be monitored by the EORTC QLQ-C30 (version 2) questionnaire developed by the European Organization for Research and treatment of Cancer; questionnaire will be completed at enrollment, every 3 week and at the end of intervention.

Body composition: The body weight and composition will be assessed at the beginning of the study, and every 3-weeks intervals until the end of study through using bioimpedance analysis (BIA). (Tanita BC-418, Illinois, USA).

Approval and Ethical considerations

Written informed consent will be obtained from all participants at recruitment,. Patients will be informed that they have the right to withdraw from the study at any time. The protocol was approved by National Nutrition and Food Technology Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences (IR.SBMU.NNFTRI.REC.1396.187).

Statistical analysis

Continuous variables will be tested for normal distribution by the Kolmogorov-Smirnov test. Student t-test or Mann–Whitney U test will be used to compare continuous variables between groups. Continuous variables in within groups will be compared using paired t-test or Wilcoxon. Categorical data will be analyzed with the Chi-square test. All analyses wil be adjusted for potential confounders. A repeated measures ANOVA will be used to evaluate differences at baseline, middle and endpoints in time-dependent variables within patient groups. All of p values in repeated measures will calculated based on Bonferroni correction for multiple comparisons. All statistical tests will be performed using SPSS 18.0 (SPSS Inc., Chicago, IL) and STATA version 13.

Discussion

Cancer cells heavily depend on glycolysis as their key source of energy, even in an aerobic environment (Warburg, 19562). Hyperglycemia, which results in an increased circulating insulin and IGF1 levels, is associated with poor prognosis in a variety of human cancers (Weiser et al., 2004; Adeberg et al., 2016). It has been indicated that high blood glucose, insulin and IGF1 levels increases tumor cell growth by activating mitogenic signal and PI3K-Akt-HIF-1 alpha pathway (Mathupala et al., 2009; Fine et al., 2012). So KD could hinder tumor growth through reversing this pathway (Allen et al., 2014). Also the KD promote a reduction in inflammation, neovascularization, and angiogenesis as well as increase in programmed cellular death by apoptosis (Wright & Simone, 2016).

The antitumor effect of KD in mice has been reported in animal studies (Lv et al., 2014; Klement et al., 2016). In addition, antitumor activity of KD have been suggested by several human studies which mostly were case reports (Klement, 2017). Colon, breast, neuroblastoma**,** gastric and prostate cancer cell line exposure to ketogenic diet resulted in tumor stability or shrinkage (Fine et al., 2009; Skinner et al., 2009; Magee et al., 1979).

In some studied compliance of this diet was good and in another trial compliance rate was lower and falling out was high. In the ERGO trial 12 /17 patients complete the study (Rieger et al., 2014). In one study just 5 out of 16 patients completed the trial (Schmidt et al., 2011). Tan-Shalaby reported modified atkins diets are safe and feasible in advanced cancer but compliance was difficult, 6 patients completed the diet for 8 weeks and 4 patients maintained for 16 week (Tan-Shalaby et al., 2016).

No adverse effect of the KD as well as its tolerability has been reported by previous studies (Fine et al., 2012; Schmidt et al., 2011; Schwartz et al., 2015). We hypothesize that the adherence to ketogenic diet would be difficult in cancer patients especially among Iranian population due to high proportion of carbohydrate in their diet. Previous studies lacked enough power due to their low sample size, not having control groups and heterogeneity. None of this studies were not evaluate effect of ketogenic diet on complete response in breast cancer patients concurrent with chemotherapy. To address this gap, we will first evaluate the feasibility and safety of KD among cancer patients. Second, we will assess the effects of KD on body composition, qulity of life, blood parameter and complete response. The findings of current study, which investigate the effectiveness of KD as an adjuvant or alternative therapy for cancer, will provide new insights into feasibility and safety of the KD as well as the potential mechanisms contributed in antitumor activity of this diet

Strength of our clinical trial are the sample size (80), duration of follow-up (3 months) and presence of control group. Also all of participants are from our own hospital and just enrolled one type of cancer (breast), Therefore, the group is very homogeneous.

Conflict of interest

The authors declare that they have no competing interests

Funding

Not applicable

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