Date: February, 2022

STUDY PROTOCOL

Administration of ONS Based on Purple Sweet Potato in Head and Neck Cancer Patients With Radiotherapy

Fuadiyah Nila Kurniasari

Universitas Brawijaya
Malang, East Java, Indonesia

INTRODUCTION

A. Background

Cancer was the first and second most common cause of death before the age of 70 in 91 of 172 countries in the world in 2015 (Bray et al., 2018). One type of head and neck cancer (HNC), namely nasopharyngeal cancer, is ranked the 4th most common cancer in men and the 5th most common in both men and women (World Health Organization, 2019).

Cancer patients are at risk of experiencing malnutrition caused by changes in metabolism and pathophysiology of cancer itself, as well as side effects of cancer treatment or therapy. Malnutrition occurs in most (74%) of HNC patients with radiotherapy. Radiation to the head and neck area causes damage to the mucosa cells and salivary glands. It changes in taste, xerostomia, mucositis, pain, and hypophagia resulting in malnutrition. Its symptoms are decreased food intake, reduced body weight by 5%, decreased upper arm circumference, and reduced serum protein and albumin levels (Citak et al., 2019; Nicolini et al., 2013).

One of the nutritional interventions that can be given to prevent and treat malnutrition is administering oral nutritional supplements (ONS). ONS is part of nutritional support or medical nutrition therapy (MNT) in the form of oral food intake supplementation given for particular medical purposes in addition to a regular diet. The ONS form is usually ready-to-drink liquid or ready-to-boil powder but can also be used as a dessert or snack bar (Cederholm et al., 2017; Lochs et al., 2006). High protein ONS enriched with omega-3 in cancer patients can increase body weight, maintain muscle mass, reduce inflammatory response by lowering C-reactive protein (CRP) levels, and improve several domains of quality of life. Such as global health scores and cognitive and physical functioning (Schueren et al., 2018).

Formulating ONS can be made using local food ingredients that have been processed and prepared in powder form, ready to be brewed so that it is easy and practical for patients without mixing food ingredients in fresh conditions (Susetyowati et al., 2019). One of the local food ingredients widely developed in Malang, East Java, is purple sweet potato. Purple sweet potato is rich in anthocyanins, where the highest anthocyanin content is found in the Antin 3 variety, containing 133.39 mg/100 g (Daniela Ticoalu et al., 2016). Anthocyanin colour pigments could modulate the activity of various targets in carcinogenesis through direct interaction or modulation of gene expression and inhibit the growth of cancer cells. The potential antioxidant capabilities of anthocyanins have been seen in vitro studies using cancer cell cultures of the ovary, colon, liver endothelial, breast, and leukemia cells. Anthocyanins have anti-proliferative and anti-carcinogenic effects (Chen et al., 2015; Diaconeasa et al., 2017).

Another food ingredient often used in making homemade ONS as a source of protein is egg white, especially in powder form (Jansen et al., 2017). Egg white consists of ovalbumin (most), ovotransferrin, ovomucoid, ovomucin, lysozyme, globulin, avidin, and cystatin. Eggs can be antibacterial, antiviral, immunomodulator, and anticancer for humans (Mine and Kovacs-nolan, 2004). Other protein sources often used are full cream milk, skim milk, or whey protein (Lestari S, Rahmawati M, Shita D, 2019). Whey protein isolate (WPI) supplementation in cancer patients receiving chemotherapy can improve nutritional status, glutathione (GSH) levels, and immunity and prevent toxicity due to chemotherapy (Bumrungpert et al. , 2018; Cereda et al., 2019; Mazzuca et al., 2019).

In addition to carbohydrates and protein, it is necessary to add food sources of fat which can be selected based on the type of fat. The type of fat that can function as an immunonutrient is an omega-3 polyunsaturated fatty acid (PUFA). High omega-3 food intake can reduce cytokines and nuclear factor-κB (NF-κB) through several mechanisms. One produces thromboxane A2, prostaglandins E2 and I3, and leukotriene B5, which provide anti-inflammatory effects. It can reduce the risk of complications in cancer patients (Freitas and Campos, 2019).

Another type of fatty acid is monounsaturated fatty acids (MUFA), which are more stable than PUFA and have health benefits (Vieira et al., 2015). Monounsaturated fatty acids are abundant in safflower oil and olive oil. Olive oil, besides containing MUFA, also contains high polyphenols, namely 150-400 mg/kg in m form virgin olive oil (Menendez and Lupu, 2006). In general, polyphenols have antioxidant, anti-inflammatory, antiallergic, antiatherogenic, antithrombotic and antimutagenic effects. Due to their ability to modulate cell death, olive oil polyphenols are also helpful as chemopreventive and therapeutic agents in cancer (Cárdeno et al., 2013; Gorzynik-Debicka et al., 2018).

Based on the results of preliminary research on the ONS formulation made from purple sweet potato, added whey protein, egg white powder, omega-3 powder, and olive oil, the ONS formula was obtained with an energy content of 440 kcal, 15.8 g protein (14% energy), 11.8 g fat (24% energy), carbohydrates 68.1 g (62% energy), dietary fibre 3.7 g, omega-3 164.6 mg and anthocyanins 32.8 mg per 100 g ONS powder. Combining these ingredients is expected to help increase food intake to positively affect the nutritional status and inflammatory response of HNC patients receiving radiotherapy.

B. Problem

Based on the above background, it appears that it is necessary to conduct a clinical trial of the effectiveness of purple yam-based ONS in head and neck cancer patients with radiotherapy, with research questions that can be formulated as follows:

"Is there any effect of local food-based oral nutritional supplements (ONS) containing immunonutrients (omega-3 and anthocyanins) on the nutritional status and inflammatory response of head and neck cancer patients receiving radiotherapy?"

C. Research Objectives

The general objectives and specific objectives of this study are described in the following points:

1. General purpose

   The general objective of this study was to identify and analyze or study the effect of local food-based oral nutritional supplements (ONS) containing immunonutrients (omega-3 and anthocyanins) on the nutritional status and inflammatory response of head and neck cancer patients receiving radiotherapy.

2. Specific goals

   This research has several specific objectives, which are described as follows:

   a. Assessing the effect of the ONS administration on nutritional status, which includes: energy and macronutrient intake, body weight, body mass index (BMI), mid-upper arm circumference (MUAC), muscle mass.
   b. Assessing the effect of giving ONS on physical function, namely grip strength.
   c. Assess the effect of the ONS administration on the patient's inflammatory response, which includes: CRP, neutrophils, lymphocytes, neutrophil/lymphocyte ratio (NLR) and IL-6.

RESEARCH METHODS

A. Research Design

This research is an experimental study using a Double-Blinded Randomized Controlled Trial design, with the intervention group receiving ONS containing immunonutrients with a composition consisting of purple yam flour, white egg flour, whey protein, olive oil, omega-3 powder, sugar, and creamer. In contrast, the control group received a standard ONS (iso-calorie) placebo with a composition of powdered skim milk, coconut oil, sugar, maltodextrin, and creamer. Each group received two packs of ONS/day @ 57 grams, brewed into 200 ml of formula with an energy content of ±500 kcal for three weeks.

B. Population/Subject/Sample

The population in this study were MPA patients at RSUP Dr. Sardjito Yogyakarta, while the research subjects were these patients who met the following inclusion and exclusion criteria.

1. Inclusion criteria
    a. Subjects diagnosed with advanced stage MPA (stages 3 and 4) who received radiotherapy or chemoradiotherapy, both as concurrent and adjuvant therapy.
    b. Subjects aged 18-80 years.
    c. Can consume food through a nasogastric tube (NGT) or orally.
    d. Willing to participate in the research by signing informed consent. However, if the patient's general condition is terrible, the family is willing to sign an informed consent and help with the research.
2. Exclusion criteria
    a. The subject has a milk or egg allergy
    b. Subjects with comorbid renal impairment
    c. Subjects with poor nutritional status with BMI < 17.0 kg/m2
    d. Subject could not stand for weighing
3. Dropout criteria:
    a. Subject died
    b. Subjects withdrew from the study
    c. Average ONS consumption <75%

The size of the study subjects was determined based on parameters of protein intake because one of the significant differences in the intervention and control formulas was the protein

content (Arnold and Richter, 1989; Dahlan, 2013). The minimum number of subjects in this study was 22 subjects, with an additional 20% of 5 subjects, so the number of research subjects in each group was 27 or 54 subjects.

C. Location and Time of Research

The location of this research will be carried out at dr. Sardjito Yogyakarta, with research time, will be carried out for 14 months, starting from March 2022 to April 2023.

D. Research Parameters

This study has several research parameters, which include nutritional status in the form of changes in body weight, BMI, MUAC, body composition (muscle mass), and albumin; handgrip strength as physical function; and inflammatory response in the form of CRP, complete blood profile (leukocytes, neutrophils, lymphocytes, neutrophil/lymphocyte ratio).

E. Materials and Procedures

1. Research Materials

   Materials for the manufacture of ONS immunonutrients

   1) Purple sweet potato flour made from purple sweet potato flour of the Antin 3 variety
   2) Ovobel white egg flour
   3) Whey protein isolate 90
   4) Bertolli brand virgin olive oil
   5) Omega-3 powder from Novotech Nutrition USA
   6) Powdered sugar obtained from refined sand from the Gulaku brand
   7) Fiber Creme brand creamer

   Materials for making standard ONS:

   1) Low-fat milk brand Tropicana Slim
   2) Barco brand coconut oil
   3) Powdered sugar obtained from refined sand from the Gulaku brand
   4) Fiber Crème brand creamer
   5) Maltodextrin

2. Research Procedures

a. Preparation of Intervention Substances (ONS Immunonutrient formula and standard ONS)

Making ONS formulas containing either immunonutrients or standard ONS is done by mixing all the ingredients using a mixer with the amount of each ingredient according to the predetermined amount. The main ingredient in this formula is purple sweet potato flour, made by drying fresh purple sweet potatoes which have been blanched beforehand.

b. Clinical Trials

A clinical trial study with a double blind, randomized controlled trial design, where there was blinding on two parties, both researchers and subjects. Disguising is done on 2 types of products by assigning a specific code to each product (eg VGYJ code for standard ONS products and KMJP code for intervention ONS products), where the coding is carried out after product packaging and labeled on aluminum foil by external parties (in outside the research team) who finalized the research product. Next, make an envelope containing each of the one codes, several research subjects to be recruited, then later 1 subject will take one envelope so that it is known that the subject belongs to a group with a specific codification. The codification is only known by external parties, which will then be stored and duplicated through hard file or soft file documents to be given to the researcher after the data has been collected and statistically analyzed. Thus, the 2 types of research products (standard ONS and intervention ONS) were unknown to either the researchers or the research subjects.

c. Storage, analysis, and reporting of research data

After collecting data, the next stage is storing, analyzing and reporting research data with the following details:

1) The researcher collects research data and stores it in a secure place, namely a locked cupboard and in a safe place, in terms of temperature, humidity, and attack by animals such as rats. In addition, periodically (once a week) researchers will enter research data in soft files and send them to email so that there is duplication of data or documents to avoid unwanted events. Both hard and soft file data storage is carried out in a closed and locked or password manner so that subject confidentiality is maintained and safe from the reach of other parties who have no interest in research. This is done until all the data has been collected from a large number of predetermined samples.
2) Researchers conducted research data analysis
3) Researchers report research data in the form of a dissertation

d. Possible risks that the subject will experience

For the measurement of research parameters in the form of nutritional status, it relatively does not pose a medical risk or trauma to the subject; while taking blood samples there is a risk in the form of phlebitis but this can be prevented by handling blood sampling, by a competent laboratory assistant. The product consumed by the subject was in the form of ONS or liquid food (such as milk) dense in nutrition (high in energy, high in protein, containing immunonutrients in the form of anthocyanin and omega 3), which was recommended by ESPEN as nutritional support for cancer patients undergoing radiotherapy. In addition, the consumption of products provided in the form of standard ONS or those containing immunonutrients carries a risk of digestive tract disorders, such as diarrhea, bloating, or constipation. If this happens, the subject will be referred to the doctor in charge of the study (DPJP or a radiation oncologist/dr. Sp. Onk. Rad) for examination and medication, the handling costs are the full responsibility of the researcher

F. Design of Data Processing/Analysis

The research data results were analyzed descriptively in the form of average ± standard deviation if the data were normally distributed or median if the data were not normally distributed. The pre and post-test dependent variable data in each treatment and control group will be analyzed using a paired t-test if the data is normally distributed. Still, if the data is not normally distributed, it will be analyzed using Wilcoxon. As for the analysis of the difference between the pre and post test data then comparison between the control and intervention groups was carried out with the Independent t-test if the data was normally distributed, but if it was not normal, then it was analyzed using Mann Whitney.

G. Research Ethics

This research has received permission for ethical research feasibility from the Health Research Ethics Commission, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada Yogyakarta Indonesia with the number KE/FK/0996/EC/2021. Then before participating in the research, there will first be an explanation of the research's aims and objectives, the course of the research, and the benefits and risks that prospective research subjects may face. If the subject understands and agrees to participate in the study, the subject is asked to sign the informed consent that has been provided.

INFORMATION SHEET FOR PROSPECTIVE RESEARCH SUBJECTS

"The Effect of Local Food-Based Oral Nutritional Supplements (ONS) Containing Immunonutrients in the form of Anthocyanins and Omega-3 on Nutritional Status and Inflammatory Response in Head and Neck Cancer Patients Receiving Radiotherapy"

I am Fuadiyah Nila Kurniasari, S.Gz, MPH, a Doctor of Medicine student, the Faculty of Medicine, Public Health, and Nursing, Gadjahmada University, Yogyakarta. I am currently researching "The Effect of Oral Nutritional Supplements (ONS) Containing Anthocyanin and Omega-3 on Nutritional Status and Inflammatory Response in Head and Neck Cancer Patients Receiving Radiotherapy" requesting you to participate in this research. Your participation can contribute to the development of science, especially nutrition and health.

A. Research objectives:
To examine the effect of nutritional intervention in a liquid formula on the nutritional status and inflammatory response of head and neck cancer patients receiving radiotherapy.

B. Why the subject was chosen:
Mr/Mrs were selected to be included in this study because you are head and neck cancer patients aged 18-80 planning to receive radiotherapy. Also, you can consume food either directly by mouth or with the help of a feeding tube and do not have allergies to milk and eggs and has no history of kidney disease and diabetes mellitus.

C. Procedures/procedures:
If you are willing to be a participant/research subject and have signed the consent form, you will:

1. Take the envelope containing the allocation for the control and treatment groups, where each group will receive:
   a. Control group: liquid formula containing milk
   b. Treatment group: liquid formula containing purple sweet potato and egg white

   Both groups contain the same energy value and taste similar but have different nutritional content
2. Measured nutritional status, physical function, and sample examination, which includes:
   a. Measurement of nutritional status and physical function:
      1) Assessment of food consumption is carried out by interviewing and filling in the liquid formula consumption monitoring sheet
      2) Body weight and muscle mass were measured by weighing with a digital scale
      3) Height is measured with a stadiometer (a device that measures height)
      4) Upper arm circumference is measured with tape
      5) Grasping strength was measured using a handgrip dynamometer
   b. A 2-3 cc blood sample was taken from a peripheral vein in the fold of the upper arm (cubital fossa) to examine the blood profile related to the study. A laboratory assistant from RSUP Dr. Sardjito Yogyakarta carried out blood sampling. Each procedure (measurement of nutritional status and blood sampling) was carried out twice before and after the study.
3. Asked to drink seven packs of liquid formula once a week with the details: 1 pack is taken 2x/day (1x drink ½ pack), where ½ pack is brewed with warm water to produce 200 ml of liquid formula during the 4-week radiotherapy period. You report it by writing it on the Liquid Formula Consumption Monitoring Sheet that has been provided. If you find it challenging to fill in and report consumption of liquid formula due to a condition, the family can help with this.

D. Risks and inconvenience:
The possible risk is very minimal, namely, the inconvenience that may be experienced due to the interruption of time to take part in this research, namely the time taken to follow all research procedures and also the possibility of having uncomfortable effects due to taking blood samples (a bit painful, swelling or redness on the skin after the injection) and consumption of liquid formulas, for example diarrhea or bloating if you are not used to consuming food ingredients in the liquid formula (eg milk, egg whites, purple sweet potatoes). Suppose there are side effects that are felt to be bothersome. In that case, Mr/Mrs/Sisters/i will be referred to the doctor in charge of the research (doctor or radiation oncologist/dr. Sp. Onk. Rad) to be examined and given medication, with the cost of handling is the responsibility of answer the researcher completely.

E. Benefits (direct to the subject and general):
The direct benefit for you in participating in this research is to get additional nutritional intake by consuming liquid formula and knowing your nutritional status and blood profile.
While the general benefits of this research are

1) For head and neck cancer patients, the results of this study are expected to be able to provide benefits regarding the importance of fulfilling nutritional needs through the administration of liquid formulas to improve nutritional status and inflammatory response to support healing and patient care;
2) For hospitals, the results of this study are expected to be used as a reference for developing hospital formulas to increase the impact of good health on patients; and
3) For future researchers, the results of this study are expected to provide input or references to develop nutritional research, especially formula development for patients.

F. Data confidentiality:
All data obtained in this research will be kept confidential and will be entirely the responsibility of the researcher in the research process and scientific publication.

G. an Estimated number of subjects:
The total number of participants/subjects who participated in the study was 54.

H. Volunteering:
Your participation in this research is voluntary; there is no coercion from anyone, but accompanied by responsibility until your participation ends.

I. Subject participation period:
Your participation in this study was during the radiotherapy period attended by you or for four weeks.

J. Subjects may be excluded/withdrawn from the study:
You can be removed from this research if you do not follow the established procedures, and you are free to withdraw at any time and without any sanctions.

K. Possibility of financing from health insurance companies or researchers:
Your BPJS membership can be used in this study if needed.

L. Incentives and compensation:

As a form of gratitude for your participation in this research, the researcher will provide incentives in the form of souvenirs consisting of a cloth bag, a mini thermos, and 1 set of stainless steel straws

You can also inquire about research to the Medical and Health Research Ethics Committee, Faculty of Medicine UGM (Tel. 0274-588688 ext 17225 or +62811-2666-869; email: mhrec_fmugm@ugm.ac.id).

Question:
If you have questions, please contact:
Name: Ardha Nurul
Address : Bale Asri G-12, RT 10, RW 46, Pereng Dawe, Balecatur, Gamping, Sleman
Phone: 083128177767

Research person in charge:
Name : Fuadiyah Nila Kurniasari, S.Gz, MPH
Address: Jl. Sumbersari III No. 182 Malang
Institutions:
1) Department of Nutrition Faculty of Medicine, University of Brawijaya Malang
2) Doctoral Study Program in Medical and Health Sciences, FKKMK - UGM
Phone : 082226868412

INFORMED CONSENT

I have read or obtained an explanation, am fully aware, understand, and understand the purpose, benefits, and risks that may arise in this research, and have been allowed to ask questions and have been answered satisfactorily, and I also can withdraw from participation at any time, then **I AGREE / DISAGREE *)** to participate in this study entitled:

"The Effect of Local Food-Based Oral Nutritional Supplements (ONS) Containing Immunonutrients in the form of Anthocyanins and Omega-3 on Nutritional Status and Inflammatory Response in Head and Neck Cancer Patients Receiving Radiotherapy"

I voluntarily participate in this research without pressure/coercion from anyone. I will be provided with a copy of the explanation sheet and consent form I have signed for my filing.

I agree:
YES/NO (choose one by crossing out the unnecessary)

If the condition of the prospective participant/research subject is not good, then the signature can be represented by the family who accompanies and can be trusted

	Date :	Sign/thumbprint
Subject Name: Age: Adress:		
Researcher:		
Witnes name:		