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CLINICAL TRIAL PROTOCOL

Title

The impact of a moderate calorie and protein restriction program (CARE-PRO) as an efficient and therapeutic strategy in patients with Barrett's Esophagus.

Approval number: CESC IOV2015/68

Version 2.0, 11/11/2014

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Compliance disclaimer
The clinical trial will be conducted in compliance with the protocol, standard operating procedures, Good Clinical Practice (CPMIP/ICH-135/95) and applicable regulatory requirements.

Study protocol

The impact of a moderate calorie and protein restriction program (CARE-PRO) as an efficient and therapeutic strategy in patients with Barrett's Esophagus.

ABSTRACT

Introduction: The increasing incidence of Esophageal Adenocarcinoma (EAC) in several Western countries can be primarily ascribed to risk factors such as obesity, chronic gastroesophageal reflux, dietary habits and alcohol intake. Nevertheless, Barrett's Esophagus (BE), the precancerous lesion, remains the main risk factor for EAC. Several studies supports the role played by the gut microbiota on the modulation of metabolic and immunological pathways. An abnormal state of the microbial ecosystem seems to be involved in the promotion and onset of various diseases, including cancer. In 2009, Yang et al. identified two types of *in situ* esophageal microbiota (type I and II). Type I microbiota was associated with the normal esophageal mucosa and type II microbiota was associated with unhealthy conditions of the esophageal mucosa typical of patients affected by GERD-related esophagitis and BE. Recent studies have shown that diet and lifestyle have an important modulatory role as protective or risk factors for oncological diseases. The World Cancer Research Fund (WCRF) and the American Institute for Cancer Research(AICR) released a review of the evidence that emerged from published studies in the field of nutrition and cancer prevention and summarized their findings into 10 recommendations that everyone should practice to prevent cancer. The concordance between adherence to such indications and risk of onset of various types of cancer (including cancer of the esophagus) has been demonstrated in a recent large prospective study. Several studies have also shown that a moderate caloric and/or protein restriction seems to be able to reduce the risk of neoplastic disease development. The primary aim of this study is to evaluate the impact of a lifestyle-oriented intervention on body weight, waist circumference, biomarkers associated with cancer risk, esophageal microbiota composition and adherence to cancer prevention recommendations after 24 months in overweight or obese BE patients.

Methods and analysis: Patients are randomly divided into two arms, a control arm (CA) and an interventional arm (IA). The CA receives information about a correct lifestyle to prevent cancer. The IA is involved in the two-year program of moderate caloric and protein restriction.

At the time of enrollment, anthropometric and body composition measurements will be recorded for each patient and they will be allocated by a data manager in the IA or CA. Blood samples will be obtained from each patient and blood glucose will be determined. Serum metabolic biomarkers will be measured in each serum sample and total proteins will be extracted from fresh frozen esophageal biopsy and will be analyzed to evaluate the insulin signal pathway.

To assess esophageal microbiota profiling, total gDNA will be extracted from matched fresh frozen biopsy. In order to determine a score of adherence to cancer prevention recommendations, participants will be asked to complete a self-administrated questionnaire reflecting WCRF/AICR recommendations. Measurements will occur also at the end point, after two years from the enrollment.

Ethics and dissemination: Ethical approval for the study has been received from the Ethics Committee for Clinical Experimentation (Comitato Etico per la Sperimentazione Clinica – CESC) of the Veneto Institute of Oncology (approval number CESC IOV2015/68). This randomised controlled trial will generate substantial information regarding the effect of multimodal approach to reduce

risk of esophageal adenocarcinoma in high-risk population. The results of the study will be disseminated through publication, reports and conference presentation.

INTRODUCTION

Despite the general improvement in living conditions in recent decades and the increased (average) median age of population, the incidence of certain diseases is increasing, especially those associated with aging. Obesity, central adiposity, type two diabetes (TD2) and insulin resistance are widespread in Western countries, and their role as a risk factor for oncological diseases is well documented.[1–3]

Among malignant cancers, Esophageal Adenocarcinoma (EAC) is steadily becoming the most common esophageal malignancy in several Western countries,[4]. The increasing incidence of this tumor can be primarily ascribed to well-known risk factors such as obesity and chronic gastroesophageal reflux,[5,6] but other lifestyle aspects, such as dietary habits and alcohol intake, are likely involved in the onset of EAC.[6–10]

Nevertheless, Barrett's Esophagus (BE) remains the main risk factor for EAC. BE is a precancerous lesion characterized by replacement of normal squamous epithelium lining the esophageal lumen with columnar-lined metaplastic epithelium. The metaplasia is mainly due to chronic reflux, commonly known as gastroesophageal reflux disease (GERD) and it is endoscopically identified in 5-15% of patients with GERD[11]. BE patients have a 30 to 125 fold greater risk to develop EAC compared with non-GERD population,[12] with a EAC risk of 0.1-0.5% *per* year.[13–15]

Despite the low rate for EAC development in BE patients, its mortality rate after 5 years from diagnosis is higher than 80% and it can be dramatically improved when cancer is diagnosed at an early stage.[16]

Nowadays, BE patients are usually enrolled in expensive endoscopic follow-up programs for early diagnosis. These programs are based on endoscopic biopsy standard protocols and/or random sampling (four quadrant biopsies taken at the gastroesophageal junction and 2 cm above it) and are of dubious effectiveness.[17–21]

Thus, other factors and approaches need to be considered in a strategy to reduce EAC risk.

Several data suggest that obesity, particularly central obesity, could play a causative role in the development of BE and its progression to EAC.[22]

Overweight and obesity are associated with a metabolic dysregulation which is part of the Metabolic Syndrome (MS).[23] MS is associated with a chronic pro-inflammatory state strictly related to an excessive amount of visceral adipose tissue. Abdominal obesity leads to alteration of the normal physiological balance of adipokines with an increase in pro-inflammatory adipokines secretion, such as leptin, and a reduction in anti-inflammatory adipokines, such as adiponectin.[24–26] Central obesity is associated with insulin-resistance, defined as a condition in which target tissues of insulin (liver, adipose tissue and skeletal muscle) have a lower sensibility to insulin action. For this reason, the pancreas produces much more insulin to maintain normal glucose levels in the blood. Chronic hyperinsulinemia, as well as an increased level of serum leptin and a reduced adiponectin secretion, are factors that could play a role in oncogenesis.[27–31]

Moreover, a growing body of evidence supports the role played by the gut microbiota on the modulation of several metabolic and immunological pathways. Gut microbiota is composed of several microbial communities with almost 10^{14} microorganisms.[32,33] An abnormal state of the microbial ecosystem in a host, defined "dysbiosis", seems to be involved in the promotion and onset of various diseases, including cancer.[34,35] The mechanisms by which bacteria can induce carcinogenesis include chronic inflammation, immuno-suppression and immuno-evasion.[35,36] Like the intestinal tract, even the esophagus is colonized by human microbiota (HM). In 2009, Yang et al.[37] identified two types of *in situ* esophageal microbiota (type I and II). Type I microbiota was associated with the normal esophageal mucosa and is characterized by prevalence (about 80%) of Gram-positive taxa belonging to Firmicutes phylum (in particular *Streptococcus* and *Gemella* genera) and low percentages of Gram-negative taxa belonging to Proteobacteria (*Haemophilus* and commensal *Neisseria* genera), Bacteroidetes (*Prevotella* genus) and Firmicutes (*Veillonella* genus) phyla. The type II microbiota was associated with unhealthy conditions of the esophageal mucosa typical of patients affected by GERD-related esophagitis and BE. Type II microbiota is characterized by high presence (more than 50%) of Gram-negative bacteria. The more abundant genera in the type II microbiota included *Veillonella* (phylum Firmicutes), *Prevotella* (phylum Bacteroidetes), *Haemophylus*, *Neisseria and Campylobacter* (belonging to Proteobacteria phylum), *Fusobacterium (Fusobacteriaphylum), Rothia and Actinomyces* (belonging to Actinobacteria phylum*)*.[32,33,37]

Recent studies have shown that diet and lifestyle in general have an important modulatory role as protective or risk factors for oncological diseases. In 2007, the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research(AICR) released a wide review of the evidence that emerged from published studies in the field of lifestyle, including physical activity, nutrition and cancer prevention.[38] WCRF/AICR summarized their findings into 10 recommendations that everyone should practice to prevent cancer. Eight out of ten are addressed to cancer primary prevention in the general population and two recommendations are appropriate for "special persons", such as mothers who breastfeed their children and cancer survivors.

The concordance between adherence to such indications and risk of onset of various types of cancer (including cancer of the esophagus) has been demonstrated in a recent large prospective study by Romaguera D., et al.[39]

Several studies have also shown that a moderate caloric and/or protein restriction seems to be able to reduce the risk of neoplastic disease development by bringing back the altered metabolic-hormonal status to a physiological condition and stimulating cell autophagy.[40–45]

Numerous studies have also shown that the effectiveness of a nutritional intervention is greatly improved when the informants, besides providing information, also use health coaching techniques, giving people the opportunity to practice and share acquired knowledge, for example by attending cooking classes, sharing meals and practicing physical activity.[46–58]

OBJECTIVES

In this study, patients are randomly divided into two arms, a control arm and an interventional arm. The aim of this two-arms randomized clinical trial is to assess the effect of a moderate CAloric-protein REstriction PROgram (CARE-PRO) on metabolic-hormonal condition (such as excessive BMI and/or waist circumference (WC), insulin resistance, unbalanced esophageal microbiota composition, chronic inflammation and adipose tissue-related hormones release) which can represent, when altered, a risk factor for the development of esophageal adenocarcinoma in patients with Barrett's esophagus. The control arm receives information about a correct lifestyle

and suggestions to increase the consumption of fiber and vegetable proteins. The interventional arm instead is involved in the two-year program of moderate caloric and protein restriction.

-Overall aim of the study

The main objective of the study is to evaluate the impact of a lifestyle-oriented (diet, physical activity, behavior change) intervention on body weight, waist circumference, biomarkers associated with cancer risk, esophageal microbiota composition and adherence to cancer prevention recommendations (WCRF/ARICR 2007) after 24 months in overweight or obese (BMI ≥ 25.0 Kg/m^2) BE patients.

-Primary aim

To examine the effect of a dietary and exercise intervention on body weight and waist circumference reduction in overweight or obese BE patients.

-Secondary aims

To assess the impact of CARE-PRO intervention on the relationship between anthropometric parameters (body weight, BMI and WC), metabolic serum biomarkers, the expression of proteins involved on insulin and IGF1 receptors signal transduction and esophageal microbiota composition associated with an increased risk of developing EAC.

i) To assess the impact of CARE-PRO intervention on the adherence to WCRF/AICR recommendations for cancer prevention.[6,38] Adherence to cancer prevention recommendations will be assessed by a validated score of adherence.[39]
ii) To evaluate possible differences on CARE-PRO outcomes between elderly (> 65 years) BE patients and younger BE patients (<65 years).
iii) To evaluate economic costs of the intervention.

METHODS AND ANALYSIS

Study design

-Study population: inclusion and exclusion criteria

Patients will be recruited from those included in the Barrett Esophagus and esophageal Adenocarcinoma Risk (EBRA – Esofago di Barrett e Rischio di Adenocarcinoma) Register.

Inclusion criteria

i) Histological confirmation of Barrett's esophagus without dysplasia or cancer aged ≥ 18 years with BMI ≥ 25.0 kg/m^2
ii) Willingness and ability to perform supervised Nordic walking session twice a month and self-planned physical activity at least 3 times a week
iii) Signed informed consent

Exclusion criteria

i) No histological confirmation of Barrett's esophagus
ii) Cancer diagnosis within one year before trial begins
iii) Presence of insulin-dependent diabetes
iv) Denied informed consent

-Sample size

The sample size is based on the primary outcome of a clinically significant change in body weight at the end of the study. A 7% weight loss has been shown to be clinically effective in reducing the risk of diabetes. According to previous data,[44] it is expected that around 30% of patients enrolled in the IA and 15% of those enrolled in the CA will achieve the aforementioned weight loss within the time allotted for trial. In order to reveal a statistically significant difference in the rates of achievement of a 7% weight loss between IA and CA, at least 95 patients are required for each group (power: 80%, type-1 error: 5%). In order to achieve this sample size, we estimate that a pool of 340 patients will be required for recruitment, to allow a recruitment rate of 70% (n=238), and a subsequent drop-out rate of 20% (n=190).

-Trial design

The study will be a two-arm, randomized control trial comparing CARE-PRO intervention with usual clinical approach to BE patients. Study enrollment was started in 2015 and will be complete at the end of 2018. The intervention will last 2 years (24 months) and will be accompanied by the normal follow up procedures.[59]

The study team, including research nurses, will be blinded to the patient group allocation until completion of the primary outcome analysis. Only the principal investigator, study administrator, dietitian and participants will not be blinded considering the nature of the intervention. None of these unblinded staff will have a role in data analysis.

Details of the collected outcomes at different time points are showed in Figure xx.

-Randomization

At the time of enrollment, prior to group allocation, anthropometric and body composition measurements (such as height, body weight, waist circumference at the umbilical level, BMI [weight/(height)^2 (Kg/m^2) will be recorded for each patient immediately before endoscopic procedure. Patients will be randomly allocated by a data manager in the intervention (IA) or control arm (CA), using a permuted-block technique, with block size of four or eight. The Data Manager will not be involved in recruitment or intervention process.

-Blood and biopsy sample collection

Immediately before endoscopy, blood samples will be obtained from each patient (after 10-12 hours fasting) and blood glucose will be determined with a glucometer (Abbott Laboratories® , TX, USA). Serum will be extracted, aliquoted and frozen in liquid nitrogen. During endoscopy, 4 quadratic esophageal biopsies – according to the Levine protocol – will be obtained, fixed in formalin and sent to the Medicine, Surgical Pathology & Cytopathology Unit for histological examination designed to confirm the presence of Barrett's Esophagus and the evaluation of the presence or absence of dysplasia cancer. Additional target biopsies will be obtained from areas affected by Barrett's Esophagus and frozen in liquid nitrogen.

-Serum analysis

Serum metabolic biomarkers such as fasting glucose (mg/dl) insulin (pg/ml), C-peptide (pg/ml), IGF1 (nmol/ml) and its binding proteins IGFBP1 (nmol/ml) and IGFBP3 (nmol/ml), leptin (ng/ml), adiponectin (μg/ml), TNF-alpha (pg/ml) and IL-6 (pg/ml) levels will be measured in each serum sample with Luminex xMAP® technology (multiplexed fluorescent bead-based immunoassay, Luminex, TX, USA); insulin resistance index (HOMA-IR index) will be calculated using the formula: [fasting plasma glucose (mg/dl) × fasting serum insulin(μU/ml)/405],according to the method developed by Matthews et al.[60] HOMA-IR index ≥2.5 will be selected as cut-off for insulin-resistance according to Capasso et al.[61]

-Esophageal biopsy analysis: Insulin/IGF1 signal transduction and microbiota composition

Total proteins will be extracted from fresh frozen esophageal biopsy and will be quantified by Bicinchoninic Acid (BCA) assay (Thermo Scientific Pierce, IL, USA). For each sample, 12.5 μg of total proteins will be analyzed to evaluate the activation state of Insulin Receptor (IR) and IGF1 Receptor (IGF1R) signal pathways using Luminex xMAP® technology. Both the metabolic PI3K/Akt pathway and the mitogenic (ERK/MAPK) pathway will be analyzed.

To assess esophageal microbiota profiling, total gDNA will be extracted from fresh frozen biopsy. Specific primers for the bacterial V3-V4 hypervariable regions of 16S rRNA will be used to amplify bacterial DNA [≈ 500 bases pair (bp)] to be sequenced by Illumina Miseq platform with 300 bp paired-end approach. BMR Genomics (Padua, Italy) will perform the sequencing.

Biopsy and serum samples stored in nitrogen will, as a routine, be managed by the Biological Bank staff who will provide for their conservation in a totally anonymity (as prescribed by law and already authorized by the Ethics Committee with prot. No. P 480 2002)

The acceptance or rejection by patients to participate in the study will not change in any way their established diagnostic-therapeutic procedures and follow-up.

-WCRF/AICR adherence score calculation

In order to determine a score of adherence to cancer prevention recommendations, participants will be asked to complete a self-administrated questionnaire reflecting six out of eight WCRF/AICR recommendations: 1) body fatness; 2)physical activity; 3) energy dense food and drink consumption; 4) plant food consumption; 5) red and processed meat consumption; 6) alcohol intake.

Some recommendations have several sub-recommendations. To each item of the questionnaire, a score of 1 will be assigned when the recommendation is met and a score of 0 will be assigned when it is not. An answer that partially satisfies the recommendation will be assigned a score of 0.5. The final score will derive from the mathematical sum of the individual scores obtained for each recommendation. The maximum expected score will be equal to 6.

Intervention Arm (IA) program

-Dietary caloric-protein restriction

Patients randomized in the IA will be given individualized dietary advice on the basis of WCRF/AICR recommendations.[6,38] The aim of healthy dietary advice will be the reduction of patient's total daily calorie intake up to 600 kcal below their energy requirements. Energy requirement for each patient in the IA will be estimated through revised Harris-Benedict formula,[62] which combines basal metabolic rate (BMR) and physical activity level. In addition to caloric restriction, dietary intervention will aim for a total protein intake to 0.8g of protein/Kg body weight mostly form plant-origin food.

Patients will be required to keep a weekly diet diary that will be analyzed with metaDieta 3.5 software (Me.Te.Da. s.r.l., San Benedetto del Tronto, Ascoli Piceno, Italy). Once a month for the first four months and bi-monthly for the rest of intervention period, patients will meet individually with the dietitian for a 45-minute nutritional counselling session. During these meetings, patients will discuss their diet diary with the dietitian in order to identify the best strategy to comply with the caloric restriction program.

-Health coaching

After the nutritional counseling session with the dietitian, patients in the IA will meet with a trained nurse (coach) for a 15-minute health coaching session. During these sessions, the role of the coach will be to empower participants to take control of their own health, to move the attention from what professionals want to the patient' s own objectives, to help patients to achieve their objectives through feasible steps, and to challenge behaviors that represent an obstacle to positive change.

-Cooking classes

Each patient in the IA will be involved in at least three 4-hour sessions of culinary practice (cooking classes). Participants will be asked to take part to the cooking classes with a relative, such as a wife or husband.

The aim of this practical part of the intervention will be to provide patients with skills and knowledge that they will be able to use to modify their dietary approach and behaviors, and to reach the goal of calorie restriction. Every session and every recipes will be developed based on the Mediterranean Diet in accordance with WCRF/AICR recommendations and with the Healthy Eating Plate proposed by the Harvard School of Public Health using healthy, mostly local and seasonal foods.[6,38,63] Cooking classes will be held by a dietitian and professional cook. At the end of each cooking class, participants will have lunch together, consuming the meal they prepared, and they will discuss the learned knowledge with the dietitian.

-Physical activity

Patients in the IA, after a medical/cardiac evaluation to assure their physical ability for exercise, will attend Nordic walking sessions of moderate intensity two times per month during the 24 months of intervention. A certified expert will supervise every Nordic walking session.

Patients will be also counselled on how they can perform physical activity of moderate-intensity by themselves or in a group in their usual living environment.

Participants in the IA will be provided with a pedometer (Omron walking style IV, Omron Healthcare Europe B.V.) to check the number of steps or distance (Km) walked each day.

Nordic walking session will be performed in groups of twelve participants. Every session will start with 10-minutes of warm-up exercises and will continue with moderate intensity walking in a green, pedestrian area.

Patients will be encouraged to maintain a good level of physical activity during the rest of the week, with 40 minute of brisk walking 4 days/week.

All patients in the IA will receive reminders, of their appointments for nutritional counseling, cooking classes and Nordic walking sessions, through phone call, e-mail or/and text message on their mobile phone and will be contacted for any change in the scheduled activities due to unexpected events, for example bad weather in the case of Nordic walking.

Control Arm (CA) program

Participants in the CA will be given information about the importance of a healthy lifestyle in reducing the risk of cancer and will receive a leaflet based on WCRF/AICR recommendations.[38]

Statistical analysis

Fisher's exact test will be used for comparison of categorical variables. Numerical variables will be expressed as median and interquartile range (Q1; Q3). The Mann–Whitney *U*-test will be used to evaluate differences between IA and CA groups. Paired Student's T test will be used to compare the difference in the parameters measured at baseline (at the time of enrolment) and at the end of the trial in the same patient. Spearman's rank correlation coefficient will be calculated to evaluate the correlation between two measured parameters. All tests will be two-tailed and a *p* value lower than 0.05 will be assumed to indicate a significant difference. Data analyses will be performed with SPSS v20 and Stats-Direct.

ETHICS AND DISSEMINATION

Ethics approval and consent

Ethical approval for the study has been received from the Ethics Committee for Clinical Experimentation (Comitato Etico per la Sperimentazione Clinica – CESC) of the Veneto Institute of Oncology (approval number CESC IOV2014/68).

The study will be conducted in accordance with the principles of the 1975 Helsinki Declaration (6threvision, 2008) and written informed consent will be obtained from all patients. The features and aims of this study will be thoroughly explained to all patients.

Confidentially

Information collected directly from participants will be in a reidentifiable form and any information collected for, used in generated by this project will not be used for any other purpose. The site principal investigator and associated research personnel will have access to information.

Dissemination policy

Results if this clinical trial aim to be disseminated through peer-reviewed journal articles, conference abstract ad presentation, as well as media publications.

Translational relevance and impact for CARE-PRO trial

The impact of diet in preventing diseases is largely underestimated. If in our BE patients' cohort we will demonstrate feasibility and efficacy of the CARE-PRO in bodyweight change and modification of biomarkers associated with increased risk of cancer and other lifestyle related comorbidities, we could propose it as a low-cost therapy to reduce mortality and morbidity, especially in older patients where endoscopic follow-up has been proven to be not cost effective. Ideally the frequency of follow-up endoscopic examinations could be dramatically reduced with a significant expense reduction for the NHS (National Health System). Moreover, the outcome parameters potentially improved with this approach are associated with an increased risk of other cancers and cardiovascular diseases as well, thus the net savings for the NHS could be larger than the already substantial ones derived from the optimization of endoscopic follow-up in BE patients due to an overall decrease in morbidity.

Competing interests

The Authors declare no conflict of interest.

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