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Food Habits and Risk of Endometrial Cancer: Development of an Assessment Questionnaire

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Abstract

Endometrial cancer is the most common gynecologic malignancy in the Western world and is strongly associated with obesity. Most of the studies investigating this relationship have been conducted by using Food Frequency Questionnaires, which carry several biases and are not easily applicable. At the Academic Department of Obstetrics Gynecology of Mauriziano Hospital, in Turin, we conducted a case-control study in which a food diary was administered to 142 women with endometrial cancer and to control healthy women with the aim of collecting eating habits of cases and controls, in terms of quantity and quality of nutrients during a period of three consecutive days. Our research revealed a real imbalance in the eating behavior between the two groups of patients. In particular in the group of cases typical diet was high in calories, proteins (especially of animal origin) and lipids. Our diary proved to be a simple innovative instrument to evaluate personal food habits of EC patients and as secondary endpoint our study succeeded in confirming the association between EC risk and the intake of certain nutrients. This paper highlights the importance of lifestyle in the prevention in endometrial cancer and represents a starting point to further investigate the theme.

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Introduction

Endometrial Cancer (EC) is the fourth most common cancer in women. While the incidence and mortality rates from several other cancers have plateaued or decreased in the last decade, rates for EC continue to rise [1]. The reasons underlying this raise are many, but the growing obesity rate is surely a major contributing factor. Obesity is a significant risk factor in the development of EC and also one of the greatest post-treatment health threats. Up to 90% of Type 1 (estrogen-dependent) EC patients are obese [2]. Although affecting Western nations more dramatically, the prevalence of obesity is increasing globally. Epidemiological studies have associated obesity with several cancer types, although the exact mechanisms by which obesity induces or promotes tumorigenesis vary a lot. These include insulin resistance and chronic hyperinsulinemia, increased bio-availability of steroid hormones and inflammation [3]. The most significant risk factors for EC and its precursor, endometrial hyperplasia, include unopposed estrogens, obesity and sedentary lifestyle [4]. Schouten et al. [5] demonstrated that obesity increases the risk of EC by 4.5 times and sedentary lifestyle by 46%, not only in postmenopausal but also in premenopausal women. The literature shows that EC survivors with obesity and diabetes have decreased life expectancy when compared to their non-obese, non-diabetic counterparts with the same malignancy [6].

Few studies have evaluated the role of nutrition as risk factor for EC, but specific types of food have been associated with higher risk, especially those high in fat and cholesterol. On the opposite, grains, vegetable and fruit are inversely associated with cancer risk [7]. A case control study of more than 500 women with EC compared to women without cancer showed a significant increase in EC risk with consumption of red meat (OR 2.07). Inverse associations were observed for coffee (OR 0.83), cereal (OR 0.92) and vegetable (OR 0.83) [8]. All these studies support a role for diet in the etiology of EC. Most of the studies investigating this relationship have been conducted by using Food Frequency Questionnaires, characterized by several biases plus being non-easily applicable. The present study aimed to develop an innovative instrument to evaluate personal food habits of EC patients in comparison to controls through a three-day food diary. As secondary endpoint our study intended to confirm the association between EC risk and the intake of certain nutrients.

Materials and Methods

Patients' selection

A case-control study was conducted at the Academic Department of Obstetrics Gynecology, Mauriziano Hospital, Torino. Patients diagnosed and treated for Endometrial Cancer (EC), endometrioid histology, were enclosed. Exclusion criteria were as follows: (1) final diagnosis of endometrial hyperplasia or EC different from endometrioid histology, (2) any qualitative or quantitative change to their eating habits until the diagnosis of cancer, (3) presence of synchronous solid cancer, (4) diagnosis of family cancer syndrome (Lynch syndrome), and (5) incomplete data. Controls were recruited among patients treated for non-neoplastic diseases of the pelvis (Clinic of menopause, Clinic of urodynamics and uro-gynecology and Service of gynecological ultrasound at Mauriziano Hospital). Women with previous hysterectomy or patients suffering from any disease requiring changes in their diet were excluded from the controls. History of each patient was collected including age at diagnosis/present age for the controls, Body Mass Index (BMI), number of pregnancies, age at menarche and at menopause, smoke, any current or previous hormone replacement therapy, diagnosis of dyslipidemia, diabetes mellitus, hypertension, thyroid diseases, treatment with tamoxifen, radiotherapy for previous cancers, and family history of malignancies. Ethics committee approval was obtained and all women included in the study gave written informed consent for data collection with research purpose.

Food diary

A food diary was prepared by the Division of Dietetics of the Mauriziano Hospital with the aim of collecting eating habits of cases and controls, in terms of quantity and quality of nutrients. The evaluation of food habits was conducted by the analysis of the intake of food and drinks during a period of three consecutive days, including at least one public holiday, to assess any changes in food at weekends compared to weekdays. Example of the three-day food diary is reported in Table 1. To get an appropriate food history of the patients, the interviews, lasting about 30 min to 40 min each, were conducted under the supervision of the same operator, following a clear and precise set list, developed point by point. Patients were asked about their habits in terms of mealtimes including snacks. They were asked to write down every type of food and all beverages (including tea, coffee and water) they consumed during the day, indicating the amount of each food they consumed. The quantity of food was expressed through conventional measures (g, l, ml, etc.) or most common measures used in cooking (glass, cup, plate, spoon, teaspoon, etc.). To simplify the evaluation of the size of the portions, we have depicted examples of some food in different sizes (e.g. small, medium and large portion) in the last pages of the food diary.

Moreover patients were asked to report the brand and the exact name of the industrial cooking products to note all the information indicated on the label. They were supposed to note in the appropriate column of the diary all seasonings added, indicating the amount and the unit of measure. Seasoning was considered any kind of ingredient added to a prepared food (parmesan cheese on pasta, sugar in coffee, etc.). They were moreover asked to note if they used any supplements (vitamin and mineral supplements) during the day. In addition, the patients were asked to give information regarding any possible physical exercise made during the day, indicating the type (e.g. walking) and duration (e.g. 30 min) of it.

WinFood software

Once the questionnaires were completed, the data were entered into the software release 2.8.0 WinFood for automatic data processing. The software elaborated a quantitative assessment of patients food supply in terms of calories, differentiating among various nutrients, based on the statements of each patient about the kind and quantity of foods they said they had during the three days (proper meals and snacks). We evaluated the food consumed by the patients by consulting an archive of about 2200 different types of food with 100 different nutritional components for each food, including 250 types of frozen food, 200 different recipes reporting the different ingredients and methods of food preparation and about 400 special foods (gluten-free, protein-free, cholesterol-free, sugar-free) showing the compositions of nutrition labels. For each food our software reported the caloric content, the percentage of carbohydrates, fats and calories (per 100 g of raw food), etc. referring to the Tables of Food Composition of the National Institute of Nutrition and Food Composition Database for Epidemiological Studies in Italy of the European Institute of Oncology [9]. The software calculated all the data and obtained an assessment of usual dietary intakes of the patients, in terms of absolute amount of food per day and the percentage of single nutrients and food components.

Statistics

The descriptive variables relating to our groups of study cases and controls were represented in contingency tables with absolute numbers and percentages for categorical variables and average values for continuous variables. We calculated Odds Ratios (ORs) (number of cases in which the phenomenon occurred)/(number of cases in which the phenomenon did not occur): as a dichotomous scale, events were represented by the cases (patient suffering from EC) and controls indicate no events. The strength of association and odds ratios were determined by univariate logistic regression analysis which selected variables differentially expressed in our cases and controls. The covariates (as regards the nutritive components of the diet) were initially introduced into the logistic model as continuous variables. The variables for which there was a significant difference level with $\alpha=0.05$ between cases and controls were subjected to correction for possible confounders and values were dichotomized as $<$ or $\geq$ the median. We produced then ORs, 95% confidence intervals and corresponding P values, corrected by introducing in our multivariate models as covariates: age (continuous numeric value), previous use of hormone replacement therapy (yes or no), BMI (continuous numeric value). The variables (diet nutrients) that were not significantly different between cases and controls have been introduced in the models as a continuous numeric variables and their values of OR were considered risk values for each unit increase of the unit of measure for the substance in the group of cases. SPSS software-release 17 (Chicago, IL, SPSS Inc., USA) was used for the analysis.

Results

We enrolled a total of 142 women, recruited during a seven-month period (November 2014 to May 2015). The case group included 61 women (43%) aged between 43 and 83 (average age 53.3). The control group included 81 women (57%) with similar age of the cases.

The clinical characteristics of all the patients are reported in Table 2. The analysis of the clinical characteristics in the two groups showed statistically significant differences for the following variables: BMI, previous HRT, BMI, and hypertension. The questionnaire resulted easy to administer and to understand, under the supervision

Figure 1: Examples of some food in different sizes (eg. small, medium and large portion).

of a dedicated operator. The registration of the amount of food each patient had during the day was the most delicate point of this type of interview. The depicted examples of some food in different sizes (e.g. small, medium and large portion) in the last pages of the food diary were helpful to bring the dosing closer to the real consumption. Collecting all the data, we were able to depict the eating behavior of all cases and controls and, thanks to the particular structure of our diary; we easily collected all the answers. WinFood allowed us to make, with easily overlapping procedures, the Food Survey and the Diet Survey. In the comparative univariate analysis of macro- and micro-nutrients introduced with food in the group of cases and controls, we observed a statistically significant difference between the two groups of patients about the nutrients reported in Table 3.

The univariate analysis of macro and micronutrients confirmed a statistically significant association between the risk of developing endometrial cancer and the intake of several nutrients, such as lipids, saturated and unsaturated fatty acids, cholesterol, animal protein, some amino acids (methionine, serine, and tryptophan), calcium, iron, and thiamine. A higher intake of alpha-tocopherol was associated with the reduction of endometrial cancer risk.

Discussion

In 2007, the World Cancer Research Fund published a report on the relationship between food, nutrition, physical activity and neoplastic disease [1]. This paper highlights the importance of lifestyle in the prevention of several types of cancers, including EC. Most of the studies about the role of diet in the pathogenesis of EC are based on Questionnaires of Frequency of Intake of Foods (FFQs) or on the "recall of 24 h"; they are used to get qualitative information on food habits of the population, but are not the ideal tool to quantify the consumption of nutrients. A study by Kowalkowska et al. compared a FFQ and a three day food diary as method of assessment of food intake in a sample of healthy subjects: it came clear that both methods, both self-compiled, have some limitations related to the difficulty in estimating the portions of food and this is evident especially for FFQs [10]. The recall of the 24 h is a method that allows operating on large groups of patients and, unlike the FFQs, to better quantify food consumption with the help of homely measures, plastic models or photographic atlas. However, this method is not convenient to evaluate the general eating habits of the population.

In the light of this evidence our study was performed by using an original investigation instrument that resulted easier to apply: it consisted of a three-day food diary where patients were requested to give information about both quality and quantity of their daily eating intake. The evaluation of patients eating habits included at least one non-working day to assess any changes in food at weekends compared to weekdays. All interviews were conducted under the supervision of the same operator who tried to make information the patients gave really representative of their usual eating behavior and not only of the three days preceding the interview. Despite the small sample size, our data analysis showed a significant difference between eating behaviors of patients with EC and controls. Our food diary appeared to be an applicable and convenient investigating tool: not only was our instrument simple to use, but was also valid and effective like more complex but less easy-to-use tools.

Thanks to our diary in people with cancer we found higher levels of daily eaten calories, total fats (OR=2.4), saturated fatty acids (OR=4.5), unsaturated fatty acids, cholesterol, glucides, alcohol, starch, animal proteins, certain amino acids (methionine, serine, tryptophan, etc.), calcium, sodium, iron and thiamine. In the

Table 1: Example of the three-day food diary.
Day of the week …….. Date … /… /…

	Time	Food and drinks	Quantity	Type of cooking	Seasoning, any adding
Breakfast					
Snack					
Lunch					
Snack					
Dinner					
Snack					
Supplements					
Physical exercise					
Notes					

Table 2: Patients characteristics.

Variables	Cases (n 61)	Controls (n 81)	OR	IC (95%)	*p*
BMI (range)	29.8 (18.59-48 .07)	24.03 (17.96-34.67)	4.6	1.8-11.5	0.001
Pregnancies					
0	13	28	1	-	
1	18	25	0.25	00-0.9	0.191
2	19	29	0.38	0.1-0.3	
2	9	9	0.48	0.1-0.7	
Age at menarche	12.4 (11-17)	12 .9 (9-15)	1	0.9-1	0.3
Age at menopause	50.9 (37-59)	50.1 (40-57)	1	0.9-1.1	0.3
Previous HRT					
no	48	77	1	-	
yes	11	4	4.2	1.2-14.0	0.009
Diabetes	15	4	4.9	1.6-14.5	0.004
Hypertension	26	18	2.6	1.2-5.4	0.01
Dyslipidemia	2	9	3.6	0.7-17.7	0.1
Adjuvant hormonal therapy	7	6	0.53	0.17-1.61	0.26
Radiotherapy	4	8	1.2	0.38-3.9	0.79
Smoke	17	16	1.5	0.7-3.4	0.2

literature four case-control studies and a meta-analysis have shown a relationship between the intake of total fats and EC (increased risk of cancer especially for high consumption of saturated fats and animal-derived fats [1,11-14], even though opposite results were obtained in some others studies [15,16]. In our study increased EC risk was associated with high intake of mono-unsaturated fatty acids (eggs, cheese, pork and beef) and also by poly-unsaturated omega 3. However, this last finding was not confirmed by other studies showing a protective effect, thanks to their anti-inflammatory effect and by balancing plasma levels of cholesterol and triglycerides [17].

Our work showed higher prevalence of obesity (OR=4.6), diabetes (OR=4.9) and hypertension (OR=2.6) in patients with EC in comparison with the healthy group: body weight excess is one of the major risk factors for type I EC, because obesity and sedentary lifestyle with high-calorie food diet lead to proliferative processes and anti-apoptotic effects on the endometrium: peripheral conversion of androgens into estrogens and increased levels of insulin. Obesity is the principal determinant of "delta-4 steroids effect": obese women have higher serum levels of androstenedione, testosterone and free testosterone [18].

Even though an inhibitory effect on the proliferation of endometrium by androgens is reported [19]. The relationship between obesity and EC appears to be also mediated by inflammatory mechanisms: cytokines and adipokines (IL1, IL6, TNF-α), whose blood levels are affected by circulating estrogens, contribute to the initiation and progression of cancer [20]. Moreover, elevated serum insulin levels enhance the effect of estrogen on endometrial cells and similarly to other studies, ours confirmed that high serum insulin levels are associated with higher risk of EC, particularly in overweight/obese women [21]. High blood levels of cholesterol (OR=1.6) and total calories introduced (OR=3.3), in addition to calcium (OR=7.5) and sodium (OR = 4.1) resulted to be associated with higher risk of EC among our patients: cholesterol is the precursor for the synthesis of steroid hormones and is therefore converted into estrogens, increasing their bioavailability. In post-menopausal age estrogens production is mainly concentrated in the fatty tissue and their production is more affected by cholesterol levels than during the pre-menopausal age.

Apart from fats, hyper caloric diet and cholesterol, we could also highlight a statistically significant association between EC and daily high intake of glucides (OR=2.5) and we observed higher percentage of women with diabetes mellitus in the group of patients: in the literature type II diabetes seems to be related to increased risk of atypical EH and EC, regardless of obesity; similar considerations also regard women without diabetes but with hyperinsulinemia, insulin resistance, or metabolic syndrome [22-25]. Insulin itself by reducing the hepatic production of SHBG is responsible for high serum levels of testosterone [26]. Increase in EC risk does not only come through being energy source for cell proliferation, but also because it generates free radicals which cause oxidative damage to DNA or enzymes for DNA's repair [27,28].

In our work high protein intake, especially of animal origin (meat, fish, eggs, milk, cheese) increased 4.2 times EC risk (p=0.001). Many different mechanisms underlie the association between red meat consumption and EC risk, including the formation of N-nitrous compounds which are potentially carcinogenic and the formation of heterocyclic amines and polycyclic aromatic hydrocarbons, formed because of high temperature when beef, poultry, pork or fish are cooked [29,30]. In particular, we observed an increased risk for certain amino acids (lysine, histidine, arginine, aspartic acid, serine, threonine, glutamic acid, proline, glycine, alanine, cystine, valine, leucine, isoleucine, tyrosine, phenylalanine, tryptophan) and for high levels of iron of animal origin (OR=2.4) in diet: free iron is able to catalyze the formation of free radicals which cause oxidative damage in specific cellular components, including DNA, proteins and membrane lipids and it is also involved in the modulation of the pro-inflammatory effects of estrogens on the endometrium [31]. As to vitamins and amino acids we report a protective association between EC and the intake of methionine (OR=2.4) or thiamine (OR=3.0): it is known from the literature that methionine has a protective effect against lung cancer and a study by the team at the Mario Negri reported our same results as regards EC [32]. Another interesting result is about alpha-tocopherol (OR=0.3) as a protective element for EC: the action of tocopherol on the growth of malignant cells is well-known and food of vegetable origin with seeds above all contains big amounts of it. Tocopherol is a powerful fat-soluble antioxidant and has an important role in preventing the oxidation of polyunsaturated

Table 3: Comparative analysis of macro- and micronutrients between cases and controls.

Nutrients	OR	IC 95%	*p*
Calories Kcal	3.3	1.4-7.8	0.006
Alcohol Kcal	1.08	1.005-1.165	0.037
Proteins g	1.8	1.04-1.1	0.001
Glucides g	2.5	1.0-5.8	0.032
Lipids g	2.4	1.06-5.6	0.001
Cholesterol mg	1.6	1.2-2.9	0.046
Saturated fatty acids g	4.5	1.8-10.9	0.001
Polyunsaturated fatty acids g	1.1	1.0-1.2	0.02
Mono unsatured fatty acids g	4.3	1.7-10.3	0.001
C4:0-C10:0 g	3.3	1.4-7.5	0.005
Lauric acid g	2.1	1.1-4.9	0.05
Myristic acid g	2.8	1.2-6.4	0.012
Stearic acid g	1.8	1.371-2.977	0.001
Calcium mg	7.5	3.0-1.8.7	0.0001
Sodium mg	4.1	1.7-9.9	0.001
Potassium mg	2.3	1.0-5.4	0.037
Iron mg	2.4	1.1-5.6	0.029
Thiamine mg	3	1.3-6.9	0.002
Starch g	2.4	1.0-4.6	0.020
Animal proteins g	4.2	1.8-10.0	0.001
Methionine mg	2.4	1.0-5.5	0.037
Lysine mg	3.9	1.6-9.5	0.002
Histidine mg	3.4	1.4-8.0	0.005
Arginine mg	3.2	1.4-7.5	0.006
Aspartic acid mg	3	1.3-7.0	0.010
Serine mg	4.5	1.9-10.9	0.001
Threonine mg	2.9	1.2-6.9	0.012
Glutamic acid mg	3.2	1.4-7.5	0.006
Proline mg	4.3	1.8-1..0	0.001
Glycine mg	3	1.3-7.0	0.008
Alanine mg	3.1	1.3-7.2	0.009
Cystine mg	2.1	1.0-4.8	0.05
Valine mg	3.5	1.5-8.1	0.003
Isoleucine mg	3.2	1.3-7.4	0.007
Leucine mg	4.1	1.7-9.7	0.001
Tyrosine mg	2.7	1.1-6.1	0.019
Phenylalanina mg	3.5	1.5-8.2	0.004
Tryptophan mg	5	2.0-12.1	0.0001
Alpha-tocopherol mg	0.3	0.1-0.7	0.009

fatty acids.

Finally, as regards fibers consumption, our result is not statistically different in the two groups, even if the amount of fibers consumed in the control group was higher than that in the group of women with EC. In the literature, results are not concordant about this topic and we require further investigation: the biggest work so far, anyway, is a meta-analysis of case-control studies that showed an inverse association between fiber intake and risk of tumor development [33].

One limitation of our study is the size of the sample. The 142 patients (61 cases and 81 controls), however, can represent a starting point from which to continue the study and to expand the sample. Among the limitations of using a food diary as method of investigation, we can recognize a not so accurate recording of consumption of food at home and the variability over the time, with differences among the seasons and days of the week. Interviews conducted on our patients, however, were balanced between the different seasons and the structure of the diary allowed the registration of the consumption of two weekdays and one holiday. Besides, it is important to point out the crucial role of the same interviewer in the guided compilation of the diary which resulted in accurate information recording. We finally also selected people belonging to the same geographical area.

Conclusion

Our research revealed a real imbalance in eating behavior between the group of patients with EC and the control group. In particular in the group of cases it has been highlighted a diet with a higher amount of calories, proteins (especially of animal origin) and lipids. These results, obtained through the guided administration of the diary, encourage us to confirm that the survey instrument chosen is agile, innovative and effective. Those requirements are an incentive to expand the future prospects of our work. Currently, in fact, there are no effective screening methods for EC. In 2001 the annual report of the American Cancer Society (ACS) has ruled out the possibility of setting up a screening program. The ACS recommends a inform women at intermediate-high risk at the beginning of menopause about symptoms and risk factors. For this reason it would be possible to apply the research to clinical reality, with appropriate guidance to patients on changes in lifestyle, and specifically in eating behavior, to implement primary prevention. In fact the food diary could be useful to individualize any necessary nutritional corrections in patients with known risk factors (previous estrogen therapy not balanced by progestin therapy, previous tamoxifen therapy, late menopause, nulliparity, infertility, anovulation, obesity, diabetes, arterial hypertension). There is no doubt that an accurate sensitization to food education proves the most effectiveness only if it is able to involve patients: a positive response, in this sense, was achieved in this study thanks to the simple, understandable and attractive structure of the food diary. This tool can also be useful to plan the diet of patients already suffering from EC, allowing the physician to make recommendations for the prevention of recurrences. Furthermore, thanks to the results of this study, we propose the construction of a multi-center trial conducted on a larger population to carry out validation studies of the questionnaire we used and of the methods we applied to data collection in a population's representative sample.

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