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Non-adherence to Mediterranean diet and synergy with lifestyle habits in the occurrence of breast cancer: a case-control study in Italy

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Abstract. – OBJECTIVE: The aim of this study was to assess the synergistic effect of non-adherence to the Mediterranean Diet (MD) and lifestyle habits on the occurrence of breast cancer (BC).

PATIENTS AND METHODS: A case-control study was carried out from September 2018 to February 2019 at the Teaching Hospital "Umberto I" in Rome. A Food Frequency Questionnaire was used for assessing the level of adherence to MD, the IPAQ Questionnaire to measure physical activity, and AUDIT-C to estimate alcohol consumption. The possible interaction between risk factors was tested using the synergism index.

RESULTS: A total of 94 cases and 88 controls were enrolled (median age 55.8 for cases and 57.9 for controls). The MD Score over 6 was associated with low odds of having breast cancer (OR = 0.29; 95% CI: 0.12-0.69). There is a clear indication for the additivity and synergism between non-adherence to MD and many risk factors on the occurrence of BC: current smoker (S = 2.02; 95% CI 0.62-8.07), physical inactivity (S = 2.14; 95% CI 0.71 2-8.28) and alcohol consumption (S = 3.02; 95% CI 0.91-12.95).

CONCLUSIONS: Primary prevention of BC can benefit from intervention targeting nutritional and lifestyle factors that act synergistically.

Key Words:
Breast cancer, Epidemiology, Synergism, Mediterranean diet, Alcohol, Physical activity, Smoking.

Introduction

Breast cancer (BC) represents one of the main issues for women' health for both morbidity and mortality all over the world. 2.1 million women are diagnosed having a breast cancer each year. Moreover, this cancer accounts for almost 15% of all cancer deaths among women[1]. There is evidence that supports the association between Mediterranean Diet (MD) and decreased risk of breast cancer[2]. Dianatinasab et al[2] found in a recent systematic review a significant inverse association between MD and invasive ductal carcinoma in case-control studies (RR = 0.47; 95% CI, 0.39-0.55), and invasive lobular carcinoma for the highest compared to the lowest category of MD (RR = 0.76; 95% CI, 0.64-0.87). Moreover, other factors, such as alcohol consumption[3] and diabetes mellitus, oral contraceptives, hormonal replacement therapy, or breast-feeding[4], are known as risk factors for BC risk. Recently, a systematic review studied the association of combined lifestyle factors (i.e., including mainly tobacco smoking, alcohol consumption, physical inactivity/sedentary behavior, overweight/obesity, diet, and sleep duration) and the risks of site-specific cancers. In this review, the role of combined healthy lifestyle factors is significantly protective (HR = 0.77) for breast cancer[5].

However, little is known about the synergistic effects of these risk factors. So, the aim of this study was to assess the synergistic effect of non-adherence to MD and lifestyle habits in the occurrence of breast cancer.

Patients and Methods

Study Design and Participants

A case-control study was carried out from September 2018 to February 2019 (Local Ethics Committee Approval Prot. 739/2018).

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The patients (cases) included in the study were incident breast cancer cases consecutively recruited at the Department of Breast Unit of the Teaching Hospital "Umberto I" in Rome (Italy), while controls were recruited, matched for age, at the Surgical and Ortophaedics wards in the same hospital.

Patients were given a questionnaire for the assessment of the influence of lifestyles on exposure to risk factors. The questionnaire consists of the following sections: a section for the collection of socio-demographic variables, a Food Frequency Questionnaire (FFQ) validated in a previous study[6] for the assessment of the level of adherence to MD, the Italian version of the IPAQ Questionnaire to verify the execution of physical activity (in terms of frequency)[7], the AUDIT-C[8] used for assessing alcohol consumption.

We calculated the MD score using FFQ, which included 12 items that correspond to the 12 elements typical of the MD (pasta, olive oil, fruits, vegetables, red meat, salami, white meat, legumes, fish, commercial sweets or pastries, wine, sweet or carbonated beverages). For each participant, the score was built by adding the single point obtained for 12 groups of foods. To each of the 12 food components, 1 point was assigned if the consumption was according to the reference daily/weekly frequency of the Pyramid of the Modern MD, so the range scores from 0 (minimum) to 12 (maximum).

For the alcohol consumption, the following scores were considered indicative of problematic use: a score of 4 for men and 3 for women on the AUDIT-C is considered optimal for identifying hazardous drinking or active alcohol use disorders[9].

To select the sample, the following inclusion criteria have been chosen:

- Age between 30 and 80;
- Female patients;
- Patients with a healthy state of consciousness.

Statistical Analysis

Sample size calculations (Epicalc2000) were made on the following parameters:

- Alpha level: 0.05;
- Beta error: 0.20;
- OR to detect the association between Mediterranean Diet and breast cancer: 0.40;
- Prevalence of MD score over 6: 40%.

Differences between cases and controls were tested using Mann-Whitney and Chi-square tests for quantitative and categorical variables, respectively. A logistic regression analysis was carried out for assessing the association between the independent variables and being a case of breast cancer. Results are reported as Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs). For the MDS a ROC was plotted, and a cut-off point was calculated on the basis of the maximum value of the sum of sensibility and specificity (MDS over 6).

Moreover, the possible interaction between risk factors was tested using the synergism index, which indicates a biological interaction and not merely a statistical interaction, calculated as follows: $S = [OR_{11} - 1] / (OR_{01} + OR_{10}) -2]$, where OR_{11} is the OR of the joint effect of two risk factors and OR_{10} / OR_{01} are equal to OR of each risk factor in the absence of the other[10]. According to Rothman procedure, S>1 indicates positive synergistic interaction (i.e., the effect of the joint exposures of 2 risk markers is greater than the sum of the separate effects), S=1, indicates no, or exactly additive, interaction, and S<1 indicates negative interaction.

The statistical analysis was performed using SPSS, release 25.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA). The statistical significance was set at $p < 0.05$.

Results

A total of 94 cases and 88 controls were enrolled, with a median age of 55.8 for the cases and 57.9 for the controls. The analysis of socio-demographic variables does not underline statistically significant differences in the groups in terms of risk (Table I).

A logistic regression model highlighted the score of Mediterranean Diet >6 have can be considered as a protective factor (Table II).

Finally, there is a clear indication for the additivity and synergism between many risk factors (Table III): non-adherence to MD and current smoker (S = 2.02; 95% CI 0.62-8.07), non-adherence to MD and physical inactivity (S = 2.14; 95% CI 0.71 2-8.28) and non-adherence to MD and alcohol consumption (S = 3.02; 95% CI 0.91-12.95). No additivity and synergism were found considering the combination between the non-adherence to MD and no smoking, physical activity, and no alcohol consumption.

Discussion

The main aim of this study was to assess the synergistic effect of non-adherence to MD and

Table I. Socio-demographic characteristics of the study participants.

Socio-demographic variable	Cases N (%) or mean (SD)	Controls N (%) or mean (SD)	*p*-value
Age	55.8 (7.8)	57.9 (8.1)	0.335
Marital status			
unmarried	11 (11.7%)	14 (15.9%)	0.199
divorced	11 (11.7%)	13 (14.7%)	
cohabitant	59 (62.7%)	46 (52.2%)	
widowed	10 (10.6%)	15 (17%)	
Type of employment			
self-employed	11 (11.7%)	13 (14.7%)	0.001
public servant	6 (6.38%)	29 (33%)	
private employee	24 (25.5%)	11 (12.5%)	
retired	17 (18.08%)	17 (19.3%)	

lifestyle habits in the occurrence of breast cancer. The study demonstrates the presence of synergism between non-adherence to MD and exposure to other risk factors and underlines the role of primary prevention for the reduction of risk, and as far as we know it is the first time that this effect was assessed. A synergistic effect does exist between non-adherence to MD and tobacco smoking (S = 2.02), physical inactivity (S = 2.14), and alcohol abuse (S = 3.02).

These results are consistent with previous studies that give evidence on the synergistic effect of risk factors in the occurrence of chronic diseases[11-13].

The prevention of BC has several options. Many authors underline the importance of periodic checks (especially screening activities of secondary prevention), but conflicting opinions do exist[14]. On the other hand, the role of primary prevention and especially the promotion of healthy diets as well as healthy lifestyles are crucial for fighting one of the most killing cancer in the world[15,16].

This case-control study has some limitations: first of all, the sample size is quite small; nevertheless, the regression analysis reveals significant results even with a small sample size, and the synergism was found for several risk factors combinations. Secondly, a recall bias cannot be excluded, even if there is no evidence that cases and controls must be different in remembering nutritional aspects as well as lifestyle habits. Moreover, in this study, we did not take into consideration endocrine and genetic factors[17-19], as well as menopausal status, parity, BMI, oral contraceptive use, hormone replacement therapy use, that are known to be associated with the occurrence of breast cancer, since our focus was to study the effect of the combinations of modifiable factors.

Another possible factor that could have an impact is the menopausal situation. One possible solution should have been limiting the study group to post-menopausal women, in order to obtain the effect of accumulation of some risk factors, especially dietary habits. However, this is a single centre study, and it is difficult to recruit post-menopausal women with breast cancer in a short period of time. Concerning the genetic factors, most cases of breast cancer are not caused by inherited genetic factors. Only about 5% to 10% of breast cancer cases are thought to be hereditary**.** Moreover, the best study design for assessing the association between diet and breast cancer is a prospective study (cohort), but this lasts several years to be carried out. Concerning the choice of the control group, this is a case-control with hospital controls, and the controls were recruited in surgical wards (General surgery and Orthopae-

Table II. Results of the logistic regression analysis – Dependent variable: breast cancer case.

Variables	OR (95% CI)
Marital status: cohabitant	1.36 (0.70-2.66)
MD score >6	0.29 (0.12-0.69)
Type of employment: public servant	0.15 (0.06-0.4)
Alcohol consumption	0.99 (0.55-1.78)
Current smoker	1.30 (0.58-2.99)
Ever smoker	1.51 (0.75-3.03)
Physical inactivity	1.20 (0.48-2.98)

Table III. Synergistic interaction between non-adherence to Mediterranean diet and lifestyle habits in influencing breast cancer in women.

Variables	Index of synergism (95% CI)
Non-adherence to Mediterranean Diet – Current smoker	2.02 (0.62-8.07)
Non-adherence to Mediterranean Diet – No smoker	0.35 (0.01-10.75)
Non-adherence to Mediterranean Diet – Physical inactivity	2.14 (0.71-8.28)
Non-adherence to Mediterranean Diet – Physical activity	0.73 (0.42-7.19)
Non-adherence to Mediterranean Diet – Alcohol consumption	3.02 (0.91-12.95)
Non-adherence to Mediterranean Diet – No Alcohol consumption	0.67 (0.39-3.83)

dics) that generally are chosen in these cases as representative of the general population.

Finally, even considering the small sample size of the study, it's worth of attention to the fact that all the synergistic indexes are above 1, indicating the presence of a biological interaction between variables. The 95% CIs of the SI indicates that at least an additive effect is present (lower level over 0, but under 1). It is to consider that we followed the approach to synergism proposed by Rothman (1976), in which the synergism is not a statistical interaction, but is a biological interaction. This kind of concept (synergy, and on the other hand, antagonism) is appropriate to capture biological relationships.

Conclusions

Concerning the generalizability (external validity) of the study results, while there is clear evidence that the Mediterranean diet supplemented with olive oil or nuts has a very good impact in terms of prevention on cardiovascular risk factors[20], we need to recognize this is the first study that addresses the synergism issue associated with the development of breast cancer, and confirms of these results are needed, also from other countries. Nevertheless, these results, for the first time to our knowledge, show a synergistic effect between nutritional factors lifestyle habits and reinforce the conviction that for breast cancer not only secondary and tertiary prevention is an issue, as outlined by the American Cancer Society[21]. For this type of cancer, primary prevention can benefit from intervention targeting both on nutritional and lifestyle factors.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Author Contributions

Conceptualization, G.L.T. and M.M.; methodology, G.L.T., A.M.; software; formal analysis, G.L.T, I.D.C.; investigation, C.S., R.A.C, L.L, O.D.B., S.C., V.D.E., M.M., V.P., A.D.L., F.F., V.A., M.I.A., M.C.; data curation, G.L.T., L.L., I.D.C.; writing—original draft preparation, G.L.T., I.D.C.; writing—review and editing, G.L.T., M.M. All authors have read and agreed to the published version of the manuscript.

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