Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

Article

The association of dietary patterns with high-risk human papillomavirus infection and cervical cancer: a cross-sectional study in Italy

Martina Barchitta [1], Andrea Maugeri [1], Annalisa Quattrocchi [1], Ottavia Agrifoglio [1], Aurora Scalisi [2], and Antonella Agodi [1,*]

[1] Department of Medical and Surgical Sciences and Advanced Technologies "GF Ingrassia", University of Catania, via S. Sofia, 87, 95121, Catania, Italy
[2] Unità Operativa di Screening Ginecologico, Azienda Sanitaria Provinciale 3, Catania, Italy
* Correspondence: agodia@unict.it

Abstract: Specific foods and nutrients help prevent the progression from persistent high-risk human papillomavirus (hrHPV) infection to cervical cancer (CC). We aimed to focus on dietary patterns which may be associated with hrHPV status and risk of high-grade cervical intraepithelial neoplasia (CIN2+). Overall, 539 eligible women, including 127 CIN2+, were enrolled in a cross-sectional study, and tested for hrHPV infection. Food intakes were estimated using a food frequency questionnaire. Logistic regression models were applied. Using the Mediterranean Diet Score, we demonstrated that, among 252 women with normal cervical epithelium, medium adherence to Mediterranean diet decreased odds of hrHPV infection when compared to low adherence (adjOR=0.40, 95%CI=0.22-0.73). Using principle component analysis, we also identified two dietary patterns which explained 14.31% of variance. Women in the 3rd and 4th quartiles of the "western pattern" had higher odds of hrHPV infection when compared with 1st quartile (adjOR=1.77, 95%CI=1.04-3.54 and adjOR=1.97, 95%CI=1.14-4.18, respectively). Adjusting for hrHPV status and age, women in the 3rd quartile of the "prudent pattern" had lower odds of CIN2+ when compared with 1st quartile (OR=0.50, 95%CI=0.26-0.98). Our study is the first to demonstrate the association of dietary patterns with hrHPV infection and CC, discouraging unhealthy habits in favour of Mediterranean-like diet.

Keywords: Cervical intraepithelial neoplasia; Mediterranean Diet Score; Principal Component analysis; western diet; prudent diet

---

1. Introduction

Prevalence of cervical cancer (CC) makes it the most frequent female cancer worldwide, second only to breast cancer [1]. Prediction models show that, without innovative preventive strategies, the cases of CC will rise up to 702,500, an alarming increase of 42%. [2]. Vaccination and safe sex education have been established as effective preventing strategies against progression from the persistent human papillomavirus (HPV) infection to cervical intraepithelial neoplasia (CIN) and/or carcinoma *in situ* [3]. However, natural history of CC hints at the possibility of achieving prevention also through nutrient-mediated programs which lead to modifications of host's immune system [4]. These strategies should be developed to stimulate host's cell-mediated immune response against the HPV oncogenic proteins E6 and E7 [3]. Epidemiological studies revealed that several food groups or nutrients could prevent the progression of precursor lesions to CC. Results from the European Prospective Investigation into Cancer and Nutrition (EPIC) study showed a significant inverse association between fruit daily intake and CC [5]. Particularly, fruit and vegetable consumption, as well as intake of nutrients (i.e. vitamins A, C, and E, folates, carotenoids, and minerals), have been associated with reduced risk of HPV infection, CIN and CC [6-14]. These findings support the role of

© 2018 by the author(s). Distributed under a Creative Commons CC BY license.

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

vitamins as protective nutrients against CC, via inhibiting cancer cell proliferation [15], stabilizing p53 [16], preventing DNA damage and reducing immune-suppression [15,17].

Although previous studies usually focused on the consumption of specific food groups and/or nutrients, intervention programs, based on dietary patterns rather than on single nutrients or food groups, may be more effective in reducing the risk of HPV infection and CC. Since lack of evidence exists about the synergic effect of food groups on CC etiopathology, we aimed to identify both *a priori* and *a posteriori* dietary patterns which may be associated with HPV status and high grade CIN risk.

2. Materials and Methods

Study design

The study protocol, approved by the ethics committee of the involved Institution (CE Catania 2; Prot. N. 227/BE and 275/BE) and performed according to the Declaration of Helsinki, has been fully described elsewhere [18]. Briefly, from 2013 to 2015, all women diagnosed with abnormal PAP test, referring to the cervical cancer screening unit (Unità Operativa di Screening Ginecologico) at the Azienda Sanitaria Provinciale (ASP 3), in Catania (Italy) for further examination by colposcopy and biopsy, were invited to participate in a cross-sectional study. Participants were fully informed of the purpose and procedures of the study, and a signed written consent was obtained. Women were classified by histological diagnosis and tested for high-risk HPV (hrHPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) using digene HC2 HPV DNA Test (Qiagen, Italy). Thus, women were classified as hrHPV positive if they were infected with any of the thirteen hrHPV types, otherwise women were classified as hrHPV negative. Notably, the specific HPV genotype is not provided by the test. According to the histological result, women were further classified as cases (CIN2+: CIN2, CIN3 or carcinoma *in situ* - CIS) or controls (≤CIN1: CIN1 or normal cervical epithelium). A structured questionnaire was used by trained epidemiologists to obtain information on socio-demographic variables and lifestyle factors. Women were classified into two categories of educational level: low-medium (primary school, i.e., ≤8 years of school) and high education level (high school education or greater, i.e., >8 years of school). Body mass index (BMI) and nutritional status were calculated based on criteria from the World Health Organization [19].

Dietary assessment

Dietary data were obtained by a 95-item semi-quantitative Food Frequency Questionnaire (FFQ), using the previous month as reference period [20]. For each food item, women were asked to report frequency of consumption and portion size. To estimate the amount of each food item and to minimize inaccuracies, an indicative photograph atlas was used. Frequencies of food consumption were classified into twelve categories ranging from "almost never" to "two or more times a day". The medium serving sizes were described by natural portions or standard weight and volume measures of the servings commonly consumed in the Italian population. Accordingly, portion size was classified into three categories: small (half a medium serving size), medium, and large (1.5 times or more than a medium serving size). The food intakes derived from the FFQ were calculated by multiplying frequency of consumption with daily portion size of each food group. Total energy intake was calculated using the USDA Nutrient Database (http://ndb.nal.usda.gov/) adapted to the Italian food consumption. Food intakes were adjusted for total energy intake using the residual method [21].

The Mediterranean diet score

Adherence to Mediterranean diet (MD) was assessed using the Mediterranean Diet Score (MDS) [22, 23]. This *a priori* score includes 9 components: fruits and nuts, vegetables, legumes, cereals, lipids, fish, dairy products, meat products, alcohol and the ratio of unsaturated to saturated lipids. For components that are more consumed in Mediterranean countries (vegetables, legumes, fruits and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

nuts, cereals, fish, and a high ratio of unsaturated to saturated lipids), women whose consumption was below or equal to the median value of the population were assigned a value of 0, and 1 otherwise. For components less consumed in Mediterranean countries (dairy and meat products), women whose consumption was below the median were assigned a value of 1, and 0 otherwise. A value of 1 was given to women consuming a moderate amount of alcohol (5 to <25 g per day). The MDS ranges from 0 (no- adherence) to 9 (perfect adherence). MD adherence was categorized, according to MDS, as follows: low adherence (MDS range: 0–3), medium adherence (MDS range: 4–6), or high adherence (MDS range: 7–9) [24]. Low adherence to MD was used as the reference for further analyses.

Principal component analysis

A posteriori dietary patterns were extracted using principal component analysis (PCA). We firstly classified the 95 FFQ food items into 39 predefined food groups, based on the similarity of nutrient profiles or culinary usage. Individual food items were preserved if they constituted a distinct item on their own (e.g., eggs, pizza, coffee or tea, etc) or if they were thought to represent a particular dietary pattern (e.g., wine, alcoholic drinks, and chips, etc). For each food group, the energy-adjusted variable was entered in the factor analysis. Factors were rotated by orthogonal transformation (varimax rotation) to maintain uncorrelated factors and to facilitate interpretability. The number of retained dietary patterns was determined according to eigenvalues (eigenvalues >2.0), Scree plot examination, and interpretability. Factor loadings with absolute value ≥ 0.2 were retained to define food groups that characterized dietary patterns. To confirm internal reproducibility, factor analysis was performed separately in two randomly selected subgroups, using the same approach of the main analysis. Factor scores for each dietary pattern were computed as the sum of products between observed energy-adjusted food group intakes and their factor loadings. For each dietary pattern, factor scores were categorized by quartiles (Q1-Q4); the lowest quartile (Q1) of each dietary pattern was used as the reference for further analyses.

Statistical analyses

Statistical analyses were performed using the SPSS software (version 22.0, SPSS, Chicago, IL). Descriptive statistics were used to characterize the population using frequencies and means ± standard deviations (SDs). The two-tailed Chi-squared test was used for the statistical comparison of proportions, whereas continuous variables were tested using Student's t test. Trend across dietary pattern categories was analyzed using generalized linear models for continuous variables and Mantel–Haenszel chi-squared tests for categorical variables. Unconditional multiple logistic regression models were used to estimate odds ratio (ORs) and corresponding 95% confidence intervals (95%CI) of hrHPV infection and CIN2+ status, associated with dietary pattern categories, as well as to one-unit increase in factor scores and MDS. ORs and 95%CIs for hrHPV infection were calculated among women with normal cervical epithelium, using the following models: age-adjusted model (Model 1); model adjusted for variables found to be significantly associated with hrHPV infection in univariate analysis (Model 2). ORs and 95%CIs for CIN2+ status were calculated among women, classified as cases or controls, using the following models: age-adjusted model (Model 1); a model adjusted for age and hrHPV status (Model 2). All statistical tests were 2-sided, and p values less than 0.05 were considered statistically significant.

3. Results

3.1. Study population

Overall, 539 women with abnormal PAP test were classified by histological diagnosis and tested for hrHPV. Among these, 252 were diagnosed with normal cervical epithelium (46.7%), 160 CIN1 (29.7%), 57 CIN2 (10.6%), 67 (12.4%) CIN3 and 3 (0.6%) CIS. With regard to hrHPV status, women were classified as hrHPV positive (hrHPV+; N=302; 56%) and hrHPV negative (hrHPV-; N=237; 44%).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at Nutrients 2018, 10, 469; doi:10.3390/nu10040469

Table 1 displays the characteristics of women diagnosed with normal cervical epithelium according to hrHPV status. Particularly, the odds of being diagnosed with hrHPV infection increased among younger women (≤median age) (OR= 2.4; 95%CI=1.0-5.8; p=0.043), smokers (OR=2.6; 95%CI= 1.1-6.2; p=0.035), underweight-normal weight (OR= 3.2; 95%CI= 1.1-9.5; p=0.028) and nulliparous women (OR=4.9; 95%CI= 1.7-14.1; p=0.002). Table 2 shows the characteristics of women according to cases/controls classification. The odds of being diagnosed with CIN2+ increased among younger (≤median age) (OR= 2.3; 95%CI=1.6-3.5; p=0.001), smoker (OR=2.0; 95%CI= 1.3-3.0; p=0.001), and nulliparous women (OR=1.7; 95%CI= 1.1-2.6; p=0.011), as well as among oral contraceptive users (OR=1.9; 95%CI= 1.0-3.5; p=0.039). However, after adjustment for hrHPV status, no statistically significant differences between cases and controls were evident (data not shown).

Table 1. Characteristics of healthy women according to hrHPV status

Characteristics	hrHPV+ (n=84)	hrHPV- (n=167)	p-value[a]
Age, mean (SD)	38.63 (10.53)	43.65 (9.62)	**<0.001**
Current smokers	47.0%	28.1%	**0.003**
BMI, mean (SD)	22.36 (4.01)	24.34 (4.68)	**0.001**
Nutritional status			
Underweight	8.4%	5.4%	**0.004**
Normal weight	75.9%	56.6%	
Overweight	10.8%	24.1%	
Obese	4.8%	13.9%	
Workers	51.2%	41.3%	0.138
Parity (≥1 live births)	58.3%	83.2%	**<0.001**
Low education level	31.0%	41.9%	0.092
Use of oral contraceptive	13.1%	7.8%	0.177

Abbreviations: SD standard deviation; BMI Body Mass Index.

[a] Statistically significant p values ($p < 0.05$) are indicated in bold font

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

Table 2. Characteristics of women according to cases/controls classification

Characteristics	Cases (n=127)	Controls (n=411)	p-value[a]
Age, mean (SD)	36.01 (8.10)	41.50 (10.21)	**<0.001**
Current smokers	53.5%	36.8%	**0.001**
BMI, mean (SD)	22.47 (3.63)	23.59 (4.53)	**0.012**
Nutritional status			
Underweight	11.0%	7.1%	0.052
Normal weight	67.5%	63.1%	
Overweight	18.3%	19.8%	
Obese	3.2%	10.0%	
Workers	46.5%	46.5%	0.998
Parity (≥1 live births)	59.8%	71.8%	**0.011**
Low education level	41.0%	38.2%	0.579
Use of oral contraceptive	14.2%	8.0%	**0.039**

Abbreviations: SD standard deviation; BMI Body Mass Index.

[a] Statistically significant p values ($p < 0.05$) are indicated in bold font

3.2. Dietary assessment

The mean MDS value was 4.2 (median 4; range 0-9). According to MDS, women were classified as follows: 33.1% low adherence; 60.0% medium adherence; 6.9% high adherence. No statistically significant differences in socio-demographics characteristics were observed across categories of the MD pattern. Based on Scree plot examination (Figure 1), we identified two major dietary patterns, with eigenvalues ≥ 2.0, which explained 14.31% of total variance among 39 food groups. The first dietary pattern, named "western", was positively characterized by high intake of chips, snacks, dipping sauces, plant oil, processed and red meat, and negatively by olive oil. The second one, named "prudent", was positively characterized by high intake of legumes, vegetable soups, potatoes, cooked, raw vegetables and olive oil (Figures 2a and 2b). The distribution of population characteristics by dietary pattern categories is reported in Table 3. High western dietary pattern

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

scores were associated with decreasing age and increasing percentages of smokers. No statistically significant differences were observed across categories of prudent dietary pattern.

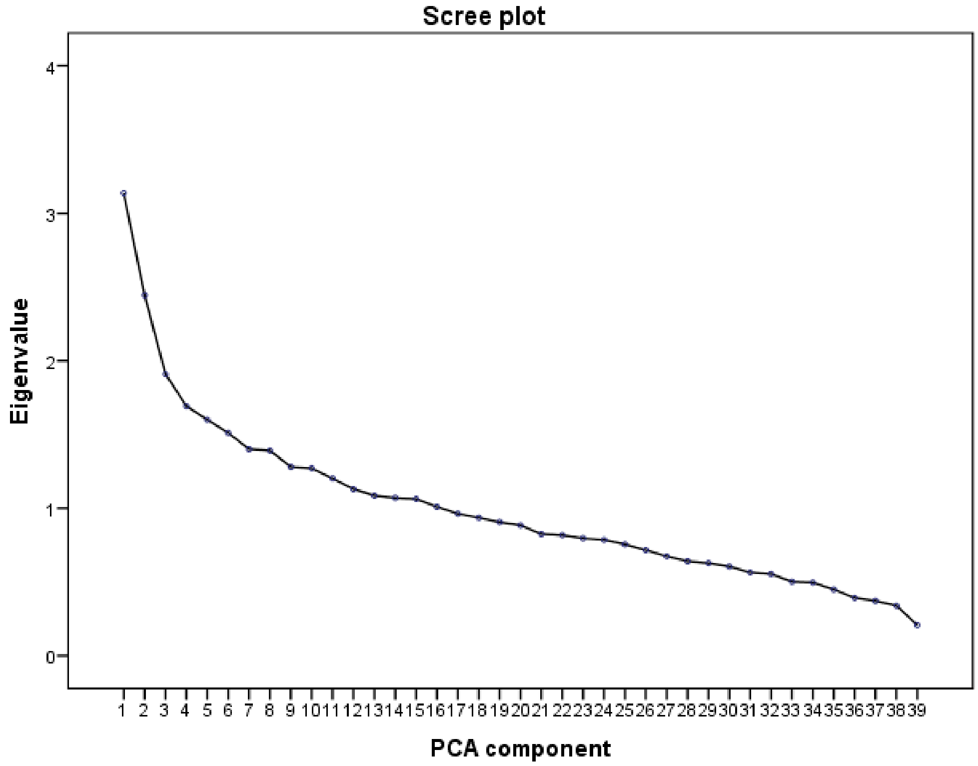

Figure 1. Scree plot of the eigenvalues

Peer-reviewed version available at Nutrients 2018, 10, 469; doi:10.3390/nu10040469

A

	Western pattern	Prudent pattern
Potatoes	0.199	**0.344**
Legumes	-0.049	**0.832**
Vegetable soup	-0.080	**0.800**
Cooked vegetables	-0.030	**0.373**
Raw vegetables	-0.144	**0.299**
Fruit	-0.139	0.009
Nuts	-0.047	-0.050
Milk	-0.081	0.020
Yoghurt	-0.100	0.027
Curd cheese	-0.100	-0.046
Ripened cheese	-0.083	0.030
Pasta	-0.034	-0.061
Rice	0.015	0.073
Bread and cereals	0.025	-0.035
Wholegrain bread and cereals	-0.081	-0.013
Breakfast cereals	-0.036	0.067
Offal	-0.015	0.019
Canned fish	0.061	-0.056
Shellfish	-0.002	-0.058
Fish	-0.051	0.040
Eggs	0.012	-0.019
Olive oil	**-0.402**	**0.238**
Butter and margarine	0.136	0.160
Sweets and processed sugar	0.123	-0.158
Fruit juice	0.147	0.105
Coffee	-0.010	-0.071
Tea	-0.067	0.037
Pizza	0.122	0.008
Wine	0.009	-0.003
Beer	0.058	-0.113
Alcoholic drinks	-0.130	0.013
Plant oil	**0.434**	-0.038
Dipping sauces	**0.734**	-0.007
Snacks	**0.594**	-0.113
Chips	**0.780**	-0.057
White meat	0.086	0.025
Red meat	**0.200**	-0.089
Processed meat	**0.242**	-0.096
Fruit salad	0.045	-0.046

Factor loading

-1 0 1

B

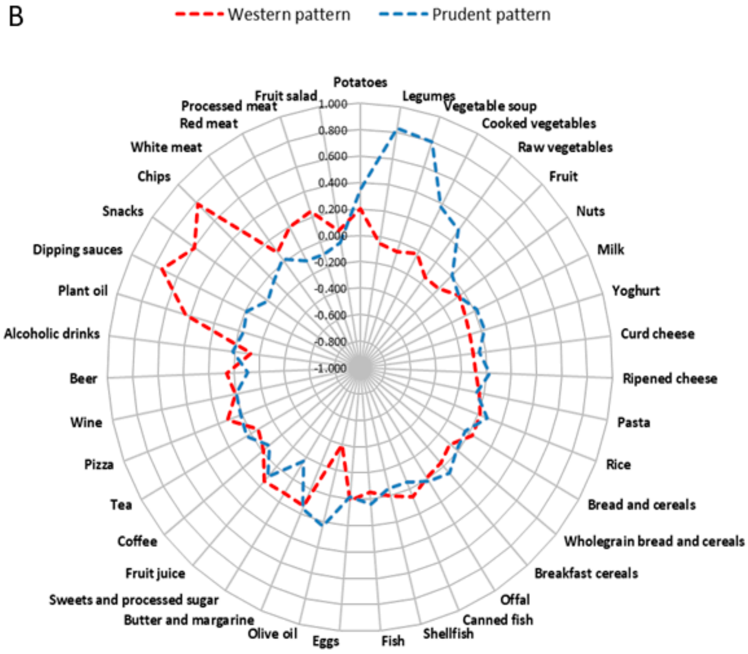

Figure 1. Factor loadings of dietary patterns derived by Principal Component Analysis: (**a**) table of factor loadings characterizing each dietary pattern (in red, food groups that negatively characterize dietary patterns; in green, food groups that positively characterize dietary patterns), factor loadings ≥ |0.2| are in bold font; (**b**) Radar graph of factor loadings that characterize dietary patterns.

Table 3. Characteristics of women according to dietary pattern quartiles

Characteristics	Western dietary pattern					Prudent dietary pattern				
	Q1 (n=134)	Q2 (n=135)	Q3 (n=135)	Q4 (n=134)	p-value[a]	Q1 (n=134)	Q2 (n=135)	Q3 (n=135)	Q4 (n=134)	p-value[a]
Age, mean (SD)	42.37 (10.87)	43.35 (9.48)	39.79 (9.25)	35.28 (8.46)	**<0.001**	40.55 (10.74)	40.68 (9.90)	40.52 (9.86)	39.05 (9.58)	0.500
Current smokers	38.1%	34.1%	37.3%	53.7%	**0.005**	41.0%	46.3%	34.8%	41.0%	0.300
BMI, mean (SD)	23.39 (4.23)	23.69 (4.19)	23.76 (4.77)	22.45 (4.13)	0.053	23.18 (4.38)	23.14 (4.30)	23.44 (4.30)	23.53 (4.48)	0.846
Workers	36.6%	50.4%	47.4%	54.1%	0.631	44.8%	45.2%	51.1%	44.8%	0.667
Parity (≥1 live births)	71.5%	77.8%	66.7%	59.7%	**0.011**	63.4%	68.9%	70.4%	73.1%	0.372
Low Education level	43.3%	34.8%	34.1%	43.3%	0.218	40.3%	35.6%	41.5%	38.1%	0.762
Use of oral contraceptive	9.0%	8.9%	11.1%	9.0%	0.906	11.9%	7.4%	9.6%	9.0%	0.644

Abbreviations: Q quartile; SD standard deviation; BMI Body Mass Index.

a Statistically significant p values ($p < 0.05$) are indicated in bold font

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at Nutrients 2018, 10, 469; doi:10.3390/nu10040469

3.3. Dietary patterns and hrHPV infection

In the age-adjusted model, increasing factor scores of the western pattern were associated with significantly higher risk of hrHPV infection (OR =1.44, 95%CI= 1.03-2.03, p=0.036). Particularly, the risk of hrHPV infection was higher among women in Q3 and Q4, than those in Q1 (OR= 1.77, 95%CI= 1.04-3.54 and OR=1.97, 95%CI= 1.14-4.18, respectively; p trend=0.039) (Table 4). Conversely, increasing MDS was associated with significantly lower risk of hrHPV infection in both age-adjusted (OR= 0.76, 95%CI= 0.64-0.92, p= 0.004) and multivariate-adjusted model (OR= 0.79, 95%CI= 0.66-0.96, p= 0.018). Compared to low adherents, women with medium adherence to MD showed a lower risk of hrHPV infection (OR= 0.40, 95%CI= 0.22-0.73; p trend= 0.004). This result was confirmed after adjustment for age, smoking, nutritional status and parity (OR= 0.40, 95%CI= 0.21-0.75; p trend= 0.015) (Table 5).

Table 4. Association between dietary patterns derived by principal component analysis and the risk of hrHPV infection

Dietary pattern	Regression model	adjOR (95% CI)						
		Q1	Q2	Q3	Q4	p-trend	continuous	p-value
Western	Model 1[a]	1.00 (ref)	1.11(0.48-2.57)	**1.77(1.04-3.54)**	**1.97 (1.14-4.18)**	**0.039**	**1.44 (1.03-2.03)**	**0.036**
	Model 2[b]	1.00 (ref)	1.33 (0.54-3.28)	1.96 (0.88-4.34)	2.06 (0.86-4.90)	**0.047**	1.39 (0.97-1.99)	0.069
Prudent	Model 1[a]	1.00 (ref)	1.18 (0.54-2.60)	0.86 (0.58-1.28)	0.85 (0.65-1.11)	0.842	0.83 (0.63-1.11)	0.215
	Model 2[b]	1.00 (ref)	1.05 (0.45-2.43)	0.86 (0.57-1.32)	0.82 (0.62-1.10)	0.226	0.83 (0.62-1.11)	0.210

Abbreviations: adjOR adjusted Odds Ratio; CI Confidence Interval; Q quartile

Statistically significant results ($p < 0.05$) are indicated in bold font

[a]Adjusted for age

[b]Adjusted for age, BMI, smoking status and parity

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

Table 5. Association between adherence to the Mediterranean diet and the risk of hrHPV infection

Regression model	**adjOR (95% CI)**					
	Low adherence	Medium adherence	High adherence	p-trend	MDS (continuous)	p-value
Model 1[a]	1.00 (ref)	**0.40 (0.22-0.73)**	0.43(0.15-1..22)	**0.006**	0.76 (0.64-0.92)	**0.004**
Model 2[b]	1.00 (ref)	**0.40 (0.21-0.75)**	0.50 (0.17-1.50)	**0.015**	0.79 (0.66-0.96)	**0.018**

Abbreviations: adjOR adjusted Odds Ratio; CI Confidence Interval;

Statistically significant results ($p< 0.05$) are indicated in bold font

[a]Adjusted for age

[b]Adjusted for age, BMI, smoking status and parity

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

3.4. Dietary patterns and cervical cancer

Table 6 shows results of logistic regression analysis of the association between dietary patterns and the risk of CIN2+. With regard to prudent dietary pattern, the risk was lower among women in Q3 than those in Q1 (OR= 0.50, 95%CI= 0.26-0.98), after adjustment for hrHPV status and age. No statistically significant differences were observed across categories of the Western dietary pattern and adherence to MD (Table 7).

Table 6. Association between dietary patterns derived by principal component analysis and the risk of CIN2+

Dietary pattern	Regression model	adjOR (95% CI)						
		Q1	Q2	Q3	Q4	p-trend	Continuous	p-value
Western	Model 1[a]	1.00 (ref)	1.35(0.73-2.51)	1.05(0.57-1.94)	1.35 (0.74-2.45)	0.560	1.11 (0.92-1.35)	0.281
	Model 2[b]	1.00 (ref)	1.28 (0.63-2.60)	0.90 (0.45-1.80)	1.04 (0.53-2.03)	0.753	1.00 (0.81-1.23)	0.990
Prudent	Model 1[a]	1.00 (ref)	0.66 (0.36-1.22)	0.57 (0.31-1.04)	0.77 (0.42-1.40)	0.352	0.87 (0.71-1.05)	0.144
	Model 2[b]	1.00 (ref)	0.62 (0.32-1.22)	**0.50 (0.26-0.98)**	0.58 (0.29-1.14)	0.076	0.83 (0.62-1.11)	0.210

Abbreviations: adjOR adjusted Odds Ratio; CI Confidence Interval; Q quartile

Statistically significant results (p< 0.05) are indicated in bold font

[a]Adjusted for age

[b]Adjusted for age and hrHPV status

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

Table 7. Association between adherence to Mediterranean diet and the risk of CIN2+

Regression model	**adjOR (95% CI)**					
	Low adherence	Medium adherence	High adherence	p-trend	MDS	p-value
Model 1[a]	1.00 (ref)	1.01 (0.65-1.57)	0.50 (0.18-1..40)	0.473	1.01 (0.88-1.15)	0.941
Model 2[b]	1.00 (ref)	1.23 (0.76-2.01)	0.76 (0.24-2.41)	0.726	1.09 (0.94-1.27)	0.272

Abbreviations: adjOR adjusted Odds Ratio; CI Confidence Interval;

Statistically significant results ($p< 0.05$) are indicated in bold font

[a]Adjusted for age

[b]Adjusted for age and hrHPV status

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at Nutrients 2018, 10, 469; doi:10.3390/nu10040469

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at Nutrients 2018, 10, 469; doi:10.3390/nu10040469

4. Discussion

Growing body of evidence suggests that dietary patterns might modulate the risk of female cancers [25,26], and several dietary intervention programs have been proposed to reduce cancer incidence and to improve health and quality of life [27-29]. However, to our knowledge, no previous studies evaluated the association between dietary patterns, hrHPV status and high grade CIN risk. Given this lack of evidence, using an *a posteriori* approach, we firstly derived two dietary patterns which mainly characterize dietary habits of study population. The first pattern, named as "western", was characterized by high intakes of red and processed meat, dipping sauces, chips and snack; the second one, named as "prudent", consisted in high intake of legumes, vegetable soups, potatoes, cooked and raw vegetables. Since dietary habits of Mediterranean populations show healthy effects in terms of overall morbidity and mortality [30], we also evaluated the adherence to MD, using an *a priori* score. According to MDS classification, only 6.9% of enrolled women reported high adherence to MD, which gives a balanced ratio of omega 6 and omega 3 essential fatty acids and high amounts of fibre, antioxidants and polyphenols found in fruit, vegetables, olive oil and wine [31].

Among healthy women, increasing adherence to western dietary pattern was associated with higher risk of hrHPV infection. Consistently, a previous study showed that unhealthy dietary pattern might put women at higher risk of developing hrHPV-related cervical lesions [32]. This is also in line with results on the protective effect of MD, which showed that increasing MDS was associated with reduced risk of hrHPV infection. Particularly, compared to low adherents, women with medium adherence to MD were less likely to be at risk of hrHPV infection. Although the paucity of women with high adherence to MD raises the need of large epidemiological studies investigating this association, recent evidence suggests that several micronutrients typical of MD may help in CC prevention by inhibiting HPV persistence [33].

Previous studies reported that women with lower intakes of vegetables and fruits, as well as vitamins A, C, and E, had higher risk of high grade CIN and CC [9,12]. Consistently, our study highlighted the protective role of the prudent dietary pattern, which was negatively associated with risk of CIN2+. Particularly, CIN2+ risk was lower among women with medium-high adherence, compared to those with low adherence to prudent pattern. The biological basis of this evidence could be explained by the activity of dietary factors that may modify the epigenome [34]. The role of epigenetics in women's health and reproductive functions has been deeply investigated [18,35,36], suggesting bioactive compounds which can affect epigenetic signatures by different mechanisms [37]. A study on a cancer free population showed that low fruit consumption and folate deficiency could modulate LINE-methylation level [36], an epigenetic marker of cancer risk [38]. Particularly, dietary patterns associated with LINE-1 methylation levels could alter the risk of developing cervical intraepithelial neoplasia [32]. Moreover, the consumption of foods which maintain normal DNA methylation levels, such as plant based foods, could potentially suppress the expression of HPV oncogenes, thereby reducing cell transformation rates and CC risk [3,17].

Weaknesses of our study included the cross-sectional design which does not allow determination of causality. Since the use of dietary pattern analysis was largely limited to cross-sectional studies, further large prospective studies should be encouraged. Moreover, food intakes were estimated using FFQ, which does not preclude measurement errors and may suffer from inaccuracies. Nevertheless, the FFQ used in this study was specifically developed for the use among our population and was previously validated against a 4-day weighted dietary record, with a correlation coefficient in accordance with other FFQ validation studies [20]. Finally, although we adjusted for several lifestyle factors, known to potentially affect hrHPV status and/or cervical cancer, we could not rule out the possibility of bias from residual confounding of unmeasured lifestyle factors.

In conclusion, to our knowledge, this study is the first to point out evidence about the association of dietary patterns with hrHPV infection and CC risk. In the context of CC prevention, our data discourage unhealthy dietary habits in favour of healthy one, such as the Mediterranean diet, which reduces the risk of hrHPV infection. However, future prospective large-scale studies are needed to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at Nutrients 2018, 10, 469; doi:10.3390/nu10040469

evaluate the association between dietary pattern and CC risk, taking into account the physiological and molecular pathways involved in this relationship.

Acknowledgments:

We are grateful to Bench Srl, University of Catania, Italy for partial financial support and assistance in data analysis

Author Contributions:

AA conceived and designed the study, reviewed the data quality, interpreted the data and drafted the manuscript and provided the final editing. MB, AM, AQ and OA conducted the statistical analyses, interpreted the data and drafted the manuscript. AS was responsible for cohort enrollment, sample collection, histological diagnosis and hrHPV identification and provided the final editing of the manuscript. All authors read, edited, and approved the final manuscript.

Conflicts of Interest:

The authors declare no conflict of interest.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

References

1. Bosch, F.X.; Burchell, A.N.; Schiffman, M.; Giuliano, A.R.; de Sanjose, S.; Bruni, L.; Tortolero-Luna, G.; Kjaer, S.K.; Muñoz, N. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine* **2008**, *26 Suppl 10*, K1-16.
2. Serrano, B.; Brotons, M.; Bosch, F.X.; Bruni, L. Epidemiology and burden of hpv-related disease. *Best Pract Res Clin Obstet Gynaecol* **2018**, *47*, 14-26.
3. zur Hausen, H. Papillomaviruses and cancer: From basic studies to clinical application. *Nat Rev Cancer* **2002**, *2*, 342-350.
4. Chih, H.J.; Lee, A.H.; Colville, L.; Binns, C.W.; Xu, D. A review of dietary prevention of human papillomavirus-related infection of the cervix and cervical intraepithelial neoplasia. *Nutr Cancer* **2013**, *65*, 317-328.
5. González, C.A.; Travier, N.; Luján-Barroso, L.; Castellsagué, X.; Bosch, F.X.; Roura, E.; Bueno-de-Mesquita, H.B.; Palli, D.; Boeing, H.; Pala, V., *et al.* Dietary factors and in situ and invasive cervical cancer risk in the european prospective investigation into cancer and nutrition study. *Int J Cancer* **2011**, *129*, 449-459.
6. Zhang, X.; Dai, B.; Zhang, B.; Wang, Z. Vitamin a and risk of cervical cancer: A meta-analysis. *Gynecol Oncol* **2012**, *124*, 366-373.
7. Zhou, X.; Meng, Y. Association between serum folate level and cervical cancer: A meta-analysis. *Arch Gynecol Obstet* **2016**, *293*, 871-877.
8. Cao, D.; Shen, K.; Li, Z.; Xu, Y.; Wu, D. Association between vitamin c intake and the risk of cervical neoplasia: A meta-analysis. *Nutr Cancer* **2016**, *68*, 48-57.
9. Hwang, J.H.; Lee, J.K.; Kim, T.J.; Kim, M.K. The association between fruit and vegetable consumption and hpv viral load in high-risk hpv-positive women with cervical intraepithelial neoplasia. *Cancer Causes Control* **2010**, *21*, 51-59.
10. Siegel, E.M.; Salemi, J.L.; Villa, L.L.; Ferenczy, A.; Franco, E.L.; Giuliano, A.R. Dietary consumption of antioxidant nutrients and risk of incident cervical intraepithelial neoplasia. *Gynecol Oncol* **2010**, *118*, 289-294.
11. Guo, L.; Zhu, H.; Lin, C.; Che, J.; Tian, X.; Han, S.; Zhao, H.; Zhu, Y.; Mao, D. Associations between antioxidant vitamins and the risk of invasive cervical cancer in chinese women: A case-control study. *Sci Rep* **2015**, *5*, 13607.
12. Kim, J.; Kim, M.K.; Lee, J.K.; Kim, J.H.; Son, S.K.; Song, E.S.; Lee, K.B.; Lee, J.P.; Lee, J.M.; Yun, Y.M. Intakes of vitamin a, c, and e, and beta-carotene are associated with risk of cervical cancer: A case-control study in korea. *Nutr Cancer* **2010**, *62*, 181-189.
13. Tomita, L.Y.; Longatto Filho, A.; Costa, M.C.; Andreoli, M.A.; Villa, L.L.; Franco, E.L.; Cardoso, M.A.; Team, B.I.i.N.a.C.C.P.B.S. Diet and serum micronutrients in relation to cervical neoplasia and cancer among low-income brazilian women. *Int J Cancer* **2010**, *126*, 703-714.
14. Piyathilake, C.J.; Henao, O.L.; Macaluso, M.; Cornwell, P.E.; Meleth, S.; Heimburger, D.C.; Partridge, E.E. Folate is associated with the natural history of high-risk human papillomaviruses. *Cancer Res* **2004**, *64*, 8788-8793.
15. García-Closas, R.; Castellsagué, X.; Bosch, X.; González, C.A. The role of diet and nutrition in cervical carcinogenesis: A review of recent evidence. *Int J Cancer* **2005**, *117*, 629-637.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

16. Reddy, L.; Odhav, B.; Bhoola, K.D. Natural products for cancer prevention: A global perspective. *Pharmacol Ther* **2003**, *99*, 1-13.
17. Field, C.J.; Johnson, I.R.; Schley, P.D. Nutrients and their role in host resistance to infection. *J Leukoc Biol* **2002**, *71*, 16-32.
18. Barchitta, M.; Quattrocchi, A.; Maugeri, A.; Canto, C.; La Rosa, N.; Cantarella, M.A.; Spampinato, G.; Scalisi, A.; Agodi, A. Line-1 hypermethylation in white blood cell dna is associated with high-grade cervical intraepithelial neoplasia. *BMC Cancer* **2017**, *17*, 601.
19. Physical status: The use and interpretation of anthropometry. Report of a who expert committee. *World Health Organ Tech Rep Ser* **1995**, *854*, 1-452.
20. Agodi, A.; Barchitta, M.; Valenti, G.; Marzagalli, R.; Frontini, V.; Marchese, A.E. Increase in the prevalence of the mthfr 677 tt polymorphism in women born since 1959: Potential implications for folate requirements. *Eur J Clin Nutr* **2011**, *65*, 1302-1308.
21. Willett, W.; Stampfer, M.J. Total energy intake: Implications for epidemiologic analyses. *Am J Epidemiol* **1986**, *124*, 17-27.
22. Trichopoulou, A.; Kouris-Blazos, A.; Wahlqvist, M.L.; Gnardellis, C.; Lagiou, P.; Polychronopoulos, E.; Vassilakou, T.; Lipworth, L.; Trichopoulos, D. Diet and overall survival in elderly people. *BMJ* **1995**, *311*, 1457-1460.
23. Couto, E.; Boffetta, P.; Lagiou, P.; Ferrari, P.; Buckland, G.; Overvad, K.; Dahm, C.C.; Tjønneland, A.; Olsen, A.; Clavel-Chapelon, F., *et al.* Mediterranean dietary pattern and cancer risk in the epic cohort. *Br J Cancer* **2011**, *104*, 1493-1499.
24. Barchitta, M.; Quattrocchi, A.; Adornetto, V.; Marchese, A.E.; Agodi, A. Tumor necrosis factor-alpha -308 g>a polymorphism, adherence to mediterranean diet, and risk of overweight/obesity in young women. *Biomed Res Int* **2014**, *2014*, 742620.
25. Si, C.J.; Shu, L.; Zheng, P.F.; Zhang, X.Y.; Yu, X.L.; Gao, W.; Zhang, L. Dietary patterns and endometrial cancer: A meta-analysis. *Eur J Cancer Prev* **2017**, *26*, 336-345.
26. Brennan, S.F.; Cantwell, M.M.; Cardwell, C.R.; Velentzis, L.S.; Woodside, J.V. Dietary patterns and breast cancer risk: A systematic review and meta-analysis. *Am J Clin Nutr* **2010**, *91*, 1294-1302.
27. Martin, M.Y.; Pisu, M.; Oster, R.A.; Urmie, J.M.; Schrag, D.; Huskamp, H.A.; Lee, J.; Kiefe, C.I.; Fouad, M.N. Racial variation in willingness to trade financial resources for life-prolonging cancer treatment. *Cancer* **2011**, *117*, 3476-3484.
28. Koutoukidis, D.A.; Beeken, R.J.; Manchanda, R.; Burnell, M.; Knobf, M.T.; Lanceley, A. Erratum to: Diet and exercise in uterine cancer survivors (deus pilot) - piloting a healthy eating and physical activity program: Study protocol for a randomized controlled trial. *Trials* **2017**, *18*, 28.
29. Koutoukidis, D.A.; Beeken, R.J.; Manchanda, R.; Michalopoulou, M.; Burnell, M.; Knobf, M.T.; Lanceley, A. Recruitment, adherence, and retention of endometrial cancer survivors in a behavioural lifestyle programme: The diet and exercise in uterine cancer survivors (deus) parallel randomised pilot trial. *BMJ Open* **2017**, *7*, e018015.
30. Sofi, F.; Macchi, C.; Abbate, R.; Gensini, G.F.; Casini, A. Mediterranean diet and health status: An updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr* **2014**, *17*, 2769-2782.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 March 2018 doi:10.20944/preprints201803.0109.v1

Peer-reviewed version available at *Nutrients* **2018**, *10*, 469; doi:10.3390/nu10040469

31. Giacosa, A.; Barale, R.; Bavaresco, L.; Gatenby, P.; Gerbi, V.; Janssens, J.; Johnston, B.; Kas, K.; La Vecchia, C.; Mainguet, P., *et al.* Cancer prevention in europe: The mediterranean diet as a protective choice. *Eur J Cancer Prev* **2013**, *22*, 90-95.
32. Piyathilake, C.J.; Badiga, S.; Kabagambe, E.K.; Azuero, A.; Alvarez, R.D.; Johanning, G.L.; Partridge, E.E. A dietary pattern associated with line-1 methylation alters the risk of developing cervical intraepithelial neoplasia. *Cancer Prev Res (Phila)* **2012**, *5*, 385-392.
33. Moga, M.A.; Dimienescu, O.G.; Arvatescu, C.A.; Mironescu, A.; Dracea, L.; Ples, L. The role of natural polyphenols in the prevention and treatment of cervical cancer-an overview. *Molecules* **2016**, *21*.
34. Hardy, T.M.; Tollefsbol, T.O. Epigenetic diet: Impact on the epigenome and cancer. *Epigenomics* **2011**, *3*, 503-518.
35. Barchitta, M.; Maugeri, A.; Quattrocchi, A.; Agrifoglio, O.; Agodi, A. The role of mirnas as biomarkers for pregnancy outcomes: A comprehensive review. *Int J Genomics* **2017**, *2017*, 8067972.
36. Agodi, A.; Barchitta, M.; Quattrocchi, A.; Maugeri, A.; Canto, C.; Marchese, A.E.; Vinciguerra, M. Low fruit consumption and folate deficiency are associated with line-1 hypomethylation in women of a cancer-free population. *Genes Nutr* **2015**, *10*, 480.
37. Ong, T.P.; Moreno, F.S.; Ross, S.A. Targeting the epigenome with bioactive food components for cancer prevention. *J Nutrigenet Nutrigenomics* **2011**, *4*, 275-292.
38. Barchitta, M.; Quattrocchi, A.; Maugeri, A.; Vinciguerra, M.; Agodi, A. Line-1 hypomethylation in blood and tissue samples as an epigenetic marker for cancer risk: A systematic review and meta-analysis. *PLoS One* **2014**, *9*, e109478.