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Original article

Combined effect of diet and cervical microbiome on the risk of cervical intraepithelial neoplasia

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SUMMARY

Background & aims: Several food groups or dietary factors and the cervical microbiota may be involved in cervical carcinogenesis, but the evidence is not clear yet. We aimed to assess the association between dietary pattern and cervical intraepithelial neoplasia (CIN) and the combined effect of dietary pattern and cervical microbiome on the risk of CIN.

Methods: The cervical microbiota and diet assessed by pyrosequencing and a food-frequency questionnaire, respectively, of 65 women with CIN and 72 control women were used in this study. Principal component analysis and cluster analysis were used to identify dietary patterns and microbiome community types, respectively. The association between dietary pattern and CIN risk was assessed using multivariable logistic regression analysis. The combined effect of dietary pattern and microbiome on CIN risk was determined using relative excess risk due to interaction (RERI) and synergy index (S).

Results: Two dietary patterns and four community types were identified: prudent diet characterized by higher intake of vegetables and fishes; semi-Western diet characterized by higher intake of bread, dairy products, eggs, and soft drinks and relatively higher fat intake ratio; and *Lactobacillus crispatus*−, *L. iners*−, *Atopobium vaginae*−, and *Prevotella bivia*−dominant types. The high-scoring group of participants with a semi-Western diet had a higher risk of CIN (odds ratio [OR] 3.44, 95% confidence interval [CI] 1.11−10.7, $p = 0.03$), compared with the low or medium-scoring group of those with a semi-Western diet. *L. iners*−dominant (OR 6.39, 95% CI 1.52−26.7, $p = 0.01$) and *A. vaginae*−dominant (OR 4.99, 95% CI 1.17−21.3, $p = 0.03$) dominant types had a higher risk of CIN, compared with the *L. crispatus*−dominant type. The synergistic effect of semi-Western diet and *A. vaginae*−dominant type on CIN risk was observed (OR 20.8, 95% CI 2.21−195.6, $p = 0.01$, RERI/S 9.64/1.96).

Conclusions: Our findings suggest that semi-Western diet and its combination with *A. vaginae*−dominant microflora may represent an important risk factor for cervical neoplasia.

1. Introduction

Cervical cancer develops from a progression of epithelial cellular changes by the multi-stages of oncogenic human papillomavirus (HPV) infection; persistent HPV infection; cervical neoplasia intraepithelial (CIN) 1, 2, and 3; and carcinoma in situ, which were precursor lesions of cervical cancer [1]. Various cofactors such as smoking, oral contraceptive use, sexually transmitted infections, parity, and diet are involved in the progression of cervical cancer in combination with HPV [2]. Recent evidences have revealed that several food groups or dietary factors could prevent the development of cervical cancer or precursor lesions of the disease [3–11]. The EPIC (European Prospective Investigation into Cancer and Nutrition) study, a large prospective study carried out in 10 European countries, showed a significant inverse association of invasive squamous cervical cancer and daily increase in the intake of total fruits [5]. Several reports—including our previous studies—also

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showed that fruit and vegetable consumption [6,7] and intake or blood levels of vitamins A, C, and E, carotenoids, and minerals are associated with a reduced risk of CIN and cervical cancer [7–11]. Higher folate intake is inversely associated with oncogenic HPV infection [12], and tumor suppressor genes display increased methylation according to more-severe cervical neoplasia [13]. Although there is a lack of evidence from controlled trials to confirm those findings, a balanced-diet strategy could be very important in the prevention of cervical cancer [3].

The association between microbial dysbiosis and several cancer types has been reported in areas with a dense bacterial population [14]. Our previous work also showed that a cervical microbial pattern characterized by high abundance of *Atopobium vagiane*, *Lactobacillus iners*, and *Gardnerella vaginalis* and low abundance of *L. crispatus* has a higher risk of CIN [15]. Bacterial vaginosis is a dysbiosis of the vaginal flora characterized by a shift from dominance by *Lactobacillus* spp. to an overgrowth of anaerobic microorganisms. Bacterial vaginosis is a very common disease but had considerable adverse reproductive and obstetric health outcomes in reproductive women. Several studies, including a meta-analysis, reported that bacterial vaginosis is associated with cervical neoplasia [16–18]. Although dysbiosis has only a moderate effect on influences modestly on cancer progression, dysbiosis over a long duration and in combination with other cofactors could exert greater clinical implications [19]. Both the dietary factor and the microbial factor seem to have important roles in the progression of cervical carcinogenesis. To our knowledge, there is no evidence to support the hypothesis that some dietary pattern influences on cervical neoplasia and the effect of those dietary patterns on the development of cervical neoplasia increase considerably when a specific dysbiosis of the cervical microflora also develops. The purpose of this study was to assess the association between dietary pattern and CIN and the biological interaction of dietary pattern and cervical microbiota, identified using pyrosequencing, on the risk of CIN.

2. Materials and methods

2.1. Study subjects and design

Subjects aged 18–65 years, participated in the HPV cohort study from 2006 to the present and were randomly selected from the gynecologic clinics of six university hospitals in South Korea. If the subjects were sexually active, were using birth control methods, were not pregnant women with an intact uterus, had no illnesses requiring referral to hysterectomy, and received no CIN treatment within the previous 18 months, they received a proposal for study participation from their physicians. However, if they had histories of gynecologic cancers, chronic diseases, drug dependencies, or psychological problems, or if there was insufficient data on the questionnaire or in specimens, they were excluded. Cervical swabs were collected for a Pap-smear, an HPV DNA test, and a microbiota analysis (Cervical Sampler, Digene Corp. Gaithersburg, MD, USA). More information on subject recruitment was presented in a previous study [6]. Among 1096 subjects enrolled in the study, 137 having both pyrosequencing data for cervical swabs and food frequency questionnaire data at enrollment were included in this case–control study. Among them, 72 had normal or atypical squamous cells of undetermined significance in Pap smear results and 65 had CIN 1 ($N = 50$) and CIN 2 or 3 ($N = 15$) at enrollment; in particular, 49 control subjects and 63 case subjects were recruited from the previous study [15], and 23 control subjects and 2 case subjects were new additions. All subjects provided written informed consent, and this study was approved by institutional review boards of the Korean National Cancer Center (NCCNCS-06-062) and the ethics committees of the Korean National Cancer Center and Korea University Guro Hospital.

2.2. Demographic questionnaires and food frequency questions

At the time of subject enrollment, a lifestyle questionnaire for collection of data on height, weight, parity, menstrual history including menopausal status, age at menopause, and age at menarche, oral contraceptive use history, medical record, and family history of cancer and a sociodemographic questionnaire for collection of data on education level, tobacco-smoking history, exposure to secondhand smoke, alcohol consumption, and other parameters were administered to each subject. In addition, epidemiological, dietary, pathological, laboratorial, and pyrosequencing results were collected in a database.

Semiquantitative food frequency questionnaire (SQFFQ) having a 95-item was completed by recording the details of food intakes during the year prior to enrollment, including usual frequencies of consumption and typical portion sizes. Frequencies of food consumption were classified into nine categories: almost never, once a month, two or three times a month, one or two times a week, three or four times a week, five or six times a week, once a day, two times a day, and three times a day. The portion size of one item was determined by the mean amount, the typical or standard value, or the natural unit as indicated in the portion size booklet provided from the Korean Ministry of Health and Welfare [20]. Portion size was classified into three categories: small (half a medium portion), medium, and large (1.5 times or more than a medium portion). The food intakes derived from the SQFFQ were calculated by multiplying frequency of consumption with daily portion size of each food group. Nutrient intake for each food item was calculated using the analysis program for nutrients which is included in a web-based management system for recoding, management, calculating, and downloading of data provided from this cohort study. Daily intake of proximate nutrients and sodium was calculated using the Korean Standard Food Composition Table [21] and that of vitamins and minerals was calculated using the database for vitamins [22] and minerals [23] in Korean standard foods.

2.3. Oncogenic HPV DNA detection, pap smear, and histological diagnosis

Oncogenic HPV DNA was detected by Hybrid Capture II system (HC II assay, Digene Corp.). Chemiluminescent HPV DNA tests were measured in relative light units (RLUs) with a probe specific for 13 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The result was read as HPV positive at 1 pg/mL or greater than the RLU/cutoff ratio (RLU of specimen/mean RLU of 2 positive controls). The cytological result for the Pap smear test was based on the Bethesda system. The histological diagnosis was made based on the fact that in cervical squamous intraepithelial lesions (SILs), the number of positive cells for Ki-67 increases with cell grade from normal to low-grade SILs or to high-grade SILs and so, positive nuclei for Ki-67 in the middle and upper-third layers of the epithelium were used for detecting moderate or severe dysplasia (CIN 2 or 3) [24].

2.4. DNA extraction, pyrosequencing, and data analysis

Metagenomic DNA samples were extracted by the Fast DNA SPIN kit (MP Biomedicals, Santa Ana, CA, USA). Target fragments of the 16S rRNA gene corresponding to the V1–V3 regions were amplified using bar-coded primers. Amplification was performed in a final volume of 50 μL containing 10 × *Taq* buffer, a dNTP mixture (Takara Bio, Shiga, Japan), 10 μM of the bar-coded fusion primers, and 2 U of *Taq* polymerase (Ex Taq, Takara Bio). The amplification conditions

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