Plasma Uric Acid Levels in Women With Cervical Intraepithelial Neoplasia

Jayasri Basu, Magdy S. Mikhail, Chul W. Ahn, Joseph Furguiele, Gloria Y. Ho, Robert D. Burk, Prabhudas R. Palan, and Seymour L. Romney

Abstract: The objective of this study was to determine the association of plasma levels of uric acid, an endogenous antioxidant, in women with cervical intraepithelial neoplasia (CIN), while controlling for the confounding effects of human papillomavirus (HPV) infection, age, smoking, and use of oral contraception. Plasma-reduced and oxidized uric acid levels were determined in 650 women by high-performance liquid chromatography, employing electrochemical technique. The findings demonstrated that 1) plasma-reduced uric acid (PRUA) levels in women with CIN (n = 311) were significantly lower (P < 0.05) compared with women in a control group (n = 339); 2) according to multiple logistic regression analysis, PRUA levels were negatively (P = 0.0113) and HPV infection were positively associated (P < 0.0001) with CIN, after controlling for the confounding effects of the studied factors; 3) according to multiple regression analysis, there was a 31% decrease in CIN risk for each incremental increase of 1mg/dl of PRUA; and 4) according to polychotomous logistic regression analysis, independent of HPV infection, PRUA level was inversely associated with the histopathological graded severity of CIN. We have previously reported decreased plasma levels of exogenous antioxidants, for example, vitamins C and E, in women with CIN independent of HPV infection. The data suggest that plasma deficiencies of several antioxidants in HPV-infected uterine cervical tissue may create an oxidative environment that renders the tissue susceptible to free radical damage. It may be speculated that chronic free radical-induced tissue damage in the context of persistent HPV infection may be involved in the pathogenesis of CIN.

Introduction

Free radical-mediated cellular disturbances, as well as dietary and plasma exogenous antioxidant deficiencies, have been implicated in the etiology of cancer. Free radicals can cause oxidative damage to DNA, proteins, and lipids, and result in mutations while antioxidants such as vitamin C, vitamin E, carotenoids, and thiols are capable of protecting against free radical-induced cell damage (1,2). In previous studies designed to investigate the potential role of nutritional factors in the pathogenesis and prevention of uterine cervix cancer, we reported decreased dietary intake of vitamin C in women with CIN (3) and also demonstrated that independent of HPV infection, plasma ascorbate and α-tocopherol levels are significantly and inversely linked to CIN (4,5). Other studies that adjusted for HPV have also reported a protective association between serum carotenoids and CIN (6,7), while other reports have failed to demonstrate any nutrient association with CIN (8,9).

The association of micronutrients with CIN primarily has been focused on the nutritional exogenous antioxidants such as vitamins A, C, and E, and carotenoids (3–10). In this report, the emphasis is on uric acid, a potent endogenous antioxidant. Uric acid is ubiquitous in body fluids and tissues, and its concentration in plasma is higher than that of most endogenous antioxidants, second only to albumin (11). In vitro experiments reportedly have shown that uric acid protects erythrocytes against damage by singlet oxygen, inhibits lipid peroxidation, and protects against free radical-induced damage to DNA (12,13). Plasma urate levels have been examined
in patients with cancer in various sites (14,15). However, plasma uric acid levels in women with CIN have never been previously reported. The objective of the present study was to investigate whether plasma levels of both reduced (PRUA) and oxidized (POUA) uric acid are independently associated with CIN, while controlling for a number of potential confounders implicated in the development of CIN.

Materials and Methods

Subject Recruitment

In this cross-sectional study, volunteer women were interviewed and recruited, with informed consent, from July 1992 through June 1996, from the gynecology and colposcopy clinics of an inner-city hospital in the Bronx, New York. The study protocol was approved by the Institutional Review Board.

All subjects came from the same catchment area and had comparable socioeconomic backgrounds. Hispanics and African Americans (84%) constituted the majority of the study population. Study subjects were either on Medicaid or some form of public assistance. The body weight of the women ranged from 110–250 lb, with an average of 150 lb.

The eligibility criteria for both case and control subjects included: 1) age of 18 to 65 yr, 2) nonpregnant state and no history of childbirth, miscarriage, or abortion within the previous 6 mo, 3) no prior or present history of malignant disease, and 4) no history of renal disease.

Cases and controls were both recruited over the same time period. It was imperative for the woman in the gynecology clinic to have a normal Pap smear on the day she was recruited and her blood and cervicovaginal samples were collected to be included in the statistical analysis as a control. Women whose Pap smears on the day of recruitment were found to be abnormal were not included in the statistical analysis as controls.

Cases were recruited from the colposcopy clinics. They all had abnormal Pap smears and underwent colposcopically directed cervical biopsies on the day of recruitment. Women who had an abnormal Pap smear but were found not to have an abnormal colposcopic finding were not biopsied or recruited. Women who had biopsies, but the histopathologically diagnoses as retrieved from patients’ clinic charts were found to be negative for CIN, were also not included in the statistical analysis as cases.

Biological Specimen Collection

Peripheral venous blood samples were collected from each participant, regardless of the clinical status, in heparinized tubes for plasma uric acid and creatinine assays. After the study was initiated, from year 1993 onward, HPV DNA assay was included in the study. As a result, a cervicovaginal (CVL) lavage sample was additionally collected from each woman for HPV DNA assay. Each participant also completed a study questionnaire, which included age, smoking history, and method of contraception used continuously during the previous 6 mo. The biological specimens and the questionnaires were all coded and were processed without any knowledge of the clinical status.

Biochemical Analysis

Uric acid: Freshly collected blood samples were analyzed for PRUA and POUA levels by high-performance liquid chromatography (HPLC), employing electrochemical detection, within 1–3 h of blood sample collection and without any knowledge of the women’s clinical status. The blood samples were not stored or analyzed in batches. The stationary phase consisted of a reverse-phase C18 column (15 cm × 3.9 mm I.D., 5 µm particle size; Waters, Milford, MA). The mobile phase consisted of: 80 mM sodium acetate buffer, 5% methanol, 0.15% metaphosphoric acid, and 1 mM n-octylamine. The final pH of the mobile phase was 3.2. The LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN) used was equipped with a 5A thin-layer glassy carbon working electrode, set at +0.7 V versus Ag/AgCl reference electrode, with a range of 20 nA. The flow rate was 0.6 ml/min. Each day, the column was equilibrated with freshly prepared mobile phase before any analysis was carried out. A standard curve was run using serial dilutions of analytical grade uric acid (0.5 to 2.5 ng/ml) to test the function of the HPLC equipment and to obtain a standard curve, which was linear throughout the range of standard uric acid concentrations measured. Plasma samples were deproteinized using 10% metaphosphoric acid. Each deproteinized plasma supernatant was passed through a 0.45µm membrane filter (Gelman Sciences, Ann Arbor, MI) to remove particulate matter. An aliquot of the filtered supernatant was injected into the chromatographic system using an autosampler (Waters, Milford, MA), maintained at 4°C. Uric acid was identified by its retention time. Results were calculated by determining the ratio of the peak areas of the extracted plasma samples to the peak areas of the standards, based on the standard curve of that day. The reduced uric acid in the plasma supernatant was stable for 6 h under the present experimental conditions. To measure oxidized uric acid, POUA was reduced to PRUA using 1% DL-dithiothreitol, quantified as total uric acid and the POUA levels determined from the difference in prereduction and postreduction quantitative values. To determine the coefficient of variation for uric acid, several freshly collected plasma samples were pooled. The intra-assay coefficient of variation for 10 replicate samples determined in duplicates was 2.3% (mean ± SD: 3.90 ± 0.09 mg/dl). Since PRUA levels depreciate when stored, the inter-assay coefficient of variation was determined using standard uric acid samples. The inter-assay coefficient of variations of standard samples run over a week’s period was 1.2% (mean ± SD: 25.0 ± 0.30 ng/dl).
**Creatinine:** Creatinine levels of the plasma samples were determined by the method of Fabiny et al (16) as a means of evaluating renal function. Creatinine levels of plasma samples stored at −80°C were assayed in batches. The intra-assay coefficient of variation of 25 replicate pooled plasma samples was 4.5% (mean ± SD: 1.10 ± 0.05) and the inter-assay coefficient of variation of 25 replicate pooled plasma samples over a week’s period was 5.08% (mean ± SD: 1.18 ± 0.08 mg/dl).

**HPV DNA positivity:** HPV DNA determination of coded samples of lavaged cervicovaginal epithelial cells was analyzed by polymerase chain reaction (PCR), using consensus primers MY09 and MY11 to a highly conserved region in the L1 open reading frame (17). A total of 512 CVL samples were analyzed for HPV DNA, 281 samples in the control group and 311 samples in the CIN group. For the remaining 138 women, the HPV DNA data are missing.

**Data Analysis**

The sample size estimate was based on our pilot study, which showed that normal controls and CIN subjects had PRUA values of 2.83 and 2.60 mg/dl, respectively, with a common standard deviation of 1.00. A sample size of 300 in each group will have 80% power to detect a difference in means of 0.23, assuming that the common standard deviation is 1.00, using a two-group t test with a 0.05 two-sided significance level. Therefore, we planned to recruit at least 300 normal controls and 300 CIN subjects for this study to have at least 80% power. In the study, we recruited 339 normal controls and 311 women with CIN.

After uric acid, creatinine, and HPV DNA assays and histopathological diagnoses were completed, the study code was broken. Pap smear and histopathological diagnoses of the biopsy specimens were retrieved from the women’s medical charts.

Of 1,050 women initially interviewed, a total of 835 (31.9 ± 9.8 yr) were comparable. Compared with controls, ages (± SD) of the control (33.7 ± 11.4 yr) and CIN groups were presented in Table 1. The age of the women ranged between 18 and 65 yr, with the median being 33 yr. The mean ages (± SD) of the control (33.7 ± 11.4 yr) and CIN groups (31.9 ± 9.8 yr) were comparable. Compared with controls, a significantly greater number of women in the CIN groups had abnormal Pap smears, had abnormal colposcopic findings, and were biopsied on the day of recruitment. Of them, 311 women were diagnosed by staff pathologists as having CIN lesions. At the hospital, both cytological and histopathological diagnoses were made according to the guidelines of the American College of Obstetricians and Gynecologists (ACOG). As retrieved from the women’s charts, the distribution of the histopathological diagnoses were: 215 CIN 1, 53 CIN 2, and 43 CIN 3.

Chi-square tests and/or Fisher’s exact tests were used to compare categorical risk factors among case and control groups. Student’s t tests were used to compare age and PRUA and POUA levels: between control and CIN cases; between nonsmokers and current smokers; between oral contraceptive pill (OCP) users and non-OCP users; and between HPV DNA-negative and HPV DNA-positive women. Analysis of variance (ANOVA) tests were used to compare PRUA and POUA levels among women with various grades of CIN. Multiple logistic regression analysis was done to examine the association between PRUA and CIN, after controlling for the confounding effects of risk factors, including age, smoking, oral contraception, and HPV infection. Polychotomous logistic regression analysis was performed to examine the contribution of risk factors to the severity of CIN grade.

**Results**

The demographic characteristics of the study population are presented in Table 1. The age of the women ranged between 18 and 65 yr, with the median being 33 yr. The mean ages (± SD) of the control (33.7 ± 11.4 yr) and CIN groups (31.9 ± 9.8 yr) were comparable. Compared with controls, a significantly greater number of women in the CIN groups

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**Table 1. Demographic Characteristics Based on Cervical Status**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal n = 339 (%)</th>
<th>CIN1 n = 215 (%)</th>
<th>CIN2 n = 53 (%)</th>
<th>CIN3 n = 43 (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>33.7 ± 11.4</td>
<td>31.8 ± 10.3</td>
<td>30.9 ± 8.6</td>
<td>34.0 ± 8.6</td>
<td>0.097</td>
</tr>
<tr>
<td>21–30</td>
<td>138 (41)</td>
<td>84 (39)</td>
<td>26 (49)</td>
<td>14 (33)</td>
<td></td>
</tr>
<tr>
<td>31–40</td>
<td>101 (30)</td>
<td>71 (33)</td>
<td>19 (36)</td>
<td>22 (51)</td>
<td></td>
</tr>
<tr>
<td>41–50</td>
<td>41 (12)</td>
<td>25 (12)</td>
<td>4 (8)</td>
<td>4 (2)</td>
<td></td>
</tr>
<tr>
<td>51–60</td>
<td>30 (9)</td>
<td>8 (4)</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>61+</td>
<td>8 (2)</td>
<td>5 (2)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Age &gt; median (33 yr)</td>
<td>143 (42)</td>
<td>65 (30)</td>
<td>16 (30)</td>
<td>20 (47)</td>
<td></td>
</tr>
<tr>
<td>OCP users (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.737</td>
</tr>
<tr>
<td>71 (21)</td>
<td>46 (21)</td>
<td>11 (21)</td>
<td>6 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker (n)</td>
<td>79 (30)</td>
<td>50 (24)</td>
<td>21 (41)</td>
<td>19 (45)</td>
<td>0.009</td>
</tr>
<tr>
<td>HPV Positivity (n)</td>
<td>76 (27)</td>
<td>103 (67)</td>
<td>34 (85)</td>
<td>30 (81)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*a: Abbreviations are as follows: CIN, cervical intraepithelial neoplasia; OCP, oral contraceptive pill; HPV, human papillomavirus.

b: P values by chi-square and/or Fisher exact tests. Smoking: P = 0.046 between normal and CIN 3; P = 0.012 between CIN 1 and CIN 2; P = 0.004 between CIN 1 and CIN 3.

c: HPV positivity: P<0.001 between normal and CIN 1, between normal and CIN 2, between normal and CIN 3; P = 0.028 between CIN 1 and CIN 2. A total of 512 cervicovaginal samples were analyzed for HPV DNA: 281 in the control group, 154 in the CIN 1 group, 40 in the CIN 2 group and 37 in the CIN 3 group.
had HPV infection ($P < 0.0001$). In the study, the women were either current smokers or had never smoked. A review of the epidemiologic questionnaires revealed that the data on 80 smoking histories were either missing or were incomplete: 68 in the control group and 12 in the CIN group. The smokers on average smoked 11.1 ± 9.4 cigarettes per day for an average smoking duration of 16.0 ± 8.7 yr. The percentage of smokers among the control and CIN groups were, however, comparable.

PRUA and POUA concentrations of women with CIN are presented in Table 2; the data show that uric acid in the plasma exists predominantly in the reduced form. The mean (± SD) plasma level of reduced uric acid (2.60 ± 0.95 mg/dl) in women with CIN was significantly lower ($P < 0.05$) compared with women in the control group (2.77 ± 1.08 mg/dl). Mean PRUA levels among women with varying grades of CIN were comparable. POUA levels were determined for all subjects. However, it needs to be pointed out that 50% of the plasma samples in the control group and 48% in the CIN group had plasma oxidized uric acid levels below the detection limit of 50 pg/dl. Nevertheless, the table depicts that POUA levels between the cases and the controls are comparable.

Univariate analysis of PRUA levels of women with normal Pap smears are shown in Table 3. The data (mean ± SD) revealed that PRUA levels of current smokers (2.88 ± 0.94, $n = 41$), OCP users (2.46 ± 0.95, $n = 16$), and women positive for HPV DNA (3.09 ± 1.13, $n = 28$) were comparable with women who were non-smokers, non-OCP users, and negative for HPV DNA (3.14 ± 1.29, $n = 95$), respectively.

Multiple logistic regression analysis identified significant independent risk factors for CIN. The results are presented in Table 4. The results indicate that plasma PRUA levels were negatively ($P = 0.0133$) and HPV infection was positively ($P < 0.0001$) associated with CIN; whereas age, smoking, and OCP use had no effect. The data additionally demonstrated that there was a 31% decrease in the risk of having CIN for an increase of 1 mg/dl of PRUA, after controlling for the confounding effects of the variously studied risk factors. Polychotomous logistic regression analysis was carried out, taking only the cases into account and excluding the controls, to identify any independent risk factors associated with the severity of CIN. The analysis showed that HPV infection ($P < 0.0001$) was significantly associated with the more severe grades of CIN. PRUA ($P = 0.054$) was marginally associated with less severe grades of CIN (Table 5).

Plasma creatinine levels analyzed to evaluate renal function of the studied women showed (mean ± SD) plasma creatinine levels of women with ($n = 311$) and without CIN (339) to be 1.10 ± 0.16 mg/dl and 1.08 ± 0.14 mg/dl, respectively. The difference was not statistically significant.

**Table 2. Plasma Reduced (PRUA) and Oxidized (POUA) Uric Acid Levels of Women With Cervical Intraepithelial Neoplasia**

<table>
<thead>
<tr>
<th>Groups</th>
<th>PRUA (mg/dl)</th>
<th>POUA (mg/dl)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.77 ± 1.08</td>
<td>0.24 ± 0.36</td>
<td>&lt;0.05</td>
<td>0.657</td>
<td></td>
</tr>
<tr>
<td>CIN</td>
<td>2.60 ± 0.95</td>
<td>0.23 ± 0.34</td>
<td>&lt;0.0001</td>
<td>5.400</td>
<td>(2.967, 9.831)</td>
</tr>
<tr>
<td>CIN 1</td>
<td>2.60 ± 0.99</td>
<td>0.20 ± 0.40</td>
<td>0.657</td>
<td>0.657</td>
<td></td>
</tr>
<tr>
<td>CIN 2</td>
<td>2.60 ± 0.80</td>
<td>0.20 ± 0.30</td>
<td>0.657</td>
<td>0.657</td>
<td></td>
</tr>
<tr>
<td>CIN 3</td>
<td>2.60 ± 0.90</td>
<td>0.10 ± 0.20</td>
<td>&lt;0.0001</td>
<td>5.400</td>
<td>(2.967, 9.831)</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Values are means ± SD. Values in parentheses indicate the number of plasma samples in which oxidized uric acid levels were detectable out of the total number of plasma samples analyzed for POUA.

**Table 3. Plasma Reduced Uric Acid Levels of Normal Women**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PRUA Mean (mg/dl)</th>
<th>SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95</td>
<td>3.14</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>Smokers&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41</td>
<td>2.88</td>
<td>0.94</td>
<td>NS</td>
</tr>
<tr>
<td>OCP users&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16</td>
<td>2.46</td>
<td>0.95</td>
<td>NS</td>
</tr>
<tr>
<td>HPV DNA positive&lt;sup&gt;e&lt;/sup&gt;</td>
<td>28</td>
<td>3.09</td>
<td>1.13</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Abbreviations are as follows: PRUA, plasma reduced uric acid; OCP, oral contraceptive pill; HPV, human papillomavirus.
<sup>b</sup>: Control: women with normal Pap smears who are non-smokers, not on OCPs, and are negative for HPV DNA.
<sup>c</sup>: Smoker: women with normal Pap smears, who are current smokers, not on OCPs, and are negative for HPV DNA.
<sup>d</sup>: OCP users: women with normal Pap smears, on OCPs continuously for 6 mo, who are nonsmoker and are negative for HPV DNA.
<sup>e</sup>: HPV DNA positive: women with normal Pap smears, who are non-smokers, are not on OCPs, and are positive for HPV DNA.

**Table 4. Multiple Logistic Regression Analysis for the Association of CIN With Risk Factors**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>P Value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>-0.0162</td>
<td>0.0149</td>
<td>0.2761</td>
<td>0.984</td>
<td>(0.956, 1.013)</td>
</tr>
<tr>
<td>OCP users (n)</td>
<td>0.0878</td>
<td>0.3959</td>
<td>0.8246</td>
<td>1.092</td>
<td>(0.502, 2.372)</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.0671</td>
<td>0.3212</td>
<td>0.8346</td>
<td>0.935</td>
<td>(0.498, 1.755)</td>
</tr>
<tr>
<td>PRUA (mg/dl)</td>
<td>-0.3718</td>
<td>0.1466</td>
<td>0.0113</td>
<td>0.690</td>
<td>(0.518, 0.919)</td>
</tr>
<tr>
<td>POUA (mg/dl)</td>
<td>0.1538</td>
<td>0.4532</td>
<td>0.7344</td>
<td>1.166</td>
<td>(0.480, 2.835)</td>
</tr>
<tr>
<td>HPV positivity (n)</td>
<td>1.6865</td>
<td>0.3057</td>
<td>&lt;0.0001</td>
<td>5.400</td>
<td>(2.967, 9.831)</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Abbreviations are as follows: OCP, oral contraceptive pill; PRUA, plasma-reduced uric acid; POUA, oxidized uric acid. Odds ratios and 95% confidence intervals (CI) of PRUA and POUA are computed based on 1 standard deviation increment of PRUA and POUA.
further revealed that the association of PRUA with CIN is in-
normal renal function. Multiple logistic regression analysis
acid levels in women with CIN could not be attributed to ab-
CIN and control groups. This suggests that alteration in uric
reflection of renal status revealed comparable levels in both
in the oxidized form. Plasma creatinine levels measured as a
determination of uric acid free of other contaminations, espe-
tial methods (14,15,30). The present findings establish the
urtic acid, in the present study, appear to be somewhat
if smoking is associated with higher grades of CIN
arette smoking to be associated with higher grades of CIN

**Discussion**

Uterine cervical cancer is the second most common ma-
lignancy worldwide, and its prevention continues to be a pub-
health challenge (18). The availability of the Pap smear, the
anatomic accessibility of the cervix, and the usefulness of
colposcopy and colposcopically directed biopsies constitute
an unusual opportunity to investigate and treat precancerous
cervical lesions. Many of the early CIN lesions, if not associ-
ated with an oncogenic strain of HPV infection, do not de-
velop into a more aggressive or invasive stage, but rather dis-
appear spontaneously (19). Better understanding of the
multifactorial aspects of CIN lesions may contribute to our
goal of cervix cancer prevention.

In the present study, plasma uric acid levels were investi-
gated in volunteer women with established CIN lesions,
while controlling for the confounding effects of HPV, age,
smoking, and OCP use, known risk factors implicated in the
development of CIN. The HPLC procedure employed specif-
ically enabled the determination of uric acid in its reduced
form, the molecular configuration in which uric acid func-
tions as an antioxidant. The method additionally allowed the
determination of uric acid free of other contaminations, es-
specially ascorbic acid. It is for this reason perhaps that plasma
uric acid levels, in the present study, appear to be somewhat
lower than that reported by other investigators using enzy-
matic methods (14,15,30). The present findings establish the
fact that, in human plasma, uric acid is measurable in both its
reduced and oxidized forms. The reduced form is predomi-
nant and is present at a 10-fold higher concentration. Com-
pared with control subjects, PRUA was significantly
lower in women with CIN, without any detectable alteration
in the oxidized form. Plasma creatinine levels measured as a
reflection of renal status revealed comparable levels in both
CIN and control groups. This suggests that alteration in uric
acid levels in women with CIN could not be attributed to ab-
normal renal function. Multiple logistic regression analysis
further revealed that the association of PRUA with CIN is in-
dependent of HPV infection. Age, smoking, and OCP use
were not found to have any effect on PRUA levels. The data
additionally demonstrated that there was a 31% decrease in the
risk of having CIN for an increase of 1 mg/dl of PRUA,
after controlling for the confounding effects of other vari-
ously studied risk factors. Polychotomous logistic regression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>P Value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>−0.0134</td>
<td>0.0135</td>
<td>0.3193</td>
<td>0.987</td>
<td>(0.961, 1.013)</td>
</tr>
<tr>
<td>OCP users (n)</td>
<td>−0.2528</td>
<td>0.3459</td>
<td>0.4649</td>
<td>0.777</td>
<td>(0.394, 1.530)</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>0.1473</td>
<td>0.2821</td>
<td>0.6016</td>
<td>1.159</td>
<td>(0.667, 2.014)</td>
</tr>
<tr>
<td>PRUA (mg/dl)</td>
<td>−0.2527</td>
<td>0.1314</td>
<td>0.0544</td>
<td>0.777</td>
<td>(0.394, 1.530)</td>
</tr>
<tr>
<td>POUA (mg/dl)</td>
<td>−0.0808</td>
<td>0.4241</td>
<td>0.8489</td>
<td>0.922</td>
<td>(0.402, 2.118)</td>
</tr>
<tr>
<td>HPV positivity (n)</td>
<td>1.6333</td>
<td>0.2877</td>
<td>&lt;0.0001</td>
<td>5.121</td>
<td>(2.914, 9.000)</td>
</tr>
</tbody>
</table>

* a: Abbreviations are as follows: OCP, oral contraceptive pill; PRUA, plasma-reduced uric acid; POUA, oxidized uric acid; HPV, human papillomavirus. Odds ratios and 95% confidence intervals (CI) of PRUA and POUA are computed based on 1 standard deviation increment of PRUA and POUA.

Epidemiological and molecular studies have identified
specific strains of oncogenic HPV infections to be the caus-
itive factor in the development of CIN and cervix cancer
(20–23). HPV DNA has been found in 99% of all cervix can-
cer cases worldwide, establishing HPV to be the primary sex-
ually transmitted etiologic factor in cervical cancer (22). It is
also reported that women who test persistently HPV positive
with oncogenic types are at a significantly greater risk of de-
veloping CIN, compared with women who are only tran-
siently infected (24). However, while most women with CIN
cervix cancer are HPV positive (20–22), only a small
percentage of women infected with HPV progress to invasive
cervical cancer. This suggests that HPV infections per se may
not be solely responsible for malignant transformation, and
other cofactors may be involved in this pathological process
(5).

Besides HPV, epidemiological and experimental studies
have for decades implicated a variety of other risk factors in
the etiology and pathogenesis of CIN. Among these are ciga-
rette smoking, low dietary intake and/or reduced blood levels
of various antioxidants, long-term use of OCPs and other
lifestyle characteristics (3–9,18–27). The present findings
suggest that in smokers the severity of CIN is significantly
increased ($P = 0.009$; Table 1). Others have also reported cig-
arette smoking to be associated with higher grades of CIN
(5,25,26). The effect of smoking, however, was not found to
be significant on multivariate analysis. Oral contraception
has not been found to have any association with CIN, both in
our study and those reported by others (5,26). After adjusting
for HPV infection, none of the following lifestyle variables
were found to have any association with CIN: age, ethnicity,
income, education, partner promiscuity, or early age at first
intercourse (27).

In human subjects, uric acid is formed from purine degra-
dation. Contribution to purine levels come from dietary
sources and from biosynthesis de novo. Case and control
subjects recruited in previous studies came from the same
catchment residential areas. In our earlier studies, dietary in-
takes were recorded, and they did not reveal significant dif-
fences in the nature of dietary intake, except for the intakes
of $\beta$-carotene and vitamin C (3,5). In another study, plasma

Table 5. Results of Polychotomous Logistic Regression Analysis for the Association of Risk Factors With the Severity of CIN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>P Value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>−0.0134</td>
<td>0.0135</td>
<td>0.3193</td>
<td>0.987</td>
<td>(0.961, 1.013)</td>
</tr>
<tr>
<td>OCP users (n)</td>
<td>−0.2528</td>
<td>0.3459</td>
<td>0.4649</td>
<td>0.777</td>
<td>(0.394, 1.530)</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>0.1473</td>
<td>0.2821</td>
<td>0.6016</td>
<td>1.159</td>
<td>(0.667, 2.014)</td>
</tr>
<tr>
<td>PRUA (mg/dl)</td>
<td>−0.2527</td>
<td>0.1314</td>
<td>0.0544</td>
<td>0.777</td>
<td>(0.394, 1.530)</td>
</tr>
<tr>
<td>POUA (mg/dl)</td>
<td>−0.0808</td>
<td>0.4241</td>
<td>0.8489</td>
<td>0.922</td>
<td>(0.402, 2.118)</td>
</tr>
<tr>
<td>HPV positivity (n)</td>
<td>1.6333</td>
<td>0.2877</td>
<td>&lt;0.0001</td>
<td>5.121</td>
<td>(2.914, 9.000)</td>
</tr>
</tbody>
</table>
urate levels were also reported not to decline when healthy males were placed on a purine-free diet (28). It, therefore, seems unlikely that the lower PRUA levels observed in this study, in women with CIN, could be attributed to purine intake alone.

In humans, uric acid is not metabolized to allantoin, the metabolic end product of uric acid in animals. Mutational events have resulted in the loss of uricase activity in humans, with the evolvement of a rather highly efficient kidney reabsorption system, responsible for reabsorption of more than 90% of uric acid (29). These evolutionary modifications in humans are proposed by Ames and colleagues (29) to be a compensatory mechanism to replace some of the functions of ascorbic acid, enabling humans to survive in an oxygen-rich environment. Because there is no enzyme capable of degrading uric acid in humans and hypouricemia is somewhat associated with increased urinary reabsorption, the present finding of lower PRUA levels in women with CIN could be ascribed to its increased utilization.

The correlation of uric acid with other micronutrients has been studied in vitro and in vivo. In cancer patients, uric acid, ascorbic acid, and \( \alpha \)-tocopherol independently were shown to be significantly lower in the plasma, but were not correlated with each other (13,14,30). Depletion of serum urate, however, was reported to result in rapid subsequent oxidation of ascorbate in in vitro studies (31) and when both urate and ascorbate were sequentially lost, the antioxidant capacity of the serum was reported to be markedly reduced (31). Hence, based on our previous and present findings of depleted antioxidant levels of vitamin C, E, \( \beta \)-carotene, and uric acid in the plasma of women with CIN, it may be suggested that the antioxidant capacity of the plasma in women with CIN may be compromised.

Recently Shi and colleagues (32) have reported uric acid to be an important component of the immune system. Danger signals released by injured cells are essential to initiate an immune response. These danger signals stimulate the immune cells to mature, enable them to present foreign antigens, and stimulate the T lymphocytes. The principal endogenous danger signal released by injured cells has been demonstrated to be uric acid. The clinical significance of Rock’s findings may be debatable, however, the findings contribute to our understandings of the mechanisms involved in immune response.

The limitations of the present study need to be noted. Although we recognize the importance of analyzing dietary purine intake and HPV typing, these two parameters were not evaluated in the present report. Any measure of diet or nutrient-related lifestyle was not included in this study because of our previous experience, in which exogenous antioxidants had been investigated, and a marked difference in the dietary intakes between the cases and the controls was not detected, except in their intakes of \( \beta \)-carotene and vitamin C (3–5,24). While HPV positivity was determined, HPV typing was not. Had HPV typing been available, some of the HPV-positive women may have been found to have non-oncogenic types and might have been reclassified. We intend to include dietary purine intake and HPV typing in future uric acid studies along with a more detailed epidemiologic questionnaire. Another limitation is the potential for reverse causality. Since plasma uric acid levels are measured after the onset of CIN, the CIN may have caused the decrease in plasma uric acid levels rather than vice versa. Furthermore, data on other potential confounders are also lacking. Nevertheless, several points regarding the present study are noteworthy: 1) women were asymptomatic when recruited into the health care system by virtue of obtaining Pap smears; 2) plasma uric acid, creatinine, HPV DNA assays, Pap smears, and tissue diagnoses of the cervix biopsies were all analyzed without knowledge of case-control status; and 3) plasma uric acid levels were assayed using HPLC, employing electrochemical technique, rather than being determined by enzymatic methods; moreover, the HPLC technique enabled the determination of uric acid, free of other contaminants, especially that of ascorbic acid, and allowed uric acid to be determined in its reduced form, the form in which uric acid functions as an antioxidant; 4) HPV DNA of the cervical cells were analyzed to detect viral infection; and 5) the studied risk factors were evaluated using multivariate analysis.

It is reported that HPV infection results in an increase in the generation of free radicals (33), and uric acid has been reported to be the principal endogenous danger signal capable of activating specific immune response (32). The present findings reveal significantly lower levels of PRUA, and our previous findings demonstrated lower levels of several other antioxidants, including reduced ascorbic acid, \( \alpha \)-tocopherol, and \( \beta \)-carotene in women with CIN. We, therefore, hypothesize that free radical-induced cell damage and antioxidant defenses may be involved in the pathogenesis of CIN. Furthermore, it may be speculated that failure to initiate an immune response, as a result of antioxidant deficiency, may facilitate the persistence of HPV infection and the development of CIN.

Acknowledgments and Notes

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