Association between cigarette smoking and consumption of daily fruits and vegetables with human papillomavirus infection among women in United States

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“In the name of Allah, the most Gracious, the most Merciful”

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Abstract

Background: The human papillomavirus is a sexually transmitted infection, which can cause several diseases including genital warts and more importantly carcinoma of the cervix. Worldwide cervical cancer is the second most common type of cancer among women after breast cancer. Although the HPV prevalence of 99.7% among diagnosed patients of cervical cancer confirms the causative role of infection towards cancer, it does not necessarily mean that every woman who is exposed to HPV will acquire the infection or progress towards cancer after being infected. There are some important risk factors thought to alter the probability of acquisition and progression of HPV infection.

Objective: The purpose of this study is to increase the understanding of the association between smoking, consumption of daily fruits and vegetables with human papillomavirus infection positivity among women.

Methods: Secondary data from the 2007 National Cancer Institute’s (NCI) Health Information National Trends Survey (HINTS) were analyzed in this study. STATA (version 10.1) was used for data analysis. Smoking status and daily fruits and vegetables consumption of women with and without HPV infection was compared by chi-square. Logistic regression analysis was used to examine the association between smoking status and women’s consumption of daily fruits and vegetables with HPV infection positivity.

Results: The analysis indicated a statistically significant association between ever and current-smokers with HPV infection positivity. Conversely a non-significant association was seen between daily consumption of fruits and vegetables with HPV infection positivity. Results from the analysis demonstrated that smoking was a significant risk factor, while daily consumption of fruits and vegetables were non-significant risk factors for the HPV infection among women.

Conclusion: Prevalence of smoking is increasing worldwide particularly in young women making it a major public health problem. The positive association of smoking with HPV infection observed in this and in other studies, may seem due to deleterious effects of smoking, which is responsible for causing changes in cervical epithelium. These kinds of changes have biological plausibility as nicotine along with other smoke metabolites has been identified in cervical mucus membrane of smokers. At present, the relationship between fruits and vegetables consumption and the risk of HPV infection is contradictory and mixed as there are several studies including the present study which describe a non-significant association while some authors also claim a positive association between these two. Therefore more prospective cohort studies with long follow up time are well warranted to prove causality and to define the relationship between consumption of daily fruits and vegetables with acquisition of HPV infection.
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ALTS</td>
<td>Atypical Squamous Cells of Undetermined Significance Low grade Squamous Intraepithelial Lesion Triage Study.</td>
</tr>
<tr>
<td>CATI</td>
<td>Computer assisted telephone interview</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CIN</td>
<td>Cervical intraepithelial neoplasia</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>FIGO</td>
<td>International federation of obstetricians and gynecologists</td>
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<td>HBV</td>
<td>Hepatitis B virus</td>
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<td>HCV</td>
<td>Hepatitis C virus</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HINTS</td>
<td>Health information national trends survey</td>
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<td>HPV</td>
<td>Human papillomavirus</td>
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<tr>
<td>HR</td>
<td>High risk</td>
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<tr>
<td>IARC</td>
<td>International agency for research on cancer</td>
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<tr>
<td>NCI</td>
<td>National cancer institute</td>
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<tr>
<td>RDD</td>
<td>Random digit dialing</td>
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<tr>
<td>UICC</td>
<td>Union for international cancer control</td>
</tr>
<tr>
<td>USPS</td>
<td>United states postal service</td>
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<td>VIA</td>
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1. Introduction

Cancer

The human body is made up of billions of living cells. Normal body cells grow, divide and then die in a systematic way. During childhood, normal cells divide quicker to allow the person to grow. This division stops when a person becomes an adult, then most cells divide only to replace damaged or dying cells. Cancer begins when cells in a specific part of the body start to grow uncontrollably. Cancer can be divided into different types, but they all start due to this out of control growth of abnormal cells. Cancer cells grow differently compared to normal cells. Instead of dying after being damaged; cancer cells keep on growing and produce more cancer cells. These cancer cells can invade surrounding normal tissues; can travel to other parts of the body through bloodstream or lymph vessels. There they start to grow and form new tumors, which afterwards replace normal tissues. This process is named “Metastasis” (1).

No matter where cancer may spread, it is always termed after the place where it originated. For example if breast cancer spreads to liver, it is still called breast cancer and not the liver cancer. All tumors are not cancerous. Tumors that are not cancerous are called benign tumors and can become very large in size thereby pressing healthy organs or tissues. But these tumors cannot grow into other tissues and can't travel to different parts of the body (metastasize). Benign tumors are almost never life threatening (1).

Around the world tobacco use is the leading known risk factor for cancer. In addition to tobacco use an unhealthy diet, alcohol use, chronic infections from hepatitis B and C (HBV, HCV), and certain types of human papillomavirus (HPV) are important risk factors. Proper awareness about the cause of cancer (e.g. radiation, chemicals, infection and ageing), avoiding potential risk factors and early detection can significantly reduce the burden of cancer (2).

According to International Agency for Research on Cancer (IARC), during the year 2008 nearly 12.7 million new cases of cancer and about 7.6 million deaths (13% of all deaths) due to cancer occurred worldwide. Of these cancer deaths, 56% new cases of cancer and 63% cancer deaths occurred in developing countries (3). More than 30% of these cancer
deaths are preventable (2). World Health Organization estimated that worldwide deaths from cancer are expected to continue to rise to more than 11 million in 2030 (2).

In United States, cancer is the second most common cause of death. An estimated 1,529,560 cases of cancer were diagnosed, and around 569,490 deaths occurred due to cancer in the year 2010. However cancer incidence is decreasing in the American population, American Cancer Society and Centers for Disease Control and Prevention reported that incidence of cancer for both men and women decreased by 0.8% per year from 1999 to 2005 (4).

**Cervical cancer**

The cervix is the lower part of uterus. Uterus has 2 parts, the upper part is called body of uterus and the part, which connects the body of uterus to the vagina, is called cervix. Cervical cancer (also called carcinoma of the cervix) originates in the lining of cervix; it takes many years for normal cells to change into cancer. These changes are called dysplasia, which can easily be detected through screening, and can easily be treated to prevent cancer. Squamous cell carcinoma and adenocarcinoma are two main types of cervical cancer (1).

With a prevalence of 2.3 million cases and around 510,000 incident cases each year, cervical cancer is the third most common cancer worldwide (5). In women after breast cancer, cancer of the cervix is the most common cancer accounting for 250,000 deaths annually, of which 80% occur in low-income countries (6). There is always much emphasis given on reducing maternal mortality, but little is written about mortality due to cervical cancer while the rates are quite similar. In low and middle-income countries approximately two thirds of healthy years lost by women are due to cervical cancer. These facts are alarming because cervical cancer is a preventable disease (5).

In United States carcinoma of the cervix is the third most common gynecological malignancy (7). The American Cancer society estimates that approximately 12,170 new cases of invasive cervical cancer are expected to be diagnosed and an estimated 4,220 deaths are expected in the country due to cervical cancer during the year 2012 (8). It is seen that over the past several decades incidence rates have decreased in both African
and white American women. From 1975 to 2003 mortality rates dropped progressively due to prevention strategies and early detection of disease but rates have been stable since 2003 (8).

**Risk factors of cervical cancer**

- **Human papillomavirus**
  In order to develop cervical cancer; a woman must be infected with one or more high-risk types of human papillomavirus (9). The association between HPV and invasive cervical cancer is well established and it is considered to be a necessary cause for the development of cancer (10,11), however not sufficient cause for the progression of disease (12). It is believed that certain cofactors should be present in order to develop cancer (7,10,12). Following are some of the important risk factors, which along with HPV infection are responsible for developing carcinoma of the cervix.

- **Smoking**
  In 1977 Winkelstein first hypothesized that smoking can be a risk factor for cervical cancer (13). Later in 2003 Plummer et al performed a pooled analysis on 10 case control studies; eight on invasive cervical cancer and two on carcinoma in situ conducted by IARC from 1985 to 1997. Their analysis showed that the risk of developing cervical cancer among ever-smokers remained higher even after controlling for the strong effect of HPV. They also suggested that cervical squamous cell carcinoma should be added to the list of cancers associated with tobacco use (13).

- **Hormonal contraceptives**
  Studies suggest that the risk of cervical cancer increases in HPV positive women if they use oral contraceptives for more than 5 years. A recent study by Smith et al showed that the relative risk of cervical cancer increases as the duration of oral contraceptives increase (14).

- **Diet**
  Role of micronutrients on invasive cervical cancer is contradictory (15). Potishman and Brinton described in their study that there was sufficient evidence on the basis of which it could be concluded that cervical cancer is related to both low levels of vitamin C and carotenoids in the body (16). While Wideroff et al concluded in their study that the risk
of cervical cancer in HPV positive women did not decrease with increased intake of vitamins or β-carotene (17).

- **Parity**
In the IARC multicentric study the authors found direct association between squamous cell cervical cancer and number of full term pregnancies. Women who stated seven or more full term pregnancies and also had HPV positive status, the risk of cervical cancer increased by four-fold compared to nulliparous women infected with HPV (odds ratio=3.8, CI=2.7-5.5) (18).

- **Additional factors**
In addition to the above-described risk factor there are some other important risk factors, which can result in increased risk of cervical cancer. Various studies described that the risk of cervical cancer increases among HPV positive women having other infections like HIV, Chlamydia trachomatis and/or herpes simplex virus-2 (19,20). Early age at first intercourse and low participation in organized screening are strongly associated with increased risk of invasive cervical cancer (21,22).

**Clinical presentation and treatment of cervical cancer**

Signs and symptoms of women with cervical cancer may differ depending on the stage of disease (7). International Federation of Obstetricians and Gynecologists (FIGO) in collaboration with World Health Organization (WHO) and the International Union against Cancer (UICC) developed the staging system, which is widely used to measure the progression of disease and for its treatment (23).

- **Symptoms**
At early stages women may seem asymptomatic thus these women can only be diagnosed on a pap smear incidentally. However some patients can have symptoms including a foul smelling discharge, vaginal bleeding, postcoital bleeding and intermittent bleeding during their menstrual cycle. In more advanced disease, symptoms may include leg swelling due to compression of lymphatic and/or venous system. Patients with advanced disease may also present with complaint of hematuria due to extension of cancer to genitourinary system (7).
• Signs and physical examination
Enlarged supraclavicular lymph nodes, inguinal lymphadenopathy, ascites, edema of lower extremities, and decreased breath sounds on auscultation indicate disease metastases. In bimanual examination enlarged uterus may be palpable due to tumor growth and invasion. During digital rectal examination (DRE) irregular, thick and less mobile or fixed mass indicates that tumor has invaded to pelvic sidewalls (23).

• Treatment and prognosis
Ligation of uterine or hypogastric arteries may be a life saving approach at emergency basis when treating a woman with vaginal hemorrhage resulting from gross ulceration in stage II–IV. Total hysterectomy is a treatment of choice in women who have completed child bearing at stage 0. Simple, extrafascial hysterectomy is done at stage IA1. Radiation and chemotherapy is used at stages IA2, IB1, and IIA. Radiation therapy plus cisplatin-based chemotherapy is used to treat stages IB2, IIB, III and IV (24).

The 5 years survival rate for cervical cancer is 100% in stage 0; stage I, 85%; stage II, 65%; stage III, 35%; and stage IV, 7% (25).

Human papillomavirus and cervical cancer

Human papillomavirus is one of the most commonly occurring sexually transmitted infections and is thought to be the essential cause of carcinoma of the cervix (9,10,11,26). Three evidences confirm the association between HPV and cervical cancer: (I) Worldwide HPV-DNA is identified in most cervical cancer biopsy specimens, (II) In case control studies the relative risk of cervical cancer is more than 70% for several high-risk HPV types, (III) In cohort studies relative risk of cervical cancer is about 10% for women with HPV infection (26).

Pathway from HPV infection to cervical cancer

Persistent infection with one or more high-risk or oncogenic types of human papillomavirus is the primary cause of squamous cell cervical carcinoma. Most common cancer causing HPV types are 16 and 18, found in 70% of all reported cases. Other HPV types (e.g. 6 & 11) are called low-risk types, which are not associated with cancer instead.
they can cause genital warts (9). HPV spreads from one person to another though skin to skin contact such as vaginal, oral or anal sex. However sex is not the only mode of transmission of infection, skin contact with the part of body infected with HPV is all that is needed. All women infected with HPV may not develop cancer, in many cases body’s immune system eradicates virus before disease progression (1).

![Figure 1: From HPV infection to cervical cancer development (9).](image)

**Epidemiology of human papillomavirus**

HPV is a member of papovaviridae family. It is 55 nm in diameter, which consists of a single molecule of double-stranded DNA and resembles a golf ball when viewed under an electron microscope. There are about 30 different types of HPV that spread from person to person through sexual contact and are responsible for infecting cervix, anus, vagina, penis, and vulva. Of these 30 types, those causing malignant transformation of cervix are types 16, 18, 31 and 45. Type 16 is responsible for infecting half of the cases while other three types 18, 31 and 45 infect additional 30% cases in Europe and United States. Overall HPV is responsible for 99.7% of squamous cell cervical cancer cases worldwide (27).

Most epidemiologic studies indicate that HPV is transmitted through sexual contact (10,11,26), however there are some studies, which show that nonsexual transmission can also occur in some cases. Richard et al concluded in their study that HPV is resistant to heat and desiccation for at least 1 week so it can spread nonsexually through fomites, including undergarments or surgical instruments like surgical gloves (28). Sexual
activity at early age increases the risk of acquisition of HR-HPV. Zana et al showed in their study that the acquisition of HPV infection is higher in women who started sexual activity before the age of 15 compared to those who started their sexual life after the age of 15. In the same study the authors also concluded that women with multiple sexual partners were at greater risk of acquiring HPV infection. Rate of HPV was 17.2% in women who had only one partner and 40.9% in women having multiple partners (29).

Age is also an important risk factor of HPV infection. Sexually active women between 18 to 30 years old are most commonly infected with HPV. The prevalence decreases significantly after the age of 30. However women older than 35 years are commonly diagnosed with cervical cancer. This suggests that cervical cancer has slow progression in women infected at younger age (27).

Although detection of HPV is a critical step and needs to be identified as soon as possible in order to prevent cervical cancer, at the same time it alone may not be sufficient for the development of the disease (12). The risk of development of cervical cancer increases among patients who have immunocompromised status such as patients having HIV-seropositive status always remain at greater risk of developing cervical cancer than those having HIV-seronegative status (30). Cofactors such as smoking and parity (as described formerly) can also play significant role in disease development (7,10,12).

Among women of reproductive age in United States, human papillomavirus (HPV) is the most common sexually transmitted infection. Centers for Disease Control and Prevention estimated that each year about 5.5 million Americans acquire a new genital HPV infection. The reported range of prevalence of HPV infection in women is extensive from 14% to more than 90%, and the annual incidence ranges from 7% to 20%. Numerous factors are identified that may contribute to the noticeable variability seen in HPV prevalence. These factors include different methods to detect HPV, intermittent nature of infection and heterogeneity of the study population (31).

Dunne et al conducted a study to determine the prevalence of HPV infection among 2482 females aged between 14 to 59 years in United States. The authors had shown a statistically significant trend for increase in HPV prevalence with each year increase in age from 14 to 24 years. After 24 years there was a steady decline in prevalence. The overall prevalence of HPV was 26.8% among females between 14 to 59 years. Among 14
to 19 years old females prevalence was 24.5%, among women aged from 20 to 24 years prevalence was 44.8%, among 25 to 29 years old women the prevalence was 27.4%, prevalence among 30 to 39 years old females was 27.5%, among women aged between 40 to 49 years prevalence was 25.2% and 19.6% among women aged between 50 to 59 years. The authors concluded that burden of prevalence of HPV was highest among women aged from 20 to 24 years (32).

**Screening for cervical cancer; detection of HPV**

Cervical cancer screening is considered to be a model for successful cancer prevention, having resulted in approximately 70% decrease in mortality due to cancer of the cervix over the last 50 years in United States (33). Following are the three methods commonly used to screen women for cervical cancer.

- **Cytology: conventional (Pap smear)**
  The main method for detection of human papillomavirus is still Papanicolaou-stained (pap) smear, introduced in 1949 by a pathologist George N. Papanicolaou even before the cause of cervical cancer was known (34). To perform this test, from the transformation zone of the cervix, a sample of cells is taken by using an extended-tip wooden brush; the sample is then spread onto a glass slide and immersed instantly in a solution to preserve the cells. For cytological examination, the slide is sent to a laboratory, where it is examined under a microscope to look for changes in the cells of the cervix (9). It is considered that these changes are usually caused by HPV (27). In developed countries or under best conditions, the sensitivity of conventional cytology can be 84% and in poor conditions it can be as low as 38%. However the specificity is usually over 90% (9).

- **HPV DNA test**
  HPV DNA test is a relatively new screening procedure, which can detect high-risk human papillomavirus DNA in cervical or vaginal smears. From the cells of cervix or vagina; a sample is collected using a small brush, which then is sent to a laboratory where it can be examined. If HPV infection is detected in a sample, it does not mean that a cancer is present; it only suggests that there is an HPV infection, which is very common in women under the age of 35 years and most of these infections resolve naturally. Most studies
report the sensitivity of HPV DNA based test is 85% or more, while the average specificity is around 84%. In females aged 35 or above this test gives better results because a positive test is due to persistent infection in younger age. In this age group the sensitivity and specificity is even higher, 89% and 90% respectively (9).

- Visual Inspection

Visual inspection is performed after applying dilute acetic acid (VIA) on cervical cells. Inspecting cervix without magnification, abnormalities in cervical cells can be recognized. When acetic acid is applied to cervical tissues, abnormal tissues momentarily turn white (acetowhite), which helps to evaluate positive (abnormal) or negative (normal) results. In limited resources, visual inspection can be used as an alternative to cytology because there is no need of laboratory or transport equipment and the assessment can be done instantaneously. VIA has an average sensitivity to detect precancer and cancer of about 77% and specificity of 86% in research settings (9).

American Cancer Society emphasizes that all women should begin screening 3 years after starting sexual activity or before the age of 21, whichever comes first (1,33).

**Prophylactic vaccines against high-risk HPV infection**

Cervarix® and Gardasil™ are licensed in more than 100 countries as prophylactic HPV vaccines. In these countries vaccination programs have been widely implemented in young and adolescent girls (35). Gardasil™ produced by Merc & Co. is a quadrivalent vaccine against HPV types 6, 11, 16 and 18, while Cervarix® produced by GlaxoSmithKline is a bivalent vaccine against HPV types 16 and 18. In clinical trials these vaccines are proven to be effective against HPV, safe and highly immunogenic and have met all expectations (36).
The association between smoking, daily consumption of fruits and vegetables and the risk of HPV infection

Smoking and the risk of human papillomavirus infection

It is now well established that smoking is a risk factor for cervical cancer and the association remains even after controlling for the strong effect of HPV (13). However the relationship between smoking and HPV is inconsistent (37). To investigate the association between smoking and HPV, in 2008 Salvatore et al performed a pooled analysis of 13 IARC studies on HPV prevalence in 11 countries carried out from 1993 to 2005. They found an increased risk of being HPV positive for women who ever-smoked (OR=1.18, CI=1.01-1.39), among current smokers as smoking intensity increased the risk of being HPV positive increased; OR for 15 and > 15 cigarettes per day was 2.01 (95% CI=1.32-3.08) compared with women who never smoked. The authors concluded that women who smoke 15 or more cigarettes per day had 2-fold increased risk of HPV positivity compared with never smokers (37).

In 2009 Xi et al presented their analysis of 1071 ALTS participants infected with HPV16 and/or HPV18 at baseline. The authors concluded that baseline HPV16 and HPV18 DNA load was significantly higher among current smokers but not former smokers compared with nonsmokers (38).

Smoking impairs HPV16 and 18-antibody response resulting in acquisition of HPV infection. Aline et al showed in their study that development of HPV16/18 antibodies was significantly less among smokers than nonsmokers. They concluded that smoking might produce impaired antibody response in high-risk HPV infected women (39).

A significant association between current smoking and incident HPV infection was also seen by Rachel et al in a cohort study on genital HPV infection. The association remains significant even after adjusting for variables related to both infection and smoking (including acquisition of new life partner, oral contraceptives and condom use) (40).
In addition to acquisition, smokers maintain an HPV infection for significantly longer period of time and have lower probability of clearing infection compared with women who never smoked (40). Anna et al in 2002 reported in their study that women who smoked for more than six years were approximately 60% less likely to clear HPV infection compared to women who were non-smokers at that time. They also observed a dose-response relationship with smoking duration (41).

In contrast to the findings described above, some studies found no association between smoking and prevalence of HPV infection. In 2010 Stuart et al reported in their longitudinal study that there was no evidence to conclude that the risk of acquiring any type of HPV infection including HPV16/18 increased with either exposure to smoking or duration of smoking. They also reported that duration of any type of HPV infection was unrelated to smoking (42).

Susanne et al found similar prevalence of HPV infection in current smokers and in never smokers (43). In 2004 Nubia et al also reported no association between smoking and HPV infection in their study (44). Tiffany et al performed a study in 2003 on cigarette smoking and Oncogenic HPV infection. The authors showed a non-significant negative association between HPV DNA positivity and smoking up to more than 10 cigarettes per day (OR=0.3, 95% CI= 0.1-0.8) (45).

A few studies have shown that smoking has a protective effect against human papillomavirus infection including the one by Gloria et al. The authors reported in their study that they had seen a protective effect of smoking against persistent HPV infection. However they were not able to define the protective mechanism whether it was due to confounding or was a biologic effect (46).

**Fruits and vegetables consumption and risk of HPV infection**

Decreased risk of cervical cancer due to high levels of micronutrients from fruits and vegetables in blood circulation is shown in various studies (16). However the exact role is contradictory and mixed (15) because there is another group of scientists who do not agree with the protective effect of these micronutrients. They conclude that the risk does not decrease with increasing vitamins or β-carotene intake (17). There are also contradictions in the results of studies, which examined the association between
micronutrients from vegetables and fruits and the risk of HPV infection. Some authors reported a non-significant association between plasma micronutrients and human papillomavirus infection while the others reported a significant association.

During the last several years, the association between both circulating and dietary micronutrients and the risk of cervical cancer has been examined in various studies. But neither of these studies has examined the role of micronutrients on HPV acquisition and prevalence. Giuliano et al were among those few researchers who reported the association between antioxidant nutrients and HPV infection. For their cohort study they enrolled nine hundred ninety-nine Hispanic women with no previous treatment of cervical cancer and who did not have any other malignancy. The authors reported that they had seen no significant association between circulating levels of micronutrients such as α-carotene, lycopene or ascorbate and the HPV status (47).

Prabhudas et al also observed a non-significant association between plasma micronutrients and human papillomavirus DNA infection status. In their randomized control trial, they found no significant association between mean plasma levels of micronutrients and HPV DNA infection status. The authors concluded that at the end of their nine months study period, no difference was noticed in plasma levels of six micronutrients (β-carotene, α-carotene, lycopene, γ-tocopherol, α-tocopherol and retinol) and HPV virus infection status between the intervention and the placebo group (48).

There are some studies, which seem to be contradictory with the above-described studies in which the authors reported a non-significant association between micronutrients from diet including carotenoids and vitamins and the risk of human papillomavirus infection. In the following a few studies are reported which showed a significant association between plasma micronutrients and the risk of HPV infection. The authors of these studies also reported that in addition to the protective effect, women with higher levels of micronutrients from diet could clear an oncogenic HPV infection more quickly from their body than women with lower levels.

To observe whether dietary and serum concentration of carotenoids and vitamins were associated with HPV persistence, Rebecca et al conducted a prospective cohort study. They showed that there was significant inverse association between vegetables and juice
consumption and the risk of HPV infection and its persistence. The authors mentioned that lycopene (a carotenoid pigment found in fruits and vegetables) had an antioxidant role in the body; these antioxidant properties of lycopene were responsible for the protective effect against human papillomavirus infection. The antioxidant properties may also help to decrease viral load, which in turn resulted in decreased HPV persistence (49).

Rebecca et al in 2003 reported a study in which they measured the association between nutritional factors with the clearance and duration of HPV infection. After adjusting for potential confounders the authors observed three-fold higher probability to clear oncogenic human papillomavirus in women with higher levels of cis-lycopene (50).

A recent paper published by Yurii et al in 2010 examined the role of micronutrients on acquisition and clearance of anal HPV infection in a longitudinal cohort of Hawaiian women. In conclusion the authors reported that women with highest levels of carotenoids in their blood circulation had significant 50% reduced risk of acquiring and clearing anal HPV infection compared with women with lowest levels (51). Although the natural history of cervical malignancy is similar to anal malignancy, (52) however the authors reported that the acquisition rate for cervical HPV infection was lower than the acquisition rate for anal HPV infection (51).
Study Aims and Objectives

The overall aim of this thesis is to increase the understanding of the association between smoking, consumption of daily fruits and vegetables with human papillomavirus infection positivity among women. The study tested the hypothesis whether cigarette smoking and consumption of daily fruits and vegetables are associated with HPV prevalence. The more specific aims are as follows:

1. To analyze the human papillomavirus infection positivity among ever and never smokers.
2. To analyze the human papillomavirus infection positivity among current and never smokers.
3. To estimate the association between consumption of daily fruits and the human papillomavirus infection positivity.
4. To estimate the association between consumption of daily vegetables and the human papillomavirus infection positivity.

Conceptual framework

Research from previous several decades has shown that infection with HPV is an essential cause of cervical cancer (9,10,11,26). The reason made for this association is that worldwide HPV-DNA is identified in almost all cervical cancer biopsy specimens (26,47). Although the risk of cervical cancer is significantly higher among women infected with HPV, the infection alone may be insufficient to cause disease (47). There are other cofactors such as smoking and antioxidant micronutrients from several fruits and vegetables, which may modify the progression of HPV infection towards cervical cancer (37,39,40,48,49).

Every woman has a different degree of immune response, which plays a key role against certain types of infections. Studies have shown that smoking and antioxidant micronutrients can cause changes in local or systematic immune system modifying the chances of acquiring HPV infection (39,48). The association between smoking, daily consumption of fruits and vegetables with HPV infection positivity is studied in this thesis.
Current understanding on the association between smoking and incidence or prevalence of HPV infection is not clear because previous studies on the association have reported inconsistent and mixed results (37). There are some studies describing a significant association between smoking and HPV infection, its persistence and clearance (37,40,41). But there is also another group of scientists who believe that there is no association between these two variables (42,43,44,45), they argue that if there was any association, which they found in their analysis, it disappeared after adjusting for potential confounders (42). There are even some scientists reporting that smoking is protective against persistent HPV infection (46).

To find out the exact relationship, there is need of further studies describing the effect of smoking on HPV infection. Cross sectional studies can be helpful to describe whether there is any relationship between these variables or not, however the effect of smoking on human papillomavirus can only be measured in longitudinal studies with specific focus on the effect of smoking on HPV acquisition. There seem to be a limited number of such studies (39,42). However there are many studies on the prevalence or incidence of HPV infection (37,40), in which the authors have seen the association between smoking and prevalence or incidence of HPV infection. Regarding the clearance and persistence of the infection there are also some longitudinal studies (40,42), but the results of these studies are mixed, so the role of smoking on HPV infection acquisition, its persistence or its clearance is still not clear and needs to be evaluated in further research.

Women with weakened immune system are at higher risk of getting the infection. A study by Aline et al (39) showed that smoking impairs HPV antibody response resulting in acquisition of HPV infection. Most studies that showed a relationship between smoking and HPV detection concluded that women who ever smoked or those women who were current smokers were at higher risk of getting HPV infection compared with women who had never smoked during their life (37,38).

Several studies have revealed that smoking behavior of women can be associated with their age, while age of a woman might also be related with acquisition of HPV infection as shown by Aline et al in their prospective cohort study (39). The authors concluded that in young females who smoked and also were infected with HPV16 or 18, smoking weakened their immune response increasing the probability to acquire high-risk HPV types than nonsmoker women. In another study Anna et al (41) found a significant
association between women who started to smoke at older age (>13 years) with a reduced probability to clear human papillomavirus infection. Therefore the role of age should be evaluated carefully while studying the association between smoking and HPV infection.

The relation between women’s level of education and the risk of acquiring HPV infection is also discussed in some studies. A similar study is reported by Eduardo et al (53), in which the authors concluded that women with less than 10 years of education were at doubled risk of acquiring HPV infection than women having more than ten years of education. While certain studies have shown that education is also associated with smoking habits of females. Such a study is reported by Giskes et al (54), in which the authors had indicated that cigarette smoking was more prevalent among women educated up to elementary level than women with tertiary level education.

In this study the effect of age and education, which are considered to have a close relationship with both exposure and outcome will also be evaluated while studying the association between smoking and prevalence of HPV infection.

The association between micronutrients from fruits and vegetables in blood and HPV infection among women is also analyzed in several studies. But the exact relationship is still unknown due to mixed results of these studies and there are not enough reports, which describe the effect of micronutrients on acquisition of HPV infection. However there are some studies, which indicate the association between circulating plasma levels of micronutrients with HPV persistence or clearance.

The reports on the association between fruits and vegetables consumption with HPV infection have shown that antioxidant micronutrients from fruits and vegetables can result in clearing infection more rapidly. Unfortunately there is lack of studies, which show the association of daily consumption of fruits and vegetables with HPV status. Cross sectional studies can be helpful to contemplate the association between these variables and then longitudinal studies can be used to measure the effect of consumption of daily fruits and vegetables on HPV acquisition.

Some studies indicated that women’s household income could be a risk factor in the association between fruits and vegetables consumption and HPV infection positivity. The
positive association between less household income and acquiring high-risk HPV infection is reported by Jessica et al (55). In their paper the authors reported that women in the lowest category of income were two times more likely to acquire high-risk HPV infection compared with women in the highest category. While lower level of household income can also be associated with consumption of less quantity of fruits and vegetables as shown by Giskes et al (56). The authors concluded in their report that women with lower household income consumed smaller quantities of vegetables and fruits.

The above-described findings suggest that women’s household income might be associated with consumption of daily fruits and vegetables as well as with the acquisition of HPV infection among women. As a result it is crucial to evaluate the role of women’s household income while reporting the association between fruits and vegetables consumption with HPV infection. In the present study the effect of women’s household income will also be evaluated while describing the association between consumption of daily fruits and vegetables with HPV prevalence.

![Conceptual framework](image)

**Figure 2:** Conceptual framework.
2. Methodology

Secondary data from the National Cancer Institute’s (NCI) Health Information National Trends Survey (HINTS) were used in this study. HINTS collects nationwide descriptive data about United States population’s use of cancer related information. The data are free for public-use and is available on HINTS’s website (57).

The purpose of HINTS is to provide information on altering disease patterns and to recognize health communication trends and practices. It also provides information about how people perceive cancer risks; and offers a platform to researchers to study new concepts in health communication. Collection of HINTS data is done every 2 to 3 years. The data set used for this study is the third round of HINTS data collection series, and is also known as HINTS 2007. The first round of this series started in 2003 and was done by using random digit dialing (RDD) telephone numbers, followed by the second cycle in 2005, also carried out by using RDD method, but for HINTS 2005 the overall response rate was lower than expected.

Data collection in HINTS 2007

This cross-sectional survey of US adults was conducted from January to April 2008. In an effort to increase response rates, HINTS 2007 data were collected using dual-frame design. The first frame was random digit dialing (RDD) by using state-of-the-art computer assisted telephone interview (CATI) techniques to increase the response rate. By using CATI, household screener was directed over the telephone. The purpose of this screening was to select a suitable person for the interview. Interviews were conducted in either English or in Spanish. The second frame was comparatively comprehensive; participants were recruited from a national listing of available addresses from United States postal service (USPS). These households were administered a pencil-and-paper based mailed survey.

This dual-frame design has some advantages over RDD alone, for example the USPS frame also covers individuals without a telephone thus increasing the possibility to reach those group of people who couldn’t be reached through RDD, resulting in increased response rate.
**Pilot Studies**

Before the full field study, pilot studies of both RDD and mail methodologies were conducted. These pilot studies were conducted to improve the methods in an effort to attain best possible data quality and to maximize the response rate. RDD pilot study was conducted from 24th September to 15th October 2007. The sample size for RDD pilot study was 1000 individuals. Mail pilot study was conducted from 23rd August to 15th October 2007 with sample size of 640 households. The purpose of these pilot studies was to test the operations and procedures for the full field study. The pilots also tested the impact of study procedures and materials on respondent’s understanding and cooperation rates.

**Advance Letters and Confidentiality**

After pilot study, selected households were sent advance letters describing the purpose of study, some facts and statistics about health seeking behavior and how they had been chosen for the survey. The participants were also told the maximum time for the completion of survey and why their participation is important for scientific purposes and for the health information, which people might need. At the end, individuals were assured that their answers would be kept confidential and were also given the right to refuse to participate in the study.

**Sample Size**

The HINTS 2007 recruited a total sample of 7,674 adults either through mail or through telephone by using random digit dialing (RDD). 3,582 respondents completed the questionnaire through mail; a pencil-and-paper study design, while 4,092 respondents completed survey by RDD mode. For mail mode, the response rate was 40% for homes that sent back at least one complete survey. For adults inside every household who completed a survey, the response rate was 77%. The overall response rate for mail mode was 31%. For RDD mode, response rates were 42.4% and 57.2% for RDD screener and for RDD interview respectively. The overall response rate for RDD mode was 24.2%.
From the total of 7,674, there were 2,969 (38.7%) male respondents; while female participants were 4,696 (61.2%) and 9 (0.1%) were classified as unknown or having missing information on gender. To provide stable estimates on race or ethnicity, Hispanics and African Americans were oversampled. Overall, the whole sample under represents minorities, male, young individuals and those with low education. Further details on the HINTS data collection methods and study design are described elsewhere (57).

**Definition of Applied Variables of Interest**

**Dependent/Outcome Variable**

- Human Papillomavirus Infection
  To evaluate if a women acquired HPV infection during her life or not, questions regarding HPV knowledge and acquisition were asked. All female participants who had heard about HPV infection were further asked; “Have you ever been told by a health care provider that you had a human papillomavirus or HPV infection?” participants were directed to answer either “Yes” or “No” to this question.

**Independent/Explanatory Variables**

- Smoking
  Smoking behavior of the respondents was assessed using a sequence of questions. Participants were first asked “Have you smoked at least 100 cigarettes in your entire life?” women who answered “Yes” to this question, were then asked second question “How often do you now smoke cigarettes?” participants were asked to choose from “every day”, “some day” and “not at all”. Finally women who specified current smoking were asked to write “On average how many cigarettes do you now smoke a day?”

  Participants were then classified as “never smokers” for those who responded “No” to the first question, as “former smokers” for those who replied “Yes” to the first question but “not at all” to the second question, and as “current smokers” for those who replied “Yes” to the first question, and either “every day” or “some day” to the second question.

- Fruits and Vegetables Consumption
  To measure fruits and vegetables consumption, participants to the mail survey were asked, “About how many cups of fruits (including 100% pure fruit juice) do you eat or
drink each day?” Respondents were provided with 1-cup of fruit could be 1-apple, 1-large banana, or 1-cup (8 oz.) of 100% juice, and “About how many cups of vegetables (including 100% vegetable juice) do you eat or drink each day?” 1-cup of vegetable could be 1-cup of cooked leafy greens, 1-large raw tomato, or 12-baby carrots. Participants were provided with seven categories starting from “none” to “4 or more cups” and asked to choose from one of these categories.

RDD participants were asked open-ended questions: “How many servings of fruits do you usually eat or drink each day? Think of a serving as being about 1-medium piece or ½-cup of fruit, or ¾-cup of fruit juice” and “How many servings of vegetables do you usually eat or drink each day? Think of a serving as being about 1-cup of raw leafy vegetables, 1/2 –cup of other cocked or raw vegetables, or ¾-cup of vegetable juice.

To ensure consistency in units of measurement during data analysis, responses provided to the fruits consumption in RDD survey were converted into cups. The both variables were then merged together to study its association with HPV infection. The similar procedure was done with vegetables consumption variable.

**Other Variables of Interest**

- **Age**
  Each participant was questioned, “What is your age?” The responses were then combined into four levels “18-34 years”, “35-39 years”, “40-44 years”, and “45 or older”.

- **Education**
  All participants were asked, “What is the highest grade or level of schooling you completed?” participants were provided with seven options, starting from “less than 8 years” to “post graduate”. All responses were then collapsed in four categories: “less than high school”, “high school graduate”, “some college” and “college graduates”.

- **Household Income**
  To measure household income of participants, all respondents were asked, “Thinking about members of your family living in this household, what is your combined annual income meaning the total pre-tax income from all sources earned in the past year?”
Responses were classified into five groups: “less than $20,000”, “$20,000 to < $35,000”, $35,000 to < $50,000”, “$50,000 to < 75,000” and “$75,000 or more”.

Exclusion criteria and selection of study participants

For the present study, following exclusion criteria were used to select individuals from HINTS 2007 dataset.

1. All men were excluded from the analysis.
2. To increase statistical power and to avoid biased estimates in results, women with non-responses in any of the variables of interest were excluded from the analysis.

After considering exclusion criteria, a total of 2,891 women were included in this study.

![Flow chart of selecting study participants from HINTS 2007 dataset.](image)

**Figure 3**: Flow chart of selecting study participants from HINTS 2007 dataset.
**Statistical Analysis**

Analysis was performed using STATA (version 10.1). Smoking status of women with and without HPV infection was compared by chi-square. The same test was applied while comparing fruits and vegetables consumption among HPV-positive and HPV-negative women. To estimate the association between smoking and consumption of fruits and vegetables with HPV prevalence, Univariate logistic regression was employed.

Multiple logistic regression analysis was used for the multivariate analysis. To measure the confounding effect of a variable, a step-up model building procedure was used. Variables with significant p-value and 95% CI were taken into account while variables which showed p-value of more than 0.05 and a non-significant confidence interval were dropped from the model. The parameters were tested by likelihood ratio test. Models, which are statistically different from the crude model, were included in the analysis. A variable, which showed 10% difference in crude and adjusted odds ratio was considered to have a confounding effect in the association between exposure and outcome.

Interaction was evaluated with a similar step-up model building procedure. 95% confidence interval and the p-value were used to estimate the statistical significance for the interaction. Variable with non-significant p-value and non-significant confidence interval were dropped from the model. Interaction was considered to be absent for the association between exposure and outcome, if the 95% CI and p-value showed non-significant results for the interaction variable.
3. Results

Descriptive Statistics

The characteristics of the outcome variable (HPV) and its risk factors (smoking, fruit & vegetables) as well as other independent variables of interest (age, education, household income) for all women [N = 2,891] are presented in Table 1. Mean age of women was 50 years (SD = 16) ranging from 18 to 93 years. Of the total participants, about 7.4% women acquired HPV infection. Nearly 44% of the women ever smoked cigarettes during their life. Smoking was more prevalent among younger women compared with women older than 45 years, with the highest prevalence of 21% in women aged 18-34 years. While among women older than 45 years 30% had quit smoking (p < 0.005).

Nearly 7% of women were not eating or drinking any kind of fruit or fruit juice while 3% refused to eat or drink vegetables or vegetable juice. About 85% women consumed fruits from \( \frac{1}{2} \) to 3 cups daily. While 88% women consumed vegetables from \( \frac{1}{2} \) to 3 cups daily. Household income was significantly associated with consumption of fruits and vegetables. Women having annual household income more than $75,000 consumed more cups of fruits and vegetables daily than women having household income less than $20,000 (p < 0.005). Almost 53% of women had income more than $50,000. Less household income was also associated with greater prevalence of smoking. Among women having income less than $20,000, the prevalence of current smokers was 7% higher compared with those having income more than $75,000 (p < 0.005).

Almost 73% women went to college and amongst them approximately 41% graduated. About 6% were never even gone to high school. Data showed (not presented here) that 61% of women who were unable to go to high school had annual income less than $20,000, while 51% women who graduated from college had household income more than $75,000 (p < 0.005). Smoking prevalence was 23% more among women with less than high school education compared with college graduates (p < 0.005).
Table 1: Characteristics of variables of interest

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency [n]</th>
<th>Percentage [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPV Acquisition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>214</td>
<td>7.4</td>
</tr>
<tr>
<td>No</td>
<td>2,677</td>
<td>92.6</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
<tr>
<td><strong>Smoking Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>523</td>
<td>18.09</td>
</tr>
<tr>
<td>Former</td>
<td>740</td>
<td>25.60</td>
</tr>
<tr>
<td>Never</td>
<td>1,628</td>
<td>56.31</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
<tr>
<td><strong>Fruits Consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>206</td>
<td>7.13</td>
</tr>
<tr>
<td>½ cup or less</td>
<td>612</td>
<td>21.17</td>
</tr>
<tr>
<td>¾ to 1 cup</td>
<td>723</td>
<td>25.01</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>792</td>
<td>27.40</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>353</td>
<td>12.21</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>134</td>
<td>4.62</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>71</td>
<td>2.46</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
<tr>
<td><strong>Vegetables Consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>75</td>
<td>2.59</td>
</tr>
<tr>
<td>½ cup or less</td>
<td>516</td>
<td>17.85</td>
</tr>
<tr>
<td>¾ to 1 cup</td>
<td>780</td>
<td>26.98</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>779</td>
<td>26.95</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>468</td>
<td>16.19</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>190</td>
<td>6.57</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>83</td>
<td>2.87</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>166</td>
<td>5.74</td>
</tr>
<tr>
<td>High school graduate</td>
<td>608</td>
<td>21.03</td>
</tr>
<tr>
<td>Some college</td>
<td>942</td>
<td>32.58</td>
</tr>
<tr>
<td>College graduate</td>
<td>1,175</td>
<td>40.65</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-34</td>
<td>556</td>
<td>19.23</td>
</tr>
<tr>
<td>35-39</td>
<td>230</td>
<td>7.96</td>
</tr>
<tr>
<td>40-44</td>
<td>251</td>
<td>8.68</td>
</tr>
<tr>
<td>45+</td>
<td>1,854</td>
<td>64.13</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
<tr>
<td><strong>Household Income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than $20,000</td>
<td>462</td>
<td>15.98</td>
</tr>
<tr>
<td>$20,000 to &lt; $35,000</td>
<td>466</td>
<td>16.12</td>
</tr>
<tr>
<td>$35,000 to &lt; $50,000</td>
<td>425</td>
<td>14.70</td>
</tr>
<tr>
<td>$50,000 to &lt; $75,000</td>
<td>581</td>
<td>20.10</td>
</tr>
<tr>
<td>$75,000 or more</td>
<td>957</td>
<td>33.10</td>
</tr>
<tr>
<td>Total</td>
<td>2,891</td>
<td>100 %</td>
</tr>
</tbody>
</table>
The relationship between women’s smoking status and HPV acquisition is presented in Table 2. A total of 214 (7.4%) women reported of being told by the medical professional about their infection, which they had acquired at any stage of their life. The prevalence of HPV infection was almost 4% higher among current smokers compared with never smokers. About 1% greater prevalence was noticed among former smokers than among women who had not exceeded 100 cigarettes during their lifetime. In women reported as current smokers, the acquisition of infection was 3% higher compared with women who quit smoking.

Table 2: Comparison of HPV by smoking status

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>HPV Acquisition</th>
<th>P-value [Chi² test]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Current</td>
<td>10.5%</td>
<td>89.5%</td>
</tr>
<tr>
<td>Former</td>
<td>7.4%</td>
<td>92.6%</td>
</tr>
<tr>
<td>Never</td>
<td>6.4%</td>
<td>94.6%</td>
</tr>
</tbody>
</table>

*P < 0.05

To further explore the relationship, smoking status was classified into two categories, “ever smokers” for those who were former or current smokers and “never smokers” for non-smokers. The results showed (graph 1) that ever smokers were at significantly higher risk (3% higher) of acquiring HPV infection compared with never smokers (P<0.05).

Graph 1: Prevalence of HPV infection among smokers and non-smokers
The prevalence of human papillomavirus among women with regard to their fruits and vegetables consumption is presented in table 3. HPV prevalence was similar among women who consumed $\frac{1}{2}$ to 1 cup and 1 to 2 cups of fruits. Prevalence increased by 1% when fruit consumption increased by 1 cup in category 5 and 6 who consumed 2 to 3 and 3 to 4 cups. Equal prevalence was seen among women who consumed no fruits and women who consumed 4 or more cups of fruit. Chi² test for this relationship showed a non-significant p-value, which suggested that the relationship between fruits consumption and HPV prevalence was statistically non-significant.

Non-significant p-value of chi² test was also seen when the role of vegetables consumption on HPV prevalence was considered. The results showed that there was no difference in prevalence among women who did not consume vegetables and women who consumed 3 to 4 cups of vegetables. The prevalence was also the same among women who consumed $\frac{1}{2}$ cup or less and $\frac{1}{2}$ to 1 cup. Higher prevalence was noticed among women who consumed 4 or more cups of vegetables than women who consumed less than 4 cups.

Table 3: Prevalence of HPV in relation to fruits and vegetables consumption

<table>
<thead>
<tr>
<th>Fruits Consumption</th>
<th>HPV Acquisition</th>
<th>P-value [Chi² test]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>None</td>
<td>11%</td>
<td>89%</td>
</tr>
<tr>
<td>$\frac{1}{2}$ cup or less</td>
<td>8%</td>
<td>92%</td>
</tr>
<tr>
<td>$\frac{1}{2}$ to 1 cup</td>
<td>7%</td>
<td>93%</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>7%</td>
<td>93%</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>5%</td>
<td>95%</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>6%</td>
<td>94%</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>11%</td>
<td>89%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vegetables Consumption</th>
<th>HPV Acquisition</th>
<th>P-value [Chi² test]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>None</td>
<td>9%</td>
<td>91%</td>
</tr>
<tr>
<td>$\frac{1}{2}$ cup or less</td>
<td>8%</td>
<td>92%</td>
</tr>
<tr>
<td>$\frac{1}{2}$ to 1 cup</td>
<td>8%</td>
<td>92%</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>6%</td>
<td>94%</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>7%</td>
<td>93%</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>9%</td>
<td>91%</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>13%</td>
<td>87%</td>
</tr>
</tbody>
</table>

*P > 0.05
Univariate Analysis

Results of simple logistic regression models for the association between smoking and HPV infection are illustrated in table 4. The table demonstrates the unadjusted effect of smoking status on prevalence of HPV infection.

In the first model, women who ever smoked during their life were matched with women who were never smokers. The results indicated a statistically significant association between ever smokers and prevalence of human papillomavirus infection. The odds of acquiring HPV infection among ever smokers were 40% higher than never smokers. This indicates that women who ever smoked during their life were almost 1.4 times more likely to acquire HPV infection compared to those who did not smoke.

In the second model, current smokers were matched with never smokers. To study this association, the analysis was restricted to women who were current smokers and never smokers. A statistically significant association was seen between women who were current smokers and the prevalence of HPV infection. The odds were 72% higher among current smokers compared with never smokers. These results indicate that prevalence of HPV infection was 1.72 times higher among women who were daily smokers at the time of study compared with never smokers.

Table 4: Association between smoking and human papillomavirus

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>OR Crude *</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never smokers</td>
<td>1.00</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Ever smokers</td>
<td>1.40</td>
<td>0.018</td>
<td>1.05 – 1.85</td>
</tr>
<tr>
<td>Never smokers</td>
<td>1.00</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Current smokers</td>
<td>1.72</td>
<td>0.002</td>
<td>1.22 – 2.42</td>
</tr>
</tbody>
</table>

* The unadjusted odds ratio for the association between smoking and HPV prevalence. OR = odds ratio.

Results from the analysis demonstrated that smoking, whether current or ever was a significant risk factor for HPV infection among women. The results were consistent with those described earlier for ever-smokers versus never-smokers and for current-smokers versus never-smokers.
Table 5 illustrates the association between consumption of daily fruits and vegetables with HPV prevalence. The results indicated that neither fruits nor vegetables consumption was significantly associated with HPV prevalence.

In the first model role of daily consumption of fruits on HPV prevalence was studied. When consumption of fruits was put as a linear variable into the model, the odds ratio for the linear effect of fruits on HPV prevalence was 0.91, which showed that with every serving or with every half-cup increase in fruit consumption, the odds of acquiring HPV infection decreased by 9%. But the p-value and 95% confidence interval showed that the association was statistically non-significant. To further explore this association, consumption of daily fruits was put as a categorical variable into the model.

**Table 5: Association between daily consumption of fruits and Vegetables with HPV prevalence**

<table>
<thead>
<tr>
<th>Fruits Consumption</th>
<th>OR Crude *</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linear effect</strong></td>
<td>0.915</td>
<td>0.091</td>
<td>0.825 – 1.014</td>
</tr>
<tr>
<td>None</td>
<td>1.00</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>½ cup or less</td>
<td>0.71</td>
<td>0.194</td>
<td>0.420 – 1.192</td>
</tr>
<tr>
<td>½ to 1 cup</td>
<td>0.60</td>
<td>0.057</td>
<td>0.359 – 1.014</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>0.59</td>
<td>0.050</td>
<td>0.356 – 0.992</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>0.45</td>
<td>0.014</td>
<td>0.240 – 0.853</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>0.51</td>
<td>0.109</td>
<td>0.220 – 1.170</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>1.01</td>
<td>0.981</td>
<td>0.430 – 2.373</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vegetables Consumption</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linear effect</strong></td>
<td>1.036</td>
<td>0.505</td>
<td>0.934 – 1.148</td>
</tr>
<tr>
<td>None</td>
<td>1.00</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>½ cup or less</td>
<td>0.80</td>
<td>0.593</td>
<td>0.342 – 1.846</td>
</tr>
<tr>
<td>½ to 1 cup</td>
<td>0.82</td>
<td>0.644</td>
<td>0.363 – 1.873</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>0.58</td>
<td>0.203</td>
<td>0.252 – 1.340</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>0.76</td>
<td>0.530</td>
<td>0.324 – 1.785</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>1.02</td>
<td>0.972</td>
<td>0.406 – 2.543</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>1.48</td>
<td>0.441</td>
<td>0.544 – 4.050</td>
</tr>
</tbody>
</table>

* The unadjusted OR for the association between fruits and vegetables consumption with HPV.

All categories of fruit consumption showed non-significant protective effect against HPV infection except category 4 and 6 with 2 to 3 cups and 4 cups or more, which showed
significant protective effect and 1% non-significant increased risk respectively. The results showed that there was no trend between the odds. $P$ for trend was 0.09.

Table 5 also illustrates the crude effect of daily consumption of vegetables on HPV prevalence. The linear effect indicated that when vegetable consumption increased by one serving or half-cup, the odds of acquiring HPV infection was increased by 4%, but the p-value and 95% confidence interval for the association were non-significant which indicated that there was statistically non-significant association between vegetables consumption and HPV prevalence.

The association was further studied by putting the daily consumption of vegetables as categorical variable into the model. The results showed that all categories of vegetables consumption were not associated with HPV prevalence. Odds ratio showed protective effect from ½ cup or less to 2 to 3 cups, but 95% confidence interval and the p-value indicated that the association was statistically non-significant. So there was statistically non-significant association between daily vegetables consumption and HPV prevalence. The p-value for score test for trend of odds was 0.5. The null hypothesis for trend of odds was no trend between the odds.

Results from table 5 demonstrated that fruits and vegetables consumption were non-significant risk factors for the HPV infection among women.
**Multivariate Analysis**

Table 6 shows the multivariate analysis between smoking and prevalence of HPV infection. The results indicated a significantly increased risk of being HPV positive among ever smokers even after controlling for age and education. The crude odds ratio for being HPV positive among ever smokers was 1.40 (95% CI = 1.05 – 1.84). The odds of having HPV infection among ever smokers were 89% higher compared with never smokers after controlling for age and education. The odds were 77% higher among ever smokers than never smokers when controlled for age alone.

The risk of being HPV positive also remained significantly higher among current smokers after controlling for age and education. The risk was almost 100% higher among current smokers compared with never smokers (95% CI = 1.39 – 2.87). The results showed that smoking remained a significant risk factor for HPV infection after controlling for age and education.

*Table 6: Multivariate analysis for the association between smoking and HPV infection*

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>Crude OR [95% CI]</th>
<th>AOR * [95% CI]</th>
<th>AOR ** [95% CI]</th>
<th>AOR *** [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Never smokers</strong></td>
<td>1.00</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td><strong>Ever smokers</strong></td>
<td>1.40 (1.05 – 1.84)</td>
<td>1.77 (1.32 – 2.37)</td>
<td>1.49 (1.12 – 1.97)</td>
<td>1.89 (1.40 – 2.53)</td>
</tr>
<tr>
<td><strong>Never smokers</strong></td>
<td>1.00</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td><strong>Current smokers</strong></td>
<td>1.72 (1.22 – 2.42)</td>
<td>1.76 (1.24 – 2.51)</td>
<td>1.96 (1.37 – 2.78)</td>
<td>2.00 (1.39 – 2.87)</td>
</tr>
</tbody>
</table>

* Adjusted for age.
** Adjusted for education.
*** Adjusted for age and education.

AOR = Adjusted odds ratio.

Results indicated (not presented here) that the effect of ever and current smoking on the prevalence of HPV infection was not different among women with different levels of education. A similar effect was also observed among women of different age groups. These findings suggested that neither age nor the education was an effect modifier in the association between smoking and prevalence of HPV infection.
Table 7 shows the multiple logistic regression analysis between fruits and vegetables consumption and prevalence of HPV infection after adjustment for women’s annual household income. The results demonstrated that there was not significant difference between crude and adjusted odds ratios and the effect of fruits and vegetables consumption on HPV prevalence remained non-significant even after adjustment. Results suggested that neither fruits nor vegetables were significant risk factors for HPV infection even after adjustment for women’s household income.

Results also indicated (data not shown) that the effect of fruits and vegetables consumption on HPV prevalence was similar among women with different levels of household income, which suggested that income was not an effect modifier in the association between fruits and vegetables consumption and HPV infection.

Table 7: Multiple logistic regression analysis between fruits and vegetables consumption and HPV

<table>
<thead>
<tr>
<th>Fruits Consumption</th>
<th>Crude OR [95% CI]</th>
<th>AOR* [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linear effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.00</td>
<td>.</td>
</tr>
<tr>
<td>½ cup or less</td>
<td>0.71 (0.420 – 1.192)</td>
<td>0.70 (0.419 – 1.190)</td>
</tr>
<tr>
<td>½ to 1 cup</td>
<td>0.60 (0.359 – 1.014)</td>
<td>0.58 (0.350 – 1.001)</td>
</tr>
<tr>
<td>1 to 2 cups</td>
<td>0.59 (0.356 – 0.992)</td>
<td>0.57 (0.342 – 0.958)</td>
</tr>
<tr>
<td>2 to 3 cups</td>
<td>0.45 (0.240 – 0.853)</td>
<td>0.43 (0.229 – 0.817)</td>
</tr>
<tr>
<td>3 to 4 cups</td>
<td>0.51 (0.220 – 1.170)</td>
<td>0.48 (0.210 – 1.122)</td>
</tr>
<tr>
<td>4 cups or more</td>
<td>1.01 (0.430 – 2.373)</td>
<td>0.93 (0.396 – 2.202)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vegetables Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linear effect</strong></td>
</tr>
</tbody>
</table>

*Adjusted for women’s annual household income.
4. Discussion

The human papillomavirus is considered to be the most common sexually transmitted infection (39). Among diagnosed cervical cancer patients worldwide, the HPV prevalence of 99.7% shows the causative role of infection towards cancer (27,58). The relationship between cigarette smoking and prevalence or incidence of HPV infection is described in some studies (37,40). There are also some studies, which show the positive association between impaired immune response due to cigarette smoking and acquisition of HPV infection (39). The subsequent decreased immunity due to cigarette smoking can also interfere with clearance of HPV infection (40,41). However it is still not clear whether smoking can increase the risk of cervical cancer by increasing the probability of acquiring or simply by the persistence of HPV infection (37).

The present study revealed that cigarette smoking was positively associated with prevalence of HPV infection among women. Women who ever smoked cigarettes during their life were at significantly increased risk of acquiring HPV infection. The risk was 40% higher among ever smokers compared with never smokers. Additionally the analysis also showed the effect of current smoking on prevalent HPV infection. Women who were current smokers were at 72% significantly increased risk of acquiring HPV infection compared with women who were non-smokers.

Our results are consistent with majority of the published studies, which describe the association between smoking and prevalence of HPV infection. Salvatore et al in 2008 reported a pooled analysis of 13 IARC studies on the association between smoking and HPV prevalence. The authors showed that among women who were current smokers, the risk of being HPV positive was higher than never smokers. They also showed that the risk increased by increasing the number of cigarettes smoked per day. They reported that women who smoke 15 or more cigarettes per day had two fold increased risk of being HPV positive compared with non-smokers (37).

Tobacco smoking is considered to be associated with generalized suppression of immune system, consequently resulting in decreased natural killer (NK) cells and NK cell activities (59). Smoking is also believed to suppress circulating levels of IgG and IgA antibodies as well as Langerhans cells in the cervix (60). Overall these findings suggest
that smoking can adversely affect a person’s defense system, hence impairing its ability to cope with infections when needed. Reduced Langerhans cells in the body cannot produce enough antigens against HPV infection, which may facilitate the acquisition and persistence of HPV infection (41).

In contrast to our findings, some studies reported no relationship between smoking and HPV infection. In 2010 Stuart et al (42) concluded that there is no relationship between smoking and HPV acquisition or HPV duration. The authors initially found an increased risk of HPV acquisition among smokers, which disappeared after adjustment for age of oldest partner and lifetime number of sexual partners. Describing such an association with respect to sexual behavior and smoking status needs careful consideration because smoking can strongly be associated with sexual behavior.

The non-significant relationship between smoking and HPV infection seen by Stuart et al might be due to under reporting of sexual or smoking behavior. The authors stated that under reporting of these two variables might be the reason for the non-significant association seen between smoking and HPV infection (42).

In a similar study in 2008 by Salvatore et al showed a significant relationship between smoking and HPV infection after controlling for lifetime number of sexual partners. The authors found significantly increased risk of being HPV positive among ever-smokers compared with never smokers (OR = 1.18, 95% CI = 1.01 – 1.18) after allowing for lifetime number of sexual partners (37).

Our findings also suggested that the risk of being HPV positive remained higher among women who were current smokers than non-smokers after allowing for age and education. The risk was two times higher among current smokers compared with never smokers after adjusting for age and education of women. The results also indicated a significantly increased risk among women with a history of ever having smoked. The odds of having an HPV infection were 89% higher among ever-smokers than never smokers after controlling for age and education (95% CI = 1.40 – 2.53). Thus cigarette smoking remained a significant risk factor for human papillomavirus among women even after adjusting for the effect of age and education.
The results of our study are also supported by several other studies, which were designed to measure the association between smoking status and the risk of HPV infection. In 2002 Anna et al showed that women who ever smoked in their life maintained HPV infection for significantly longer period of time as compared to non-smoker women. They also described that the probability of clearing an HPV infection is lower among smokers than non-smokers (41).

The association between cigarette smoking, HPV infection and the development of cervical intraepithelial neoplasm has been a subject of controversy for the past several decades due to inconsistent results described by various epidemiologic studies. A likely explanation for this relationship could be that smoking changes the normal epithelial cell proliferation and can induce metaplasia by increasing cell division (45). Several studies on lung cancer showed this mechanism could induce metaplasia in lung epithelium, which leads to carcinoma (61). The deleterious effect of smoking on cervical epithelium has also been discussed in various studies and smoke metabolites and nicotine have been identified in mucus membrane of the cervix (62,63).

Therefore it is suspected that smoking may act on the transformation zone of the cervix by increasing cell turnover and resulting in metaplastic changes in the cervical epithelium (45). Smoking is also believed to enhance the rate of squamous metaplasia in cervical columnar epithelium (45). These evidences clearly show the harmful effect of cigarette smoking, which may facilitate the acquisition of HPV infection or its sustenance, hence working towards disease development.

In the current study we also examined the association between consumption of fruits and vegetables and the prevalence of HPV infection. Our results for this association suggested that neither fruits nor the vegetables consumption was significantly associated with the prevalence of HPV infection. The results demonstrated that fruits and vegetables consumption were non-significant risk factors for HPV infection.

We described the relationship between quantity of fruits and vegetables consumed by a woman and the prevalence of HPV infection in this study. There are several studies, which measured the association between circulating levels of micronutrients obtained from fruits and vegetables (e.g. beta-carotene, lycopene, retinol etc.) and the HPV status. But not enough studies observed the role of consumption of raw fruits and vegetables in
the form of servings or cups on HPV status. Although the level of micronutrients in blood circulation and their effect on HPV status has been reviewed in some studies, however the results are inconsistent and are unable to offer a satisfactory explanation for the role of these nutrients on HPV infection positivity.

The results of this study are consistent with previous studies describing the association between circulating levels of micronutrients and HPV status. Prabhudas et al conducted a nine-month randomized controlled trial and examined the relationship between beta-carotene and 5 other micronutrients in plasma and the HPV status. The authors reported a non-significant association between mean plasma levels of six micronutrients (β-carotene, α-carotene, lycopene, γ-tocopherol, α-tocopherol and retinol) and HPV DNA virus infection status at baseline and after nine months in placebo and beta-carotene supplemented groups. Additionally they also concluded that these micronutrients did not play any role in spontaneous resolution of chronic persistent HPV infection among previously HPV infected women (48).

Giuliano et al also reported a non-significant association between circulating levels of micronutrients and the HPV infection. They enrolled a cohort of ninety-nine Hispanic women for their study and reported a non-significant association between plasma micronutrients (α-carotene, lycopene, ascorbate) and the HPV status (47).

The present study also indicated that fruits and vegetables remained non-significant risk factors for the human papillomavirus infection even after adjusting for women’s annual household income. Not significant difference was seen between crude and adjusted odds ratios and the effect of fruits and vegetables consumption on human papillomavirus infection was similar among women with different levels of household income. The findings of this study indicated that women’s annual household income does not play any significant role in the association between consumption of daily fruits and vegetables and the HPV infection positivity.

In contrast to our findings, there are some studies that reported a significant association between circulating plasma micronutrient levels with HPV persistence and clearance (49,50). Rebecca et al conducted a study in 2003 to determine the association between nutrients and duration and clearance of HPV infection. They reported three-fold higher probability to clear an oncogenic HPV infection among women with highest levels of
lycopene than women with lowest levels (49). In 2007 Marc et al conducted a similar study to examine the association between serum carotenoids, retinol, lycopene and tocopherol with the clearance of HPV infection. They reported that the likelihood of clearing a cervical HPV infection was three to seven times greater among women with higher levels of carotenoids and lycopene than women with lower levels of these micronutrients in their body (50).

The contradiction of our results with studies describing a positive association is ambiguous. However there are some elements, which could explain this inconsistency. One reason behind this contradiction could be non-response values in HPV variable, which might be the reason to induce uncertainty because of not responding to a sensitive question. These could be women with multiple risk factors of HPV infection and/or actually having HPV infection, or these non-respondents might be healthy women free of disease and not at risk of getting a disease.

Another reason, which might cause the estimates towards null value, could be due to failure to convert quantity of daily consumption of fruits and vegetables into number of servings or amount of cups by women. Under reporting of consumption of fruits and vegetables might also lead to biased estimates.

The studies that report a positive association between fruits and vegetables consumption with HPV infection argue that a diet with sufficient quantity of fruits and vegetables is protective against numerous diseases. The main nutrients of fruits and vegetables believed to provide protection against diseases include antioxidants (64). During the initial stages of infection, there is a constant creation of free radicals. These free radicals are thought to be hunted by antioxidants from fruits and vegetables. Therefore antioxidants can result in reducing the destruction caused by free radicals (48).

Like other epidemiological studies, there are limitations to this study, which need to be addressed. First it is important to note that this is a cross-sectional study and cannot assess temporality. So the findings of this study should be properly interpreted as the association between smoking, fruits and vegetables consumption with prevalence of HPV infection rather than being interpreted as causal relationships. Second, information on sexual behavior and number of sexual partners of women is not available in our study. Sexual behavior of women is found to be a strong risk factor for acquisition of a new HPV infection in some studies (40).
In this study we analyzed data which was self-reported by women through mail or telephone to gain information about HPV infection, smoking status and consumption of fruits and vegetables. Most epidemiologic studies use self-reported data in order to reduce the cost and to save time of study. However there is always a concern about self-reported data that it might be biased or inaccurate (65). There are studies available, which indicate that self-reported data can be quite accurate (65,66,67).

Several studies validated self-reports of cancer screening tests with medical records including a study by Lee et al in 2003. The authors compared women’s self-reports of screening for cervical and breast cancer with the medical records and reported that the percentage of overall agreement between self-reported and the recorded information for Pap smear test was 87.2% and for mammography it was 88.4%. The authors concluded that self-reporting for the screening of cervical and breast cancer was quite accurate (65). Another study compared interview reports of participants with their hospital records. The authors found that women who reported themselves, as breast cancer patients were 100% accurate when compared with their hospitalization records (66).

Vartiainen et al examined the validity of women’s self-reported smoking with their plasma cotinine concentrations and found 95% women who reported themselves as regular smokers were confirmed right from their plasma tests. Among never-smoker reporters, 97% did not have detectable level of cotinine in their serum. The authors reported that they had seen very high validity of self-reported smoking behavior (67).

Overall our findings are consistent with the majority of previous studies. The positive association seen between smoking and prevalence of HPV infection has a strong biologic plausibility and is also consistent with available studies on the association between smoking and HPV prevalence. Moreover the non-significant association seen between fruits and vegetables consumption with prevalence of HPV infection is also consistent with a number of published studies. Additionally, the data used in this study were collected from a large nationally representative sample of US adults. Advanced techniques and enhanced data collection methods were used while collecting data in an effort to reduce the probability of getting biased results.
5. Conclusion and Recommendations

At the existing level of knowledge, the injurious effect of smoking on cervical epithelial cells has been proved in various studies. Prevalence of smoking is increasing worldwide particularly in young women (13) making it a major public health problem. Women who start to smoke at younger age are less likely to either develop HPV antibodies or maintain HPV antibody positivity in their body. How smoking influences HPV antibody response is unknown. However the impaired HPV antibody response is responsible for the delay in clearing the persistent infection or acquisition of a new HPV infection (39,41).

The positive association of smoking with HPV infection, which has been seen in this and in several other studies, may seem due to the adverse effects of smoking which is responsible for causing changes in cervical mucus membrane. These kinds of changes have biological plausibility as nicotine and other smoke metabolites have been identified in cervical mucus membrane of smokers (42,62,63).

To date, the role of fruits and vegetables in HPV infection is contradictory. Several studies including the present study report a non-significant relationship; while at the same time various researchers also explain the protective effect of fruits and vegetables against HPV infection. The mechanism of action by which the nutrients from fruits and vegetables provide protection against diseases is very complex and requires sophisticated procedures to elucidate the basic biology, chemistry and other possible mechanisms by which antioxidants might play any role against HPV infection acquisition or persistence. Also, there is need for cohort studies to further evaluate the relationship between fruits and vegetables with HPV infection while controlling for potential confounders.

At present, there are some available studies that describe the effect of cigarette smoking on HPV persistence and clearance. However there are very few published studies explaining the effect of tobacco exposure on acquisition of cervical HPV infection. Similarly the effect of fruits and vegetables on HPV acquisition is not described in detail. Therefore prospective cohort studies with long follow up time are needed to measure the effect of tobacco exposure and consumption of daily fruits and vegetables on acquisition of HPV infection. Attention must be given to other risk factors, which could distort this
association. Since human papillomavirus is a sexually transmitted infection, careful observation on women’s sexual behavior must be given to get unbiased estimates.

There is also a lack of studies describing the effect of ethnicity and physical activity on the risk of HPV infection. The association between these risk factors with human papillomavirus should be examined in larger prospective cohorts especially among women of varying ages and different socio-economic groups.
References


