The Correlation between Lifestyle, Nutrition, Vitamin Deficiency and Human Papillomavirus (HPV) Cervical Changes

by

AnnMarie Breda


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CHAPTER 1: Introduction to the Problem or Issue

Statement of the Problem

Genital Human Papillomavirus (HPV) infections are sexually transmitted infections of increasing public health importance (1), affecting both men and women. The highest rate of infection occurs in women between the ages of 15 and 25.

There are over 80 different strains of HPV and many do not pose any health risks. However, some of the strains of HPV, especially types 16, 18, 31, and 33, can cause cellular changes that may lead to cervical cancer in women.

The Division of Sexually Transmitted Disease (STD) Prevention of the Center for Disease Control (CDC), estimates that approximately 20 million Americans (both men and women) are infected with HPV; 5.5 million new cases are diagnosed annually. Approximately one-fourth (1/4) of these new infections are in teenagers (2).

Studies have consistently shown that HPV is associated with age and directly related to the number of sexual partners one has had. However, studies have also shown that HPV infections have risen in women who have never experienced sexual intercourse (3). It would appear that other additional variables have a role in the disease. The impact of these other risk factors such as demographics, and dietary factors has been less consistent and is poorly understood.
The association between cervical cancer risk and various dietary components has been studied but the research is problematic. Many studies were conducted before HPV testing was refined, and few studies controlled for co-factors such as smoking and oral contraceptive use.

The results of this research project should help increase awareness and understanding of the effects of these risk factors to young women. These results should also compare and contrast variables such as lifestyle, diet and vitamin deficiency on infected and non-infected women.

Inadequate dietary intake is among the causes of vitamin deficiency. Vitamins are required for maintenance of optimal health and prevention of chronic diseases. Multiple deficiencies of vitamins occur more frequently than a vitamin deficiency in a single vitamin. Therefore, this research included the testing of three major antioxidants, namely vitamin C, E, and beta Carotene. In the last few years, increasing number of reports have suggested that the use of antioxidants may act to prevent the development of various malignancies (5).

**Background and History**

Oncogenic Human Papillomavirus (HPV) has been implicated in the pathogenesis of cervical, vulvar, penile and anal cancers. Cervical cancer is the number one
gynecological cancer in the world and most commonly takes 10 to 20 years or more to develop. The primary detector of HPV is the pap smear screen. Other cases are detected by the visual appearance of genital warts. There are two kinds of abnormal tissue caused by HPV: Condyloma (warts) and dysplasia (pre-cancer).

Condyloma (genital warts) are wart-like growths found in the genital area, most commonly on the outside of the vulva (see illustration #3). They are usually painless, but may cause itching, burning, or slight bleeding. Warts can also be found around the urethra and anus. Inside the vagina and on the cervix, warts are usually flat and it is the pap smear that alerts us of their presence. The treatment depends on the size and location of the wart. Treatments include prescription ointments, cryo-therapy (freezing), laser therapy or excision by surgery.

Dysplasia is the presence of abnormal cells on the surface of the skin of the genital organs. Dysplasia is not cancer, but may turn to cancer over a period of years. The only way to tell if dysplasia is present on the skin of the genital organs is by the pap smear. Treatment can get rid of dysplasia so that the tissue cannot turn into cancer. A colposcopy and ECC (endocervical curettage) and/or cervical biopsy (piece of tissue) are performed as standard of care after the pap smear has detected an abnormality. Dysplasia is evaluated through a magnifying device called a colposcope, but only the laboratory results of the ECC and cervical biopsy can give the final diagnosis (see table #4 for treatment protocols).
The pap smear is labeled the best cancer test available. Early detection and treatment of lesser dysplasias are very effective in the prevention of cervical cancer. In the United States, where pap tests are widely available, about 2.5 million women are diagnosed annually with low-grade cervical abnormalities and each year 15,800 women develop and 4,900 die of cervical cancer.

The Bethesda System is the most commonly used system to describe pap smear results. This system is a categorical method of stratifying results into various grades of severity. The following are the most common categories used to describe abnormal cervical cells:

- **ASCUS (Atypical Squamous Cells of Unknown Significance)**- minor changes of unknown cause.
- **ASGUS (Atypical Squamous Glandular Cells of Uncertain Significance)**- abnormal changes in glandular cells of unknown cause.
- **LGSIL (low grade squamous intraepithelial lesion)**- Infection with Human Papilloma Virus (HPV), which in some instances, can be a risk for cervical cancer.
- **HGSIL (high grade squamous intraepithelial lesion)**- Very atypical cells that may result in cancer.
- **Squamous Cell Carcinoma**- cancer is evident and requires immediate attention.
Statistical studies of nearly 28,000 patients showed the following rates of progression of the disease within 24 months without treatment:

- 68% of ASCUS pap smears regressed to normal
- 47.39% of LGSIL regressed to normal (low grade)
- 35% of HGSIL regressed to normal (high grade)
- 7.13% of ASCUS pap smears progressed to HGSIL
- 20.81% of LGSIL progressed to HGSIL
- 0.25% of ASCUS progressed to invasive cancer
- 0.15% of LGSIL progressed to invasive cancer
- 1.44% of HGSIL progressed to invasive cancer

The American College of Obstetrics and Gynecology indicates that malignant transformation requires other factors in addition to the presence of HPV. It is hypothesized that there may be a specific genetic event involving a suppressor gene or an oncogene or other environmental factors. The importance of cellular immunity in the pathogenesis of HPV infection is suggested from studies of persons with genetic, induced or acquired cell-immune deficiencies.

Although much has been learned about HPV during the last decade, little progress has been made toward the prevention of infection or the education of young women regarding the infection. This lack of progress is largely due to the fact that none of the
available treatments are curative, and a vaccine is not yet available. When the vaccine
does become available, it will not help the women who are already infected.

Other methods of prevention are required in order to lower the number of women with
HPV induced cervical changes, and ultimately affect the reduction of the development of
cervical cancer. If an HPV infection is persistent past the age of 30, there is a greater risk
of developing cervical cancer.

**How HPV works:**

In the normal cell, the p53 protein is a negative regulator of cell growth that functions as
a tumor suppressor protein. It halts cell growth after chromosomal damage and allows
DNA repair enzymes to function. Certain viral proteins, including E6 produced by HPV,
disrupt this normal process. Following E6 binding of p53, p53 is degraded, allowing
unchecked cellular cycling and permitting the accumulation of chromosomal mutations
without DNA repair. In fact, the p53 gene on chromosome 17p is the most commonly
mutated gene in human cancer.

This anti-apoptotic process may be the major cause of chromosomal instability in high-
risk HPV containing cells since the HPV genome encodes DNA sequences for six of
these (E) proteins. The role of HPV infections in the etiology of cervical cancer is related
to its expression of these (E) proteins which bind to p53, preventing it from suppressing
tumor growth.
Etiology of Cervical Cancer:

Although the cause of cervical cancer is unknown, there has been an association of the disease with several types of Human Papillomavirus (HPV), all of which are transmitted sexually. Evidence of HPV is found in nearly 90% of cervical carcinomas. It has also been established that women who smoke and use oral contraception are twice as likely to develop cervical cancer.

The literature suggests that cigarette and cigarette by-products as well as oral contraception, may affect the early evolution of HPV-related lesions, possibly by increasing the rate of cell tumors. Smoking decreases plasma levels of beta carotene, vitamin C and folate and the use of oral contraception decreases beta carotene, ascorbate and red-blood cell folate.

It is likely that the actual cause of the disease is multifactorial and that the presence of the HPV virus induces the development of premalignant cells which, under the influence of oxidative stress, genetic and nutritional factors, will further evolve into malignant and eventually invasive lesions.

Some of these enabling factors may be reversible by the use of appropriate antioxidants. The literature clearly establishes both a protective and a therapeutic advantage in selected antioxidants. Nutrients such as vitamins, C, E, and beta carotene are antioxidants that can protect cells from the damaging effects of reactive oxygen molecules by quenching
these reactive oxygen molecules and enhancing immune function. The biological antioxidant defense system provides protection against carcinogens and is an integrated array of enzymes, antioxidants and free radical scavengers. Therefore, adequate levels of these antioxidants are required for the prevention of cell damage and the enhancement of immune function (20).

Researchers have suspected that socioeconomic status relates to quality and type of food intake. Lack of education and motivation often results in poor food choices and ultimately, health consequences.

High intake of fruits and vegetables, including vegetable soups and juices effectively increases plasma levels of important antioxidant nutrients (carotenoids, vitamins C & E) and have been associated with a relatively low incidence of such diseases as cardiovascular disease, cataracts and cancers. Exposure to a high fruit-and-vegetable diet increases antioxidant concentrates in blood and body tissue and potentially protects against oxidative damage to cells and tissue, thus providing protection from persistence and progression of diseases (23).

Since morphology itself does not predict which lesion will progress or regress, further efforts to seek factors other than morphology to determine the prognosis seems to be essential.
**Research Questions:**

Could dietary modifications such as the increase of fruits and vegetables (dark green and yellow), reduced consumption of junk foods (foods low in micronutrients and high in fat and calories), and the avoidance of excessive alcohol, lower the number of women with HPV induced cervical changes and ultimately reduce the risk of the development of cervical carcinoma?

Are there additional variables such as vitamin deficiency, smoking, drug use and other lifestyle behaviors, such as age at first coitus that may contribute to poorly defined immunologic factors that are the major determinants of viral outcome?

Do young women who live outside the jurisdiction of their parents, eat less healthy, indulge in more high risk behaviors, and put themselves at higher risk for disease because of these poorly defined immunologic factors?

Would supplementation of antioxidants to young women, in spite of these risk factors, provide protection from the HPV virus and ultimately reduce the risk of cervical cancer?
**Hypothesis:**

To determine whether a correlation exists between abnormal pap smears associated with HPV cervical changes and lifestyle, nutrition, and vitamin deficiency.

**The Significance of the Study:**

The young cervix undergoes developmental changes from the time of menarche to young adulthood. Teenage girls are at higher risk for acquiring HPV due to biologic vulnerability of the adolescent cervix. Mucosal immunity is an important component of a woman’s defense mechanism against bacterial and viral pathogens such as HPV.

Differences in the adolescent and adult cervix (see illustration #5), leaves young women susceptible to infection as the adolescent cervix is undergoing increased cell turnover and transformation from columnar to squamous epithelium. This transformation period enhances the ability of HPV to affect DNA synthesis and thus, cell replication of damaged cells and carcinogenesis occurs.

Although young women look and act healthy, the newly diagnosed Human Papillomavirus (HPV) cases in young women are at epidemic proportions. A study published in *Pediatrics* (1998) indicates that 15 to 19 year olds had a rate of SIL (squamous intraepithelial lesion) second only to 20 to 24 year olds, and 10 to 14 year olds had a higher rate of SIL than women over the age of 30.
Since both sexually active and virgin women are diagnosed with the virus, it is imperative to look at any and all factors such as lifestyle, nutrition, and the possibility of a vitamin deficiency or lower levels of important antioxidants (due to poor diet), which may contribute to reasons that this virus is so prevalent in young women.

Millions of marketing dollars are spent each year on the promotion of the use of condoms, yet young women continue to have unprotected sex; 144 out of the 164 participants (87.8%) admitted to having unprotected sex, increasing their risk of both disease and unwanted pregnancy. There is a lack of knowledge regarding the high prevalence and serious complications associated with HPV infection, and therefore, any new information that would aid in the protection of women’s health is essential.

It is believed that an attempt to assess and analyze all variables, nutrition, vitamin deficiency, behavioral risk factors and lifestyle is likely to result in information that could improve the overall health of young women, and therefore, impact the number of new cases of HPV. The information of “new” known risk factors would likely result in a decrease in the number of cases and in the transformation of cases to cervical cancer. Also, information regarding the overall population of young women with decreased levels of antioxidants could be acted upon early on in order to protect women from virus and ultimately DNA damage.
Summary:

Young women infected with HPV have contracted a virus that may lead to a fatal cancer in their future. It is imperative to investigate the possibilities that may have led to this epidemic.

Nutrients such as beta carotene, vitamin C and vitamin E are antioxidants that protect cells and enhance immune function. Poor eating habits put young women at risk for disease by creating a deficiency of these important nutrients that affect immune function and the inability to repair DNA damage in the future.

Infected women require closer surveillance for cytological abnormalities than women in the general population. Infection, combined with poor nutrition, high risk behaviors (early unprotected sexual activity which increases the number of sexual partners one has) and other risk factors such as drug use, smoking and the use of oral contraceptives, further increases the risk of future cancers.

The goal of parents, family physicians and school curriculums should be to educate children and teen-agers increasing their knowledge and awareness of HPV and the many complications associated with this infection prior to their first sexual experience. New research is likely to result in information that will improve the number of new cases and preserve the health of our young women.
CHAPTER 2: Review of Related Literature and Research

Introduction:

There have been many studies related to HPV and cervical cancer. These studies vary by population, ethnic group, category of abnormal pap smears and the progression to cervical cancer. Other studies have associated disease entities, diet and vitamin levels found in different populations. Many of these studies have been unable to support their hypothesis and so there is not enough clinical evidence to make radical statements and changes.

Although the American Academy of Pediatrics feels that most children receive adequate vitamins through their diet and that a daily supplement is not harmful but probably not necessary, it seemed essential to investigate the probability of vitamin deficiency or vitamin inadequacy in young women; and the relationship between immune function and exposure to the HPV virus.

A stronger educational campaign for schoolgirls is needed, beginning with the family physician, who does not adequately speak about prevention of diseases to parents or the young teenager. School curriculums avoid these issues, and therefore, little or nothing is being done at the elementary or middle school level in order to increase knowledge of the affects of risk behaviors on overall health. Everyone from family physicians to school educators prance very lightly around the subject of sexual activity, and therefore HPV has
escalated to epidemic proportions. An increased understanding of the consequences of today’s behavior on girl’s future health seems to be paramount.

**Literature Review:**

Drs. Oster and Melnikow are among those who have written and reviewed the history of cervical squamous epithelial lesions and their regression, persistence and progression. The approximate likelihood of regression of CIN I is 60%, persistence 30%, progression to CIN 3,10% and progression to invasion 1%. The corresponding approximations for CIN 2 are 40%, 40%, 20% and 5%. The likelihood of CIN 3 regressing is 33% and progressing to invasion greater than 12%. (Oster, AG., Department of Anatomic Pathology and Cytology, Royal Women’s Hospital, Melbourne, Australia, Int J Gynecol Pathol. 1993 Apr; 12 (2):186-92)

Another study of 27,929 patients reported the following rate of progression without treatment to high grade SIL at 24 months: ASCUS 7.13%, low grade SIL, 20.81%. The following rates of invasive cancer at 24 months were found: ASCUS, 0.25%. Low Grade SIL, 0.15% and High Grade SIL, 1.44%. The following rates of regression were found: ASCII, 68.19%, Low Grade SIL, 47.39% and High Grade SIL, 35.03%. (Melnikow J, Nuovo J., Willan AR. Chan BK, Howell LP., Obst Gynecol. 1998 Oct; 92 (4 pt 2): 727-35)

Based on key studies on high-grade squamous intraepithelial lesions and cervical cancer conducted among HPV-positive women, it was concluded that high parity, smoking and
long term oral contraception use are cofactors that may modulate the risk of progression from HPV infection to high grade squamous intraepithelial lesions and cervical cancer. (Castellsague X, Munoz N., J Natl Cancer Inst Monogr. 2003;(31):20-8)

Since smoking was associated with both CIN 1 and CIN2, cigarette by-products may affect the early evolution of HPV related lesions, possibly by increasing the rate of cell turnover. (Harris TG., Kulasingam SL., Kineat NB., Agoff SN., Feng Q., Kontsky LA., American Journal of Epidemiology.2004 May1; 159(9): 834-42

Epidemiologic nutritional studies suggest that higher dietary consumption and levels of certain micronutrients may be protective against cervical cancer. Low levels of essential antioxidants in the circulation have been found to be associated with an increased risk of cancer. It is possible that the HPV infection induces an oxidative stress in cervical cells, which then, in association with other factors, proceeds in the development of premalignant cell, including cervical intraepithelial neoplasia (CIN) and thence to malignant dedifferentiation. Antioxidants are theorized to reverse this process by a variety of mechanisms. (Keegan L., Keegan G., Cervical Cancer and Antioxidants, Alternative Therapies in Women’s Health. Vol. 6; Aug 2004:57-64)

A study of Mackerras et al used daily oral administration of 30mg beta-carotene and/or 500 mg vitamin C to 141 women with minor squamous atypia or CIN 1 identified with colposcopy of histology. Over more than two years of follow-up, 43 lesions regressed to normal and 13 progressed to CIN 11. The regression rate was not significantly different
among groups. This study shows that neither beta-carotene nor vitamin C, even at high
doses, promotes regression or progression of minor atypia and CIN 1. (Mackerras D., et
al. Randomized double-blind trial of beta-carotene and Vitamin C in women with minor

A multicenter study in the Netherlands randomized 333 women with CIN 1, 11 or 111 to
placebo or beta-carotene (10mg for 3 months), after which Pap tests, colposcopies and
biopsies were obtained. HPV analysis was not done. Groups were comparable for
cervical cancer risks, but the placebo group had a slightly higher dietary intake of beta-
carotene. Eighty-three percent of the participants completed the study. By three months,
32% of both groups’ lesions had regressed to normal. Differences in CIN regression or
progression showed no association with treatment or by beta-carotene intake. (de Vet HC,
et al. The effect of beta-carotene and the regression or progression of cervical dysplasia:

Another study recruited 98 women with moderate dyplasia (CIN 11), randomized them to
beta-carotene 30mg or placebo daily for nine months, and did re-checks at 1.5, 3, 6 and 9
month by cytology, colposcopy, plasma carotenoids, and tests for HPV infection. A
colposcopic biopsy was done at the final visit. Unfortunately, at the baseline visit, 70%
of the placebo group had a lesion <CIN 11 compared with only 35% of the intervention
group; 74.5% of the participants completed the trial. The intervention group achieved
beta-carotene levels four times higher than the controls, although 25% of the controls
took a multivitamin or beta-carotene. Among the controls, more than 60% with initial
lesions <CIN 11 regressed, and more control than intervention subjects (46% vs. 23.1%) had complete regression to normal. Also, CIN regression was not related to serum beta-carotene levels. (Romney SL, et al. Effects of beta-carotene and other factors on the outcome of cervical dysplasia and human papillomavirus infection. Gynecologic Oncology. 1997;65:483-492)

A study of Southwestern American Indian women, a group with high rates of cervical pre-invasive lesions, proved to be at increased risk for CIN 1 and CIN 11/111, when their serum micronutrients were lowest. (Yeo AS, Schiff MA, Montaya G, Masuk M, van Asselt-King L, Becker TM, Nut Cancer: 2000; 38(2):141-50)

An Australian study monitored the dietary habits of 59 healthy, middle-aged men and women to assess the effect of supplementation with a natural phytonutrient preparation from fruits and vegetables on plasma levels of various antioxidant micronutrients and oxidative stress. Results found significant increases in blood nutrient levels after active supplementation for beta-carotene, vitamin C, vitamin E, selenium and folate. Ranges measured after supplementation often fell into those associated with a reduced risk for disease. The researchers concluded that supplementation with mixed fruits and vegetable juice concentrates increased plasma levels of important antioxidant nutrients. (Kiefer I, et al. Supplementation with mixed fruit and vegetable juice concentrates increased serum antioxidants and folate in healthy adults. Journal American College of Nutritionists 2004;91:1005-1011)
Another study was performed to evaluate the vitamin E status in Taiwan using biochemical markers on 1728 males and 1886 females. The study concluded that the prevalence rate of vitamin E deficiency in Taiwan was low as their diet is rich in fresh fruits and fresh juices. (Kang MJ, Lin YC, Yeh WH, Pan WH., Eur J. Nutr: 2004 Apr; 43(2):86-92. Epub 2004 Jan 06)

Studies also revealed that a high level of knowledge of sexually transmitted disease and their prevention failed to induce appropriate behavior among sexually active schoolgirls. A study of 603 female university students in Washington State between 1990-2000 revealed that smoking, oral contraceptive use and report of a new male partner, one known <8months before sex occurred or one reporting other partners were predictive of infection. Always using male condoms with a new partner was not necessarily protective. Infection in virgins was rare, but any type of non-penetrative sexual contact was associated with increased risk and a plausible route of transmission in virgins. (Winer RL, Lee SK. Hughes JP., Adam De, Kiviat NB, Koutsly LA. Am J Epidemiol 2003; 157(3):218-26)

A study was conducted in Vermont for middle and high school educators (teachers and nurses) to survey the type of information taught to adolescents about HPV and address the specific needs for effective HPV education. The survey addressed knowledge level, behaviors, attitude, enabling factors, motivators and barriers. The results showed that <60% of respondents gave correct answers. It was concluded that a high school curriculum was needed but that educators lacked both basic knowledge and resources for

A study of North Carolina college students surveyed students’ knowledge of Human Papillomavirus and measured the effectiveness of a brief educational intervention. Since past STD programs focused on HIV knowledge and transmission had been effective, a similar model was established for HPV. After 3 months, this focused HPV educational intervention which could be readily implemented in the family physician’s office at a routine visit, was found to be effective at improving HPV knowledge, indicating that more HPV education is needed for young adults. (Lambert EC., Journal Family Board of Family Pract.2001, May-June;14(3):178-183.  Additional comments in same journal, Sept-Oct;14(5):401)

A study to identify sexual behavior in Spanish adolescents (both male and female) 15 and 16 years of age indicated that 22.2% had had 2 sexual partners, 8.9% had had 3 partners and 40% had had 4 or more partners. Condoms were used in 83.3% of relationships with penetration. Since this study population was sexually active and therefore susceptible to sexually transmitted diseases and unwanted pregnancies, health education on the use of condoms needs to be encouraged.(Mesa Gallardo MI., Barella Balboa JL., Cabera Manzorro M., Aten Primaria.2004Apr30; 33(7): 374-80)
**Summary:**

The literature states that behavior, number of sexual partners, and the effects of smoking play important roles in the transmission of the HPV virus and the progression of the disease. It is also unclear whether women who become HPV negative actually clear the virus from the body. This information could be extremely useful when educating young women on the subject of sexual behavior, prevention of STDs, smoking, proper eating, HPV and its association to cervical cancer. Since the age of first coitus is reported to occur at age 15 for 18.6% of girls and at age 13 for 3%, proper diet and supplementation with antioxidants may improve the overall rate of infection, as the immune system plays an imminent role in the protective effect of miscoding and DNA damage.

Teenagers may already be infected by the time they have reached the suggested age of first Pap smear screen which is approximately 3 years after first sexual intercourse or by age 21, whichever comes first (see table #7). A two year prospective study of sexually transmitted disease in 98 healthy 16 year old schoolgirls showed human papilloma (HPV) infection to have spread rapidly among sexually active girls. A high level of knowledge of STDs and their prevention failed to induce appropriate behavior among the sexually active. (Lakartidningen. 1997 Feb 19;94(8);619-21). Therefore additional educational programs to pass on knowledge of known risk factors, possible new risk factors, and behavior modifications would aid in the prevention and spread of disease.
A HPV vaccine that prevents persistent HPV will dramatically reduce the 400,000 annual cervical cancer related cases worldwide, 13,000 of which occur in the United States alone. The global toll is much higher because women in developing countries cannot afford the cost of a pap smear. While the vaccine has years of testing ahead to prove its efficacy, the yet approved vaccine is estimated to be 75 percent effective per Stanford University researchers, and could be used to vaccinate all girls at age 12. Males could be vaccinated as well, to reduce the spread from one partner to another and to prevent some cancers of the anus, penis and scrotum.

A corroborative effort of early HPV educational programs for students and their parents, medical advice and treatment, behavior modifications, dietary changes and supplementation of nutrients may be of some assistance with this epidemic.
CHAPTER 3: Design of the Study

Introduction:

All female gynecologic patients seen in the LH outpatient clinic or private physician practices, between the ages of 18 and 35, with normal or abnormal findings, were eligible for this two-part study. A power analysis was performed prior to the initiation of the study. This analysis showed that one hundred forty-six (146) women were required to demonstrate a difference in response rate from 30% in the control group to 50% or more in the treatment group with a probability (power) of 0.80 or a significance level of 0.05.

Methodology:

Serum collection for antioxidant levels, specifically vitamins E, C, and beta carotene provided the blood result data required for the investigation. Quest Laboratory provided the reference ranges for normal and abnormal blood results. A special research account was established with Quest Laboratory and a discounted processing fee was negotiated. These processing fees were paid for by a research grant from LH Hospital.

Three (3) specific antioxidants, vitamins E, C, and beta carotene were chosen for testing as they are identified as the antioxidants which are associated with the greatest protection against cell and tissue damage.
A specifically designed data collection questionnaire would provide the information to make the correlation between the multi-factorial variables and the infection. This questionnaire was provided to all the women at the time of entry into the study and requested information regarding their lifestyle, behavior and nutritional habits. Each woman gave written informed consent prior to participation.

Patients were selected by age. Recruitment of patients was in an open-ended manner to maximize the number of patients available for data collection. Pap smear results (normal/abnormal) nor socioeconomic status were considered in the recruitment process. However, all females had to have had a pap smear prior to entry into the study. Most subjects were volunteers from the Resident’s out-patient Clinic. Private Ob/Gyn attending physicians made referrals to the study as well. The hospital showed their support by placing an article in the hospital’s newsletter regarding prevention of HPV and requesting volunteers for this research project. The newsletter was distributed by mail to members of the community. However, the hospital’s efforts did not ascribe any outside community volunteers.

Anonymity of the questionnaire was an option, as many of the questions were delicate in nature. The choice to identify herself was with the participant. Most of the participants provided their names and telephone numbers in order to correlate the data and blood serum results. The participants were notified of results by the investigator. Those who opted to submit an anonymous questionnaire telephoned their doctors for the lab results,