AN ABSTRACT OF THE DISSERTATION OF

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Title: Plasma Carotenoids and Retinol and Dietary Intake: Association with In situ and Invasive Cervical Carcinomas in Bangkok, Thailand.

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Chunchuie Chi

There is a strong evidence that human papillomavirus (HPV) is the key causative agent of cervical cancer. However, the majority of women who are exposed to the oncogenic HPV do not develop invasive cervical cancer, suggesting that other factors could play a role in the cause and progression of cervical cancer. Epidemiological studies have suggested carotenoids and retinol as possible risk co-factors in cervical cancer etiology. However, the evidence is not conclusive. The study was design to achieve five objectives: 1) to validate the food frequency questionnaire (FFQ) used in a case-control study in Bangkok, Thailand; 2) to examine the association of individual plasma carotenoids and retinol with the risk of in situ cervical carcinoma; 3) to examine association of individual plasma carotenoids and retinol with the risk of invasive cervical carcinoma; 4) to investigate the association between individual plasma carotenoids and retinol and the risk of progression from in situ carcinoma to invasive disease; 5) to investigate the association between concordant quartiles of the β-carotene as measured

from plasma and dietary intake with risk of invasive cervical cancer. The FFQ measures reflect longer-term usual intake while plasma carotenoid measures reflect recent intake. Included in the study were 50 in situ cases with abnormal Pap smears and pre-admission diagnosis of carcinomas in situ and 103 clinic controls with normal Pap smear test. Also included in the study were 201 invasive cases with histologically confirmed invasive cervical carcinoma and 302 hospital controls admitted to Siriraj Hospital with other unrelated diseases to cervical cancer. Plasma carotenoids and retinol were measured by high performance liquid chromatography (HPLC). Human papillomavirus infection was detected using a standard PCR-based test. Food frequency questionnaire (FFQ) was used to capture the intake of the β-carotene.

Results showed that:

1. After adjusting for age and body mass index (BMI), selected plasma carotenoids were moderately correlated with fruits and vegetable intake as well as nutrient intake.
2. In the age-adjusted and multivariate logistic regression models we found no significant associations between any of the plasma measures (carotenoid and retinol) and either risk of in situ or invasive disease as compared to controls or for in situ carcinomas versus invasive disease, a measure of disease progression.
3. Women high in both measures of exposure (dietary and plasma) for β-carotene were at significantly reduced risk of invasive cervical cancer after confounders were adjusted for in the multivariate logistic regression.
4. Women high on dietary intake and low on plasma for β-carotene were also at the lowest risk of invasive cervical cancer after adjusting for confounders.

Based on the results above, the study suggests that the FFQ used in Bangkok, Thailand is an acceptable tool for long-term dietary intake assessment. However, an additional validation study is warranted using large a sample size and participants that are more representative of the general population in Thailand. Furthermore, this study does not support the possibility of individual carotenoids and retinol as risk factors for in situ and invasive cervical carcinomas as well as the progression of cervical cancer to invasive disease. Lastly, the study suggests that long-term increased consumption of fruits and vegetables may provide some protection in women in Thailand from having invasive cervical cancer. However, additional research is needed to support this finding from our study.

Plasma Carotenoids and Retinol and Dietary Intake: Association with In situ and Invasive Cervical Carcinomas in Bangkok, Thailand

by
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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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CHAPTER 1

INTRODUCTION

In developing countries, death from cervical cancer is one of the greatest health risks a woman faces during her lifetime. Statistics on cervical cancer in these countries is staggering especially in light of the achievement made in providing cervical screening services in developed countries. Each year, approximately 471, 000 cases are newly diagnosed and 233, 000 women die from cervical cancer, most of them in less-developed countries (Parkin, 2000). The impact of cervical cancer on women is far reaching. Not only do women die from it, the morbidity among women is devastating with repercussions on the family and the community. In light of these consequences, there is the need to focus efforts on identifying modifiable risk factors for this disease in developing countries.

Globally, cervical cancer prevention has focused on screening women for abnormal cervical tissues and treating the condition before it advances. Principally, screening efforts have relied on the extensive use of the Pap smear test to detect abnormal cell changes. While the test has achieved enormous success in developed countries, it has failed to produce the same success in developing countries due to the complexity and costliness of the Pap smear programs (Sabu et al., 2004). As a result, in countries with poor

health systems and infrastructure, programs have failed to reach meaningful proportions of women. Before an effective program can be put in place, it is necessary to evaluate other ways that could effectively minimize the etiology of cervical cancer.

Since the revelation that human papillomavirus (HPV) infection is the main causative agent in the etiology of cervical cancer, interest in cervical cancer research has increased (Walboomers et al., 1999). Although there is an agreement among researchers that HPV is very common among women of child-being age, cervical cancer is very rare and that other factors must been involved in the development of the disease (Schiffman et al., 1995; Walboomers et al., 1999; Thomas et al., 2002). Risk factors that have been linked to the disease include smoking, oral contraceptives, number of sexual partners, age at first intercourse, parity, alcohol, and diet.

Recently, the focus of research has been on the role of diet, independently or as a cofactor to HPV, in the etiology of cervical cancer. The most provocative attention has been on antioxidants such as carotenoids because of the revelation of the role of fruits and vegetables in the prevention of cancer including that of the cervix. Carotenoids as antioxidants play a role in the defense and maintenance of DNA. It is also involved in the inhibition of cellular proliferation and radical scavenging mechanisms. With regard to HPV infection, in vitro experimental studies have demonstrated that β-carotene reduces the persistence and the ability of the infection to promote carcinogenesis (Giuliano et al., 1999). In addition, prospective studies have suggested that women with higher carotenoid intake are more likely to clear the virus infection (Giuliano et al., 2003, Yeo et al., 2002). Furthermore, in a number of early observational studies, particularly

those from circulating carotenoids have observed a somewhat consistent inverse association with cervical cancer. Thus, high dietary intake and high circulating carotenoids could decrease a woman's risk for the development and progression of cervical cancer.

Results from studies have consistently reported no association between dietary intake of vitamin A (Marshall et al., 1983; La Veechia et al., 1988; Verreault et al., 1989) or blood retinol levels (Orr et al., 1985; Palan et al., 1988) and risk of cervical cancer. Despite this consistency, retinol was included in this study population because of the vitamin A deficiency in Southeast Asia, particularly Thailand. A large proportion of precursor carotenoids are converted to provitamin or retinol when retinol levels are low. Hence, there is a need for simultaneous assessment of these nutrients.

Several studies have assessed dietary intake of carotenoids in relation to cervical cancer but have reported inconsistent findings. For example, some studies suggested the protective effects of individual carotenoids (Potischman et al., 1991; Goodman et al., 1998), whereas others did not (Palan et al., 1998; Nagata et al.,1999). In addition, only a few investigations have examined possible associations of specific carotenoids other than β-carotene with the risk. Only a limited number of studies with different sample size and study population have investigated the association between carotenoids and retinol plasma concentrations and risk of cervical cancer. To our knowledge, none has looked at the development and progression of the cervical cancer to invasion by using both dietary intake and circulating levels of the carotenoids and retinol in the same study population.

Furthermore, many studies concentrated at one specific stage in the disease process such as in situ or invasive. In this study we looked at two stages of the disease, in situ and invasive in the same study population. To better understand the probability of

the disease progression to invasive stage, the study estimated the odd ratios for in situ and invasive as compared to the control groups and also by directly comparing the two sets of case group, in situ and invasive. So far, the majority of studies linking cervical cancer to carotenoids and retinol have been conducted in developed countries, whereas relatively few studies have been done in developing countries where the disease continues to pose a public health problem. This study was the first to investigate the association between dietary and plasma carotenoids and retinol with the risk and progression of in situ and invasive cervical carcinomas in Bangkok, Thailand.

Objective of the study

The overall objective of the study was to test the association of serum, retinol and dietary intake with risk and progression of in situ and invasive cervical carcinomas. The specific objectives for the study were:

Objective 1

To determine the correlations between the estimated dietary intake of total vitamin A, β-carotene, fruit and vegetable from the food frequency questionnaire with plasma carotenoids and retinol.

Hypothesis: There will be no correlation between plasma carotenoids and retinol and dietary intake as estimated by the food frequency questionnaire.

Objective 2

To determine the association between plasma carotenoids and the risk of *in situ* and invasive cervical cancer.

Hypothesis: There is no significant association between plasma carotenoids and retinol and the risk of in situ cervical carcinoma (A and B in figure 1)

Objective 3

To determine the association between plasma carotenoids and retinol levels and risk of invasive cervical cancer.

Hypothesis: There will be no significant association between plasma carotenoids and retinol and risk of invasive cervical cancer.

Objective 4

To determine the association between plasma carotenoids and retinol levels and the progression of cervical cancer from in situ carcinoma to invasive disease

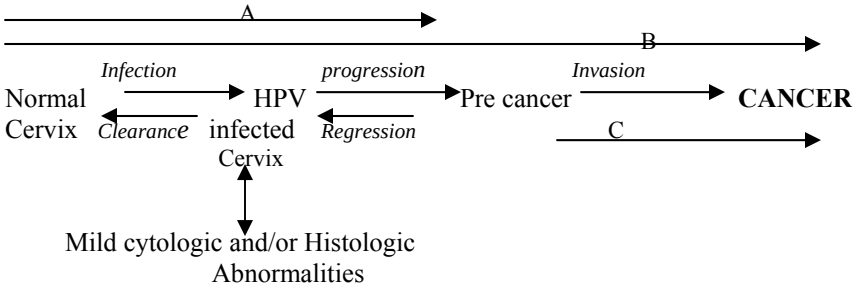
Hypothesis: Carotenoids and retinol are important primarily in cancer promotion and thus there will be no significant association between plasma carotenoids and retinol levels and risk of invasive as compared to *in situ* disease. (C in figure 1)

Objective 5

To determine the association between invasive disease and carotenoids among women in the concurrent quartiles as determined via a food frequency questionnaire and plasma concentrations.

Hypothesis: The association between β-carotene and risk of invasive disease will be more pronounced among women in the concordant quartiles as determined by a food frequency questionnaire.

Figure 1



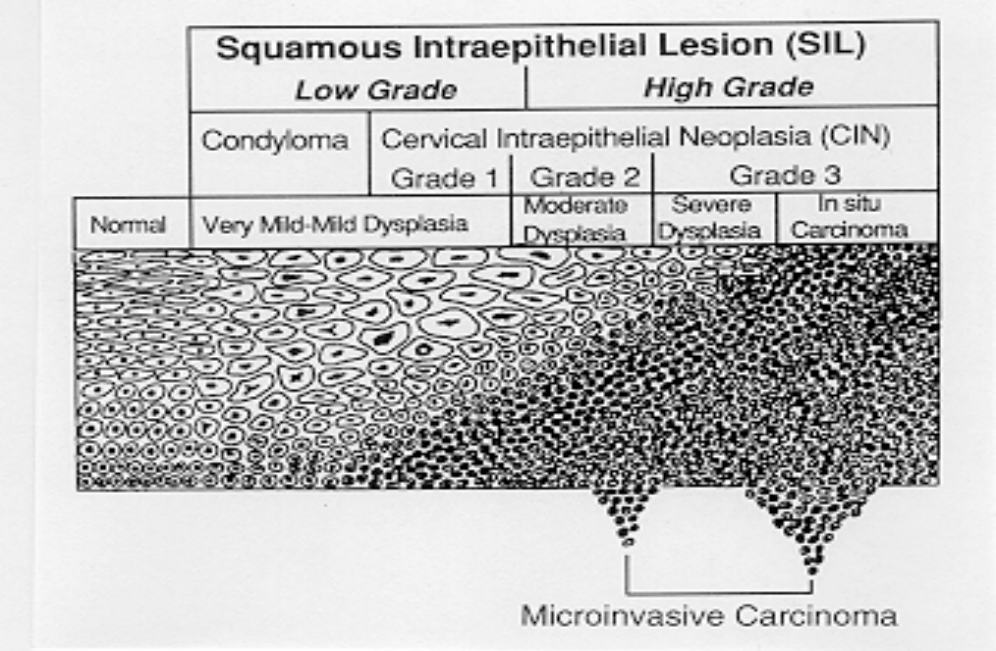
This dissertation is composed of two papers (chapter 2 and chapter 3). The first paper presents results from a comparison study between carotenoids measured by the food frequency questionnaire and serum carotenoids. The second paper examined the association between plasma carotenoids and retinol and risk of in situ cervical carcinomas, invasive cervical carcinomas and progression of cervical cancer to invasive cervical carcinomas. The extensive review of the literature describing the etiology of cervical cancer, risk factors for cervical cancer, functions and mechanism of the carotenoids, and epidemiologic exposure assessment in diet and disease is presented as part of the introduction. This literature background enhances the significance of this study.

LITERATURE REVIEW

1.1 **Pathogenesis of cervical cancer**

Pathogenesis is the description of the stages in the development of a disease. Squamous carcinoma of the cervix is a multistage disease which has a long precursor stage known as cervical intraepithelial neoplasia (CIN), which represents a spectrum of changes limited to the squamous epithelium on the surface of the cervix. CIN is usually categorized into grades CIN 1, CIN II, CIN II on the basis of the whether abnormal cells comprise one third, two thirds or the entire thickness of the epithelium respectively (Franceschi, 2005). Invasive cervical cancer develops from a progression of benign cervical cell abnormalities that change across a continuum of lesions called cervical intraepithelial neoplasia (CIN) I, II, III, and carcinoma in situ, which are earlier stages of the disease. The disease if untreated could become an invasive disease (Franceschi, 2005). Figure 1 shows the stages of cervical cancer. Human papillomavirus infection result in most CIN 1 lesions. There is belief that the majority of cases of CIN 1 will return to normal over a period of 6 to 12 months if left untreated (Moreno et al, 1995). However, over a period of many years, a proportion of CIN 1 and increasingly higher proportions of CIN II and CIN II lesions will progress to invasive cancer. Though timescale is unknown, evidence suggests it could be in the region of 10 years (Stuver et al, 2000). In addition, the likelihood of regression decreases with increasing severity of CIN and there is no way of predicting the clinical outcome of any individual lesion.

Figure 2



(Adopted from Baustein's Pathology of the Female Genital Tract)

1.2 **Risk factors for cervical cancer**

According to cancer etiology studies, cervical cancer has been linked to a number of risk factors such as number of sexual partners and age at first intercourse, smoking, use of oral contraceptive, parity, history of herpes and warts, low education attainment and nutritional status (Munoz et al, 1992a, b; Kyellberg et al, 2000; Thomas et al, 2001; Green et al, 2003; Williams et al, 1994; Chandrika J, 2004; Potischman, 1998).

However, over a decade now, it has been revealed that human papillomavirus (HPV) plays a tremendous role in the development of cervical cancer. In fact, researchers strongly believe that HPV is the causative agent to the development of the disease, and that cervical cancer can not develop in the absence of it. Although infection

of the cervix with HPV is extremely common among women, cervical cancer is relatively rare (Walboomers et al, 1999; Thomas et al, 2002; Castellsague et al.2003). Thus, additional critical determining factors must be involved with the HPV in the development and progression of the cancer (Konno et al, 1998).

Although HPV is clearly a key risk factor for cervical cancer, interest in the possibility that diet plays a role in disease etiology has been growing. Numerous epidemiologic study results suggest that nutritional factors, such as carotenoids, may also play a role in determining risk of cervical cancer (Potischman et al, 1996). Observational studies quite consistently suggest a possible beneficial role for carotenoids and consumption of vegetables and fruits in the lowest risk and progression of cervical cancer (Herrero, 1991; Palan, 1991, Potischman et al, 1991). Carotenoids as antioxidants are involved in inhibition of cellular proliferation and radical scavenging mechanisms.

However, observational epidemiological studies relating to intakes of carotenoids or fruits and vegetables to risk of cervical cancer are somewhat inconsistent and not entirely positive. Contrary, studies of associations based on serum or circulating carotenoids are more consistently supportive. Moreover, there was a significant beneficial association evident for several carotenoids including α-carotene, β-carotene, lutein, and lycopene when these compounds were examined (Wideroff et. al, 1998; Nagata et. al, 1999).

Experimental studies have demonstrated in vitro that β-carotene, a carotenoid which has been extensively studied, reduces the persistence of HPV infection and reduces the ability of HPV to promote carcinogenesis through inhibition of HPV induced transformation of keratinocytes (Giuliano et al. 1999). A human study has also demonstrated that women with higher carotenoid intake are more likely to clear an HPV

infection (Giuliano et al 2003). Case-control studies of carotenoids, in particular, β-carotene, have also shown a somewhat consistent inverse association with cervical cancer risk. Therefore, low intake and circulating carotenoid levels could increase a woman's risk for the development and progression of cervical cancer.

1.3 Carotenoids functions, biological mechanisms and cancer

The natural occurring preformed vitamin A is commonly referred to as retinol, which is the physiological active form of vitamin A. Retinol is found mainly in foods from animal sources. In plant kingdom, green leafy vegetables and yellow fruits and vegetables contain the provitamin A commonly known as the carotenoids some of which can be converted to retinol within the human cells. Beta-carotene, α-carotene, lycopene, β-cryptoxanthin are the predominant carotenoids in the diet (Crews et al 2001; Wildman et al 20000. Beta-carotene is the most active form of provitamin and as such has received tremendous attention in studies. Lutein, zeaxanthin and lycopene which do not have vitamin A activity are also well studied because of their high concentration in serum (Liaaen-Jensen, 2004).

A cell differentiation abnormality is the basis to many malignancies in humans. Retinol and carotenoids have long been known to play a key role in the regulation of cell differentiation. In animal studies, for instance, vitamin A deficiency has lead to the occurrence of induced tumors and metaplasia of the gastrointestinal (Hill et al., 1982; Berger et al., 1991). Metaplasia is believed to be a precursor stage in the malignant degeneration of the normal cell (Stalh et al., 2004). Hence, insufficient dietary levels of vitamin A have been linked to increased susceptibility to carcinogen-induced cancer such as cancer of the stomach (Romppamen et al., 1985).

Despite their shared physiological function with retinol, carotenoids have other unique activities. As antioxidants, carotenoids are capable of quenching singlet oxygen and free radicals that could initiate reactions such as lipid peroxidation (Krinsky et al., 1998). Oxygen species such as singlet oxygen have been implicated in the etiology of a wide array of human diseases such as cancer. Thus, carotenoids as antioxidant play a key role in the protection of the cells against several diseases such as cardiovascular diseases and cancer among others having cancer-prevention activity (Stahl, 2004).

Another possible basis for the protective effect of the carotenoids towards the development of cancer comes from their capability to induce gap junctional communication (Zhang et al, 1992; Zhang et al, 1991). Gap junctions are cell-to-cell channels that allow the flow of low molecule weight compounds, for example nutrients, between connected cells. Gap junctional induction is correlated with growth inhibition of chemically transformed cells, which has been demonstrated by carotenoids (Hossain et al, 1993). Thus the ability of carotenoids to protect this activity may be important in prevention of cancer.

In the interpretation of observational studies, a very important consideration that suggests relation between carotenoids intake and cancer is that these compounds reflect intake of fruits and vegetables which are the major sources of these compounds. However, fruits and vegetables contain other constituents that have several biological activities, thus it is unwarranted to assume the association based on one specific carotenoid. Moreover, it is not clear whether the association between diet and disease is due to the combined effect of several of the active ingredients. It is worthy to know that fruits and vegetables are the main source of carotenoids and that serum carotenoids are generally accepted as markers of recent fruits and vegetable intake (Yeum et al., 1996;

Drewnoski et al., 1997; Polsinell et al., 1998). Tissue carotenoids are believed to be indicators of long-term carotenoid consumption patterns and tissue concentration increase in response to feeding these foods (Parker. 1989).

Another highly relevant issue that needs to be considered when linking cancer and carotenoids intake relates to the quality of data for foods. The quality of data for these compounds, especially for nonprovitamin carotenoids, constrains the ability to estimate collectively the intakes of the carotenoids. There have been some improvements in the carotenoids database over the past few years. In addition, carotenoids actual amount in a given food is influenced by plant maturity, climate and other growing conditions, resulting substantial variability in the carotenoid content of the food as reported in observational studies (Beecher et al., 1991).

1.4 **Metabolism of the Beta-carotene**

Beta-carotene is considered an essential nutrient when the dietary intake of retinol is inadequate. This is especially important in areas where dietary intake of retinol which is mainly from animal sources is inadequate such developing countries. The absorption of the β-carotene takes place in the intestinal mucosa. There are a number of conditions that affects the rate of absorption of the β-carotene such as the amount of lipids, protein an calories in the diet. The major carries of the β-carotene in the fasting state are the low-density lipoproteins (LDLS) and the High-density lipoproteins (HDLS). During absorption, β-carotene should provide two molecules of retinol, however, this does not happen because of the content of beta-carotene in foods varies with the growing conditions and the post-harvest storage of foods. Beta-carotene molecule will provide about 50% of its quantity as vitamin A. The number one pathway for the conversion of

β-carotene to retinal in healthy individuals is oxidative cleavage at the central 15:15' double bond. Two molecules of retinal which can either be reduced to retinol or oxidized to retinoic acid are formed per molecule of β-carotene cleaved.

1.5 **Epidemiologic exposure assessment in diet and disease**

In any epidemiologic study, assessment of the exposure is very critical because accurate results depend on how the information was collected. For that reason, epidemiologic studies such as dietary assessment require techniques that are as accurate as possible at the individual level (Nelson, 1991). In chronic diseases such as heart disease and cancer, relevant exposure is long-term diet consumed many years prior to disease diagnosis because these diseases develop over 10 to more years. Food frequency questionnaire (FFQ) assessment has been the method of choice in assessing usual food and nutrient intake for an extended period of time in such diseases (Willet, 2000). The main advantage of using FFQ is that it can capture individuals usual, long-term diet; either current or in the past. The method is also inexpensive and simple.

Although FFQ is the most commonly used method in dietary assessment exposure, it has been associated with inaccuracy and misclassifications. Since FFQ rely on self-report and interviews, it is associated with potential errors and biases. The complexity of foods that people eat brings some errors in the reporting of the actual foods and nutrients (Wild et al, 2001). Study participants can not recall correctly the foods eaten some time back due most of the times to the complexity of the foods. Hence, there is apparent tendency to overestimate or underestimate dietary intake, which can distort the scale on which differences in intakes of food or nutrients are measured (Kaak, 1997; Black et al., 1993).

Also, food recall practices pose a concern in case-control studies because of individuals who have been diagnosed with a disease, for example cancer, may consistently under or overestimate their dietary intake. Dietary report biases in case-

control studies can inflate or deflate the odd ratios. The problem is compounded when the source of the bias in dietary intake data is also related to the disease outcome of interest (Tarasuk et al., 1997).

Food composition database are mainly used to calculate the nutrient content of each food that has been reported. In food frequency questionnaires for example, foods are weighted by their frequency of use and nutrient content to calculate nutrient intake for each subject in a study. Although food composition databases and tables have been used for decades in epidemiological studies, biases have been associated with these sources of information. Lack of sufficiently detailed information from food composition tables for specific components has been of major concern. These database and tables have normally been created for nutrients and not inevitably for components of interest in studies of diet and health such as antioxidants levels (Wild et al., 2001). For example, for decades β-carotene was the only carotenoid content in the food supply found in the database (Knekt, 1988). The other carotenoids have recently been added into the carotenoids database. Riboli et al, (1996) adds that food composition tables may be inaccurate because the same food item may contain different levels of compounds due to natural variations in growing conditions. Due to the complexity of the food matrix, it is impossible to predict with certainty the health effects of food solely based on the content of one specific nutrient.

Food reporting can also be affected by factors such as culture, age, gender, education and type of food consumed. Moreover, recipes and menus tend to vary across sociocultural groups. Lastly, cooking process can affect the dietary nutrients, which is difficult to assess by questionnaire-based approaches and use of the standard food composition tables (Wild et al., 2001). Therefore, poor dietary or nutrient assessment

questionnaire may obscure diet-disease relationships that would become apparent had it been that more accurate techniques were used (Nelson, 1991).

In order to interpret results with confidence, questionnaires have been validated, which is the ability of a questionnaire to measure what it is intended to measure. Assessing the true validity of an FFQ would require measuring the usual self-selected diet of individuals with high accuracy over an extended period of time, which is not visible. Alternatively, researchers assess relative validity by comparing the FFQ with reference method with its own limitations. To this end, it is important that the reference method used be independent of the original method with regard to measurement errors.

In recent years, biomarkers of nutrients in blood have been used as reference standards for validation of FFQ. However, there are still few studies that have been conducted relating nutrient intake assessed by food frequency questionnaires to biomarker measures of the nutrient status. The chief advantage of using biomarkers in dietary assessment is that the potential errors associated with biomarkers are different from those of the questionnaire measurement of intake (Kaaks, 1997; Cade et al., 2001; Jacques et al., 1993). These methods are dissimilar because FFQ measures intake while biomarker measures circulating concentrations that are influenced not only by intake but by other physiological and environmental factors. Biochemical measures also provide a more proximal measure of nutrient status for disease outcome because it serves as an integrated measure of metabolism of the nutrient of interest (Potischman, 2003). Although biomarkers can provide estimates of dietary intake that is independent of subject's reported intake errors or poor memory of subjects, they are often expensive and nutrient specific, as such may only be used to compare one nutrient at a time. Other

potential problems related to the use of biomarkers come from the absorption and metabolism of nutrients. Estimates from biochemical measures are unlikely to be influenced by dietary intake alone because generally individuals differ to some degree in the absorption and metabolism of most nutrients (Bates et al., 1991). The technical errors associated with laboratory measures may also contribute to the variation in the estimated biochemical levels. biomarkers are subject to sample collection; processing and storage errors which if not corrected may alter the results of sample composition and deterioration of sample quality (Wild et al., 2001) Fluctuation in biochemical indicator levels may also be influenced by the day to day variation in dietary intake. Moreover, differences in bioavailability of nutrients from food to food could cause further weakening of the correlation between dietary intake estimates and biochemical estimates. Therefore, due to errors in both dietary intake estimates and serum measures, combining questionnaire data with serologic measures can provide a powerful tool for estimating the exposure of interest (Potischman et al., 1991)

1.6 **Bangkok Preliminary studies**

A few studies have been conducted to identify risk factors that could be associated with cervical cancer. The first study by Thomas et al. (2001) looked at the risk factors for invasive cervical cancer with human Papillomavirus (HPV). In this study, oncogenic HPV types 16 and 18 were strongly associated with risk of squamous and adenomatous lesions, respectively. In the second study by Thomas et al. (2001) again investigated risk factors for in situ and invasive squamous cervical carcinomas. HPV types 16 and 18 were associated with fourfold increase in the risk of progression to invasive from in situ disease, as determined using a case-case analysis. After HPV was

controlled for, the risk of developing invasive disease was not related to any of a large number of sexual factors or smoking suggesting that other cofactors are involved before the development of in situ carcinoma. In the same study, socioeconomic status indexes, for example, low level of education were associated with a reduced risk of only invasive disease suggesting the existence of unknown factors that operate after the cancer develops.

In another preliminary Bangkok study by Shannon et al. (2002), the possible effects of specific dietary factors on cervical carcinogenesis were evaluated using three analyses 1) in situ cases versus clinic controls 2) invasive cases versus hospital controls and 3) invasive cases versus in situ cases. This type of analysis allowed evaluating the association between dietary intake and risk of in situ cervical cancer, invasive cervical cancer and the progression from in situ to invasive disease within the same group and using the same study protocol. The results of this study revealed that foods high in retinol and total vitamin A were consistently associated with a reduced risk of in situ and invasive cervical cancer. There were no significant associations between high vitamin C foods, high folate foods, high vitamin E foods, high β-carotene foods and risk of either in situ or invasive cervical cancer.

CHAPTER 2

Comparison of the dietary intake Carotenoids with plasma levels: Validation of the Food Frequency Questionnaire used in Bangkok, Thailand.

Abstract

Although culturally sensitive food frequency questionnaires (FFQ) are widely used in epidemiologic studies, little has been done to validate the tool in Thailand using biochemical markers. The purpose of the study was to validate the FFQ used in the cervical cancer case-control study by comparing specific dietary measures as estimated by the FFQ with individual carotenoids and retinol as measured from the plasma. Included in the study were the 50 in situ cases with abnormal Pap smears and pre-admissions of carcinomas in situ and 103 family clinic controls with normal Pap smear tests. For validation, this study specifically used the 103 family clinic controls only. Plasma carotenoids and retinol were analyzed using high performance liquid chromatography (HPLC). Human papillomavirus infection was detected using a standard PCR-based test. The food frequency questionnaire was used to capture the intake for total vitamin A, retinol and β-carotene. After adjusting for age and body mass index, plasma carotenoids were moderately correlated with fruit and vegetable intake as well as selected nutrient intake measures from the FFQ. The study, therefore, reveals an agreement between plasma carotenoids and dietary intake estimated from the FFQ. Therefore, we suggest that the FFQ used in Bangkok, Thailand case-control study can be used to assess long-term dietary intake.

INTRODUCTION

In epidemiologic studies, a food frequency questionnaire (FFQ) has increasingly become a widely used tool in dietary assessment because it is relatively inexpensive and easy to administer (Lee et al., 1996; Gibson, 2005). The primary objective of the instrument is to estimate the usual dietary intake over a long-term period. Over the years, however, this instrument has generated much controversy and discussion due to the inherent imprecision that can profoundly affect the results of epidemiologic studies in nutrition. These imprecisions stem from the misclassification of study subjects, where exposed people are classified as unexposed and unexposed people are classified as exposed. This may distort the true picture of exposure, tending to lead to underestimation of the impact of the exposure. As a result, this bias may even cause sizable odds ratio to appear quite unimpressive (Marshall, 2003). Some common errors associated with the FFQ arise from the assessment of the frequency of food consumption, use of food composition tables and portion sizes of certain foods. Also, the FFQ may be subject to response bias because current diet may influence recall of the dietary intake in the past, particularly for individuals with diet-related diseases (Dweyer et al., 1997; Malila et al., 1998). Thus, the presence of these errors could attenuate the estimate of disease relative risk and obscure the relation between diet and disease (Kipnis et al., 2002, Ocker et al., 1997). Elucidation of the diet-disease relationship requires dietary instruments that could provide accurate estimates of the dietary intake.

Numerous validation studies have been conducted to assess the accuracy of the FFQ by comparing it with another instrument as a reference method (Anderson et al., 2005; Cade et al., 2002) with the assumption that errors of the test method be

independent of the errors of the reference method (Bingham, 2002). However, there is a lack of consensus on the most appropriate standard method for validating the FFQ.

Although in many FFQ validation studies, the 24 hour recall and the multiple diet records as reference methods (Kaaks et al., 2002; Mayer-Davis et al., 1999; Zhao et al., 2002), there is a concern with regard to the use of these methods because of their common source of error (Jasques et al., 1993). This error stems from the report biases from these methods. It is for the above reason that the use of biochemical markers of dietary intake has become popular for validating the FFQ despite its own drawbacks. These drawbacks come from poor absorption and metabolism of nutrients, poor samples collection, processing and storage to technical problems associated with laboratory processes. The advantage of using a biochemical marker is that it gives objective estimates of intake whose errors are independent of the errors associated with dietary intake measurements like the FFQ (Kipnis et al, 2002; Kaaks et al., 2002; Bingham, 2002; Borgers et al., 2004). In addition, plasma carotenoids are a good marker because they are very responsive to intake, hence directly reflect actual intake of these compounds (Yuem et al., 1996; Drewnoski et al., 1997; Polsinelli et al., 1998; Van Kappel et al., 2001; Broekmanns et al., 2000). However, there remain the drawbacks as stated above.

Over the years, culturally sensitive FFQ have been developed and validated for use in different regions of the world (Rodriguez et al., 2002). However, literature reveals that few have been validated in a Southeast Asian region (Sasaki et al., 2000) and no information is available about validity of the FFQ specifically for Thailand. In addition, very few studies have used biochemical markers as a validation tool for the accuracy of the dietary assessment method in Asia (Sasaki et al., 2000; Jacques et al., 1993). Hence,

validating studies are urgently required to evaluate dietary questionnaires for a Thai population using biochemical markers such as serum carotenoids.

As a way of validating the FFQ, the objective of the study was to determine the correlations between the estimated intake of total vitamin A and β-carotene and fruit and vegetables from the FFQ with plasma carotenoid and retinol

2.1 MATERIALS AND METHODS

2.1.1 Study population

In situ cases and controls

Patients who came into the family planning and gynecology clinics associated with Siriraj Hospital with an abnormal Pap smear and pre-admission diagnosis of carcinomas in situ or severe dysplasia were approached to be a part of the study as in situ cases. A woman was eligible to be in the study if she was born in 1930 or later and had lived in Thailand for at least one year. If the woman chose to be a part of the study, she was interviewed through a questionnaire and blood was drawn while in the clinic. Once a case was selected, two clinic controls were selected from the next two women with appointments in the same clinic for a Pap smear follow-up, whose results revealed no suspicion of neoplasia change. Eligible controls were frequency matched to cases by age (± 5 yr) and region of residency. As soon as these controls were selected and agreed to be part of the study, the individuals were interviewed through a questionnaire and blood drawn.

Invasive cases and controls

Women admitted to public wards of Siriraj Hospital with histological confirmed invasive cervical carcinoma were recruited to be a part of the study as invasive cases. A

woman was eligible to be in the study if she was born in 1930 or later and had lived in Thailand for at least a year. If selected, the woman was interviewed through a questionnaire and blood was drawn while in the hospital.

Once a case was selected, two inpatient hospital controls were selected from one otolaryngology and two general surgery wards of Siriraj Hospital. The women selected as controls were the first two women admitted during the previous 24 hours. Eligible controls were frequency matched to cases by age (± 5yr) and region of residency. After the selection, the control women were interviewed and blood drawn.

2.1.2 **Exclusion criteria**

Conditions for exclusion criteria for the controls included: use of steroid contraceptives such as circulatory or cardiovascular diseases, diagnosis with diabetes, chronic renal disease, benign breast disease, a previous diagnosed cancer, chronic liver disease and any other obstetrical. Most hospitalized controls were being treated for cataracts, non-toxic nodular goiter or gallstones.

2.1.3 **General Questionnaire.**

Demographic and reproductive risk factor data from the previous study (Shannon et al, 2002) were collected at the time of enrollment. The data included information on sociodemographic characteristics, sexual and reproductive history, tobacco and alcohol use, Pap smear history. All study participants were interviewed in person on the day they were selected. Clinic or outpatient women were interviewed at the Clinic, while invasive cases and hospital control women were interviewed at Siriraj hospital.

2.1.4 **Food Frequency Questionnaire (FFQ)**

The FFQ data from the previous study by Shannon et al. (2002) was available for the present study. The FFQ was designed specifically to capture β-carotene and fruit and vegetable intake. Briefly, dietary intake information was collected by FFQ with 80-item of foods commonly eaten in Thailand. Respondents were asked to recall how often, on average, they consumed a given amount of each food during the previous year. The average frequency of intake per day, week, or month of each food item was estimated from the food frequency questionnaire. Frequency of consumption for each food item in the FFQ was converted to intake of grams per day. In Thailand, a single dish comprises of many ingredients. In light of this, participants were not asked to estimate portion sizes of individual foods. A Thai Nutritionist, instead, purchased all the food listed on the questionnaire and prepared them in the same way food is prepared in Thailand. The FFQ was designed specifically to capture β-carotene and fruit and vegetable intake. Dietary nutrient content of selected nutrients such as total vitamin A, and β-carotene were calculated by using a Thailand food composition table. This FFQ was administered by interview to all participants by a trained interviewer.

2.1.5 **Plasma carotenoids and retinol extraction**

Non-fasting 15ml blood samples were collected from each woman on the same day as the interview. The samples were centrifuged and stored at -70°C within three hours of blood drawing. Two aliquots were retained in Bangkok and two were shipped to Seattle, Washington, on dry ice for further analysis at a later time. Samples were then transferred from Fred Hutchinson Cancer Research Center to the General Clinic Research Center laboratory, Oregon Health and Science University on dry ice. Upon arrival, the

samples were promptly frozen at -80°C. The protocol for this study was approved by the Institutional Review Board of Fred Hutchinson Cancer Research Center, Oregon Health and Science University and Oregon State University.

All carotenoid analyses were conducted under dim light because of the sensitivity of carotenoids and retinol to light. The laboratory staff did not know whether a sample belonged to a case or a control. Plasma samples were allowed to thaw at room temperature before extraction. Carotenoids were extracted using a non-enzymatic extraction method borrowing elements from Lin et al (2003) and Yuem et al (1998). 20 µl of echinenone (Hoffman-La Roche, Basel, Switzerland) and 20 µl of retinol acetate (Sigma Chemical) (internal standards) were added to 450 ul of plasma in a glass tube and after the addition of 450 µl ethanol vortexed for 5 seconds. The sample was then extracted by the addition of 600 µl of hexane and vortexed vigorously for 60 seconds. After centrifugation at 1000 x g at room temperature for 5 minutes, 400 µl of the upper layer was removed and evaporated to dryness at 40° C under a gentle stream of nitrogen. The residual was redissolved in 150 µl of ethanol and vortexed for 30 seconds.
A 50 µl aliquot was injected onto the column for high-performance liquid chromatography (HPLC) analysis.

2.1.6 **HPLC analysis of plasma carotenoids and retinol**

Although several method are available for the assay of carotenoid levels in serum or plasma, a reverse-phase HPLC has become the most widely used method for the separation of plasma or serum carotenoids and retinol. HPLC is precise at low concentrations (de Pee et al, 2002) and it is specific and easy to use. The HPLC system consisted of a HP series 1050 pump and degasser, a Spectrasystem AS3000 autosampler,

an Agilent 1100 series Diode Array multiple wavelength detector and a C30, 5 μm, 4.6x250mm carotenoid YMC column. The HPLC mobile phase consisted of solvent A) methanol: methyl-*tert*-butyl ether: water (83:15:2, by vol, with 1.5% ammonium acetate in the water), and solvent B methanol: methyl-tert-butyl ether: water (8:90:2, by vol with 1.0% ammonium acetate in the water). At a flow rate of 1 ml/min the gradient procedure consisted of the following: 1) 100% A for 1 min; 2) a 7 min linear gradient to 70% A ;3) 70% A for 5 min; 4) a 9 min linear gradient to 45% A; 5) 45% A for 2 min; 6) a 10 min linear gradient to 5% A; 7) 5 % A for 4 min; 8) a 2 min linear gradient to 100% A; 9) 100% A for 5 min. The relative amounts of plasma carotenoids and retinol were determined by measuring peak areas in the chromatograms at the optimal absorption wavelengths as follows: for retinol and retinyl acetate 325 nm, for lutein and α-carotene 445 nm, for zeaxanthin, β-cryptoxanthin, echinenone, β-carotene 450 nm and for lycopene 472 nm.

Peak areas were quantified and their concentrations calculated based on the external standards α-carotene, lutein, zeaxathin, β-cryptoxanthin, lycopene (Hoffman-La Roche, Basel, Switzerland), β-carotene and retinol (Sigma Chemical). Concentrations were corrected for procedural losses by adjusting the concentrations in proportion to the average of the two internal standards. To monitor assay acceptability a pooled plasma control was included at the beginning and the end of each assay run. The lowest external standard for each carotenoid analyzed was: retinol 200 nmol/L, lutein 50 nmol/L, Zeaxanthin 10 nmol/L, beta-cryptoxanthin 20 nmol/L, alpha carotene 20 nmol/L, beta carotene 50 nmol/L and lycopene 50 nmol/L.

2.2 Statistical Analyses

Continuous dietary nutrients and plasma carotenoid variables were assessed for normality. Positively skewed nutrients were log-transformed before applying further analyses. For the categorical variables, demographic characteristics and risk factors for cervical cancer, proportions and percentages were calculated for the invasive cases vs. hospital controls and in situ cases vs. clinic controls. Statistical significance was determined using Chi-square and fisher's exact as appropriate. Dietary groups for fruit and vegetable and concentrations of plasma carotenoids were also categorized into quartiles.

We compared agreement between the two methods. For this comparison, non-parametric spearman rank was used to assess the correlation between categorical variables from the FFQ and the plasma carotenoid and retinol measures. Thus, quartiles for fruit and vegetable intake were correlated with quartiles from the seven plasma carotenoids. For the continuous variables, Pearson correlation coefficient was used to assess the correlation between plasma carotenoid concentrations and dietary carotenoids intake from the FFQ. Due to lack of nutrients data for the other carotenoids; α-carotene, lutein, zeaxanthin, cryptoxanthin and lycopene, correlations were performed between total vitamin A, retinol and β-carotene only from dietary intake against the seven carotenoid concentrations from plasma. Partial correlation coefficients were adjusted for age and other confounding factors for serum carotenoids reported in literature such as body mass index (BMI) and smoking (Stryker et al,1988; Tucker et al,1999; Albanes et al, 1997; Pamuk et al, 1994). Smoking status was not considered as a confounder in these analyses because only 3% of the subjects were current smokers and only 1% of the

women were past smokers. A significant level of 5 percent was used. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Inc. Cary North Carolina).

2.3 Results

General characteristics of the study population

Table shows the distribution of number of known factors for cervical cancer for both cases and controls. In situ cases likely resided in the city than the clinic controls. Clinic controls were less likely to have more pregnancies and live total births than the case group. There was no difference in body mass index, age at menarche and number of sexual partners between in situ cases and clinic controls.

Hospital control women were more likely to reside in the city and more likely to have had chest x-ray screenings than women with invasive cervical cancer. The number of women with oncogenic type of HPV was significantly higher among invasive cases group than among the control group. Women with invasive cervical cancer were significantly more likely to have had their first intercourse before the age of 16 years and had more live total birth and pregnancies than the hospital control women. The hospital control women were more likely to have used an intrauterine device and more likely to have smoked than women with invasive cervical cancer. There was no difference between women with invasive cervical cancer and their control group with regard to use of oral contraceptives, history of induced abortion and spontaneous abortions, Pap smear test in the past 12 months, alcohol consumption, number of sexual partners, age at menarche and body mass index.

Table 1: **Demographic characteristic of the study subjects**

Factor	Invasive cases (n = 132		Hospital controls (n = 201)		In situ cases (n = 50)		clinic controls (n = 103)	
	No.	%	No.	%	No.	%	No.	%
Age(years)								
≤29	4	3	9	4	11	22	27	26
30-34	19	14	37	18	11	22	28	56
35-39	27	2	56	28	9	18	16	16
40-44	28	21	48	24	8	16	14	14
45-49	19	14	16	8	6	12	10	10
≥50	35	27	35	17	5	10	9	9
Current residence[pq]								
city	39	30	110	56	23	46	65	63
town	49	37	52	26	19	38	15	15
rural	44	33	39	19	8	16	24	23
Attended school								
No	18	14	15	7	3	6	7	6
Yes	114	86	185	92	47	94	97	94
unknown	0	0	0	0	0	0	0	0
Ever had a chest x-ray								
No	123	93	142	71	31	62	76	74
Yes	9	7	58	29	19	38	28	27
Unknown	0	0	0	0	0	0	0	0
Ever smoked								
Never	122	92	198	98	46	92	101	98
Past smoker	2	2	3	1	1	2	1	1
Current smoker	7	5	3	1	3	6	3	3
unknown	0	0	0	0	0	0	0	0
Ever drank alcohol beverage								
No	59	45	114	57	30	60	55	53
Yes	73	55	87	43	20	40	49	48
Body mass index								
≤19	16	12	19	9	4	8	13	13
20-21	33	25	37	18	12	24	33	32
22-25	50	38	84	41	19	38	38	37
≥26	33	25	57	28	15	30	20	19
unknown	0	0	0	0	0	0	0	0
Ever used an IUD[q]								
No	114	86	135	67	47	94	86	83
Yes	18	14	66	33	3	6	18	17
Ever used oral contraceptive								
No	67	51	101	50	20	40	49	48
Yes	65	49	100	50	30	60	55	53
Age at menarche (years)								
≤13	28	21	50	25	15	30	30	29
14-16	79	60	117	58	22	44	55	53
≥17	25	19	34	17	13	26	19	18

P: Significant differences between the in situ case group and the clinic group ($p<0.05$)
q: Significant differences between the invasive case group and the hospital control group ($p<0.05$)

Table 1 (continued)

Factor	Invasive cases (n = 132)		Hospital controls (n = 201)		In situ cases (n = 50)		clinic control (n = 103)	
	No.	%	No.	%	No.	%	No.	%
Age at first intercourse (years)[q]								
≤16	25	19	17	8	8	16	5	5
17-18	34	26	27	13	13	26	21	20
19-20	39	30	44	22	12	24	20	19
21-23	22	17	44	22	9	18	31	30
≥24	12	9	69	34	8	16	27	26
Total live birth[pq]								
0	5	4	5	2	3	6	21	20
1-2	38	29	128	64	24	48	62	60
3-4	53	40	44	22	17	34	18	17
≥5	36	27	24	12	6	12	3	3
Total pregnancy[pq]								
0	4	3	4	2	3	6	17	17
1-2	29	22	56	21	21	42	63	61
3-4	58	44	27	16	16	32	21	20
≥5	41	31	30	15	10	20	3	3
Induced abortions								
None	128	97	198	98	47	94	100	97
1 or more	4	3	3	1	3	6	4	4
Spontaneous abortions								
None	99	75	172	86	39	78	91	88
1	27	20	23	11	7	14	11	11
2 or more	6	5	6	3	4	8	2	2
Ever had a tubal ligation								
No	88	67	30	15	88	176	144	139
Yes	44	33	20	19	44	88	57	55
Number of sexual partners								
1	119	90	190	95	46	92	95	92
>1	13	10	11	5	4	8	9	9
Months since last Pap smear								
No Pap smear	107	81	84	42	36	72	71	69
1-12	9	7	65	32	8	16	13	13
>12	3	2	36	18	6	12	8	8
Unknown	0	0	0	0	0	0	1	1
HPV[pq]								
Oncogenic	92	70	5	2	25	50	4	4
Non-oncogenic	10	8	6	3	11	22	3	3
Negative	19	14	129	64	13	26	94	91
Unknown	11	8	61	30	1	2	2	2

P: Significant difference between the in situ case group and the clinic control group ($p < 0.05$)
q: Significant difference between the invasive case group and the hospital control group ($p < 0.05$)

Table 2 gives the relationship between quartiles of plasma carotenoids and quartiles of fruit and vegetable intake. Plasma lycopene was significantly correlated with the fruit group (r = 0.39; *P* 0.0001); however, after adjustment for age and BMI, this correlation decreased to 0.27, but remained significant. Plasma α-carotene concentration was found to be significantly correlated with the fruit group(r = 0.37) after controlling for the confounding factors. After adjusting for age and BMI, fruit group was weakly correlated with plasma β-carotene, plasma zeaxanthin and plasma cryptoxanthin and the magnitude for the coefficients ranged from 0.13 for zeaxanthin to 0.23 for β-cryptoxanthin. Plasma lutein remained poorly correlated with the fruit group after controlling for age and BMI (r = 0.07).

Table 2**. Spearman correlations between plasma carotenoids and retinol, and fruit and vegetables**

	DIETARY GROUPS			
PLASMA	Fruit		Vegetable	
	r	r[§]	r	r[§]
α-carotene	0.27**	0.38**	0.08	0.05
β-carotene	0.15	0.16	0.02	-0.02
Lutein	0.12	0.07	0.22**	0.21
Zeaxanthin	0.1	0.13	0.21	0.27**
β-cryptoxanthin	0.2	0.23	0.18	0.18
Lycopene	0.39**	0.27**	0.24**	0.15

[§] Adjusted for age, BMI

** Significant at <0.05

There was a modest correlation of the vegetable group with plasma lutein levels (r = 22, *P* = 0.005), which changed only slightly after adjusting for factors known to affect plasma concentrations of the carotenoids. The crude correlation of dietary zeaxanthin intake was 0.21, which improved to 0.27 (*P* = 0.05) after the adjustment for age and BMI. Plasma lycopene was significantly correlated with the vegetable group (r = 24, but after adjustment of the confounders, the coefficient dropped to 0.15. There was a weak correlation for plasma β-cryptoxanthin with vegetable group and no correlation for α-carotene and β-carotene with the vegetable group. The correlation coefficients ranged from -0.02 to 0.18.

Table 3. **Pearson correlations between plasma carotenoids and retinol and total vitamin A and β-carotene dietary intake**

PLASMA		**DIETARY INTAKE** Total vitamin A		β-carotene
	R	r[§]	r	r[§]
Retinol	0.007	-0.03	-0.03	-0.04
β-carotene	0.02	0.04	0.16	0.19
α-carotene	0.019	0.19	0.26**	0.27**
Lutein	0.15	0.16	0.14	0.11
zeaxanthin	0.12	0.15	0.23**	0.24**
β-cryptoxanthin	0.1	0.15	0.17	0.19
Lycopene	0.21	0.19	0.15	0.13

[§]Adjusted for age, BMI

** Significant at <0.05

Pearson correlation and coefficients were calculated for plasma carotenoids levels, retinol and dietary intake from vitamin A and β-carotene estimated from the FFQ (Table 3). When dietary vitamin A calculated from the FFQ was correlated with the plasma carotenoids, we found weak correlations with ranges from -0.02 for β-carotene to 0.21 for lycopene. These correlations coefficients did not change much after adjusting for age and BMI. We observed no correlation for plasma retinol and vitamin A. Adjusting for confounders did not change the pattern (r = -0.03 adjusted)

In Table 3, intake for β-carotene was significantly correlated with plasma concentrations for zeaxanthin (r = 0.23 P= 0.02) and α-carotene (r = 0.27 P = 0.007) before and after adjusting for age and body mass index. Dietary β-carotene intake estimated by the FFQ was weakly correlated with plasma lutein, plasma lycopene, and plasma β-carotene and plasma β-cryptoxanthin. This pattern was not changed after adjusting for confounders. We observed a negative correlation between β-carotene and plasma retinol. Correlation between dietary intake for total vitamin A and β-carotene was also calculated. We observed a significant correlation coefficient between dietary intakes of vitamin A and β-carotene (r = 0.51 P<0.0001).

2.4 DISCUSSION

Of the published validation studies, most used food records as the method of reference. Presently studies relating nutrient intake estimated by the FFQ to biomarkers are limited (Kant, 2000). In the present study, results were compared mostly with findings from a few similar studies that used plasma or serum carotenoids measures among nonsmoker subjects.

Studies have suggested moderate correlations in magnitude (r = 20-30) between plasma or serum carotenoids and fruit and vegetable intakes (Forman et al., 1993;Campbell et al., 1994; Tucker et al.,1999; Van Kappel et al., 2001; Block et al., 2001). In this study, modest to poor correlations were observed between selected plasma carotenoids and fruit and vegetables groups. The fruit group was only significantly correlated with α-carotene and lycopene after adjusting for other factors. Vegetable group was modestly correlated with zeaxanthin and lutein and poorly correlated with the rest of the plasma carotenoids.

Vegetable group was found to be negatively correlated with the concentration of plasma β-carotene. A study by Campbell et al. (1994) among nonsmoker women found higher correlations between consumption of fruits and vegetables and biomarkers from carotenoids. In that study however, the selection of participants was based on their high and low fruit and vegetable intake which may explain the high correlations observed. Moderate to high correlations were also found between total vegetable and fruit intake, and β-carotene, lutein and α-carotene (r = 0.48. 0.60 and 0.73 respectively) (Polsinelli et al., 1998). However, inclusion of supplement users in the study may have inflated these correlations.

The lack of correlations for plasma lutein, β-carotene, and zeaxanthin with fruit group is consistent with other reports (Block et al, 2001). This can be explained by the fact that lutein and zeaxanthin are predominantly found in vegetables and not in fruits (Ascherio et al, 1992). Similarly, β-carotene is relatively predominant in vegetables than in fruits.

Correlations between carotenoid intake and plasma or serum β-carotene varies between 0.21 (Roidt et al, 1988; Herbeth et al, 1989) and 0.41 (Stryker et al, 1988) among nonsmokers. In the present study, we observed similar correlations for selected carotenoids: zeaxanthin and α-carotene. Plasma or serum carotenoids are thought to directly reflect intake of these compounds (Broekmans et al, 2000; McEllgot et al, 1999; Willet et al, 1983b). Nonetheless, plasma β-carotene was not associated with β-carotene as estimated by the FFQ. Similarly, Ascherio et al (1995) did not find a strong correlation between plasma β-carotene and dietary β-carotene estimated by the FFQ. Goodman et al (1996) and Campbell et al (1994), however, found some correlations between FFQ estimate of β-carotene and plasma levels of β-carotene ($r = 0.24$ and 0.41, respectively). However, in Campbell et al's study, 29 % of the subjects were supplement users. Similarly, Coates et al. (1991) who found significant and positive correlations between intake of β-carotene and corresponding serum level among nonsmokers, more than half of the subjects studied used vitamin supplements. Hence, the use of supplements may have strengthened the relationship between the nutrient intake and biomarker nutrients in these studies. The lack of correlations between plasma and dietary intake β-carotene calculated from the FFQ in our study may mean that vegetables consumed by the study population are poor sources for the nutrient. It may also mean the study population consumed less vegetables. It has been reported that in Thailand

consumption of vegetables is less than in other Southeast Asian regions. In view of this fact, it is not a surprising that dietary β-carotene was not reflected in the corresponding plasma of the study population.

The weak correlations observed in the present study between total vitamin A intake estimated by the FFQ and plasma concentration of the carotenoids including retinol are consistent with the results from previous studies (Jacques et al., 1993). Jacques et al. (1993) observed no correlation between plasma retinol and vitamin A intake. The lack of association could be attributed to the fact that plasma retinol is only responsive to total vitamin A intake in individuals with inadequate vitamin A status (Jacques et al., 1993). Thus, we would expect no correlation in well nourished subjects. Additionally, the lack of correlations between dietary total vitamin A as estimated by the FFQ and plasma retinol could be due to the homeostatic control of the circulating retinol Ribaya-mercado et al., 2000; Napoli, 1999). Despite the fact that plasma β-carotene and total vitamin A dietary intake were poorly correlated, interestingly, we noted a significant correlation between FFQ dietary intakes of the total vitamin A and dietary β-carotene ($r = 0.51$) (data not shown). It is well known that β-carotene is the most common carotenoid in food and account for more than 50% of the total intake of vitamin A.

Worth mentioning is the fact that discrepancy in results for validation studies could be due to the difference in sample size, difference in plasma carotenoid analysis methods, difference in the populations studies and differences in the structure and size of the questionnaire used in these studies. Also, differences adjusted confounders may produce discrepancies among studies.

As stated earlier, the advantage of using biomarkers as reference for validating a FFQ lies in its uncorrelated errors with those from the FFQ. Thus, the uncorrelated errors

increase correlation coefficients between the two instruments. Despite the uncorrelated errors, the use of plasma concentration as a biomarker such as carotenoids is limited by the fact that these biomarkers may be influenced by other factors. There are several factors that could influence and contribute to the reduced or lack of correlation between plasma concentration biomarkers and dietary intake. Individual differences in the absorptions of dietary carotene may reduce the relationship between β-carotene from diet and plasma. Also, differences in the bioavailability of carotene from different sources may attenuate β-carotene correlations from dietary and plasma. Hence, this may cause correlations to be modest with fruits and vegetable intake (Mayne, 2003). Furthermore, biological confounders may cause variations in levels of biomarkers unrelated to the dietary component and hence, attenuate the correlations between the biomarker and true dietary intake levels. These confounding factors may include body mass index, cholesterol, smoking and age, genetic background and supplement use (Block, 2001; Gibson, 2005). Beta-carotene concentrations, for example, are reduced by smoking and alcohol intake (Forman et al., 1995).

In this study, the effect of smoking was not addressed as a confounder because the number of users was low (2% for past smokers and 5% current smokers). Additionally, vitamin supplement which easily increase plasma carotenoids was not considered in the present study because the data was not available.

Several issues should be considered when interpreting results from this study. Most studies that we compared with were not case-control studies. However, because in this study we employed a case-control study design, potential sources of bias should be considered. Selection bias may have occurred since participants were those who reported to the clinic for Pap smear follow-up. Hence, they are unlikely to represent the general

population in Bangkok, Thailand. The use of clinic controls may also have introduced recall bias because it is likely that most of the women may have been motivated to complete the FFQ more accurately than would have been the case in the general population. This study is further limited by the small sample size. Dietary intake estimates depends on the quality of the food composition tables, which provide average values for nutrient content in specific foods. The food composition table used in this and previous studies (Shannon et al., 2002), was old and outdated (a 1972 food composition table). Therefore, it may not have reflected the up- to- date nutrient content values for the foods consumed in Thailand and thus, systematic biases may have been introduced due to the use of the old composition table.

In conclusion, the results presented in this study revealed a moderate agreement between plasma carotenoids and dietary intake estimated from the FFQ. The correlation patterns found in this study indicate that the food frequency questionnaire used in Bangkok, Thailand can be an acceptable assessment instrument for a long term dietary intake. Nevertheless, we recommend another food frequency questionnaire validation study using large sample size and participants that are more representative of the general population in Thailand. In addition, this study has revealed that women classified high on both measures of exposure; plasma levels and dietary intake, of the β-carotene have a reduced risk of developing invasive cervical cancer.

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CHAPTER 3

Association of plasma carotenoids and retinol and dietary intake with in situ and invasive cervical in carcinomas in Bangkok, Thailand.

Abstract

The purpose of the study was to determine the association of plasma carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, lycopene) and retinol with either risk of in situ cervical carcinomas or invasive cervical carcinomas or progression from in situ cervical carcinomas to invasive cervical cancer. The study further examined the association between concordant quartiles for β-carotene as measured from plasma and dietary intake and risk of invasive cervical cancer. Included in the study were 50 in situ cases with abnormal Pap smears and pre-admission diagnosis of carcinomas in situ and 103 clinic controls with normal Pap smear test. Also included in the study were 201 invasive cases with histologically confirmed invasive cervical carcinoma and 302 hospital controls admitted to Siriraj Hospital with other unrelated diseases. Plasma carotenoids and retinol were measured by high performance liquid chromatography (HPLC). Human papillomavirus infection was detected using a standard PCR-based test. The food frequency questionnaire (FFQ) was used to capture the intake of the β-carotene. The results revealed that in situ cases did not differ significantly from clinic controls in median plasma levels of all the carotenoids and retinol. Invasive cases had significantly lower plasma lycopene levels compared to the hospital controls. Invasive cases did not differ significantly from the hospital controls in median plasma levels for the other carotenoids and retinol. Multivariate analysis revealed no association between any of the plasma carotenoids and retinol and either risk of in situ or invasive cervical cancer as compared to controls or the progression of in situ disease to invasive disease after adjusting for confounders. Furthermore, women classified as high on both measures of exposure were significantly at a reduced risk of invasive cervical cancer (OR =0.29 95% CI = 0.09-0.98) compared to women who had low measures on both dietary intake and plasma β-carotene after controlling for confounders. We also observed that women

classified as high on dietary intake and low on plasma level were at a lowest risk of invasive cervical cancer (OR= 0.28 95% CI= 0.10-0.79). Therefore, this study does not provide evidence of an association between individual plasma carotenoids and retinol and either risk of in situ or invasive or for the progression from in situ disease to invasive disease. However, there is evidence from this study that high β-carotene from both dietary intake and plasma could be associated with the reduction in risk for invasive cervical cancer. There is also strong evidence that long-term intake of the β-carotene, possibly with other carotenoids, may reduce the risk of cervical cancer, in particular invasive cervical carcinomas. In light of this, long-term intervention efforts to increase consumption of fruits and vegetables may protect women in Thailand from developing cervical cancer. However, further studies are recommended to support this in Thailand.

INTRODUCTION

Cervical cancer remains a very significant contributor to the morbidity and mortality of women in the world, especially in developing countries. Despite numerous research studies, the role of diet in the risk and progression of this disease remains unclear. Cumulative evidence from studies strongly suggests that carotenoids and retinol independently or as a cofactor play a role in the development and progression of cervical cancer (Rock, 2004). Researchers do suggest that carotenoids and retinol exhibit several biological activities that could prevent or slow the development and progression of cancer. Carotenoids exhibit antioxidant activities in vitro and that may influence carcinogenesis via effects on cell growth regulation. One such activity is in the inhibition of growth and malignant transformation and the promotion of apoptosis in transformed cells (Kerensky, 1998; Bertram, 1999).

Early studies that linked carotenoids with risk of cervical cancer suggest that carotenoids could help slow the growth of the disease in its early stages (Potischman, 1993; Palan, 1988). In vitro experimental studies have demonstrated that β-carotene reduces the persistence of HPV infection through inhibition of HPV induced transformation of keranocytes (Giuliano et al., 1999). Also, a study has shown that women with higher carotenoid intakes are more likely to clear an HPV infection (Giuliano et al., 2003; Potischman et al., 2001).

Studies that have looked at dietary intake and risk of cervical cancer, both in early and invasive stages of the disease have produced mixed results (Brock et al., 1988, La Vet et al., 1991; Herrero et al., 1991; VanEenwyk et al., 1991; Palan et al., 1988; Ziegler et al, 1990). Although the approach of examining serum or plasma carotenoids with the risk of cervical cancer has revealed some inverse associations, there are some

inconsistencies among studies. These inconsistencies could be caused by several factors. Some studies may be limited by inadequate control for confounding factors such as HPV, smoking, oral contraceptives and sexual behaviors (Rock, 2004). Studies may also be limited by the specific time point in the disease process when the specific study was conducted. The majority of studies evaluated associations at a single point in disease progression. Results may not be the same from studies conducted at different time points in disease progression. In addition, the majority of previous research has focused on β-carotene, whereas relatively few studies have looked at the other carotenoids with relation to cervical cancer. The reliance on dietary self report has also been a major limitation in many observational studies due to biases associated with self-reports. Contrary, the use of serum or plasma carotenoid assessment adds more confidence in the observed associations (Mayne, 2004).

In order to determine conclusively whether nutrients such as carotenoids are associated with risk of disease progression, study subjects would need to be recruited with early stage of the disease and be allowed to progress to late stage. However, such studies would be clearly unethical. A couple of studies have compared women in the early stages of the disease to those with invasive disease. In one such preliminary study, Shannon et al. (2002) examined the possible effect of dietary carotenoids intake on cervical carcinogenesis by comparing in situ to invasive cases. This analysis allowed evaluation of the association between dietary carotenoid intake and the progression of the disease by directly comparing in situ to invasive cases within the same study.

Despite the accumulation of studies of diet and risk of cervical cancer, no study has been conducted to examine the relation between plasma concentrations of carotenoid and retinol with risk and progression of in situ and invasive cervical carcinomas within

the same study population. Many previous serum and plasma carotenoids studies focused mainly at a single point in disease development, such as the early or invasive stage. Furthermore, previous case-control studies looked mainly at one specific form of the carotenoids, such as β-carotene. Contrarily, this study looked at both in situ and invasive stages in the same study using the same protocol. It also looked at five other carotenoids, β-carotene, α-carotene, lutein, zeaxanthin, lycopene and β-cryptoxanthin to the risk of in situ and invasive cervical cancer.

With this background in mind, the present case-control study had four objectives: 1) to determine the association between plasma carotenoids and retinol concentration levels and risk of in situ cervical carcinoma; 2) to determine the association between plasma carotenoids and retinol concentration levels and risk of invasive cervical carcinoma, 3) to examine association between the risk of progression of in situ to invasive cervical cancer and plasma carotenoids and retinol concentration levels; 4) to investigate the association between cervical cancer and carotenoids among women in the concordant quartiles as determined via a food frequency questionnaire and plasma concentration levels. Results from this study could have strong implications in the reproductive health of women with regard to the risk and progression of cervical cancer, particularly in developing countries where many women are still victims of the disease.

3.1 MATERIALS AND METHODS

3.1.1 Study population

This study is part of a case-control study conducted in Bangkok, Thailand. The main data was collected between 1991 and 1993 in Bangkok. The study methodology has been described in detail elsewhere (Thomas et al., 2001 and Shannon et al., 2002).

In situ cases and controls

Carcinoma in situ case group consisted of women with incidents of histological cervical intraepithelial neoplasia grade III. This group of women was recruited from the family planning and gynecology clinic associated with Siriraj hospital in Bangkok. The cases had an abnormal Pap smear and were admitted to the gynecology wards with a pre-admission diagnosis of severe dysplasia. Women born after 1929 and who had resided in Thailand for at least one year were qualified for the study. For each in situ case, two clinic control women were selected from women with appointments in the same clinic for a Pap smear follow-up and for whom results revealed no suspicion of neoplastic change. Eligible controls were in the same five-year age group and resided in the same region of the country as the cases. In situations where controls refused to participate, the next two women were selected.

Invasive cases and controls

Women admitted to the public ward of Sirarij hospital in Bangkok, Thailand between September 1991 and September 1993 with new histologically confirmed diagnosis of invasive cervical cancer were eligible as cases for the study. Invasive cases were women born after 1929 and had lived in Thailand for a year prior to the study. For each invasive case woman, two women from the otolaryngology and the general surgery ward were selected as controls. The control women were in the same 5-year age group and resided in the same region for a year as the case.

3.1.2 **Exclusion criteria**

Conditions for exclusion criteria for the cases and controls included: use of steroid contraceptives such as circulatory or cardiovascular diseases, diagnosis with diabetes, chronic renal disease, benign breast disease, a previous diagnosed cancer, chronic liver disease and any other obstetrical. Most hospitalized controls were being treated for cataracts, non-toxic nodular goiter or gallstones.

3.1.3 **General Questionnaire**

All women completed a structured risk factor questionnaire to elicit information regarding age, sexual and reproductive history, prior Pap smears, use of tobacco and alcohol and indices of prior use of medical resources and socioeconomic status.

3.1.4 **HPV DNA assays**

On the day cases were selected and interviewed, cervical scrapings for HPV DNA assays were obtained, prior to treatment. Surgeons obtained Pap smears and cervical scrapings from hospitalized and clinic controls on the day they were selected and interviewed. Teflon swab was used to scrap the cervix, including the cervical os, the end of which was broken off into a vial containing 2 ml of Virapap transport medium. The resultant specimen were stored at - 70º C and shipped to Seattle on dry ice. Assays for type-specific HPV DNA were performed using standard PCR-based technology (Thomas et al., 2001)

3.1.5 **Plasma carotenoids and retinol extraction**

Non-fasting 15ml blood samples were collected from each woman on the same day as the interview. The samples were centrifuged and stored at -70°C within three hours of blood drawing. Two aliquots were retained in Bangkok and two were shipped to Seattle, Washington, on dry ice for further analysis at a later time. Samples were then transferred from Fred Hutchinson Cancer Research Center to the General Clinic Research Center laboratory, Oregon Health and Science University on dry ice. Upon arrival, the samples were promptly frozen at -80°C. The protocol for this study was approved by the Institutional Review Board of Fred Hutchinson Cancer Research Center, Oregon Health and Science University and Oregon State University.

All carotenoid analyses were conducted under dim light because of the sensitivity of carotenoids and retinol to light. The laboratory staff did not know whether a sample belonged to a case or a control. Plasma samples were allowed to thaw at room temperature before extraction. Carotenoids were extracted using a non-enzymatic extraction method borrowing elements from Lin et al. (2003) and Yuem et al. (1998). 20 µl of echinenone (Hoffman-La Roche, Basel, Switzerland) and 20 µl of retinol acetate (Sigma Chemical) (internal standards) were added to 450 µl of plasma in a glass tube and after the addition of 450 µl ethanol vortexed for 5 seconds. The sample was then extracted by the addition of 600 µl of hexane and vortexed vigorously for 60 seconds. After centrifugation at 1000 x g at room temperature for 5 minutes, 400 µl of the upper layer was removed and evaporated to dryness at 40° C under a gentle stream of nitrogen. The residual was redissolved in 150 µl of ethanol and vortexed for 30 seconds. A 50 µl aliquot was injected onto the column for high-performance liquid chromatography (HPLC) analysis.

3.1.6 **HPLC Analysis of Plasma Carotenoids and retinol**

Although several method are available for the assay of carotenoid levels in serum or plasma, a reverse-phase HPLC has become the most widely used method for the separation of plasma or serum carotenoids and retinol. HPLC is precise at low concentrations (de Pee et al, 2002) and it is specific and easy to use. The HPLC system consisted of a HP series 1050 pump and degasser, a Spectrasystem AS3000 autosampler, an Agilent 1100 series Diode Array multiple wavelength detector and a C30, 5 μm, 4.6x250mm carotenoid YMC column. The HPLC mobile phase consisted of solvent A) methanol: methyl-*tert*-butyl ether: water (83:15:2, by vol, with 1.5% ammonium acetate in the water), and solvent B methanol: methyl-tert-butyl ether: water (8:90:2, by vol with 1.0% ammonium acetate in the water). At a flow rate of 1 ml/min the gradient procedure consisted of the following: 1) 100% A for 1 min; 2) a 7 min linear gradient to 70% A ;3) 70% A for 5 min; 4) a 9 min linear gradient to 45% A; 5) 45% A for 2 min; 6) a 10 min linear gradient to 5% A; 7) 5 % A for 4 min; 8) a 2 min linear gradient to 100% A; 9) 100% A for 5 min. The relative amounts of plasma carotenoids and retinol were determined by measuring peak areas in the chromatograms at the optimal absorption wavelengths as follows: for retinol and retinyl acetate 325 nm, for lutein and α-carotene 445 nm, for zeaxanthin, β-cryptoxanthin, echinenone, β-carotene 450 nm and for lycopene 472 nm. Peak areas were quantified and their concentrations calculated based on the external standards α-carotene, lutein, zeaxathin, β-cryptoxanthin, lycopene (Hoffman-La Roche, Basel, Switzerland), β-carotene and retinol (Sigma Chemical). Concentrations were corrected for procedural losses by adjusting the concentrations in proportion to the average of the two internal standards. To monitor assay acceptability a

pooled plasma control was included at the beginning and the end of each assay run. The lowest external standard for each carotenoid analyzed was: retinol 200 nmol/L, lutein 50 nmol/L, Zeaxanthin 10 nmol/L, beta-cryptoxanthin 20 nmol/L, alpha carotene 20 nmol/L, beta carotene 50 nmol/L and lycopene 50 nmol/L.

3.2 **Statistical analysis**

Plasma data was assessed for normality. Plasma carotenoid and retinol distributions were right skewed; therefore, the median plasma carotenoid and retinol concentration in cases and controls were compared using nonparametric Wilcoxon rank test. For the categorical variables, demographic characteristics and known risk factors for cervical cancer, proportions and percentages were calculated for the invasive vs. hospital controls and in situ vs. clinic controls. Statistical significance was determined using the chi-square and fisher's exact tests as appropriate.

In order to achieve our objectives, three models were developed. The first model evaluated the risk of situ carcinoma vs. clinic controls as a function of plasma carotenoids and retinol. The second model evaluated the risk of invasive carcinoma vs. hospital controls as a function of plasma carotenoids and retinol. The third model evaluated the risk of progression of the cervical cancer from in situ stage to invasive in relation to plasma carotenoids and retinol. In this third model, cases were women with invasive cervical carcinomas while controls were women with in situ cervical carcinomas (invasive cases vs. in situ cases). This was the best model because elevated odds ratios would indicate the risk of invasion relative to the risk of in situ carcinomas, thus could be interpreted as the risk of progression of the disease. The case to case model has been tested in other case-control studies in which women with invasive carcinoma were

directly compared with women with in situ carcinoma to investigate the progression of cervical cancer (Thomas, 1993; Moreno et al., 1995; Thomas et al., 2002; Shannon et al., 2002). The fourth model evaluated the risk of invasive cervical carcinoma in relation to combined measures from the FFQ and the plasma. In this fourth model, the analysis was restricted to invasive cases vs. hospital controls only due to small sample size for the in situ cases vs. clinic control group. Furthermore, since this model utilized data from both the dietary intake and plasma measures, it was discovered that β-carotene was the only carotenoid with data from both dietary intake and plasma measures. Therefore, the analysis of this model was restricted to β-carotene.

The multivariate analysis for the last three models was conducted in two phases with all phases using the unconditional logistic regression (Allison 1999; Homer et al., 2000) to examine the association between the risk of in situ, invasive and progression of in situ carcinoma to invasive disease. In the first phase, the effect of each of the plasma carotenoids and retinol: β-carotene, α-carotene, lutein, zeaxanthin, β-cryptoxanthin, lycopene and retinol on cervical cancer status was tested by entering the plasma concentration level in the logistic regression model as a continuous, logarithmically normalized variable. The second phase of the multivariate analysis, the risk of in situ carcinoma, invasive carcinoma and progression of cervical cancer was evaluated by using quartiles of the plasma carotenoids and retinol concentration levels. Plasma quartiles for these compounds were created based on untransformed data among controls. The lowest quartile was used as the referent category for computing odds ratios.

Based on previous knowledge, we evaluated confounding factors in all models which included: total birth, total pregnancy, age at first intercourse, use of oral contraceptives, induced abortion, age at menarche, tubal ligation, number of sexual

partners, number of pap smears in the past 12 months, urban versus rural residence, education, number of chest x-ray screening, smoking, HPV status, alcohol consumption, body mass index and age. Covariates or confounders that changed the β-coefficient of the main predictor by 10% or more were included in the final models (Maldonado et al., 1993; Rothman et al., 1998; Greenland et al., 1998; Newman, 2001). Age, total pregnancy, age at first intercourse, recent Pap smear and urban versus rural were entered into the in situ versus clinic control final model for adjustment. Confounders added in the final model for invasive versus hospital control were age, total pregnancy, frequency Pap smear, age at first intercourse, number of sexual partners and urban versus rural. The invasive versus in situ model (test of progression of the disease) was adjusted for HPV, chest x-ray and age.

Testing for linear trend for the first three models was performed by using the most efficient method of entering the exposure variable as a continuous variable in a logistic model (Lagakos, 1985; Zhao et al., 1992; Greenland, 1995a). Confidence intervals (CIs) that excluded 1.0 were considered significant (Breslow et al., 1980).

Using cross-classification analysis, women were classified into quartiles based on combined measures from the FFQ and plasma for the β-carotene. Thus, to determine which group of women in the concordant quartiles will be at greatest risk for invasive cervical cancer, we performed logistic regression controlling for age, number of sexual partners, age at first sexual intercourse, total pregnancy, frequency papanicolaou smear and human papillomavirus (HPV) 16. A significant level of 5 percent was used. All reported *P* values were two tailed. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Inc. Cary North Carolina).

3.3 Results

3.3.1 General characteristics of the study population

Table shows the distribution of known risk factors for cervical cancer for both cases and controls. In situ cases likely resided in the city than the clinic controls. Clinic controls were less likely to have more pregnancies and live total births than the case group. There was no difference in body mass index, age at menarche and number of sexual partners between in situ cases and clinic controls.

Hospital control women were more likely to reside in the city and more likely to have had chest x-ray screenings than women with invasive cervical cancer. The number of women with oncogenic type of HPV was significantly higher among invasive case group than among the control group. Women with invasive cervical cancer were significantly more likely to have had their first intercourse before the age of 16 years and had more live total birth and pregnancies than the hospital control women. The hospital control women were more likely to have used an intrauterine device and more likely to have smoked than women with invasive cervical cancer. There was no difference between women with invasive cervical cancer and their control group with regard to use of oral contraceptives, history of induced abortion and spontaneous abortions, Pap smear test in the past 12 months, alcohol consumption, number of sexual partners, age at menarche and body mass index.

Table 1: **Demographic characteristics of the study subjects**

Factor	Invasive cases (n = 132		Hospital controls (n = 201)		In situ cases (n = 50)		clinic controls (n = 103)	
	No.	%	No.	%	No.	%	No.	%
Age(years)								
≤29	4	3	9	4	11	22	27	26
30-34	19	14	37	18	11	22	28	56
35-39	27	2	56	28	9	18	16	16
40-44	28	21	48	24	8	16	14	14
45-49	19	14	16	8	6	12	10	10
≥50	35	27	35	17	5	10	9	9
Current residence[pq]								
city	39	30	110	56	23	46	65	63
town	49	37	52	26	19	38	15	15
rural	44	33	39	19	8	16	24	23
Attended school								
No	18	14	15	7	3	6	7	6
Yes	114	86	185	92	47	94	97	94
unknown	0	0	0	0	0	0	0	0
Ever had a chest x-ray								
No	123	93	142	71	31	62	76	74
Yes	9	7	58	29	19	38	28	27
Unknown	0	0	0	0	0	0	0	0
Ever smoked								
Never	122	92	198	98	46	92	101	98
Past smoker	2	2	3	1	1	2	1	1
Current smoker	7	5	3	1	3	6	3	3
unknown	0	0	0	0	0	0	0	0
Ever drank alcohol beverage								
No	59	45	114	57	30	60	55	53
Yes	73	55	87	43	20	40	49	48
Body mass index								
≤19	16	12	19	9	4	8	13	13
20-21	33	25	37	18	12	24	33	32
22-25	50	38	84	41	19	38	38	37
≥26	33	25	57	28	15	30	20	19
unknown	0	0	0	0	0	0	0	0
Ever used an IUD[q]								
No	114	86	135	67	47	94	86	83
Yes	18	14	66	33	3	6	18	17
Ever used oral contraceptive								
No	67	51	101	50	20	40	49	48
Yes	65	49	100	50	30	60	55	53
Age at menarche (years)								
≤13	28	21	50	25	15	30	30	29
14-16	79	60	117	58	22	44	55	53
≥17	25	19	34	17	13	26	19	18

p: Significant differences between the in situ case group and the clinic control group ($p<0.05$)

q: Significant differences between the invasive case group and the hospital control group ($p<0.05$)

Table 1 (continued)

Factor	Invasive cases (n = 132)		Hospital controls (n = 201)		In situ cases (n = 50)		clinic control (n = 103)	
	No.	%	No.	%	No.	%	No.	%
Age at first intercourse (years)[q]								
≤16	25	19	17	8	8	16	5	5
17-18	34	26	27	13	13	26	21	20
19-20	39	30	44	22	12	24	20	19
21-23	22	17	44	22	9	18	31	30
≥24	12	9	69	34	8	16	27	26
Total live birth[pq]								
0	5	4	5	2	3	6	21	20
1-2	38	29	128	64	24	48	62	60
3-4	53	40	44	22	17	34	18	17
≥5	36	27	24	12	6	12	3	3
Total pregnancy[pq]								
0	4	3	4	2	3	6	17	17
1-2	29	22	56	21	21	42	63	61
3-4	58	44	27	16	16	32	21	20
≥5	41	31	30	15	10	20	3	3
Induced abortions								
None	128	97	198	98	47	94	100	97
1 or more	4	3	3	1	3	6	4	4
Spontaneous abortions								
None	99	75	172	86	39	78	91	88
1	27	20	23	11	7	14	11	11
2 or more	6	5	6	3	4	8	2	2
Ever had a tubal ligation								
No	88	67	30	15	88	176	144	139
Yes	44	33	20	19	44	88	57	55
Number of sexual partners								
1	119	90	190	95	46	92	95	92
>1	13	10	11	5	4	8	9	9
Months since last Pap smear								
No Pap smear	107	81	84	42	36	72	71	69
12-Jan	9	7	65	32	8	16	13	13
>12	3	2	36	18	6	12	8	8
Unknown	0	0	0	0	0	0	1	1
HPV[pq]								
Oncogenic	92	70	5	2	25	50	4	4
Non-oncogenic	10	8	6	3	11	22	3	3
Negative	19	14	129	64	13	26	94	91
Unknown	11	8	61	30	1	2	2	2

P: Significant differences between the in situ case group and the clinic control group ($p<0.05$)
q: Significant differences between the invasive case group and the hospital control group ($p<0.05$)

3.3.2 **Comparison of the cases and controls in relation to levels of plasma carotenoids and retinol**

Table 4 shows the median plasma carotenoid concentrations. There were no significant differences between in situ cases and clinic controls in any of the individual carotenoids measured or retinol. When invasive cases were compared with hospital controls, median plasma levels for retinol, and α-carotene were higher in invasive cases than controls, but the difference was not significant. Plasma lycopene levels were significantly lower among invasive cases as compared to the controls ($P = 0.009$). Invasive cases had lower plasma zeaxanthin, β-crytoxanthin and lutein but the differences were not statistically significant.

Table 4 Comparison of plasma carotenoids and retinol levels between case groups and their control groups

Plasma Nm/L	Median (25th, 75th percentile)					
	In Situ			Invasive		
	cases, n=50	controls, n=103	p-value	cases, n=132	controls, n=201	p-value
Retinol	1796.65(1507,2102.10)	1740.70(1479,1979.10)	0.35	1715.70(1389,2124.95)	1692.6(1397.7,2090)	0.39
Lutein	202.30(139.50,271.90)	196.3(128.5,300.00)	0.84	181.40(128.9,240.4)	199.70(138.8,283.1)	0.06
α-carotene	40.15(23.00,63.70)	40.35(29.40,55.55)	0.75	34.70(24.75,52.35)	35.3(23.9,48.8)	0.68
β-carotene	311.2(193.0,432.0)	277.60(196.50,404.60)	0.63	246.35(164.40,437.55)	270.00(161.1,391.9)	0.97
Zeaxanthin	70.2(54.10,93.60)	69.50(46.9,89.6)	0.39	59.60(42.55,83.10)	66.50(47.7,85.4)	0.11
β-cyryptoxa	221.7(110.6,278.7)	192.60(118.5,254.4)	0.48	177.15(94.55,266.7)	187.70(122.3,272.4)	0.11
Lycopene	84.10(44.4,139.3)	93.10(48.2,153.6)	0.43	58.00(36.25,115.95)	73.20(44.7,132.5)	0.009

P value for Wilcoxon rank sum test (two-sided)

In situ cases were also compared with invasive cases in terms of plasma carotenoid and retinol concentration levels (data not shown). The levels for lutein, β-carotene, α-carotene and retinol were not significantly different for in situ cases as compared with invasive cases. Plasma levels for zeaxanthin, β-cryptoxanthin and lycopene were marginally significantly lower for in situ cases as compared with invasive cases (p=0.06 for lycopene; p=0.05 for zeaxanthin and 0.07 for β-cryptoxanthin).

3.3.3 **Plasma carotenoids and retinol as continuous variables and risk of in situ invasive and progression of in situ carcinoma to invasive disease.**

Age-adjusted and adjusted for confounders odds ratios (ORs) and 95% confidence intervals for plasma carotenoids and retinol as continuous variables are presented in Table 5. Plasma carotenoid and retinol concentrations were not significantly associated with risk of in situ cervical carcinomas after adjusting for age. Further adjusting for age, total pregnancy, age at first intercourse, recent Pap smears and urban versus rural did in the multivariate model, the lack of association persisted. We, therefore, report a nonsigficant association between carotenoids and retinol and risk of in situ cervical carcinoma

Furthermore, in the invasive versus hospital control model (Table 5), lycopene was significantly associated with lower risk of invasive cervical cancer (OR =0.70; 95% confidence interval 0.52-0.95). After further adjustment for age, total pregnancy, Pap smears, age at first intercourse, number of sexual partners, urban versus rural in the multivariate logistic regression model, the odd ratios for lycopene were attenuated to nonsignicant (OR=0.77; 95% confidence intervals 0.53-1.12). None of the other carotenoids and retinol was associated with risk of invasive cervical carcinoma after adjusting for age. Adjusting for additional confounders, did not change the pattern.

Therefore, we report a nonsignificant association between carotenoids and retinol and risk of invasive cervical cancer.

Table 5. Odds ratios (ORs) and 95% confidence intervals for continuous plasma carotenoids and retinol concentrations and risk of in situ, invasive and progression of cervical cancer.

	IN SITU		INVASIVE		INVASIVE VERSUS IN SITU	
PLASMA	OR 95% CI[§]	OR 95% CI[a]	OR 95%CI[§]	OR 95%CI[b]	OR 95%CI[§]	OR 95%CI[c]
Retinol	2.51(0.83-7.59)	3.25(0.85-8.34)	1.17(0.62-2.23)	1.02(0.47-2.24.	0.51(0.21-1.26)	0.57(0.16-1.03)
Lutein	1.02(0.59-2.07)	1.36(0.61-3.02)	0.69(0.46-1.03)	0.86(0.51-1.45)	0.73(0.38-1.31)	0.75(0.32-1.66)
Zeaxanthin	1.52(0.70-3.27)	2.75(1.04-7.29)	0.69(0.43-1.09)	0.93(0.51-1.70)	0.46(0.22-0.96)	0.70(0.28-1.77)
β-cyptoxanthin	1.16(0.68-1.95)	1.58(0.82-3.05)	0.81(0.58-1.11)	0.87(0.57-1.32)	0.63(0.37-1.06)	0.75(0.38-1.45)
β-carotene	0.97(0.60-1.59)	1.06(0.57-1.99)	1.04(0.78-1.39)	1.12(0.76-1.66)	0.89(0.57-1.43)	0.66(0.36-1.23)
α-carotene	0.88(0.48-1.64)	1.13(0.50-2.55)	1.12(0.76-1.67)	1.37(0.82-2.29)	0.89(0.52-1.52)	0.78(0.40-1.51)
Lycopene	0.84(0.52-1.36)	0.82(0.46-1.47)	0.70(0.52-0.95)	0.77(0.53-1.12)	0.72(0.47-1.09)	0.84(0.48-1.47)

[§] Age-adjusted

[a] Adjusted for age, total pregnancy, age at first intercourse, recent Pap Smears and urban versus rural

[b] Adjusted for age, total pregnancy, frequency Pap smear, age at first intercourse, number of sexual partners, urban versus rural

[c] Adjusted for age, chest x-ray and HPV

Furthermore, plasma carotenoids were examined as continuous variables with risk of invasive cervical carcinomas as compared to in situ cervical carcinoma, an indirect measure of progression (Table 5). Progression of cervical cancer was defined as the risk of invasive disease as compared to in situ carcinoma. In the age-adjusted model, zeaxanthin was significantly associated with a decrease in risk of the progression of the in situ carcinoma to invasive disease (OR = 0.46; 95% confidence interval 0.22-0.96). These results were attenuated in the multivariate model after controlling for age, number of chest x-ray screenings and HPV. All the other carotenoids and retinol were nonsignificantly associated with the risk of the progression of the in situ carcinoma to invasive disease in age-adjusted model (Table 5). Adjusting for confounders, did not change the pattern. Therefore, we report a nonsignificant association between plasma

carotenoids and retinol and the risk of progression for in situ cervical carcinomas to invasive cervical cancer.

3.3.4 **Plasma carotenoids and retinol as quartiles with risk of in situ carcinomas**

The odds ratios and 95% confidence interval for the risk of in situ cervical carcinoma with quartiles of plasma carotenoids and retinol are presented in Table 6. After controlling for age in the multivariate logistic regression model, no significant associations were observed between plasma carotenoids and retinol and the risk of in situ cervical carcinoma. Further controlling for confounding by age, urban versus rural, age at first intercourse, total pregnancy and recent Pap smear yielded similar results with the lowest quartile as the referent group. Therefore, we report a nonsignificant association between carotenoids and retinol and the risk of in situ cervical carcinoma.

Table 6 . Odds ratios (ORs) and 95% confidence intervals (CIs) of in situ cervical carcinoma according to quartiles of plasma carotenoids and retinol

Plasma	Q1	Q2	Q3	Q4	*OR; p* for trend
α-carotene(nmol/l)					
# of cases/controls	18/28	8/25	8/25	16/26	
OR (95% CI)	1.00	0.49(1.19-1.34)	0.49(0.19-1.34)	0.99(0.42-2.36)	OR =0.87; p 0.52
OR (95% CI)[§]	1.00	0.29(0.08-1.07)	0.62(0.17-2.18)	1.13(0.38-3.38)	OR=1.08; *p* = 0.65
β-carotene(nmol/L)					
# of cases/controls	12/26	17/26	10./26	25/11	
OR (95% CI)	1.00	1.42(0.57-3.55)	0.83(0.31-2.26)	0.95(0.36-1.55)	OR=0.93 p=0.52
OR (95% CI)[§]	1.00	1.01(0.32-3.16)	0.74(0.21-.2.57)	1.12(0.35-3.63)	OR=1.01; *p* =0.97
Lutein(nmol/L)					
# of cases/controls	12/26	9/26	21/26	8/25	
OR (95% CI)	1.00	0.75(0.27-2.08)	1.72(0.72-4.28)	0.69(0.24-1.98)	OR=1.0; p=0.94
OR (95% CI)[§]	1.00	1.41(0.38-5.22)	3.06(0.92-0.25)	0.91(0.24-3.47)	OR=0.74; *p* =0.66
Zeaxanthin(nmol/L)					
# of cases/controls	10/26	15/26	11/27	14/24	
OR (95% CI)	1.00	1.50(0.57-3.946)	1.06(0.39-2.91)	1.52(0.57-4.05)	OR=1.09; p=0.58
OR (95% CI)[§]	1.00	2.24(0.63-7.96)	1.81(0.50- 1.93)	2.85-0.83-9.83)	OR=1.33; *p* =0.14
β-cryptoxanthin					
# cases/controls	13/26	8/26	12./26	17/25	
OR (95% CI)	1.00	0.62(0.29-1.73)	0.92(0.36-2.39)	1.36(0.55-3.37)	OR=1.14; p=0.37
OR (95% CI)[§]	1.00	0.59(0.16-2.31)	2.08(0.59-7.26)	2.26(0.73-7.01)	OR=1.42; *p* = 0.06
Lycopene					
# cases/controls	17/29	14/25	10/25	9/24	
OR (95% CI)	1.00	0.96(0.39-2.32)	0.68(0.68-1.76)	0.64(0.24-1.69)	OR=0.85; *p*=0.28
OR (95% CI)[§]	1.00	0.86(0.28- 2.63)	0.47(0.14-1.54)	0.50(0.15-1.68)	OR=0.76; *p* = 0.16
Retinol					
# cases/controls	12/25	9/26	13/27	16/25	
OR (95% CI)	1.00	0.72(0.26-2.08)	1.00(0.39-2.61)	1.33(0.53-3.38)	OR=1.13;p=0.42
OR (95% CI)[§]	1.00	0.55(0.14-2.20)	1.27(0.39-4.17)	1.31(0.39-4.29)	OR=1.19; *p* =0.36

[§] Adjusted for age, age at first intercourse, urban versus rural , total pregnancy and recent Pap smears

3.3.5 Plasma carotenoids and retinol as quartiles with risk of invasive cervical cancer

Table 7 shows age-adjusted and adjusted for confounders' odds ratios (ORs) and 95% confidence intervals (CIs) of invasive carcinoma by quartiles of plasma carotenoids and retinol. Compared with women with the lowest levels, those with the highest quartile levels of β-cryptoxanthin had a dose-responsive reduced risk of invasive cervical cancer (age-adjusted OR= 0.64; 95% confidence interval 0.35-1.16; *p* for trend =0.04). However, after further adjustment for age, total pregnancy, age at first intercourse, number of sexual partners and urban versus rural, the dose-response diminished. Similarly, in the age-adjusted multivariate model, high quartiles of lycopene were significantly associated with decreased risk of invasive cervical cancer with a significant dose-response (*p* for trend = 0.0106), compared to the lowest quartile. However, adjusting for additional confounders in the multivariate model attenuated the results and the pattern disappeared. In the age-adjusted model, no significant associations were observed for the other carotenoids and retinol. Similar results persisted after controlling for additional confounders. We therefore report no significant association between plasma carotenoids and retinol and the risk of invasive cervical cancer.

Table 7. **Odds ratios (ORs) and 95% confidence intervals (CIs) of invasive cervical carcinoma according to quartiles of plasma carotenoids and retinol**

Plasma	Q1	Q2	Q3	Q4	OR (CI) *p* for trend
α-carotene					
#cases/controls	43/51	32/50	29/50	28/50	
OR (95%CI)	1.00	1.17(0.61-2.14)	0.66(0.33-1.21)	1.22(0.65-2.27)	OR=1.01;*p*=0.88
OR (95%CI)[§]	1.00	1.39(0.66-2.93)	0.81(0.35-1.85)	1.66(0.76-3.59)	OR= 1.04; *p*=0.77
β-carotene					
#cases/controls	33/51	38/50	23/50	38/50	
OR (95% CI)	1.00	1.07(0.57-2.01)	0.67(0.34-1.33)	1.05(0.64-2.16)	OR=0.97; *p*=0.80
OR (95% CI)[§]	1.00	1.05(0.49-2.19)	0.72(0.31-1.64)	1.25(0.58-2.68)	OR= 1.04; *p*=0.77
Lutein					
#cases/controls	37/51	40/50	30/50	25/50	
OR (95% CI)	1.00	1.10(0.61-1.99)	0.83(0.45-1.54)	0.68(0.36-1.31)	OR=0.88; *p*=0.17
OR (95% CI)[§]	1.00	1.54(0.74-3.23)	1.34(0.61-2.94)	1.13(0.49-2.62)	OR=1.04; *p*=0.78
Zeaxanthin					
#cases/controls	43/51	32/50	29/50	28/50	
OR (95% CI)	1.00	0.76(0.42-1.39)	0.69(0.37-1.27)	0.66(0.36-1.23)	OR=0.89; *p*=0.20
OR (95% CI)[§]	1.00	1.09(0.31-3.82)	1.05(0.21-3.45)	0.70(0.17-2.84)	OR=1.05; *p*=0.80
β-cryptoxanthin					
#cases/controls	49/51	34/50	17/50	32/50	
OR (95% CI)	1.00	0.66(0.39-1.27)	0.37(0.17-0.67)	0.64(0.35-1.16)	OR=0.80; *p*=0.04
OR (95% CI)[§]	1.00	1.19(0.57-2.52)	0.39(0.18-0.89)	0.85(0.39-1.85)	OR=0.85; p=0.23
Lycopene					
#cases/controls	57/52	26/50	24/50	25/49	
OR (95% CI)	1.00	0.45(0.24-0.84)	0.43(0.23-0.80)	0.47(0.25-0.86)	OR= 0.77; *p*=0.0106
OR (95% CI)[§]	1.00	0.51(0.24-1.07)	0.45(0.20-1.01)	0.68(0.31-1.49)	OR=0.85; *p*=0.20
Retinol					
#cases/controls	34/51	25/50	38/50	35/50	
OR (95% CI)	1.00	0.65(0.39-1.43)	1.09(0.62-2.09)	0.93(0.49-1.74)	OR=1.03; *p=0.12*
OR (95% CI)[§]	1.00	0.43(0.18-1.00)	0.98(0.45-2.13)	0.89(0.41-1.96)	OR=1.04; *p*=0.76

[§] Adjusted for age, total pregnancy, frequency Pap smear, age at first intercourse,

number of partners, urban versus rural residence

3.3.6 Plasma carotenoids and retinol as quartiles with risk of progression of cervical cancer

Results for the risk of progression of carcinoma in situ to invasive disease using quartiles of plasma carotenoids and retinol are presented in Table 8. Compared with those in the lowest quartile of plasma, women with the highest levels of plasma carotenoids and retinol were not significantly associated with risk of invasive cervical cancer in the age-adjusted multivariate logistic regression model. Furthermore, the dose-response was not statistically significant. After further controlling for confounders: age, number of chest x-ray screenings and HPV, the lack of significant association continued between quartiles of plasma carotenoids and retinol and risk of progression of in situ carcinoma to invasive cervical cancer. We, therefore report no significant association between quartiles of the carotenoids and retinol with the risk of progression of in situ carcinoma to invasive disease.

Table 8. **Odds ratios (ORs) and 95% confidence intervals (CIs) of the progression of cervical cancer according to quartiles of plasma carotenoids and retinol**

Plasma	Q1	Q2	Q3	Q4	OR (CI) *p* for trend
α-carotene(nmol/L)					
#cases/controls	32/14	46/11	34/14	20/11	
OR (95% CI)	1.00	1.83(0.74-4.54)	1.06(0.44-2.57)	0.79(0.30-1.09)	OR=0.89; *p*=0.47
OR (95% CI)[§]	1.00	2.08(0.60-7..26)	1.10(0.34-3.58)	0.64(0.21-1.94)	OR= *p*=0.83;p=32
β-carotene (nmol/L)					
#cases/controls	47/13	25/12	22/13	38/12	
OR (95% CI)	1.00	0.58(0.23-1.45)	0.47(0.19-1.18)	0.88(0.36-2.14)	OR=0.94; *p*=0.64
OR (95% CI)[§]	1.00	0.36(0.11-1.24)	0.31(0.08-1.15)	0.46(0.13-1.60	OR=0.81; *p*=0.26
Lutein (nmol/L)					
#cases/controls	37/13	40/12	29/13	26/12	
OR (95% CI)	1.00	1.17(0.48-2.89)	0.78(0.32-1.95)	0.76(0.30-1.93)	OR=0.89; *p*=0.42
OR (95% CI)[§]	1.00	1.26(0.36-4.41)	0.99(0.32-3.06)	0.62(0.19-2.05)	OR=0.85;p=0.40
Zeaxanthin (nmol/L)					
#cases/controls	59/13	18/12	33/13	22/12	
OR (95% CI)	1.00	0.33(0.13-0.85)	0.56(0.23-1.35)	0.40(0.16-1.02)	OR=0.78;p=0.06
OR (95% C)[§]	1.00	1.09(0.31-3.73)	0.61(0.19-1.95)	0.74(0.23-2.33)	OR=0.86; *p*=0.44
β-cryptoxanthin(nm/L)					
#cases/controls	41/13	49/12	10./13	32/12	
OR (95% CI)	1.00	1.29(0.53-3.15)	0.24(0.08-0.69)	0.84(0.34-2.10)	OR=0.84; *p*=0.22
OR (95% CI)[§]	1.00	1.74(0.49-6.17)	0.61(0.18-1.99)	0.76(0.25-2.29)	OR=0.85; *p*=0.39
Lycopene (nmol/L)					
#cases/controls	56/14	27/12	28./12	21/12	
OR (95% CI)	1.00	0.56(0.23-1.38)	0.58(0.24-1.43)	0.44(0.17-1.09)	OR=0.77;*p*=0.07
OR (95% CI)[§]	1.00	0.77(0.24-2.47)	0.59(0.18-1.93)	0.61(0.18-2.01)	OR=0.84; *p*=0.36
Retinol (nmol/L)					
#cases/controls	39/13	31/12	27/13	35/12	
OR (95% CI)	1.00	0.86(0.35-2.15)	0.69(0.28-1.72)	0.97(0.39-2.41)	OR=0.97; *p*=0.83
OR (95% CI)[§]	1.00	1.96(0.55-7.02)	1.32(0.43-3.06)	1.42(0.43-4.64)	OR=1.08; *p*=0.66

[§] Adjusted for age, chest x-ray and HPV

We further examined the association between risk of invasive cervical cancer and β-carotene based on the concordant quartiles created from the FFQ and plasma measures. As shown in Table 8, women classified as high on both measures of exposure (quartile D) were at a significantly reduced risk of invasive cervical cancer (OR = 0.29 CI =0.09-0.98) compared to women who had low measures on both dietary and plasma β-carotene (quartile A) after adjusting for age, number of sexual partners, age at first intercourse, total pregnancy, frequency papanicolaous smears and HPV 16. We also found that, women classified as high on dietary intake and low on plasma level (quartile B) were at a lowest risk of invasive cervical cancer (OR = 0.28, CI =0.10-0.79). This risk reduction was considerably more pronounced compared to women who had low measures on both dietary and plasma β-carotene.

Table 9**. Adjusted ** odds ratios for invasive cervical cancer by dietary and Plasma indicators of β-carotene**

		DIET				
		Low			High		Odds ratio
PLASMA	No. of cases	No. of controls	odd ratios (95% CI)	No. of cases	No. of controls	Odd ratios (95% CI)	Plasma Only
Low	88	100	1.00§ **(A)**	69	111	0.28 (0.10-0.79) **(B)**	1.00§
High	44	101	0.53 (0.19-1.49) **(C)**	63	90	0.29 (0.09-0.98) **(D)**	0.94 (0.31-2.83)
Odds ratio Diet Only			1.00§			0.41 (0.13-1.29)	

** Adjusted for age, number of sexual partners, age at first intercourse, total pregnancy and frequency of papanicolaous smear, hpv16

§ Reference group

3.4 Discussion

The present study comprised of three analyses: 1) In situ cases versus clinic controls, 2) invasive cases versus hospital controls and 3) invasive cases versus in situ cases. In the third analysis, in situ cases were compared with invasive cases. This was the best model because women with invasive disease were directly compared to women with in situ disease without letting women with in situ progress to invasive prospectively.

In this study, there were no appreciable differences between in situ cases and controls with regard to plasma concentrations of any of the measured carotenoids or retinol. In a case-control study which included 147 squamous intraepithelial lesion (SILS) cases and 191 clinic controls, plasma levels of lycopene and α-cryptoxanthin were lower among cases than controls. In a population-based nested case-control study of 32 carcinoma in situ cases and 50 matched controls, Batieha et al. (1993) reported lower mean serum concentrations of α-carotene, β-cryptoxanthin and lycopene among cases than controls. In a cross-sectional study, Palan et al. (1996) noted lower levels of serum β-carotene and lycopene in cervical intraepithelial neoplasia (CIN) cases than in controls. The mean plasma levels for retinol were comparable among groups in the same study.

Inconsistent results in plasma or serum carotenoids and retinol concentration levels have also been observed among few studies that compared invasive cervical cancer cases with controls.

One of the studies was done by Harris et al. (1986) in which cases had lower levels for β-carotene compared with controls. In a study of 387 invasive cases and 670 controls in Latin America, the mean serum levels for retinol, cryptoxanthin, lycopene, α-carotene and lutein in cases did not differ significantly from controls. The mean level of beta-carotene was lower among invasive cases as compared with controls (Potischman, 1991). In this present study, invasive cervical carcinoma cases exhibited significantly higher levels of plasma lycopene compared with the control group and the levels for lutein were marginally significant among the invasive case group than the hospital control group. No significant difference in levels was observed for the other individual carotenoids and retinol between invasive cases and the control group. Potischman et al. (1991) found higher nutrient carotenoid levels among stage II cases as compared with stage I cases and pointed out the metabolic alteration in disease process as the reason. In our study, levels of certain carotenoids and retinol among in situ cases as compared with invasive cases were comparable (data not shown). A few carotenoids showed some fairly nonsignificant differences between the two case groups. This could suggest that mild metabolic alteration resulted from the disease process.

In this study, none of the plasma carotenoids and retinol was significantly associated with either risk of in situ or invasive cervical cancer or for the progression of cervical cancer from in situ to invasive disease after adjusting for confounders.

This study confirms the results of the majority of studies which reported no or nonsignificant association between dietary intake or serum carotenoids β-carotene and the risk of cervical cancer (Wideroff et al., 1998; Kanetsky et al., 1998; Schiff et al., 2001; Ho et al., 1998; Goodman et al., 1998; Nagata et al., 1999). Two studies (Potischman et al., 1991; Batieha et al., 1997) suggested a protective effect of β-carotene with cervical

neoplasia risk. With regard to HPV persistence, a weak and nonsignificant protective effect of dietary intake or serum concentration of β-carotene has been reported in three prospective studies (Giuliano et al., 1997; Sedjo et al., 2002; Giuliano et al., 2003).

Beta-cryptoxanthin has shown inverse association with cervical neoplasia in two studies (Batiaha et al., 1993). In a nested case-control study of women in Washington County, Maryland, Batieha et al. (1993) compared 50 cases who developed in situ carcinomas or invasive cervical cancer with two controls for each case and found that cryptoxanthin was significantly associated with a lower risk of cervical cancer when examined as a continuous variable.

With regard to lutein and zeaxanthin, a case-control study in Chicago involving 102 women with cervical intraepithelial neoplasia and 102 women with normal Pap smear reported no association between cervical neoplasia and dietary lutein as well as serum lutein concentrations (Van Eenwyk et al., 1991). Similarly, in Hawaii, Goodman et al. (1998) examined 147 multiethnic women with squamous intraepithelial lesions of the cervix and 191 clinic controls. The results indicated that plasma lutein was not significantly associated with risk of cervical dysplasia. In a survey in four Latin American countries, Potischman et al. (1994) found no association between serum lutein and invasive cervical cancer when 387 cases were compared with 670 controls.

With respect to α-carotene, two observational studies reported significant associations between high levels of α-carotene and neoplasia (Nagata et al., 1999; Schiff et al., 2001). Nagata et al. (1999) observed that high serum levels of α-carotene was significantly associated with decreased risk of cervical cancer after controlling for HPV infection and smoking status for the highest as compared to the lowest tertile (OR = 0.16, 95% confidence interval 0.04-0.62). Similarly, in Southwestern American Indian women,

increasing levels of α-carotene were associated with decreasing risk of cervical neoplasia. Regarding HPV persistence, none of the studies that examined the relation with α-carotene found a significant protective effect (Giuliano et al., 1997; Sedjo et al., 2002; Giuliano et al., 2003; Palan et al., 1998).

The possibility that lycopene may be protective against cervical cancer has been investigated in a number of studies. Results from serological studies have been fairly consistent, suggesting that higher serum lycopene levels are associated with reduced risk of cervical neoplasia or cervical cancer. After controlling for HPV and smoking, Nagata et al. (1999) reported a decreased risk for the highest tertile of serum lycopene, although it was marginally significant. However, there was no association between lycopene dietary intake and risk of HPV persistence in the same study.

With respect to retinol, this study observed no association between plasma retinol with either in situ or invasive cervical carcinomas or the progression of cervical neoplasia to invasive disease. Dietary intake studies of the association between retinol and risk of cervical cancer have reported conflicting results. Among the three observational studies (Kanetsky et al., 1998; Wideroff et al., 1998; Shannon et al., 2002) that looked retinol and risk of cervical neoplasia, two observed inverse association of retinol intake with cervical neoplasia. One (Shannon et al., 2002) of the two studies reported a strong significant protective effect of high-retinol foods on cervical neoplasia. However, one limitation of the study should be mention. High percent of the cases completed the food frequency questionnaire a year after the initial diagnosis allowing for potential recall biases and the influence of disease status on food intake. With respect to plasma or serum retinol and risk of cervical neoplasia, a protective effect was reported in two (Yeo et al., 2000; Nagata et al., 1999) of the five observational studies (Kanetsky et al., 1998;

Ho et al., 1998; Goodman et al., 1998; Nagata et al., 1999; Yeo et al., 2000), though it was nonsignificant. None of the studies that looked at the persistence of HPV showed a protective effect of serum or plasma retinol (Sedjo et al., 2002; Palan et al., 1998). A cohort study found an interaction between HPV 16 seropositivity and low serum retinol after adjustments (Lehtinen et al., 1999).

Very few case-control studies have investigated the progression of cervical cancer by directly comparing in situ carcinoma cases to invasive carcinoma cases in relation to carotenoids. One such case-control dietary intake study that investigated the risk of progression from in situ cervical carcinomas to invasive disease was done in Bangkok, Thailand (Shannon et al., 2002). The results from the study indicated that elevated intake of food rich in vitamin A, particularly those rich in retinol, was associated with a decrease in risk of in situ and the study suggested a possible decrease in the progression of disease to invasion. One prospective study reported that intake of retinol and serum retinol levels were 4.5 times lower among women with cervical cancer dysplasia who progressed to invasive disease as compared to those women whose disease regressed. Results from our study and previous studies suggest that retinol may play a role of protection against cervical cancer probably in the late stage of the disease and that it may also help in the inhibition of the progression of in situ cervical cancer to invasion.

It has been claimed that oxidative stress plays a role in various stages of the carcinogenesis and that by virtue of their antioxidant capacity, carotenoids afford cancer prevention. Carotenoids are efficient scavengers of singlet oxygen species, a reactive product of the oxidative stress. However, the activities of carotenoid against oxidative stress depend on the concentration and distribution of the compounds (Krinsky et al., 2004). This could be the reason as to why a number of studies have suggested low

plasma concentrations levels of the specific antioxidants in cases as compared to control group to be associated with advanced cervical cancer (Batieha et al., 1993; Goodman et al, 1998). In this study, no significant differences were found in the plasma levels of the carotenoids in cases compared to the controls. This finding could mean that cervical cancer cases did not exhibit depletion of plasma carotenoids and retinol. Thus, the lack of association between cervical cancer and plasma carotenoids in this study may simply reflect the fact that compared to controls, cases did not show any significant depletion in most of individual carotenoid levels induced by oxidative stress.

We further observed that women classified high for both measures of exposure for β-carotene were at reduced risk for invasive cervical cancer as compared to women classified low for both measures of exposure. In agreement with results from the present study, a number of epidemiological studies have shown that high β-carotene intake and high levels of circulating β-carotene in the blood are associated with reduced risk of cancer and cardiovascular diseases (Omenn et al., 1996; Block et al., 1992). The association between high intake of β-carotene and decreased risk of invasive cervical cancer was stronger than that of high plasma level of the β-carotene. Thus, long-term consumption of β-carotene probably other carotenoids, may be an important factor in the carcinogenesis of cervical cancer.

3.5 Strengths of the study

In the majority of epidemiological studies that examine the relationship between dietary carotenoids, and risk of cervical cancer, dietary β-carotene has been the focus. A limited number of studies have investigated the association between plasma or serum carotenoid concentrations and risk of cervical cancer. In addition, the majority of

previous studies concentrated on the early stage of cervical cancer, negating the invasive stage. The strength of the present study, therefore, lies in its inclusion of other carotenoids like lutein, β-cryptoxanthin, zeaxanthin, lycopene, α-carotene and retinol. Additional strength of the study is its ability to combine two different stages of cervical cancer: in situ carcinoma and invasive carcinoma.

In conclusion, this study suggests that although carotenoids and retinol have been related to risk for cervical cancer, results from this study does not support such an association. This could indicate that individual carotenoids may not be the factors responsible for the protective effect against the risk of cervical cancer. There are many food-derived phytochemicals in fruits and vegetables that may also contribute to the protection of cervical cancer from DNA damage (Collins et al., 1998). Therefore, a complex mixture of carotenoids and other phytochemicals in foods may be more effective at protection than individual carotenoids. Therefore, is it recommended that further research be conducted to look at the association of total carotenoids and phytochemicals found in foods consumed in Thailand to the risk of cervical cancer. There is also some indication in our study that long-term intake of β-carotene, possibly with other carotenoids, may reduce the risk of cervical carcinomas especially invasive cervical cancer. In light of this, long-term intervention efforts to increase consumption of fruits and vegetables may protect women from developing cervical cancer in Thailand. However, further studies are recommended to support this in Thailand.

CHAPTER 4

CONCLUSION AND LIMITATIONS OF THE STUDY

The possibility that antioxidants are involved in the prevention of the cervical cancer has generated a lot of attention in the field of research. There is a consensus among researchers that carotenoids as antioxidants play a major role in the defense against reactive oxygen produced during oxidative stress and hence, reduce the possibility of cervical cancer development and progression. As of now, however, the specific mechanism by which carotenoids and retinol may reduce risk and progression of cervical cancer risk has not been established. Furthermore, the specific stage at which carotenoid's biological activities may inhibit progression is unknown. Thus, determination of the mechanism and stage at which carotenoids and retinol may reduce risk and progression would be an important step toward understanding the carcinogenesis of cervical cancer.

Numerous studies conducted previously that have tempted to associate carotenoids with possible prevention in the development of cervical cancer relied on reported dietary intake assessed from the food frequency questionnaire (FFQ). However, as stated earlier on, the tool is associated with errors. In light of this, the first objective of the study was to validate the FFQ by comparing plasma carotenoids and retinol with fruit and vegetable groups as well as selected carotenoid dietary nutrients.

The study found that plasma carotenoids were moderately correlated with fruit and vegetable intakes as well as selected nutrients indicating that the FFQ used in a Bangkok case-control study is an acceptable tool for a long-term dietary intake. However, due to

the lack of a large sample size and the lack of a more representative group in this study, we recommend conducting another FFQ validation study using a sample size that is varied enough to represent the diverse population in Thailand.

Also, we investigated the associations between plasma levels of carotenoids β and α-carotene, lutein, zeaxanthin, β-cryptoxanthin, lycopene and retinol, and risk of in situ and invasive carcinoma as well as progression from in situ cervical carcinoma to invasive disease. In order to assess the risk of progression, women with in situ carcinoma were compared to women with invasive carcinoma. To our knowledge, this is the first study to examine cervical cancer by combining both stages of the disease, in situ and invasive in the same study population using plasma and dietary measures. Based on the multivariate models and after confounders were controlled for, the study did not find any association between individual carotenoids and risk of either in situ or invasive or the progression of carcinoma in situ to invasive disease.

There are a number of limitations in this study that need to be discussed because they may have contributed to the weak results especially that of the lack of association between carotenoids and retinol and risk of either in situ carcinoma or invasive carcinoma or progression of the cervical cancer to invasion. To begin with, plasma carotenoids are transported by cholesterol-rich lipoproteins. During analyses, carotenoids are adjusted for plasma lipid levels due to the fact that the higher the concentration of lipoprotein is higher the concentration for plasma carotenoids. However, due to unavailability of plasma lipid levels in our study, the adjustment was not performed. This lack of adjustment may have introduced some random errors into exposure measurements and attenuate the result towards the null. However, others who adjusted for lipids reported little effect on their findings.

Beside, plasma carotenoids analyzed in this study were based on a single sample of blood. Consequently, if a single measurement is used, nondifferential misclassification of the women may occur which attenuate estimates of disease could risk towards the null.

In addition, a single measurement of a biomarker has been related to within – person variability which may introduce random error and attenuate estimates of association (Cantilena et al.,1992; Gibson, 2005; Block et al.,2006). This imprecise single measurement can be avoided by using two or more measurements per person to increase the ability to detect associations between biomarkers and disease.

Also, it is important to note that this study had a small sample size, which may have resulted in losing power and attenuating the carotenoid and disease relationship towards the null due to nondifferential misclassification (Szeklo et al., 2004)

Moreover, the blood samples used in this study were collected between 1991 and 1993. There is a possibility that long –term freezer storage may reduce carotenoid concentration to an extent of not being able of detecting the right amount of the carotenoids. Review on the effect of the carotenoid concentrations reveals that length of storage does affect carotenoids content in plasma (Comstock et al., 1993). This could be a serious problem especially when samples are stored at temperatures warmer than -70°C and are subjected to repeated defrosting and refreezing. Review of literature relevant to long-term storage effects on micronutrients reveal that concentrations of micronutrients in plasma appear to be stable at -70°C or colder for more than 15 years (Comstock et al., 1993). However, the samples for this study were stored at -70°C, thus we do not expect carotenoid to have been degraded much by the length of time frozen.

Again, this study is limited by the way blood samples were collected from the participants. In the present study, blood was drawn without fasting. Literature indicates that fasting blood samples are preferred for carotenoids analysis because intake of a meal prior to blood drawing may affect nutrient levels (Gibson, 2005).

It is worth to note that there were high correlations among the carotenoids we measured, such as lutein and zeaxanthin (r = 60). These high correlations made it difficult to isolate the effect of one individual carotenoid compound, thus limiting our conclusion about the effect of individual carotenoids on the risk of cervical cancer (Schiff et al., 2001).

Despite these limitations in our study, we still concur with the other investigators that fruits and vegetables do have a role in the development and progression of cancer, probably that of the cervix too. However, other protective compounds in fruits and vegetables independent of or in combination with the carotenoids may exert the protective effect against cervical carcinogenesis. Right now, investigations are going on the impact of these other compounds on cancer. In summary, we found that plasma carotenoids were moderately correlated with fruits and vegetables and weakly correlated with selected nutrients from fruits and vegetables as estimated from the FFQ. We also found that there was no significant association between plasma carotenids and rertinol with either in situ carcinoma or invasive carcinoma or the progression of cervical cancer to invasion.

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APPENDICES

BANGKOK STUDY OF PAPILLOMA VIRUSES AND CERVICAL CANCER

DIETARY QUESTIONNAIRE DV(1) = Universal

Study Number:

1. Study number ___ ___ - ___ ___ ___ ___
DV(2) DV(3)

2. Name of subject:

_____(family)/ _____(given) DV(4) = RT

_____(others)

(Read the following paragraphs to the woman:)

We would like to find out about your usual eating habits one year before you developed the problem that resulted in your coming to the hospital/clinic. To help you remember them, think back to where you were living then, what your daily routine was, how often you ate meals outside your home, and any special diets you may have followed. It may be difficult to remember some of this information, but please just do the best you can.

I am going to ask you how many times you ate certain foods or groups of foods. Think about how often you ate them, not only raw, but when they were steamed, stir fried, in soup, or curried. You may give your answer as the number of times per day, per week, or per month. When I ask you about a group of foods (such as cauliflower or Brussels sprouts), please think about the number of times you ate each food in that group and add them together. If you ate a food item for only part of the year because the food is seasonal, please answer how many times you ate the food when it was in season.

Type of Food	Average Use							
	Number of Times	Never or less than once per month (1)	Per Month (2)	Per Week (3)	Per Day (4)	No.	Freq.	
Main Dishes								
Glutinous rice						DV(5)	DV(6)	3.1
Cauliflower or Brussels sprouts						DV(7)	DV(8)	3.2
Collards, leaves and stems						DV(9)	DV(10)	3.3
Cabbage, Chinese cabbage or mustard greens						DV(11)	DV(12)	3.4
Celery cabbage						DV(13)	DV(14)	3.5
Chinese leek or crowdaisy leaves						DV(15)	DV(16)	3.6
Carrot						DV(17)	DV(18)	3.7
Lettuce						DV(19)	DV(20)	3.8
Green Onion						DV(21)	DV(22)	3.9
Kidney beans or hyacinth beans (young pods and immature beans, cooked)						DV(23)	DV(24)	3.10
Yard-long beans, green or red; or winged beans, young pod						DV(25)	DV(26)	3.11
Broad beans or sugar peas (young pods)						DV(27)	DV(28)	3.12
Swamp cabbage, chinese						DV(29)	DV(30)	3.13
Swamp cabbage, white stem						DV(31)	DV(32)	3.14
Tomato, ripe						DV(33)	DV(34)	3.15
Tomato, cherry						DV(35)	DV(36)	3.16
Eggplants						DV(37)	DV(38)	3.17
Angled-type gourd, fruit						DV(39)	DV(40)	3.18
Snake gourd						DV(41)	DV(42)	3.19
Waxgourd						DV(43)	DV(44)	3.20
Bitter melon, Thai						DV(45)	DV(46)	3.21
Okra, fruit						DV(47)	DV(48)	3.22
Roselle, red sorrel, leaves						DV(49)	DV(50)	3.23
Leadtree leaves or young mango leaves						DV(51)	DV(52)	3.24
Cassia, sesbania, taro, or Indian mulberry leaves						DV(53)	DV(54)	3.25
Young tamarind leaves						DV(55)	DV(56)	3.26
Neem or vinespinach leaves						DV(57)	DV(58)	3.27
Horseradish leaves, Sauropus androgynus, or amaranth (spineless leaves and stems)						DV(59)	DV(60)	3.28
Ivygourd or chili pepper leaves, or cha om						DV(61)	DV(62)	3.29
Bracken, fern fronds, tender						DV(63)	DV(64)	3.30
Indian penny wort leaves or Piper sarmentosum						DV(65)	DV(66)	3.31
Sweet potato, leaves and tender tips						DV(67)	DV(68)	3.32
Squash, young leaves						DV(69)	DV(70)	3.33
Chili pepper (small var.)						DV(71)	DV(72)	3.34
Chili pepper, green						DV(73)	DV(74)	3.35
						DV(75)	DV(76)	3.36

Type of Food	Average Use							
	Number of Times	Never or less than once per month (1)	Per Month (2)	Per Week (3)	Per Day (4)	No.	Freq.	
Snacks								
Pigeon pea, seeds, fresh						DV(149)	DV(150)	7.1
Peanut, boiled or roasted						DV(151)	DV(152)	7.2
Jackfuit, watermelon, durian, or pumpkin seeds						DV(153)	DV(154)	7.3
Roselle, red sorrel, fruits						DV(155)	DV(156)	7.4
Mango, raw						DV(157)	DV(158)	7.5
1. On average, how many times did you eat yellow sweet potatoes prepared in a main dish, or as a dessert, or as a snack?						DV(159)	DV(160)	8.1
2. On average, how many times did you eat yellow corn prepared in a main dish, or as a dessert, or as a snack?						DV(161)	DV(162)	8.2
On average, how many times did you eat squash prepared in a main dish or in a dessert?						DV(163)	DV(164)	8.3

9.1. *Continue to think about your usual eating habits one year before you developed the problem that resulted in your coming to the hospital/clinic.* We would like to know how many vegetables in total you ate per day. For example, if in a typical day, you usually ate noodle soup with bean sprouts in the morning, a stir fry with cauliflower, cabbage, tomato, yard long beans, and green onion at mid-day, and steamed squash, steamed yard-long beans, and cucumber with dipping sauce in the evening, you would answer that you ate nine vegetables per day. Notice that since yard-long beans were eaten twice during the day, they are counted two times.

So, <u>on average</u>, how many vegetables did you eat per day?

9.2. *Now think about the number of times you ate fruit in a typical day. For example, if you usually ate a banana in the morning and an orange in the evening, you would answer that you ate two fruits. If you ate a banana in the morning and another banana in the evening, you would also answer that you ate two fruits.*

So, <u>on average</u>, how many fruits did you eat per day?

10.1. Are your current dietary habits different from the usual dietary habits that you had before developing the problem that resulted in your coming to the hospital/clinic? (Do not include changes resulting from eating hospital foods)

(If no, code 0 and if unknown code 99 in 10.2 and go to question 11)

____ no (0) ____ yes(1) ____ unknown (9) DV(167)

10.2. (If yes, ask:) About how long ago did you change your diet?

_____ months ago DV(168)

10.3. What has changed?

11.1. Again, think back to one year before you developed the problem that resulted in your coming to the hospital/clinic. Did you take any vitamins before that time? (Do not count vitamins given to the woman when she was a child)

_____ no (0) _____ yes (1) DV(169)

(If no, skip Q 11.2, draw a line through the table in Q 12 and go to Q 13.)

11.2. (If yes), please tell me all the different kinds of vitamins you took. (Record the woman's answer on the lines below and for each kind reported, code 1 in Q 12.1 and ask Q 12.2 - Q 12.5 in the corresponding row in the table in Q 12. Use 9's for unknown answers in Q 12.2 - Q 12.5. Only code a 1 for those vitamins that the woman said she took. Leave the rest of the table in Q 12 blank.)

_____

_____

Have you taken any vitamins in the last month?

_____ no (0) _____ yes (1) DV(242) ___13

(If no, draw a line through Q 13.1 - Q13.9 below and go to Q 14.)

(If yes) Which kinds? (Mark all responses.)

		No (0)	Yes (1)	
13.1	Multivitamins	____	____	DV(243)___ 13.1
13.2.	Vitamin C	____	____	DV(244)___ 13.2
13.3.	Vitamin E	____	____	DV(245)___ 13.3
13.4.	Vitamin A	____	____	DV(246)___ 13.4
13.5.	Vitamin B complex	____	____	DV(247)___ 13.5
13.6.	Cod liver oil	____	____	DV(248)___ 13.6
13.7.	Folate	____	____	DV(249)___ 13.7
13.8.	Other known type (Specify)_____	____	____	DV(250)___ 13.8
13.9.	Unknown type	____	____	DV(251)___ 13.9

14. (Record any vitamins the woman is taking which are written on the hospital or clinic chart. If unable to review chart, code 9's in Q 14.1 - Q 14.9) DV(252) ___

		No (0)	Yes (1)	Unable to Review Chart (9)	
14.1.	Multivitamins	____	____	____	DV(253)___ 14.1
14.2.	Vitamin C	____	____	____	DV(254)___ 14.2
14.3.	Vitamin E	____	____	____	DV(255)___ 14.3
14.4.	Vitamin A	____	____	____	DV(256)___ 14.4
14.5.	Vitamin B complex	____	____	____	DV(257)___ 14.5
14.6.	Cod liver oil	____	____	____	DV(258)___ 14.6
14.7.	Folate	____	____	____	DV(259)___ 14.7
14.8.	Other known type (Specify)_____	____	____	____	DV(260)___ 14.8
14.9.	Unknown type	____	____	____	DV(261)___ 14.9

(Record height and weight from hospital or clinic chart. If not available, attempt to weigh and measure the woman. If this is not possible,ask her and record her answer.)

15.1. Height _____ cm. DV(262) ___ 15.1

15.2. Weight _____ kg. DV(263) ___ 15.2

15.3. Obtained from:

_____ chart (1)

_____ taken at interview (2)

_____ self-reported (3) DV(264) ___ 15.3

Thank you for your participation in this study.

INTERVIEW

GENERAL INFORMATION

I would like to start by asking you a few general questions about you and your background.

14 When were you born? (PROBE)	day / month / year	14
	V(28) / V(27) / V(29)	
15. That means you are how old at your last birthday?	Years V(30)	15
15.1 (Record accuracy of estimate of date of birth.	V(31)	15.1

0 = exact date of birth known

1 = year of birth determined

2-8 = year of birth believed by interviewer to have been estimated to within ± 2, 3 ... 8 years of actual year

9 = year of birth believed to have been estimated to no closer than ± 9 years of actual year)

16. Where were you born? _____ V(32)__
(name of country or locality)

(1 = city; 2 = town; 3 = rural area; 4 = foreign born)

MEDICAL HISTORY

Now I am going to ask you some questions about illnesses that you may have had in the past, and the kinds of treatments that you may have received.

17. First, I am going to read a list of diseases to you. Please tell me whether you have ever had any of them [before you developed the problem that brought you into the hospital now]. (For healthy controls, omit the portion of the last sentence in square brackets):

	No(0)	Yes(1)	DK(9)		
A). high blood pressure	___	___	___	V(34)	17A
B). heart trouble or heart disease	___	___	___	V(35)	17B
C). blood clots in your legs (thromboembolism)	___	___	___	V(36)	17C
D). stomach ulcer	___	___	___	V(37)	17D
E). stroke	___	___	___	V(38)	17E
F). diabetes	___	___	___	V(39)	17F
G). cancer	___	___	___	V(40)	17G
H). gallbladder disease or gallstones	___	___	___	V(41)	17H
I). jaundice during pregnancy	___	___	___	V(42)	17I
J). jaundice at any other time	___	___	___	V(43)	17J
K). tuberculosis	___	___	___	V(44)	17K
L). ovarian cyst	___	___	___	V(45)	17L

(If answer to G is No, code 0s and if DK, code 9s in Q17.1, 17.2 and 17.5, and go to Q18)

17.1 What kind of cancer did you have?_____ V(46)__ __ 17.1
(use WHO ICD-9 code) (site or type)

17.2 When did you first learn that you had that cancer?_____ V(47)__ __ 17.2
(98 = unknown year) (year) Year

17.3 Where and when were you treated for this condition?_____

_____

17.4 Validation_____

17.5 Source of information coded in Q17.1 and Q17.2 ..... V(48)__ 17.5
(code 1 = interview only, 2 = validated from medical records)

Now I would like to ask you about any operations that you may have had [prior to this hospitalization]. PROBE

	Answer	When (Yr)	Where was this done?	Validation (diagnosis, operation, year & Path No.)
18. Have you ever had an operation to prevent you from becoming pregnant, such as a tubal ligation or your tubes tied?	___ no ___ yes ___ DK	19 __		
19. Have you ever had an operation to remove your womb or uterus? (If no or DK, go to Q20.)	___ no ___ yes ___ DK	19 __		
19.1 Reason _____				
19.2 Did they take out either of your ovaries at that time? (If no or DK, go to Q20.)	___ no ___ yes ___ DK			
19.3 How many? _____				
20. Have you ever had any [other] operation to remove either of your ovaries? (If no or DK, go to Q21.)	___ no ___ yes ___ DK	19 __		
20.1 How many ovaries were removed then? _____				
20.2 Reason _____				
21. Have you ever had an operation to scrape out and examine the lining of your womb? This is sometimes called a "D&C" or dilatation and curettage. (If no or DK, go to Q22.)	___ no ___ yes ___ DK	first ____ year last ____ year		
21.1 How many? _____				
21.2 Reason(s) _____				
22. Have you ever had an operation on your tubes because you were unable to have a baby (tubal surgery for infertility)?	___ no ___ yes ___ DK	19 __		

	Answer	When (Yr)	Where was this done?	Validation (diagnosis, operation, year & Path No.)
23. Have you ever had a baby delivered by Caesarian section (include hysterotomy for third trimester abortion)? (If no or DK, go to Q24.) 23.1 How many times? _____	___ no ___ yes ___ DK	first ____ year last ____ year		
24. Have you had any other operations on your female organs? (If no or DK, go to Q25.) 24.1 Reason(s) _____	___ no ___ yes	19 __		
25. Have you had any other operations on your abdomen or stomach? (If no or DK, go to Q26.) 25.1 Reason(s) _____	___ no ___ yes	19 __		

(Code the following:)

A) Tubal ligation:

1) 0 = No, 1 = Yes, 9 = Unknown ..... V(49)__ A1

2) year of tubal ligation (00 = no tubal ligation, 98 = unknown year, 99 = unknown if had tubal ligation) ..... V(50) Year __ __ A2

4) source of information (1 = interview only, 2 = validated, 9 = no information) ..... V(51)__ A4

B) Hysterectomy:

1) 0 = No, 1 = Yes, 9 = Unknown ..... V(52)__ B1

2) year of hysterectomy (00 = no hysterectomy, 98 = unknown year, 99 = unknown if had hysterectomy) ..... V(53) Year __ __ B2

3) reason (0 = no hysterectomy, 1 = benign condition, 2 = cancer, 8 = unknown reason, 9 = unknown if had hysterectomy) ..... V(54)__ B3

4) source of information (1 = interview only, 2 = validated, 9 = no information) ..... V(55)__ B4

C) Oophorectomy:

1) number of ovaries removed (0 = none, 1 = one, 2 = two, 8 = one or two but number unknown, 9 = unknown if ovaries removed) ..... V(56)__ C1

2) year of most recent oophorectomy (00 = no oophorectomy, 98 = unknown year, 99 = unknown if had oophorectomy) ..... V(57) Year __ __ C2

3) Reason for oophorectomies (0 = no oophorectomy, 1 = benign ovarian condition, 2 = ovarian cancer, 3 = both, 4 = above uterine condition, 5 = other, 8 = unknown reason, 9 = unknown if had oophorectomy) ..... V(58)__ C3

4) source of information (1 = interview only, 2 = validated, 9 = no information) ..... V(59)__ C4

D) Dilatation and curettage:

1) number of D and Cs (0 = none, 1 = one, 2 = two, ... 6 = six, 7 = seven or more, 8 = unknown number, 9 = unknown if had D and Cs) ..... V(60)__ D1

2) reasons (0 = no D and C, 1 = for induced or after spontaneous abortion, 2 = diagnosis of previous uterine cancer, 3 = both, 4 = benign uterine condition such as hyperplasia or polyp, 5 = 1 and 4, 6 = 2 and 4, 7 = 1 and 2 and 4, 8 = unknown reason only, 9 = unknown if had D and C) ..... V(61)__ D2

4) source of information (1 = interview only, 2 = validated, 9 = no information) ..... V(62)__ D4

E) Tubal surgery for infertility:

1) 0 = No, 1 = Yes, 9 = Unknown ..... V(63) __ E1

2) year of operation (00 = no surgery for infertility, 98 = unknown year, 99 = unknown if had surgery for infertility). ..... V(64) __ __ (Year) E2

4) source of information (1 = interview only, 2 = validated, 9 = no information) ..... V(65) __ E4

F) Caesarian sections (including hysterotomies):

1) code number of caesarian sections (0 = none, 1 = one, ... 7 = seven or more, 8 = unknown number, 9 = unknown if ever had caesarian section) ..... V(66) __ F1

4) source of information (1 = interview only, 2 = validated, 9 = no information) ..... V(67) __ F4

G) Other operations on female organs:

(from item 24.1) 0 = no, 1 = yes, 9 = unknown ..... V(68) __ G1

H) Other abdominal operations: 0 = No, 1 = Yes, 9 = unknown ..... V(69) __ H1

REPRODUCTIVE HISTORY

27. How old were you when you first began to menstruate? (PROBE)..... V(75) ___ ___ Years 27

28. Are you pregnant now? ___ no (0) ___ yes (1) ___ DK (9)..... V(76) ___ 28

29. Now I would like to ask you about any <u>previous</u> pregnancies that you may have had. I would like to know about any time that you were pregnant, even if the pregnancy did not end with the birth of a live baby. Have you ever had any type of pregnancy? (PROBE)

___ no (0) ___ yes (1) ___ DK (9)..... V(77) ___ 29

(If no, code 0s and if DK code 9s in items A-R following the table, and in Q30 and Q30.1, and go to Q31).

Let's begin with your first pregnancy (PROBE and complete the following table):

(1) Pregnancy No.	(2) How old were you when this pregnancy ended? (years)	(3) Did this pregnancy end with the birth of a live baby? (If not ask How did it end?) (PROBE)*	(4) Were you given pills or injections to help you to keep from losing your baby?	(5) For how many months did you breast feed the child?	(6) Were you given any pills or injections to dry up your breast milk?
1					
2					
Total					

* Record outcome of each pregnancy as follows:

live birth	abortion, unknown type
stillbirth	ectopic pregnancy (tubal pregnancy)
miscarriage (spontaneous abortion)	hydatidiform mole
induced abortion	unknown (unknown outcome)

30. (If the first pregnancy ended in a live birth ask) What was the date of birth of your first child..... day ___ month ___ year ___ 30
V(97) V(96) V(98)

(Code 888888 if first pregnancy did not end with a live birth)

30.1 Was it a boy or a girl? (1 = boy, 2 = girl, 3 = twins of opposite sex, 7 = first pregnancy did not end in a live birth, 8 = unknown sex..... V(99) ___ 30.1

31. Are you currently nursing [breast feeding] a child?

___ no (0) ___ yes (1) ___ DK (9)..... V(100) ___ 31

(If yes, code 66 in Q29Q and go to Q32.)

32. Have you ever tried for over a year to become pregnant but were unable to become pregnant?

___ no (0) ___ yes (1) ___ DK (9)..... V(101) ___ 32

(If no, code 0s and if DK, code 9s in Q32.1 to 32.3 and go to Q33)

32.1 How old were you when you first had this problem? (98 = unknown age)..... V(102) ___ Years ___ 32.1

32.2 Did you ever consult a doctor about this problem?

___ no (0) ___ yes (1) ___ DK (9)..... V(103) ___ 32.2

32.3 How many years was it before you finally got pregnant?..... V(104) ___ Years ___ 32.3

(Code: 97 = the woman never did get pregnant after trying,
98 = unknown number of years)

33. Have you ever had an operation or X-ray treatment that stopped you from having any more menstrual periods?..... V(105) ___ 33

___ no (0) ___ operation (1) ___ X-rays (2) ___ DK (9)

(If answer is operation, check to see if details are recorded in Q19-20.2. If answer is no or operation, code 0s, and if DK, code 9s in Q33.1, 33.2, 33.5, and 33.6 and go to Q34.)

33.1 Why did you receive the X-ray treatments?..... V(106) ___ 33.1

___ cancer (1) ___ other reason (2) _____ (specify)

___ unknown reason (6)

(If cancer, check to see that this is recorded in Q17-17.5.)

33.2 When did you have these treatments? _____ (year)
(98 = unknown year) ..... Year V(107)__ __ 33.2

33.3 Where did you receive these treatments? _____

_____

_____

33.4 Validation: _____

_____

_____

33.5 Source of information coded in Q33
(Code 1 = interview only, 2 = validated from medical records) ..... V(108)__ 33.5

33.6 Source of information coded in Q33.1 and 33.2
(Code 1 = interview only, 2 = validated from medical records) ..... V(109)__ 33.6

34. [Before you developed the problem that brought you to the hospital/clinic] *have you ever had any [other] X-ray or radiation treatments?* ..... V(110)__ 34

__ no (0) __ yes (1) __ DK (9)

(If no, code 0 and if DK, code 9 in Q34.1 and go to Q35.)

34.1 *What was the reason for these treatments?* (PROBE) ..... V(111)__ 34.1

__ Tinea (1)

__ Acne (2)

__ Enlarged tonsils (3)

__ Thyroid enlargement (goitre) (4)

__ Previous cancer (5) _____

__ Other (6) _____ (specify)

__ Unknown reason (8)

(If previous cancer, check to see that the details are recorded in Q17-17.5.)

35. When was the last time that you had a menstrual period? month year
(99) = unknown month or year) ..... V(112)__/__ V(113) 35

35.1 How old were you then? Years
(99 = unknown) ..... V(114)__ __ 35.1

CONTRACEPTIVE HISTORY

36. Many different methods of birth control are used by women or by their male partners to keep the woman from getting pregnant. I am going to name some of these methods, and I want you to please tell me whether you have ever used any of them.

	No(0)	Yes(1)	DK(9)		
A) Oral contraceptives or the "pill"	___	___	___	V(115)	36A
B) Injectable contraceptives	___	___	___	V(116)	36B
C) IUD or loop	___	___	___	V(117)	36C
D) Implantations under the skin	___	___	___	V(118)	36D
E) Vaginal ring	___	___	___	V(119)	36E
F) The safe period or rhythm method	___	___	___	V(120)	36F
G) A diaphragm	___	___	___	V(121)	36G
H) Condom by your partner	___	___	___	V(122)	36H
I) Withdrawal	___	___	___	V(123)	36I
J) Jelly or foam	___	___	___	V(124)	36J
K) Douching after intercourse	___	___	___	V(125)	36K
L) Your partner having a vasectomy or sterilization procedure	___	___	___	V(126)	36L
M) Having your tubes cut or tied or other sterilization procedure (Does answer correspond to response in Q18?)	___	___	___	V(127)	36M
N) Any other method_____ (specify)	___	___	___	V(128)	36N

36.1 Have you ever taken oral contraceptives for any reason other than for birth control? ___ no, ___ yes, ___ DK (if yes, code 1 in Q36A).

If answer to:	is:	then:
36A	yes(1)	complete Q37.1-Q37.8
36A	no (0)	draw a line through table on page 14-15 and code 0s in Q37.7-37.8
36A	DK (9)	draw a line through table on page 14-15 and code 9s in Q37.7-37.8
36B	yes(1)	complete Q38.1-Q38.10
36B	no (0)	draw a line through table on page 16 and code 0s in Q38-8-38.10
36B	DK (9)	draw a line through table on page 16 and code 9s in Q38.8-38.10
36C	yes(1)	complete Q39.1-Q39.8
36C	no (0)	draw a line through table on page 18 and code 0s in Q39.7-39.8
36C	DK (9)	draw a line through table on page 18 and code 9s in Q39.7-39.8

(Code the following:)

A) Age at end of first pregnancy (98 = unknown age) ..... V(78) __ __ Years 29A

B) Age at first live birth (97 = previously pregnant but no live birth, 98 = unknown age) ..... V(79) __ __ Years 29B

C) Age at last (most recent) pregnancy (98 = unknown age) ..... V(80) __ __ Years 29C

D) Age at first breast feeding (97 = previously pregnant but never breast fed, 98 = unknown age) ..... V(81) __ __ Years 29D

E) Outcome of first pregnancy (1 = live birth, 2 = stillborn, 3 = miscarriage, 4 = induced abortion, 5 = abortion, unknown type, 6 = ectopic pregnancy, 7 = hydatidiform mole, 8 = unknown outcome). ..... V(82) __ 29E

F) Number of known live births ..... V(83) __ __ 29F

G) Number of known stillbirths ..... V(84) __ __ 29G

H) Number of known miscarriages (spontaneous abortions) ..... V(85) __ __ 29H

I) Number of known induced abortions ..... V(86) __ __ 29I

J) Number of known abortions of any kind ..... V(87) __ __ 29J

K) Number of known ectopic pregnancies ..... V(88) __ __ 29K

L) Number of known hydatidiform moles ..... V(89) __ __ 29L

M) Total number of known pregnancies ..... V(90) __ __ 29M

N) Number of pregnancies during which pills or injections were known to have been given for maintenance of pregnancy ..... V(91) __ __ 29N

O) Number of pregnancies after which child was known to have been breast fed ..... V(92) __ __ 29O

P) Total known months of breast feeding ..... V(93) __ __ __ 29P

Q) Number of years since woman last breast fed (66 = less than 1 year, 01 = from one up to but not including two years, 02 = from two up to but not including three years, etc., 77 = never breast fed, 88 = unknown number of years since last breast fed, 99 = unknown whether ever breast fed) ..... V(94) __ __ 20Q

R) Number of pregnancies after which pills or injections were known to have been given to suppress lactation (0 = none, 1 = one, 2 = two, etc., 8 = eight or more) ..... V(95) __ 29R

	37.1	37.2	37.3	37.4	37.5	37.6
Periods of use (begin with first period)	When was the 1st [2nd, etc] time you began taking birth control pills?	What kind of pills were they? (Record brand name)	Where did you get these pills? (Record exact source)	For how long did you take them? (months)	That means that you stopped taking them about when?	Validation (0 = no 1 = yes)
7	month year __ __/__ __ 37.1.7	__ __ __ 37.2.7		__ __ 37.4.7	month year __ __/__ __ 37.5.7	__ 37.6.7
8	month year __ __/__ __ 37.1.8	__ __ __ 37.2.8		__ __ 37.4.8	month year __ __/__ __ 37.5.8	__ 37.6.8
9	month year __ __/__ __ 37.1.9	__ __ __ 37.2.9		__ __ 37.4.9	month year __ __/__ __ 37.5.9	__ 37.6.9
10	month year __ __/__ __ 37.1.10	__ __ __ 37.2.10		__ __ 37.4.10	month year __ __/__ __ 37.5.10	__ 37.6.10

RT 1

37.7 [Before you developed the problem that brought you to the hospital/clinic] did a doctor ever advise you to stop taking oral contraceptives? ..... V(134)__ 37.7

__ no (2) __ yes (1) __ DK (8)

37.8 (Code whether women used oral contraceptives within one month prior to the date in Item 10. Code 0 = no, 1 = yes, 9 = DK.) ..... V(135)__ 37.8

V(136) = Inj. start month
V(137) = Inj. start year
V(138) = Inj. end month
V(139) = Inj. end year
V(140) = Total mgs.
V(141) = Inj. total months

38. (Ask only of women who have ever received injections for birth control.)
I would like to ask you more about the injections that you received to keep from getting pregnant. Sometimes women take injections for a while, then stop to get pregnant or for other reasons, and then take them again. We want to know about each period of time that you had injections. (Ask Q.38.1 to 38.6 for each period of time injections were received. PROBE. Use calendar. See instructions for codes.)

	38.1	38.2	38.3	38.4	38.5	38.6	38.7
Period of use (begin with first period)	When was the 1st [2nd, etc] time that you began receiving injections?	What was the name of the injection? (record brand)	Where did you get these injections? (record source)	What dose did you receive? How often did you receive an injection?	How many injections did you receive?	About when did you stop getting these injections?	Validation? (0 = no 1 = yes)
1	month/year __ __/__ __ V(175) V(176) 38.1.1	V(177) 38.2.1		mgs __ __ V(178) Every __ __ V(179) (months) 38.4.1	__ __ V(180) 38.5.1	month/year __ __/__ __ V(181) V(182) 38.6.1	__ V(183) 38.7.1
2	month/year __ __/__ __ 38.1.2	__ 38.2.2		mgs __ __ __ Every __ __ (months) 38.4.2	__ __ 38.5.2	month/year __ __/__ __ 38.6.2	__ 38.7.2
3	month/year __ __/__ __ 38.1.3	__ 38.2.3		mgs __ __ __ Every __ __ (months) 38.4.3	__ __ 38.5.3	month/year __ __/__ __ 38.6.3	__ 38.7.3
4	month/year __ __/__ __ 38.1.4	__ 38.2.4		mgs __ __ __ Every __ __ (months) 38.4.4	__ __ 38.5.4	month/year __ __/__ __ 38.6.4	__ 38.7.4
5	month/year __ __/__ __ 38.1.5	__ 38.2.5		mgs __ __ __ Every __ __ (months) 38.4.5	__ __ 38.5.5	month/year __ __/__ __ 38.6.5	__ 38.7.5

38.8 A) When you were receiving these injections were you ever given pills to bring on a menstrual period?

___ no (2) ___ yes (1) ___ unknown (8) V(142)__ 38.8A

B) Validation _____

C) Source of information in A)
(1 = validation, 2 = interview only) ..... V(143)__ 38.8C

38.9 [Before you developed the problem that brought you into the hospital/clinic this time] did a doctor ever advise you to stop getting injections for birth control?

___ no (2) ___ yes (1) ___ unknown (8) V(144)__ 38.9

38.10 (Code whether the woman had an injection within the three months prior to the date in Item 10. Code 0 = no, 1 = yes, 9 = DK). ..... V(145)__ 38.10

V(146) = IUD start month
V(147) = IUD start year
V(148) = IUD end month
V(149) = IUD end year
V(150) = IUD total months

39. (Ask only of women who ever had an intrauterine device.)
I would like to ask you more about your use of IUDs. Sometimes a woman may use one for a while, then it may accidentally come out or the woman may have it removed for some reason, and later she may use one again. I want know about each time that you had an IUD. (Ask Q39.1 to 39.5 for each period of IUD use. PROBE. Use calendar. See instruction codes.)

	39.1	39.2	39.3	39.4	39.5	39.6
Periods of use (begin with first period)	When was the 1st [2nd, etc] time you had an IUD put in?	What kind of IUD was it?	Where did you get the IUD? (Record source)	For how long did you wear it? (months)	About when then, did you stop wearing it?	Validation (0 = no 1 = yes
1	month year __ __/__ __ V(190) V(191) 39.1.1	__ __ V(192) 39.2.1		__ __ V(193) 39.4.1	month year __ __/__ __ V(194) V(195) 39.5.1	V(196) 39.6.1
2	month year __ __/__ __ 39.1.2	__ __ 39.2.2		__ __ 39.4.2	month year __ __/__ __ 39.5.2	__ 39.6.2
3	month year __ __/__ __ 39.1.3	__ __ 39.2.3		__ __ 39.4.3	month year __ __/__ __ 39.5.3	__ 39.6.3
4	month year __ __/__ __ 39.1.4	__ __ 39.2.4		__ __ 39.4.4	month year __ __/__ __ 39.5.4	__ 39.6.4
5	month year __ __/__ __ 39.1.5	__ __ 39.2.5		__ __ 39.4.5	month year __ __/__ __ 39.5.5	__ 39.6.5

39.7 [Before you developed the problem that brought you to the hospital/clinic this time] did a doctor ever advise you to stop using an IUD?

___ no (2) ___ yes (1) ___ DK (8) V(151)___ 39.7

39.8 (Code whether the woman wore an IUD within one month prior to the date in Item 10. Code 0 = no, 1 = yes, 9 = DK) ..... V(152)___ 39.8

40. Have you ever been advised by a doctor to not begin using:

	No(0)	Yes(1)	DK(9)	
40.1 oral contraceptives?	___	___	___	V(153)___ 40.1
40.2 injectable contraceptives?	___	___	___	V(154)___ 40.2
40.3 an IUD?	___	___	___	V(155)___ 40.3

V(197) = Universal ID
NV(197) = Subj. Type
V(200) = Subj. ID
V(201) = RT

RT5

OTHER HORMONES AND MEDICATION

41. Female hormones are sometimes given in the form of pills or injections to test whether a woman is pregnant, or to cause a pregnancy to end before a baby develops. Have you ever had pills or injections for either of these reasons?

___ no (0) ___ pills (1) ___ injections (2) ___ both (3) ___ DK (9) V(202)___ 41

(If no, code 0s and if DK, code 9s in Q41.1 and Q41.2 and go to Q42)

41.1 Were they for a pregnancy test or to end a pregnancy early, or did you have them for both reasons?

___ abortion ___ pregnancy test ___ both ___ unknown which reason

A) (If any given for abortion ask) How many times did you receive them to end a pregnancy early? (Code 0 if none given for abortion.) ..... V(203)___ 41.1A

B) (If any given as pregnancy test ask) How many times did you have a pregnancy test like this? (Code 0 if none given for pregnancy test.) ..... V(204)___ 41.1B

C) (If unknown which reason ask) About how many times did you have these hormones? (Code 0 if none given for unknown reason) ..... V(205)___ 41.1C

(Codes for A, B and C: 0 = none, 1 = one, ... 7 = seven or more, 8 = unknown number of times, 9 = unknown whether woman ever received hormones for either of these purposes.)

41.2 How old were you when you first received these hormones? (98 = unknown age) ..... V(206)___ Years 41.2

42. Have you ever taken pills or received injections after having sexual relations to prevent you from becoming pregnant?

___ no (0) ___ yes (1) ___ DK (9) V(207) ___ 42

(If no, code 0s and if DK, code 9s in Q42.1 and 42.2 and go to Q43)

42.1 About how many time have you attempted to keep from becoming pregnant by this method? (98 = unknown number of times) ..... V(208) ___ 42.1

42.2 How old were you when you first used this method? (98 = unknown age) ..... Years V(209) ___ 42.2

43. Have you ever taken female hormone pills for treatment of problems related to the menopause or the "change of live?"

___ no ___ yes ___ DK

(If no, code 0s and if DK, code 9s in Q43.1 to 43.4 and go to Q44.)

43.1 What was the name of the pills? (PROBE) ..... V(210) ___ 43.1

___ Premarin or other conjugated estrogens (1)

___ Diethylstilbesterol (DES) or stilbesterol (2)

___ Other (4) _____ (specify)

___ Unknown type (8)

(Code: 1 = conjugated estrogens, 2 = DES or stilbesterol, 3 = both, 4 = other types, 5 = other and conjugated, 6 = other and DES or stilbesterol and other, 8 = unknown type only, 9 = unknown if ever took estrogens, 0 = never took estrogens.)

43.2 When did you first take them? (98 = unknown year) ..... Year V(211) ___ 43.2

43.3 When was the most recent time that you took them? (98 = unknown year) ..... Year V(212) ___ 43.3

43.4 For about how long did you take them? (PROBE to ascertain total months of use) (97 = 97 or more months of use; 98 = unknown number of months) ..... Months V(213) ___ 43.4

44. Have you ever taken female hormone pills or injections for treatment of any menstrual or ovarian problems? ..... V(214) ___ 44

___ no (0) ___ yes (1) ___ DK (9)

(If no, code 0s and if DK, code 9s in Q44.1 and Q44.2 and go to Q45)

44.1 Were these oral contraceptives, other types of pills, or injections? ..... V(215) 44.1

___ oral contraceptives (1)

___ other pills (2)

___ both oral contraceptives and other pills (3)

___ injections (4)

___ both oral contraceptives and injections (5)

___ both other pills and injections (6)

___ unknown whether oral contraceptives or other pills (8)

(If oral contraceptives were used, details should be recorded in Q36A and 37.1 to 37.8.)

44.2 What kind of problem was it? ..... V(216) 44.2

___ heavy blood loss (1)

___ very light flow (2)

___ no menstrual flow (3)

___ irregular menses (4)

___ painful menses (5)

___ ovarian cyst (6)

___ other (7) _____
(specify)

___ unknown reason (8)

45. Have you ever used female sex hormones/estrogens for any other reasons? ..... V(217) 45

___ no (0) ___ yes (1) ___ DK (9)

(If no, code 0 and if DK, code 9 in Q45.1 and go to Q46.)

45.1 What was the reason? ..... V(218) 45.1

_____
(see instructions for code)

48. Have you ever had a chest X-ray?

___ no ___ yes ___ DK

(If no, code 0 and if DK, code 9 in Q48.1 and go to Q49.)

48.1 About how many chest X-rays do you think that you have had? ..... V(226) __ 48.1
Would you say that it was:

___ only one (1)

___ more than 1 but less than 10 (2)

___ 10 or more but not as many as 20 (3)

___ 20 or more (4)

___ unable to estimate number (8)

49. Do you ever drink alcoholic beverages? ___ no (0) ___ yes (1) ___ DK (9)..... V(227) __ 49
(If no, code 0s and if DK, code 9s in Q49.1 and Q49.2 and go to Q50.)

49.1 Which of the following kinds of alcoholic beverages do you usually drink?

		No(0)	Yes(1)	DK(9)	
A)	Beer	___	___	___	V(228) __ 49.1A
B)	Wine	___	___	___	V(229) __ 49.1B
C)	Distilled spirits	___	___	___	V(230) __ 49.1C
D)	Other local alcoholic drinks	___	___	___	V(231) __ 49.1D

(specify)_____

49.2 About how many drinks do you have per week (PROBE) ..... V(232) __ __ 49.2

FAMILY HISTORY

50. How many sisters have you had that lived to the age of 18 years? ..... NV(232) __ __ 50

I would like to ask you a few questions about illness that your sisters, mother, or grandmothers may have had. Have any of these members of your families ever had any of the following diseases?

		No(0)	Yes(1)	DK(9)	
A)	Heart disease	___	___	___	V(233) __ 50A
B)	Tuberculosis	___	___	___	V(234) __ 50B
C)	Stroke	___	___	___	V(235) __ 50C
D)	Cancer	___	___	___	V(236) __ 50D
E)	Diabetes	___	___	___	V(237) __ 50E

50.1 (If answer to D is no, code 0s and if DK, code 9s in the following table and go to Q51.

Who had cancer? (PROBE)	What kind of cancer was it? (PROBE) None (0)	Breast (1)	Ovary (2)	Corpus (3)	Cervix (4)	Liver (5)	Gall-bladder (6)	Other (specify) (7)	Unknown Type (8)	Unknown (9)	
A) ___ Mother's mother	___	___	___	___	___	___	___	_____	___	___	V(238) 50.1A
B) ___ Father's mother	___	___	___	___	___	___	___	_____	___	___	V(240) 50.1B
C) ___ Mother	___	___	___	___	___	___	___	_____	___	___	V(242) 50.1C
D) ___ Sister 1	___	___	___	___	___	___	___	_____	___	___	NV(242A) 50.1D
E) ___ Sister 2	___	___	___	___	___	___	___	_____	___	___	NV(242B) 50.1E
F) ___ Sister 3	___	___	___	___	___	___	___	_____	___	___	NV(242C) 50.1F
G) ___ Sister 4	___	___	___	___	___	___	___	_____	___	___	NV(242D) 50.1G
H) ___ Sister 5	___	___	___	___	___	___	___	_____	___	___	NV(242E) 50.1H

RESIDENTIAL AND SOCIOECONOMIC FACTORS

51. Now I am going to ask you about the places that you have lived in the past 15 years (starting with the current place of residence and working backward in time. (PROBE and complete the following table.)

-1- Residence	-2- Name of foreign country, part of city, town, or rural area	-3- Dates (years)	-4- Number of years of residence	-5- Type of area (check 1) Foreign Country	Major City	Town	Rural Area
Current residence		from: to: present					
Most recent prior residence		from: to:					
Second most recent		from: to:					

(Code the following:)

51. A) Type of area of current residence (1 = major city, 2 = town, 3 = rural area, 9 = unknown ..... V(244) __ 51A

B) Number of the past 15 years in area of current residence (99 = unknown years) ..... V(245) __ 51B

C) Number of different known areas of residence in past 15 years ..... V(246) __ 51C

D) Number of the past 15 years in a foreign country ..... V(247) __ 51D

E) Number of the past 15 years in a major city ..... V(248) __ 51E

F) Number of the past 15 years in a town ..... V(249) __ 51F

G) Number of the past 15 years in a rural area ..... V(250) __ 51G

52. What is your race or ethnic background?_____ ..... V(251) __ 52

53. What is your religion? _____ ..... V(252) __ 53

54. What is your current marital status? (PROBE) __ single (never married) (0), __ married (1), __ separated or divorced (2), __ widowed (3), __ unknown (9) ..... V(254) __ 54

(If never married, code 0s and if unknown, code 9s in Q54.1-54.2 and go to Q55.)

54.1 How old were you when you were first married? (99 = unknown age) ..... V(255) Years __ __ 54.1

54.2 What was the date of your first marriage? ..... day /month/year __/__/__ 54.2 V(257) V(256) V(258)

55. Have you ever attended school? __ no __ yes

(If no, code 00 and if unknown, code 99 in Q55.1 and go to Q56.)

55.1 For how many years did you go to school? (PROBE; record such things as matriculation, completion of middle school, degrees of certificates received) ..... V(259) Years __ __ 55.1

_____

_____

(Code: 00 = none or less than one year, 01 = from one up to but not including two years, etc., 98 = unknown years of schooling, 99 = unknown whether ever attended school)

56. (If woman has never been married, code 77 and go to Q57; otherwise ask:)
For how many years did your most recent/present husband go to school?
(PROBE as in Q55.1) ..... V(260) __ __ Years 56

(Code: 00 = none or less than one year, 01 = from one up to but not including two years, etc., 77 = woman never married, 98 = unknown years of schooling, 99 = unknown whether ever attended school.)

57. What kind of work do you usually do? _____

_____ ..... V(261) __ __ 57
(Occupation code from instructions)

58. (If woman has never been married, record 00; if she has, ask:) What kind of work does [did] your most recent/present husband usually do?

_____ ..... V(262) __ __ 58

GYNECOLOGIC HISTORY

(Begin this section of the interview with the following statement, or a similar one, to prepare the woman for the sensitive questions that will be asked.) We are almost finished with the interview, and you have been very patient. We are going to end with some rather personal questions, and you may find some of them a little embarrassing. I hope you will understand that we are asking them only because they are very important for the success of this project. Please remember that whatever information you give us will be kept strictly confidential.

59. Women can go to a doctor and have a Pap test to find out whether she may have cancer of the cervix. This is also called a cervical smear or cancer smear or cervical cytology. Many women who we have been talking to have had Pap tests.

(Ask the version of the question checked below.)

A) ___ Not counting any abnormal Pap smears that resulted in your diagnosis of cervical cancer, how long ago was it when you had your last normal Pap smear?

B) ___ How long ago was it when you had your last Pap smear?

C) ___ Not counting your most recent Pap smear that you got as a part of your evaluation for your present condition, how long ago was it when you had your last Pap smear?

D) ___ Not counting your most recent Pap smear, how long ago was it when you had your last Pap smear?

(Record answer here): _____ months/years (98 = unknown months) NV(262) Months ___ ___ 59

(If never had any prior Pap smears, code 0s, and if not known whether any prior Pap smears, code 9s in Q59, 59.1, 59.2, and 59.4,and go to Q60.)

(If A), C), or D) above is checked, begin questions 59.1 to 59.4, with the same underlined words as used above.)

59.1 About how many times have you had a Pap smear? (Code number as ascertained from the respondent; 98 = unknown number of times.) ..... V(263) ___ ___ 59.1

59.2 About how frequently did you usually have a Pap smear? _____ (Code: 7 = more frequently than once a year, 1 = about once a year, 2 = about once every two years, 3 = about once every three years, ... etc., 5 = about once every five years or less frequently, 6 = only one prior Pap smear, 8 = unknown frequency.) ..... V(264) ___ 59.2

59.3 Where was [were] this [these] Pap smear(s) done?

_____

59.4 How old were you when you first had a Pap smear? (Code respondent's answer; 98 = unknown age) ..... V(265) Years ___ ___ 59.4

59.5 Validation _____

_____

_____

(Code number of previous Pap smears that were confirmed from medical records. 0 = none, records checked, 1 = one, 2 = two, ... 7 = seven, 8 = eight or more, 9 = no validation.) ..... V(266)__ 59.5

60. Have you ever had to go to a doctor, or other practitioner because you had an abnormal vaginal discharge? ..... V(267) __ 60

___ no (0) ___ yes (1) ___ DK (9)

(If no, code 0s and if DK, code 9s in Q60.1 to Q60.4 and go to Q61.)

60.1 How old were you when you first went to a doctor for this problem? (98 = unknown age) ..... V(268)__ __ Years 60.1

60.2 How many times have you gone to a doctor for this problem? _____ (Code: 1 = one, 2 = two, etc., 7 = 7 or more, 8 = unknown number of times.) ..... V(269) __ 60.2

60.3 Did you ever have a fever or lower abdominal cramps when you had this problem? PROBE ..... V(270) __ 60.3

___ no (5) ___ fever (1) ___ cramps (2) ___ both together (3)

___ both but at different times (4) ___ DK (8)

60.4 When did you most recently have this problem? ____ PROBE (Code 98 for unknown month, estimate year, use calendar.) ..... Month/Year __ __/__ __ 60.4 V(271) V(272)

61. How many marriages or other sexual relationships have you had in your life? ..... (Code: 0 = none, 1 = one, ... 7 = seven or more, 8 = unknown number, 9 = no information obtained.) V(273)— 61

62. How old were you when you had the first of these relationships [first had this relationship]? (Code: 00 = never had a relationship, 99 = unknown age.) ..... V(274)__ __ Years 62

63. Have you ever gone to see a doctor and were told that you had a venereal disease?.....

___ no (0) ___ yes (1) ___ DK (9)

(If no, code 0s and if DK, code 9s in the following table. If yes, ask the following questions and complete the table.)

	Did you have either of these diseases?	How many different times did you have this problem?*
63.1	___ Gonorrhea	_____
63.2	___ Syphilis	_____

*(Code: 0 = none, 1 = one, ... 7 = seven or more, 8 = unknown number of times, 9 = no answer obtained, or woman uncertain whether she had the disease.

Region/Province Code

(Taken from info provided address).

BANGKOK STUDY OF PAPILLOMA VIRUSES AND CERVICAL CANCER

V(458) = Univesal

Smoking History

Study Number

1. Study number..... ___ ___ - ___ ___ ___ ___ NV(458) V(461)

2. Name of subject: V(462)

_____(family)/ _____(given)

_____(others)

3. Have you smoked a total of 100 cigarettes in your lifetime..... V(463)

___ 0 No
___ 1 Yes
___ 9 Unknown

(If no, code 0s and if unknown, code 9s in Q.4-12 and go to Q.13)

4. Have you ever smoked cigarettes regularly for six months or longer?..... V(464)

___ 0 No
___ 1 Yes
___ 9 Unknown

(If no, code 0s and if unknown, code 9s in Q.5-12 and go to Q.13)

Years

5. How old were you when you started smoking cigarettes regularly?..... V(465) ___ ___

(98 = unknown age)

6. Do you still smoke cigarettes?..... V(466) ___

___ 2 No
___ 1 Yes (record present age in Q.7)
___ 8 Unknown

Years

7. How old were you when you stopped smoking?..... V(467) ___ ___

(98 = unknown age)

8. Between the time you started smoking and the (time you stopped/present), were there any periods of a year or more when you did not smoke?..... V(468) ___

___ 2 No (record 0s in Q.9)
___ 1 Yes
8 Unknown (record 98 in Q.9)

9. For how many years did you not smoke?..... V(469) Years

10. (When you smoked), how many cigarettes do/did you usually smoke per day?..... V(470)

(98 = unknown number)

11. Did/do you usually smoke filter or non-filter cigarettes?..... V(471)

___ 1 filter
___ 2 non-filter
___ 3 both
___ 8 unknown

12. When you smoke/smoked, did/do you usually:..... V(472)

___ 1 inhale into your chest
___ 2 inhale only into your mouth or throat
___ 3 not inhale at all
___ 8 don't know

13. Have you ever used any other type of tobacco regularly for six months or more?..... V(473)

___ 0 No
___ 1 Yes (If no, code 0s and if unknown, code 9s in Q.14 and go to Q.15)
___ 9 Unknown

14. Before one year ago, did you use any of the following tobacco products regularly for six months or more?

		No (0)	Yes (1)	Unknown (9)	
A.	pipe	___	___	___	V(474)
B.	chewing tobacco	___	___	___	V(475)
C.	Keyo (local cigar with sugar and pounded tamarine bark)	___	___	___	V(476)
D.	cigars	___	___	___	V(477)
E.	other _____ (specify)	___	___	___	V(478)

15. Did you ever go to a doctor because you had warts or condylomata in your genital or anal area?..... V(479)

___ 0 No
___ 1 Yes (If no, code 0 and if unknown, code 9 in Q.16)
___ 9 Uknown

16. Where were they?..... V(480)

___ 1 around the anus
___ 2 in the genital area
___ 3 both