COMPREHENSIVE CERVICAL CANCER PREVENTION AND CONTROL: POTENTIAL STRATEGIES AT DIFFERENT STAGES OF CERVICAL CANCER PROGRESSION

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COMPREHENSIVE CERVICAL CANCER PREVENTION AND CONTROL: POTENTIAL STRATEGIES AT DIFFERENT STAGES OF THE CANCER CONTINUUM

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ABSTRACT

Advancements in the continuum of cervical cancer care, including risk factor assessment, human papillomavirus (HPV) vaccines, and screening programs, have reduced the cervical cancer burden. However, cervical cancer remains an important public health issue, particularly in developing countries. This research investigated three potential prevention opportunities along the cervical cancer continuum, including: factors associated with HPV natural history in middle-aged women which may influence current vaccination and screening recommendations; testing for carcinogenic HPV serotypes to assess the feasibility of 'catchup' HPV vaccination in populations who missed the conventional adolescent vaccination window; and protective dietary patterns. First, we measured the incidence and clearance of HPV, and associated risk factors, in the HIP (HPV in Perimenopause) Study, a U.S. clinic-based cohort of women aged 35-60 years old. Next, we measured the quadrivalent vaccine-specific HPV seroprevalence in a population-based, cross-sectional analysis of young, married, postpartum, rural Indian women. Lastly, we evaluated the associations of soy and tea consumption on risk of cervical cancer in a large Singapore population of women aged 45-74. Each of these studies yielded findings of public health significance. First, the majority of new HPV detections in U.S. older women occurred during periods of sexual abstinence or monogamy, lifetime number of sexual partners modified

incident HPV risk, and the majority of incident HPV cleared within 18 months. Our findings suggest that although HPV vaccination may provide some protection the overall benefit may be limited and considering a risk-based approach to cervical cancer screening may be valuable. Second, we found that seroprevalence of HPV-6, HPV-11, HPV-16, and HPV-18 was relatively low in the postpartum Indian women, 6.6%, 10.1%, 10.1%, and 3.9%, respectively, suggesting that 'catchup' HPV vaccination in this population may be effective in preventing cervical cancer. Third, we found that high soy intake was associated with a decrease cervical cancer risk among Chinese green tea drinkers, but not among non-drinkers of green tea. Developing nutritional interventions utilizing soy and tea components may disrupt cervical carcinogenesis. Further research is needed to continue to develop and implement effective cervical cancer interventions at various points on the cancer continuum.

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PREFACE

Completing this dissertation has be a collaborative experience and shaped by a number of excellent researchers, outstanding mentors, and caring individuals that have guided me through this experience since my first days at the University of Pittsburgh Graduate School of Public Health. First and foremost, I would like thank my academic and dissertation advisor, Dr. Clare Bunker for being a greater listener, providing valuable perspective, advocating on my behalf, and supporting me in the pursuit of my own research interests even in the face of some unforeseen challenges. I am also truly thankful and honored to have worked and collaborated with the rest of my dissertation committee members, Dr. Lesley Butler, Dr. Ada Youk, and Ms. Joanne Russell. Their expertise and perspective, thoughtful questions, and intellectual engagement pushed me to be a better epidemiologist and public health practitioner. I would also like to thank Dr. Patti Gravitt for being a mentor and colleague to me for over 10 years. Her continued support and encouragement of my academic and professional endeavors helped me remain focused and positive through this whole doctorate process. Part of this dissertation research was made possible through funding provided by the Department of Epidemiology Small Grants Program and Global Health Travel Scholarship at the University of Pittsburgh.

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both in and out of the classroom. Interacting with the Center for Global Health challenged me to think about what is global health practice and ignited my interest in strengthening global health education.

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Finally, I am immensely lucky to have family and friends who have always believed in me. They were my most vocal cheerleaders and pillars of strength. Whether it was to listen to me vent or to give me space to decompress and process the situation or simply to make me laugh, they were there for me. They inspired me to work hard toward my academic and professional goals and I would not have reached the end of this journey without their unwavering support.

1.0 INTRODUCTION

Even with the advancements of screening programs focused on early detection and surgical treatment of pre-invasive malignancies, cervical cancer persists as an important public health issue for women. Cancer of the cervix is the fourth most common cancer among women and the most common gynecological cancer (Ferlay et al., 2014; R Sankaranarayanan & Ferlay, 2006). Of the 528,000 incident cases and 266,000 mortalities in 2012, approximately 85% of new cases (445,000) and deaths (230,000) were in less developed regions, underscoring the marked geographic disparity in the worldwide cervical cancer disease burden (Ferlay et al., 2013). Although the overall disease burden is declining, disparities in cervical cancer burden are also apparent within industrialized countries. In the US, Blacks and Hispanic have one-third higher age-adjusted incidence rates compared to Whites (U.S. Cancer Statistics Working Group, 2014). In Australia, the indigenous women have a nearly 2.5-fold higher age-adjusted incidence rate than non-indigenous women are (reviewed in (Shannon, Franco, Powles, Leng, & Pashayan, 2011)).

Interventions exist across the entire cervical cancer continuum from prevention to survivorship and palliative care. Historically, cervical cancer interventions were focused solely on improving secondary prevention methods that detected neoplastic growth early, before it became invasive, and treated the precancerous lesions. Although implementing organized population-based screening programs are credited for a remarkable reduction in the global

invasive cervical cancer burden, improvements in screening tests and algorithms continue to be explored to reduce false-negativity rates and to ensure cost-effective strategies are being utilized. Although there is agreement on when cervical cancer screening should be initiated, the age at which cervical cancer screening is no longer beneficial continues to be debated. More recently, the focus of cervical cancer prevention is by vaccination against oncogenic human papillomavirus (HPV), which are known to confer high risk for cervical cancer (F. Xavier Bosch, Lorincz, Muñoz, Meijer, & Shah, 2002; Walboomers et al., 1999). Additionally, there may be interventions focused on dietary factors, such as soy and tea that may modify risk for cervical cancer.

Cervical cancer screening programs in industrialized and low-resourced countries are exploring the use of testing for carcinogenic HPV, either as a primary screening method or as a co-test with cytology (a recommended method in the US). Based on the natural history of HPV, HPV-based screening strategies are recommended for women 30 years and older, after the peak prevalence (Shi, Devarakonda, Liu, Taylor, & Mills, 2014). There are multiple HPV tests, the most common and well studied is the hybrid capture 2 (HC2) test. HC2 is more sensitive than Pap smear for detecting precancerous malignancies and early cancer, with similar specificity (Cuzick et al., 2006; Dillner et al., 2008; Elfström et al., 2014; Gravitt, Belinson, Salmeron, & Shah, 2011; Sherman et al., 2003). The high predictive value of a single negative HPV result provides stong evidence that high-grade disease risk is negligible for at least 5-10 years (Thomsen et al., 2014), allowing for screening intervals to be increases and potentially alleviating some of the burden on the health system.

Current US guidelines recommend women should stop screening at age 65 provided they do not have a recent history of abnormal screening (“Cervical Cancer Screening Guidelines for

Average-Risk Women," 2015). In other countries, the upper age for screening varies from 59 years to 70 years or older (Castañón, Landy, Cuzick, & Sasieni, 2014). Age-specific curves suggest that HPV prevalence may increase in women past their reproductive age (>45 years). Although there may be an increase in the HPV burden of older women, there is a clear gap of knowledge on whether the HPV detected in mid-adult and older women is due to a new exposure or reactivation of a latent infection. New infections could be transient and provide little risk for cervical cancer diagnosis later in life. While recurrent latent infection may be more likely to persist, thereby increasing the risk of developing cervical cancer. Therefore, better understanding of the dynamics of the second HPV prevalence peak observed in older women will add to the evidence needed to reevaluate recommendation regarding screening in older women.

Immunization with highly effective and safe HPV vaccines against HPV types 16 and 18, which cause over 70% of all cervical cancers, has become an important primary prevention strategy. High coverage of an HPV immunization program is thought to have an even greater impact on the cervical cancer burden than screening programs (F X Bosch, Castellsagué, & de Sanjosé, 2008). In order for vaccination to precede HPV exposure, young adolescent girls are the primary target population. Logistical concerns and cultural barriers about vaccinating adolescent girls may hinder the feasibility of reaching the desired coverage. 'Catch-up populations' for vaccinations, i.e. older populations which may have limited prior exposure to HPV, may play an important role in ensuring that sufficient immunity is attained in the population. A recent review suggests catch-up HPV vaccination among females 16 years and older populations is beneficial (Couto, Sæterdal, Juvet, & Klemp, 2014). In countries where adolescents have limited access to the health system, young postpartum women are a potential

target group for catch-up immunization programs if previous exposure to HPV is low. However, data are needed to understand HPV burden in this population.

Immunization prevents the causal infectious agent; early detection and treatment programs intervene after cervical cancer starts to progress. Although development of cervical cancer is linked to persistent HPV infection, cofactors may be required to trigger the development of cervical lesions that progress to cancer. Consequently, understanding promoting and inhibiting cofactors is also necessary to develop interventions that disrupt the cervical cancer continuum and may be as important as the prevention of HPV. Multiparity and long-term oral contraceptive use are believed cofactors for HPV and cervical cancer (reviewed in (Castellsague & Munoz, 2003)), combined with experimental evidence that implicate estrogen and other sex hormones in cervical carcinogenesis (reviewed in (Delvenne et al., 2007)) suggests that hormonal changes may influence risk of cancer progression. Epidemiologic evidence supports that diets rich in plant-based foods containing phytochemical, such as phytoestrogens found in soy and tea, play an important role in cancer chemoprevention of other reproductive cancers, including breast, prostate, and ovarian (Batra & Sharma, 2013; Butler & Wu, 2011; Miller & Snyder, 2012), but there are inadequate data on how these dietary factors are associated with cervical cancer. Dietary interventions that address risk or promote benefit may hold additional promise of reducing the mortality and morbidity associated with cervical cancer. It may be beneficial to study the potential effect of soy and tea on cervical cancer risk, given that data from experimental studies suggest classes of phytoestrogens suppress cellular growth and stimulate apoptosis in human cervical cancer cells, as well as HPV-infected cells (Butler & Wu, 2011; Dhandayuthapani, Marimuthu, Hoermann, Kumi-Diaka, & Rathinavelu, 2013; Guo, Kang, Xiao,

Liu, & Zhang, 2004; Hussain et al., 2012; E. Y. Kim, Shin, Park, & Kim, 2014; S.-H. Kim, Kim, Lee, & Song, 2009; Xiao, Huang, Geng, & Qiu, 2011).

1.1 SPECIFIC AIMS

The following three aims address gaps in literature related to (1) the natural history of HPV dynamics among mid-adult women, (2) the potential benefit of catch-up vaccination among young postpartum women, and (3) the influence of phytoestrogen-rich diets on risk of cervical cancer.

Specific Aim for manuscript 1

To estimate the incidence and clearance rates of HPV infections among mid-adult women, stratified by recent and past sexual activity, and to calculate the potential fraction of HPV infections that are associated with a recent new sexual partnership and with lifetime sexual partnerships. This aim will use data from a cohort of women participating in the clinic-based HPV in Perimenopause (HIP) Study. We hypothesize that the incidence and clearance rates are higher for women who reported a new sex partner, compared to non-sexually activity women. A substantial proportion of the newly detected HPV will be attributed to lifetime sexual partners, which will be higher than those attributed to recent sexual activity. Clearance of a newly detected infection will be associated with recent sex with new partner; persistence of a newly detected infection will be strongly associated with high number of lifetime sex partners.

Specific Aim for manuscript 2

To measure the quadrivalent vaccine-specific HPV seroprevalence among postpartum women age 18-35 years old to determine if a catch-up vaccination program among this population is appropriate. This aim will use data and archived specimens from postpartum women participating in the LIFE (Longitudinal Indian Family Health) Study. We hypothesize the seroprevalence of oncogenic vaccine-specific HPV (HPV-16 and HPV-18) seroprevalence is 5% or less, similar to the seroprevalence of the usual adolescent target populations for HPV vaccination. We will also identify factors associated with HPV seropositivity in this population.

Specific Aim for manuscript 3

To investigate the relationship of soy, green tea, and black tea consumption in relation to cervical cancer risk among older women. We will also assess the potential joint effects of green tea on the association of soy and cervical cancer. This aim will use data from the Singapore Chinese Health Study. We hypothesize that soy and green tea intake will have independent protective effects against cervical cancer risk. We hypothesize that black tea will not be associated with cervical cancer risk.

2.0 CERVICAL CANCER BACKGROUND

Cancer of the cervix is the fourth most common cancer among women and the most common gynecological cancer (Ferlay et al., 2014; R Sankaranarayanan & Ferlay, 2006). Squamous cell carcinoma (SCC) accounts for the vast majority of cervical cancers, followed by adenocarcinoma (AC) (reviewed in (Seoud, Tjalma, & Ronsse, 2011)). Of the 528,000 incident cases and 266,000 mortalities in 2012, approximately 85% of new cases (445,000) and deaths (230,000) were in less developed regions, underscoring the marked geographic disparity in the worldwide cervical cancer disease burden (Ferlay et al., 2013). Compared to an age-standardized incidence rate (ASIR) of less than 10 per 100,000 in more developed regions, a combined ASIR of 21.5 per 100,000, places countries in Latin America, South and Southeast Asia and sub-Saharan Africa at highest risk (Ferlay et al., 2013). Socioeconomic disparities in cervical cancer burden are also apparent within industrialized countries. In the US, Blacks and Hispanic have one-third higher age-adjusted incidence rates compared to Whites (U.S. Cancer Statistics Working Group, 2014). In Australia, the indigenous women have an estimated 16.9 per 100,000 age-standardized cervical cancer incidence rate, which is nearly 2.5-fold higher than non-indigenous women are (reviewed in (Shannon et al., 2011)).

The discovery of human papillomavirus as the etiologic agent for cervical cancer has advanced prevention and control strategies. HPV vaccines and early detection and treatment of precancerous lesions are effective prevention and control strategies, but wide variations in

implementation, as well as policy recommendations based on an incomplete understanding of HPV natural history, especially among women past the their reproductive years, are responsible for cervical cancer remaining a public health problem (Gakidou, Nordhagen, & Obermeyer, 2008).

2.1 ROLE OF HPV IN CERVICAL CARCINOGENSIS

Cervical cancer forms in the cells lining the lowest part of the uterus known as the cervix uteri or cervix. As part of the human female reproductive system, the cervix connects the uterus and vagina through the endocervical canal (Garcia, Hatch, & Berek, 2012). Two main types of cells form the endocervical canal of the cervix: (1) the columnar epithelium covers the part of the cervix closest to the uterus (endocervix) and (2) the squamous epithelium lines the part that is near the vagina (ectocervix) (Garcia et al., 2012). The squamocolumnar junction forms where the squamous epithelium and columnar epithelium meet.

Age, parity, and hormonal state are factors that affect the appearance of the cervix. At birth, the squamocolumnar junction (SJC) is located on the ectocervix, but gradually changes as the original squamocolumnar junction moves inward due to metaplasia in the columnar epithelium and forms a new squamocolumnar junction (Garcia et al., 2012). This metaplastic process, which is triggered by the production of estrogen during puberty, establishes an area between the original and new SCJ known as the transformation zone (Garcia et al., 2012).

The cells in transformation zone are more prone to human papillomavirus (HPV) infection and explains why most cervical cancer occurs in the transformation zone (Schiffman, Castle, Jeronimo, Rodriguez, & Wacholder, 2007). The cellular composition of the cervical

epithelium during adolescence and early adulthood is a mixture columnar, metaplastic, and squamous epithelial cells. As a women matures to adulthood the cervical epithelium is predominately comprised of squamous epithelial cells (Moscicki et al., 2012).

HPV establishes infection at the transformation zone through microscopic cuts in the endocervical mucosa, allowing access to the epithelial basal layer cells. There are more than 150 different HPV types, which are classified into two groups: high-risk (oncogenic type) and low-risk (non-oncogenic type).

Virologist and Nobel Prize winner Harlad zur Hausen first indicated a link between HPV and cervical cancer in the late 1970s and early 1980s, contradicting the prevailing belief at the time that herpes infection caused cervical cancer (zur Hausen, 2002). Since this original discovery, a number of epidemiological studies have confirmed this association and have established HPV as a basic causal factor for cervical oncogenesis (F. Xavier Bosch et al., 2002; Walboomers et al., 1999). In addition to cervical cancer, other anogenital cancers (e.g., vulvar, penile, and anal cancers), cutaneous and genital warts, and oral lesions are attributed to HPV. Direct skin-to-skin contact during vaginal, anal, or oral sexual contact is the most common mode of transmission; however, non-sexual transmission, such as mother-to-child transmission, autoinoculation, direct contact of infected area, or indirect contact of contaminated surface, is also possible (Ryndock & Meyers, 2014).

Of the 40 anogenital-related HPV infections, 15 are frequently detected among cervical cancer cases (Clifford, Franceschi, Diaz, Muñoz, & Villa, 2006). Although the distribution of oncogenic and non-oncogenic types varies across geographic regions (Clifford et al., 2006), HPV-16 and HPV-18 are the most prevalent in the population, are the most common high-risk types, and are responsible for 70% of cervical cancers and 50% of high-grade cervical

abnormalities (Clifford et al., 2006). Another 20% of cervical cancers are attributed to HPV types 31, 33, 35, 45, 52, 58 and 59 (Clifford et al., 2006). Combined, these nine high-risk genotypes account for approximately 90% of the cervical cancers worldwide. In addition, International Agency for Research on Cancer (IARC) also classifies HPV types 39, 51, and 56 (and possibly 68 and 73) as cancer-causing types (Doorbar et al., 2012).

The HPV genome codes for eight genes that are designated early (E1, E2, E4-E7) or late (L1, L2) based on stage of expression. E1, E2, E4, and E5 are early genes involved in replication, transcription, viral release and immune evasion. E6 and E7 are the primary oncoproteins for HPV and are expressed early in differentiation and bind with p53 and retinoblastoma protein (pRB), respectively (reviewed in (Doorbar et al., 2012)). Current thinking suggests that oncogenic and non-oncogenic E6 and E7 proteins behave differently, and the rising E6 and E7 levels are directly correlated with severity of cervical abnormalities (reviewed in (Doorbar et al., 2012)). Expressed in late differentiation, L1 is the major capsid protein and L2 is the link to the plasmid DNA (Doorbar et al., 2012).

Although the lifetime risk of HPV is estimated to be 75-80%, only 10% establish persistent infection, and the remaining are considered transient infections (Syrjanen, 1994). Over 50% are undetectable within the first year of infection and more that 80% are undetectable within three years (Jaisamrarn et al., 2013; Moscicki et al., 2012). Establishment of viral persistence is important for the cervical carcinogenesis. Persistently HPV-infected epithelium's progress to pre-invasive disease is marked by genetic instability. Cervical invasion occurs if infected cells are not controlled by the immune system or are left untreated (Doorbar et al., 2012). In addition to the oncogenic potential of the HPV genotypes, the viral load of HPV in the

cervix is predictive of high-grade cervical neoplasia and cancer, even in women without cervical abnormalities (Grabowski et al., 2014; Steben & Duarte-Franco, 2007; S.-M. Wang et al., 2013).

Time from persistent HPV infection to precancer or worse condition is approximately 10-15 years (Moscicki et al., 2012). HPV-associated cervical abnormalities in squamous epithelial cells are grouped using various classification systems, compared in Table 1. The Bethesda classification is related to cytology, while the CIN nomenclature is based on histologic diagnosis. Severe or high-grade precancerous conditions signify the presence of cervical lesions that are likely to progress to cancer or indicate an underlying cancer. Although cervical intraepithelial neoplasia of grade 3 (CIN3) and high-grade squamous intraepithelial lesion (HSIL) are not equivalent, in the literature, high-grade precancerous changes often refer to cytological HSIL, histological CIN3, severe dysplasia, or carcinoma *in-situ*. Various excision (e.g., loop electrical excision, cold-knife conization) and ablation (cryotherapy, cold coagulation) surgical techniques are used to treat high-grade precancerous cervical lesion by removing or destroying, respectively, the abnormal cervical tissue (PATH Cervical Cancer Team, 2013).

Mild squamous intraepithelial lesions or atypical squamous cells, including cervical intraepithelial neoplasia of grade 1 are not actively treated since the risk of progression to high-grade disease or worse condition is 10% or less (T. C. Wright et al., 2007). Conversely, cervical intraepithelial neoplasia of grade 2 (CIN2), which is not highly predictive of cervical malignancy development, is managed and treated similar to high-grade precancerous lesions (PATH Cervical Cancer Team, 2013; T. C. Wright et al., 2007). It is estimated that only one-fifth of CIN2 lesions develop into cervical carcinoma *in-situ* and 1 in 20 CIN2 lesions progress to invasive cancer (Latendresse, McCance, & Morgan, 2010).

Table 1. Comparison of classification systems for HPV-related squamous cell cervical abnormalities

Severity	CIN Nomenclature	Bethesda System (2001)	Dysplasia Nomenclature
Normal	Negative/Normal	Negative for intraepithelial lesion and malignancy	Negative/Normal
Mild	Squamous atypia	• Atypical squamous cells of undetermined significance (ASCUS) • Atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion (ASC-H)	Squamous atypia
	Cervical intraepithelial neoplasia of grade 1 (CIN1)	Low-grade squamous intraepithelial lesion (LSIL)	Mild dysplasia
Moderate	Cervical intraepithelial neoplasia of grade 2 (CIN2)	High-grade squamous intraepithelial lesion (HSIL)	Moderate dysplasia
Severe	Cervical intraepithelial neoplasia of grade 3 (CIN3)	HSIL	Severe dysplasia Carcinoma *in-situ* (CIS)
Invasive carcinoma	Squamous cell carcinoma	Squamous cell carcinoma	Squamous cell carcinoma

Adapted from reference (Safaeian, Solomon, & Castle, 2007)

Spontaneous regression of cervical intraepithelial neoplasia of grade 1, grade 2, and less frequently grade 3 can occur (Latendresse et al., 2010). In the case of CIN2, it is estimated that about half the women with a CIN2 lesion regress if left untreated (Castle, Schiffman, Wheeler, & Solomon, 2009; Garcia et al., 2012; Latendresse et al., 2010; S.-M. Wang et al., 2013). A precursor state of cervical cancer persists, meaning the precancerous condition neither progresses to a worse stage nor regresses to a lesser stage, in one-third or more of cervical intraepithelial lesions (Latendresse et al., 2010). Whether cervical paraneoplastic lesions have the potential to

regress, persist, or progress may be related to HPV viral load or the HPV genotype attributed to the lesion (Castle et al., 2009; S.-M. Wang et al., 2013).

Adenocarcinoma, representing approximately 20% of invasive cervical cancers, is also associated with HPV infection (Seoud et al., 2011). HPV 16, 18, and 45 are detected in the majority of the adenocarcinoma cases (Castellsagué et al., 2006; Pirog et al., 2014; Seoud et al., 2011). Results from a multi-center case-control analysis suggest that HPV-18 is associated with the highest adenocarcinoma risk (Castellsagué et al., 2006). Table 2 lists the severity of glandular epithelium abnormalities based on the Bethesda classification nomenclature. There is limited knowledge on the natural history of adenocarcinoma compared to the body of literature that describes the progression of cervical intraepithelial lesions to the development squamous cell carcinoma. Although adenocarcinoma *in-situ* (AIS) is often described as a precursor to adenocarcinoma, there remains a scientific debate on whether AIS proceeds the development of cervical adenocarcinoma (Seoud et al., 2011).

Table 2. Description of glandular abnormalities, Bethesda (2001) nomenclature

Classification	Type of abnormality
Atypical glandular cells (AGC)	• Atypical endocervical cells • Atypical endometrial cells • Atypical glandular cells not otherwise specified (NOS)
AGC, favor neoplasia	• Atypical endocervical cells
Endocervical Adenocarcinoma *in-situ* (AIS)	• Pre-invasive lesion of endocervical glandular cells
Adenocarcinoma	• Invasive cancer

2.2 EPIDEMIOLOGY OF HPV ACROSS THE LIFE COURSE STAGES

In general, there is an inverse relationship of age and HPV among reproductive-aged women 15-45 years old, with the peak HPV-DNA prevalence occurring among adolescent and young women <25 years old, which is around the time of sexual debut (L Bruni, Barrionuevo-Rosas, Albero, et al., 2014; Laia Bruni et al., 2010, 2015; J. S. Smith, Melendy, Rana, & Pimenta, 2008; Tiggelaar, Lin, Viscidi, Ji, & Smith, 2012). Among 18-20 year old women, it is estimated that one-third of women acquire HPV within 1 year of sexual initiation and another 20-25% will acquire an incident HPV infection within 5 years of first sexual intercourse (Winer et al., 2003). Given this, it makes sense that the greatest benefit from the HPV vaccine would be if vaccinations occur before the population is highly sexually active.

The shape of the age-specific HPV prevalence curves among middle- and older-aged women varies across different countries (Laia Bruni et al., 2015; J. S. Smith et al., 2008). For example, in Asian and some European countries HPV prevalence continue to decrease or plateau in women ≥45 years old. Alternatively, the age-specific prevalence curves in Central and South American and some African countries are U- or reverse J-shaped with a second peak prevalence around among women ≥55 years old (Laia Bruni et al., 2010, 2015). Two explanations are attributed to the second peak observed in older women. The first explanation is that new exposures are due to sexual behaviors later in life. As the vast majority of women become infected with HPV within a few years of sexual debut, sexual risk in older women is predicated on the notion that type-specific HPV infections previously acquired in life clears, but the initial immune response does no confer complete protection against reinfection (reviewed in (Gravitt, 2011)). Therefore, a woman continues to be susceptible to HPV types that she previously acquired. An alternative explanation is that a previously acquired infection does not completely

resolve, but becomes undetectable and latent. This latent infection reactivates in older women, possibly due to some event or condition that modulates the immune response and results in a recurrent HPV infection (reviewed in (Gravitt, 2011)). Evidence for both explanations exist (Gonzalez et al., 2010; Rositch et al., 2012; Trottier et al., 2010), however more research is needed to understand the proportion of newly HPV detected in older women attributable to new exposure or reactivation. Characterizing incident HPV detection in older women could help identify high-risk populations for cervical cancer screening (e.g., if the risk of cervical cancer is high following a reactivated infection), inform the optimal age to stop screening, and understand the benefit of other cervical prevention strategies, such as HPV vaccination (e.g., the proportion of the population truly at risk of new exposure of a vaccine-specific type).

Accepted to be very low in prevalence because sexual activity is rarely reported in this population, genital HPV infection has been detected in infants and young children. In a study that followed 324 infant from birth to 3 years of age as part of the Finnish HPV Family Study (Rintala, Grénman, Järvenkylä, Syrjänen, & Syrjänen, 2005), at birth 15% of the infants were HPV-DNA positive. The prevalence at various time points fluctuated between 4% (at 36 months) and 15% (at birth, 2, and 12 months) over the 3 years of follow-up. Nearly half of the infants were HPV negative during the entire follow-up period, while less than 2% of the infants were persistently HPV positive by the end of the follow-up period. Persistence was significantly associated with a recent history of genital warts in the mother (Rintala et al., 2005).

Following an HPV infection, only 60% of women will develop detectable levels of type-specific antibodies in the serum. Although the scientific literature has inconsistent results regarding the associations of seropositivity and protection against reinfection (reviewed in (Gravitt, 2011)), persistent infections are more likely to seroconvert (Tiggelaar et al., 2012).

HPV seroprevalence is generally higher than DNA prevalence, however the relationship between HPV detected in the serum and cervix varies greatly across different studies and populations (Dondog et al., 2008; Ermel et al., 2014; Markowitz, Sternberg, Dunne, McQuillan, & Unger, 2009; Tiggelaar et al., 2012; Vaccarella et al., 2010). Based on a recent review of vaccine-type specific seroprevalence, less than 5% of girls younger than 15 years old were positive for HPV-16 serotype. Seroprevalence generally increases through childhood and peaks between 25 and 50 years old (Markowitz et al., 2009; Newall et al., 2008; Tiggelaar et al., 2012). Although few studies estimate seropositivity among older women, women older than 50 have lower rates of HPV seropositivity compared to younger ages (reviewed in (Tiggelaar et al., 2012)).

2.2.1 HPV burden among pregnant and postpartum women

Understanding the burden of HPV infection in pregnant and postpartum women may help inform additional prevention strategies, such as identifying young pregnant or postpartum women as a catch-up vaccine population or vaccinating during pregnancy to prevent infection in the infants. In observational studies, HPV prevalence varied widely among pregnant women and postpartum women, ranging from 10-60% (Jalil et al., 2013; Y. H. Kim et al., 2014; reviewed in (Liu, Xu, Sun, & Wang, 2014)). As expected HPV prevalence decreases with increasing maternal age (Liu et al., 2014). Among 15-24 year old postpartum women after first pregnancy in Brazil, the HPV prevalence was estimated to be 58%, however only 13% were positive for either of the two most common oncogenic types (Rama, Villa, Pagliusi, Andreoli, Costa, Thomann, Alves, et al., 2010). A recent meta-analysis of 28 observational studies estimated a 12-18 fold higher prevalence of HPV among pregnant women compared to non-pregnant women (Liu et al., 2014), however other studies indicated no difference in prevalence between pregnant and non-pregnant women

(Kemp, Hakenewerth, Laurent, Gravitt, & Stoerker, 1992; Nicol et al., 2013; Nobbenhuis et al., 2002). It appears that the detection of any HPV or high-risk HPV peaks during the 2nd trimester and then decreases during the postpartum period (Y. H. Kim et al., 2014).

Seroprevalence studies in pregnant populations are limited and do not focus on Asian or South Asian populations. The studies are summarized in Table 3. Seroprevalence for the HPV genotypes included in the quadrivalent vaccine ranged from 9-35% of HPV-16, 6-23% for HPV-18, 0-53% for HPV-6, and 0-22% for HPV-11. Only one study specifically looks at the postpartum group. In this study, only 1% of the 15-24 postpartum women were HPV-16 and HPV-18 seropositive (Rama, Villa, Pagliusi, Andreoli, Costa, Thomann, Longatto-Filho, et al., 2010). No woman was seropositive for all four vaccine-specific HPV types, supporting the notion that catch-up vaccination in this population could still be beneficial. More data from various countries and settings are needed to help countries assess if vaccinating in this population is appropriate.

Table 3. Studies reporting seroprevalence among pregnant and postpartum women

Reference	Location	Pregnancy stage	Sample size	Age (years)	HPV seroprevalence (%)				
					6	11	16	18	Other
(Af Geijersstam et al., 1998)	Stockholm, Sweden	1st trimester	1963: 826 1983: 1719 1989: 967	Mean=24.9 Mean=28.3 Mean=29.3			16.0 22.0 21.0		
(Nicol et al., 2013)	Chagas, Brazil	1st trimester	HIV-: 164 HIV+: 89	Mean=36.7	8.5	8.5	22.6	22.6	all-HPV : 2.4 any-HPV: 68.5
(Hagensee et al., 1999)	New Orleans, USA	2nd trimester	2597	18-40 Mean=23.4			28.0		
(Syrjänen et al., 2009)	Turku, Finland	3rd trimester	290	18-38 Mean=25.6	53.3	21.5	34.8	21.5	HPV-45 : 9.0
(Heim et al., 2007)	Innsbruck, Austria	3rd trimester	104	17-42 Mean=27.0	23.1	2.9	8.7	5.8	HPV-31: 9.6
(Heim et al., 1995)	Innsbruck, Austria	Delivery	68	17-40 Mean=27.8		15.9			
(Smith et al., 2010)	Iowa, USA	Delivery	333	18-44 Mean=29.0	0.0	0.0	14.1	13.8	HPV-31 : 12.3 HPV-33 : 12.0 any-HPV: 19.3
(Rama, et al., 2010)	San Paulo, Brazil	2-months postpartum	301	15-24	5.0	2.7	9.0	7.0	HPV 16+18: 1.0 HPV 6+11: 0.7 all-HPV: 0.0 any-HPV: 19.3

2.3 RISK FACTORS TO HPV EXPOSURE AND PERSISTENCE

Since the primary mode of anogenital HPV transmission is sexual contact, the risk of exposure or acquisition of HPV occurs after initiation of sexual activity and it is influenced by risky sexual behaviors practiced by the woman, as well as their male partners. This section summarizes the risk factors related to HPV exposure and persistence.

2.3.1 Current and lifetime male sexual partners

The most obvious HPV risk factor is the number of sexual partners a woman has. Multiple current male sexual partners, as well as the total number of male sexual partners over the course of a lifetime, are strongly associated with female HPV infection across many populations and geographic settings (Althoff et al., 2009; Burk et al., 1996; Dondog et al., 2008; Moscicki, Palefsky, Gonzales, & Schoolnik, 1990; Vaccarella et al., 2006; Vinodhini, Shanmughapriya, Das, & Natarajaseenivasan, 2012). For example, *Burk et al.* observed that college-age women with two regular sexual partners in the last month 6-months have a 3-fold increase risk of HPV, compared to women who did not report having a current male sexual partner (Burk et al., 1996). *Vaccarella and colleagues* found prevalent HPV infection was positively associated with increasing number of lifetime partners in a pooled analysis of 11 HPV prevalence surveys representing a range of developed and developing countries (Vaccarella et al., 2006). Women who reported two sexual partners had a 1.9 higher odds of HPV positivity compared to women with only one partner, and the odds increased to 2.6 among women who reported four or more sexual partners (Vaccarella et al., 2006).

High number of lifetime sexual partners may be associated with HPV persistence, as well as acquisition. In a recent cohort study in Brazil, persistence was associated with a 2.5 increased odds among women with a history of four or more lifetime sexual partners compared to three or fewer (Rosa et al., 2008).

2.3.1.1 Male partner characteristic

High-risk sexual behaviors, such as young age of sexual initiation, greater number of recent or lifetime sex partners, and frequent sex increase the likelihood of HPV infection in men. (Dunne, Nielson, Stone, Markowitz, & Giuliano, 2006). Women having sexual relationships with high-risk male partners undoubtedly increase their exposure to HPV; therefore, the characteristics of the male partner are important HPV risk factors for heterosexual women.

Women attending college in the United States had an increased risk of HPV associated with having sexual relations with a non-monogamous partner or if the partners' sexual history was unknown by the women (Winer et al., 2003). In a pooled HPV prevalence analysis of 11 studies conducted by the International Agency for Research on Cancer (IARC), representing nine countries in Asia, Latin America, and Africa, husband's risky sexual behavior was associated with higher HPV positivity in the wife. The odds of HPV was 1.5 greater compared to women who reported no extramarital affair of the husband (Vaccarella et al., 2006).

Findings from a Dutch study show that increased age difference between a woman and her first sexual partner is positively associated with HPV acquisition (Kjaer et al., 2001). It makes sense that the older age of the male partner increases the likelihood of his own positive HPV status. After adjusting for a woman's number of sexual partners and age, women with a 10 or more year age difference between herself and her first sexual partner had a 5-fold increased prevalence compared to 4 years or fewer in partner age difference (Kjaer et al., 2001).

Conversely, male circumcision, which significantly reduces high-risk penile HPV prevalence by 30% in males (Tobian, Kacker, & Quinn, 2014), may confer protection in female partners. Female partners of circumcised males have a significantly lower risk of high-risk HPV incidence, prevalence, and viral load (Castellsagué et al., 2002; Davis et al., 2013; Tobian et al., 2014; Wawer et al., 2011). In a male circumcision randomized control trial where men in the intervention arm were circumcised and men in the control arm remained uncircumcised, female partners of the male trial participants showed a significant 1.4-fold reduction in cervical HPV prevalence in the intervention arm compared to the control arm (reviewed in (Tobian et al., 2014)). This reduction in cervical HPV may explain the observed lower risk of cervical cancer among monogamous women with circumcised partners, especially among those with male partners who have a history of risky sexual behavior (Castellsagué et al., 2002).

2.3.2 Age of sexual initiation

Early age of sexual debut also appears to increase risk of HPV infection in both high income countries and low- and middle-income countries (Almonte et al., 2008; Kahn, Rosenthal, Succop, Ho, & Burk, 2002; Vaccarella et al., 2006; Vinodhini et al., 2012). A pooled analysis stratified by developed or developing counties found the odds of prevalent HPV infection was 1.8 greater among women who initiated sex younger than 18 years old compared to women who initiated sex after the age of 21 in developed countries (Vinodhini et al., 2012). In developing countries, there is a clear positive association of HPV infection and lower age at first sexual experience. Compared to women who had sex for the first time after age 21, the odds of HPV was 1.7, 2.6, and 3.3 for women who initiated sex at 17-18 years old, 15-16 years old, and less than 15 years old, respectively (Vinodhini et al., 2012). Early age of sexual initiation may

simply be a predictor of lifetime partner number (Almonte et al., 2008). Kahn et al. specifically explored the sexual risk behaviors and partner characteristics as mediators of the association of first sexual encounter and HPV. The authors found that the relationship of age at first sex and HPV infection may be mediated by current number of sexual partners, history of sexually transmitted infection, alcohol and drug use, partner's age, and partner's lifetime number of sexual partners (Kahn et al., 2002). However, risky sexual behaviors and partner characteristics did not fully explain the effect of age at first intercourse.

2.3.3 Sexual transmitted infection

Both past exposure (seroprevalence) and current infection (DNA prevalence) of chlamydia are higher among HPV-positive populations (reviewed in (Silva, Cerqueira, & Medeiros, 2014)). Multiple studies have also found higher HPV incidence, prevalence, and a broader range of HPV-types among HIV-positive women compared to HIV-negative women (reviewed in (De Vuyst, Lillo, Broutet, & Smith, 2008)). The HPV-types detected in HIV-positive population are more likely to be oncogenic (reviewed in (De Vuyst et al., 2008)). However, it remains unclear if risk of HPV acquisition is specifically related to HIV-infection or if HIV and HPV are acquired concomitantly ((Adler et al., 2015; Grabowski et al., 2014; E. M. Jalil et al., 2013); reviewed in (De Vuyst et al., 2008)). High-risk and any HPV persistence has also been shown to be positively associated with HIV-status (Adler et al., 2015; Grabowski et al., 2014; E. M. Jalil et al., 2013). Increased HPV persistence among HIV-positive women aligns with the increased risk of cervical cancer progression, compared to HIV-negatives, observed in epidemiologic studies (reviewed in (De Vuyst et al., 2008; Denslow, Rositch, Firnhaber, Ting, & Smith, 2014).

The positive association of HPV and other sexually transmitted infections may be a marker of high-risk sexual behavior. Another biological plausible explanation is that STIs influence HPV cervical carcinogenesis by directly affecting the local environment of the cervix immunologically and physiologically (e.g., inducing chronic cervical inflammation) or have immunosuppressive effects, such as with HIV infection (reviewed in (Castle & Giuliano, 2003; De Vuyst et al., 2008; Ferenczy, Coutlée, Franco, & Hankins, 2003; Silva et al., 2014)).

2.4 OVERIVEW OF FACTORS RELATED TO CERVICAL CANCER NATURAL HISTORY

The overall risk of a women developing cervical cancer is 1.4%, but ranges from lower than 1% in developed regions to 3-5% in resources-poor settings in Asia, Central Europe, Latin America, and sub-Saharan Africa (Institiute for Health Metrics and Evaluation, 2011). The discrepancy between the high lifetime risk of HPV infection and the low risk of cervical cancer supports the notion that other factors either affect HPV persistence and progression or directly influence cervical cancer.

Worldwide, a lower socio-economic status is associated with increased risk of precancerous lesions and cervical cancer (Newmann & Garner, 2005; Parikh, Brennan, & Boffetta, 2003) and is a strong predictor of health seeking behaviors related to cervical cancer prevention and treatment (Newmann & Garner, 2005). Cervical cancer risk varies across difference racial and ethnic group (Downs, Smith, Scarinci, Flowers, & Parham, 2008; Newmann & Garner, 2005; Vasilevska, Ross, Gesink, & Fisman, 2012), although it is often attributed to structural barriers such as access to health care or individual barriers to getting

screening (Downs et al., 2008). However, structural barriers may not be the sole contributor factor to this disparity. Black women in the United States have higher cervical cancer incidence compared to white, non-Hispanic women even though screening rates are similar between the two groups (Downs et al., 2008), suggesting that other host factor, such as longer HPV persistence in black women compared to white women, may contribute to this disparity (Banister et al., 2014).

Traditional gynecologic environmental factors like smoking behavior and reproductive factors, such as parity and oral contraception use, have been well studied in relation to cervical cancer risk (reviewed in (Almonte et al., 2008; Castellsague & Munoz, 2003; Moore et al., 2003; Seoud et al., 2011; J. S. Smith et al., 2003)). High parity is a risk factor for cervical cancer (Castellsague & Munoz, 2003; International Collaboration of Epidemiological Studies of Cervical Cancer et al., 2006; Jensen et al., 2013; La Vecchia & Boccia, 2014; Muñoz et al., 2002). The declining trend of parity in many countries is often considered a contributory factor in the global reduction of cervical cancer burden (Mccormack & Boffetta, 2011; Muñoz et al., 2002; Vaccarella, Lortet-Tieulent, Plummer, Franceschi, & Bray, 2013). Duration of oral contraception appears to be a cofactor for cervical carcinogenesis (reviewed in (Castellsague & Munoz, 2003; Del Rosario-Raymundo, Sillero-Arena, & Galvez-Vargas, 1992; La Vecchia & Boccia, 2014; J. S. Smith et al., 2003)). Specifically, oral contraceptive use for more than five years is associated with increased risk of carcinoma *in-situ* and invasive cancer, especially among current users (Appleby et al., 2007). A recent review of dietary factors suggests that foods rich in antioxidant or antiviral nutrients and minerals, such as carotene, lycopene, and vitamins A, C, and E, help explain the observed association on high intake of fruits and vegetables and reduced risk of high-grade cervical intraepithelial neoplasia and invasive cancer

(reviewed in (Chih, Lee, Colville, Binns, & Xu, 2013)). The effect of foods that contain phytoestrogens like soy and teas are not well characterized for cervical cancer risk.

2.4.1 Potential benefit of soy and tea against cervical cancer

Establishing multiparity and long-term oral contraception as risk factors for high-grade and invasive cancer risk suggests that hormonal influences may affect HPV carcinogenesis. Soy and tea contains various classes of plant-derived compounds with weak estrogenic function. There is compelling experimental evidence that phytoestrogens, like isoflavones and flavonols, have a variety of chemopreventive properties ((Guo et al., 2004; Xiao et al., 2011), reviewed in (Butler & Wu, 2011)), suggesting that soy and tea intake may protect against cervical cancer. Tea also contains polyphenols and antioxidants that lower the risk of cervical cancer (reviewed in (Batra & Sharma, 2013)).

Soy isoflavones interact with estrogen receptors and have been shown to inhibit tumorgenesis in prostate and breast cancers (Chen et al., 2014; Hsu et al., 2012; Miller & Snyder, 2012; Anna H. Wu, Koh, Wang, Lee, & Yu, 2008; Yan & Spitznagel, 2009). A recent meta-analysis of epidemiological evidence on the relationship of tea and gynecological cancers found a decreased risk of ovarian and endometrial cancer and green tea intake (reviewed in (Butler & Wu, 2011)). Few observational studies explore the relationship of foods rich in phytoestrogens and risk of cervical cancer.

Evidence from two case-control studies provide the epidemiologic evidence about the relationship between cervical neoplasia and soy consumption, tea intake, and specific phytoestrogens. Both studies suggest that the dietary intake of tofu and other soy products was not associated with high-grade cervical malignancies (Hernandez, McDuffie, Franke, Killeen, &

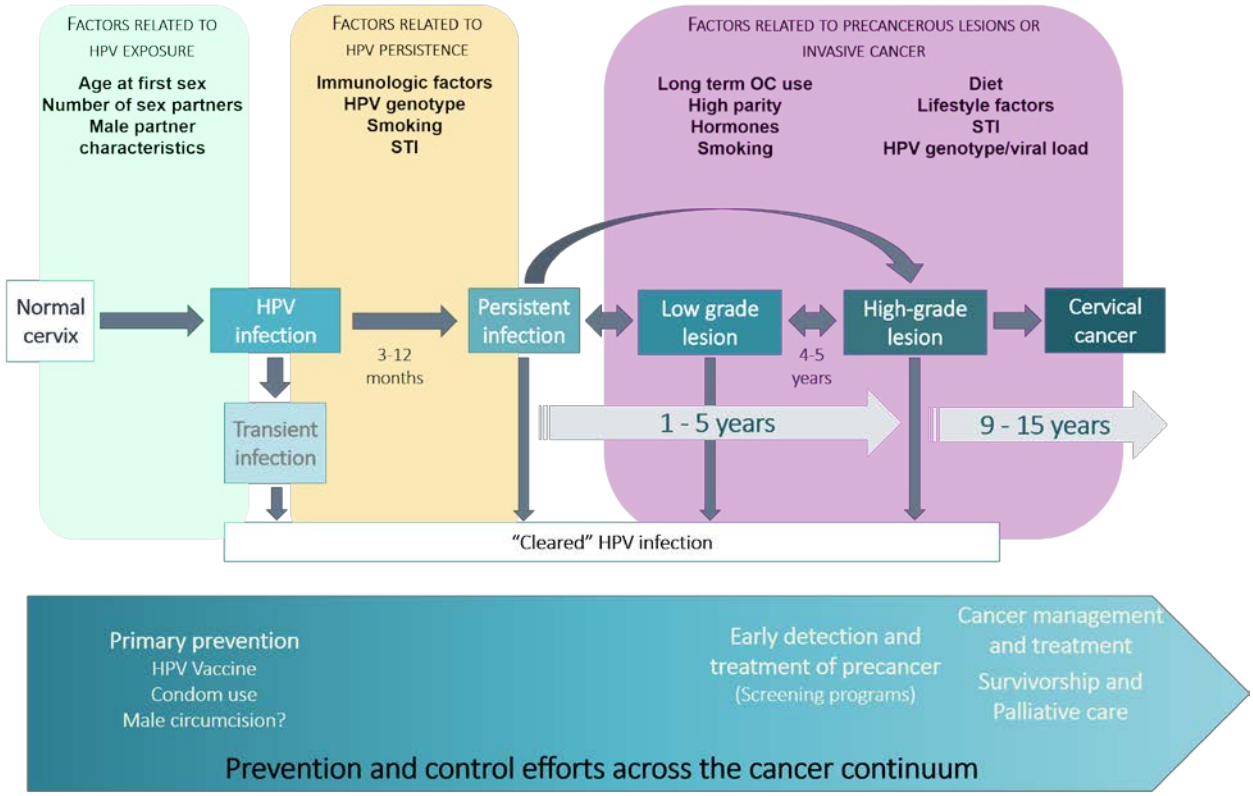
Goodman, 2004; Jia et al., 2012). The effect of tea consumption on pre-invasive and invasive cervical cancer risk is inconclusive. *Jia et al*. reported a protective effect among green tea drinkers (Jia et al., 2012), while *Hernandez et al*. show no significant association of squamous intraepithelial lesions and increasing consumption of green tea, black tea, or any tea (Hernandez et al., 2004).

Based on the results of a single study, plasma levels of daidzein, genistein, and glycitein isoflavones appear to have no association on squamous intraepithelial lesions, while increasing plasma levels of equol (metabolite of daidzein) and enterolactone (metabolite of lignan) were positively associated with squamous intraepithelial lesions (Hernandez et al., 2004). The positive association observed in this study is unexpected because equol and daidzein treatment inhibits cell growth in human cervical cancer HeLa cells and simulates apoptotic cell death (Guo et al., 2004; E. Y. Kim et al., 2014). Additionally, the use of plasma concentrations of soy isoflavones may not be an appropriate marker since plasma concentration measures short-term exposure. Short-term and long-term exposure of isoflavones may not be correlated. Measuring isoflavones using food-frequency questionnaires may be more reflective of long-term exposure, since they represent regular dietary habits and, therefore, more appropriate measure to assess cervical cancer risk.

Given the limited number of studies, the inconsistency of the data, and the potential issues with exposure assessment, a cohort or nested case-control study designs measuring soy isoflavones from validated food-frequency questionnaires could be a better design to establish if consumption of foods rich in phytoestrogens affects cervical carcinogenesis. If additional evidence supports that foods rich in phytoestrogens protect against cervical cancer, then dietary intervention promoting the consumption of foods like soy and green tea may be beneficial.

3.0 CONCEPTUAL FRAMEWORK FOR CERIVCAL CANCER PREVENTION STRATEGIES

As discussed in Chapter 2, the development of cervical cancer progresses through distinct and discernable stages, which provides the framework to develop strategies that optimize the control and prevention effort throughout the disease. Figure 1 illustrates cervical carcinogenesis, currently practiced interventions and factors related to cervical cancer development that could indicate additional opportunities for prevention and control efforts. Current comprehensive cervical cancer prevention and control efforts throughout the cancer continuum includes HPV vaccine, early detection and treatment of precancerous lesions, and improving access to pain management and other palliative care. Available primary and secondary prevention approaches are summarized below, with a focus on HPV vaccination and HPV-based screening methods and algorithms.



Adapted from references (Centers for Disease Control and Prevention, 2012; Moore et al., 2003).
HPV: human papillomavirus; STI: sexually transmitted infection; OC: oral contraception.

Figure 1. HPV oncogenesis, associated risk factors, and prevention and control strategies

3.1 PRIMARY PREVENTION

Primary prevention efforts reduce the opportunity to be infected with HPV. Limiting number of sexual partners, consistent condom use, and having a circumcised partner are ways to protect against HPV infection and cervical cancer (reviewed in (Harper & Demars, 2014)); however, the main primary prevention strategy focuses on HPV vaccines. The two first generation vaccines, Cervarix® (GlaxsoSmithKline Biologicals, Rizensart, Belgium) and Gardasil® (Merck & Co., Whitehouse Station, NJ, USA), are subunit vaccines that use non-infectious and non-oncogenic

virus-like particles. Cervarix® is a bivalent vaccine containing vaccine-like particles for oncogenic HPV-16 and HPV-18; Gardasil® contains vaccine-like particles for HPV types 16 and 18, as well as, non-oncogenic HPV types 6 and 11, the etiologic agent for 90% of genital warts in men and women. Results from the multi-country clinical trials for the bivalent and quadrivalent vaccine show the vaccine to be highly efficacious (>90%) in the prevention of HPV-16/18-related high-grade disease, using the standard 3-dose schedule (reviewed in (Harper & Demars, 2014; Schiller, Castellsagué, & Garland, 2012)). While the benefit is greater among a completely HPV-naïve population, vaccinated women with documented DNA or serologic evidence of HPV still have lower rates of cervical intraepithelial neoplasia and other HPV-related diseases, compared to women who did not receive the HPV vaccine (reviewed in (Berenson, Patel, & Barrett, 2014; Schiller et al., 2012)). The bivalent vaccine has also been shown to be efficacious in protecting against 1-year persistent infection of HPV16/18 among HPV-DNA negative women (reviewed in (Schiller et al., 2012)). Recently, at the April 2014 Strategic Advisory Group of Experts (SAGE) on immunizations meeting, the committee recommended that a 2-dose schedule for either the bivalent or quadrivalent HPV vaccine is immunologically non-inferior to the standard 3-dose schedule for girls less than 15 years old (World Health Organization, 2014b). However, the standard 3-dose schedule should still be considered for females 15 years and older and immunocompromised individuals due to the relatively lower antibody titers following vaccination, as compared to girls less than 15 years old ((World Health Organization, 2014b), reviewed in (Castellsagué, Schneider, Kaufmann, & Bosch, 2009)).

Both of these vaccines have similar safety profiles in the clinical trials and during the post-licensure phase. Injection-site related adverse event were the most commonly reported and

usually resolved within 2 days of vaccination (reviewed in (Harper & Demars, 2014; Schiller et al., 2012)). Due to a high prevalence of syncope or fainting after immunization that was documented by passive surveillance systems, it is recommended that the HPV vaccine is given while seated and the vaccinee should be observed post-immunization for 15 minutes before leaving the vaccination location (reviewed in (Harper & Demars, 2014)).

With the established vaccine effectiveness against HPV16/18 related disease, preventing a wider-range of oncogenic-HPV types has been gaining importance. Cross-protection of non-vaccine specific oncogenic-HPV infections and related disease are observed for both vaccines, especially for the genotypes that are related to HPV-16, such as HPV-31 (both), HPV-33 (bivalent only), and HPV-45 (bivalent only) ((Malagón et al., 2012), reviewed in (Schiller et al., 2012)). Although the bivalent vaccine may confer more cross-protection against non-vaccine HPV types than the quadrivalent vaccinate, the benefit may wane over time (Malagón et al., 2012). Recently, the US Food and Drug Administration (FDA) approved the 9-valent HPV vaccine (Gardasil 9®, Merck & Co., Whitehouse Station, NJ, USA), which includes five additional high-risk HP types that were not previously covered by the quadrivalent vaccine; this nanovalent vaccine now protects against the HPV types that are responsible for 90% of cervical cancers (Joura et al., 2015; U.S. Food and Drug Administration, 2014).

In 2007, Australia was the first country to adopt a national HPV immunization program, but since then over 22 countries have introduced vaccination programs (reviewed in (Markowitz et al., 2012)). The initial adoption was primarily among high-income countries, however more low-income countries are considering including HPV vaccine as part of their national immunization strategy. This has primarily been facilitated by Gavi, the Vaccine Alliance, which helped reduce the price of the bivalent and quadrivalent vaccines to less than US$5 per dose and

offers financial support for program start-up related expenses in Gavi-eligible countries (Gavi The Vaccine Alliance, 2015; "Unicef Vaccine Price Data," 2015). Implementation of the immunization programs have varied in each country; however the primary target for HPV vaccination is young adolescent girls, between that ages of 9 and 15, characterized by a highly sexually naïve population therefore limited exposure to HPV. Since the introduction of HPV vaccination, it is estimated that HPV immunization programs can reduce HPV 16/18 prevalence by 64% and 31% among adolescent girls aged 13-19 years and young women age 20-24 years, respectively (Drolet et al., 2015). Data from Australia suggests an overall 25% reduction in the risk of high-grade cervical intraepithelial neoplasia 4 years after the start of HPV vaccination program (Gertig et al., 2013). Similar reductions in cervical lesions post-HPV vaccination programs have also been observed in Denmark and Scotland (Baldur-Felskov, Dehlendorff, Junge, Munk, & Kjaer, 2014; Pollock et al., 2014).

Health and economic studies have shown that the greatest impact of the HPV vaccine is fully appreciated only with high vaccine coverage. However in many countries, including the United States, vaccination rates are less than 60% (reviewed in (Markowitz et al., 2012)). The limited contact of adolescent girls with the health system and sociocultural barriers about vaccinating adolescent girls may hinder the feasibility of reaching the desired coverage. 'Catch-up populations' for vaccinations, i.e. older populations which may have limited prior exposure to HPV, may play an important role in ensuring that sufficient immunity is attained in the population. Implementation of catch-up programs has varied from targeting 15-18 year old girls to as high as 23-26 years old women. A recent meta-analysis suggests that catch-up HPV vaccination among females 16 years and older populations has the potential to reduce the prevalence of high-grade cervical intraepithelial lesions by at least 20% (Couto et al., 2014).

In countries or other under-served settings where adolescents have limited access to the health system, capturing young pregnant women for HPV vaccines may be a potential catch-up strategy. Although HPV vaccines are not currently approved for administration during pregnancy, post-hoc analyses of pregnant women who received the vaccine during the clinical trial or post-vaccine licensure suggest that administration of the HPV vaccine is safe (reviewed in (Berenson et al., 2014)). While studies are currently underway to understand the implications of HPV vaccination during pregnancy, the current vaccines can be administered during the postpartum period. Combined with the benefit of the vaccine among sexually active women, young postpartum women may be an appropriate catch-up population, particularly if previous exposure to HPV is low. The sparse data on the need (e.g., HPV prevalence among young postpartum women), feasibility, and acceptability of such a program make it an important research question to explore further.

3.2 SECONDARY PREVENTION

The goal of cervical cancer secondary prevention is to detect precancerous lesions and treat them less invasively before invasive cervical cancer develops (see Figure 1). Early detection of cervical cancer precursors with organized cytology-based screening, such as conventional Pap test or liquid-based cytology, has successfully lowered the global cervical cancer burden, mostly in industrialized, high-resource settings (Kitchener, Castle, & Cox, 2006). Given that the sensitivity of an abnormal cytology result (atypical squamous cells of undetermined significance or greater) to detect high-grade precancerous lesions is 50-60% with poor reproducibility and technician-agreement, high clinical performance of these screening programs is dependent on

repeat testing every 1-3 years (Kitchener et al., 2006; Schiffman et al., 2011). Infrastructural and logistical barriers have been difficult to overcome with traditional Pap screening programs in limited-resourced settings and have contributed to the widening socioeconomic differences in the burden of cervical cancer. Alternative screening methods and strategies have been and continue to be developed to improve the accuracy of screening, such as HPV-DNA testing and other biomarker-based tests (reviewed in (Sahasrabuddhe, Luhn, & Wentzensen, 2011)), and to address the health system limitations of cytology, such as self-sampling and visual techniques (reviewed in (Safaeian et al., 2007)).

HPV-DNA testing was introduced initially as a sequential test for women with minor or low-grade cytology results. The detection of high-risk HPV as a triage of women with atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions improved the sensitivity of detecting cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) by 20-30% over repeat cytology within 12-months of initial abnormal cytology result (Arbyn et al., 2012, 2013). There is also consistent evidence that primary screening with the Hybrid Capture® 2 (HC2) assay (Qiagen Inc., Gaithersburg, MD, USA) has higher sensitivity than cytology for the detection of CIN2+, but similar or lower specificity (Arbyn et al., 2012; Cuzick et al., 2006; Dillner et al., 2008; Elfström et al., 2014; Gravitt et al., 2011; Sherman et al., 2003). A recent meta-analysis comparing the test characteristics of HC2 and cytology estimates the sensitivity among women 35 years and older as 94.5% and 59.5%, respectively, and specificity as 93.3% and 97.1%, respectively (Cuzick et al., 2006). Further follow-up of four randomized control studies conducted in Europe suggests that primary screening with HPV-tests provides 60–70% greater protection against cervical cancer compared with cytology-based screening (Ronco et al., 2014).

A number of countries have adopted or are considering to adopt primary HPV screening for their national screening and treatment programs. In the US, screening guidelines were updated in 2012 and recomend that HPV-based screening be offered simulatenously with conventional or liquid-based cyotolgy for women age 30-65 years old, known as HPV co-testing ("Cervical Cancer Screening Guidelines for Average-Risk Women," 2015). Co-testing extends the screening interval from 3 years for cytology only programs to 5 years. Primary HPV testing was not recommended at that time. However, recently the US FDA approved an HPV test, the cobas HPV test (Roche Molectular Systems, Pleasanton, CA), to be used as a primary screening test. The test performace of a primary HPV screening with the cobas HPV test is superior to cytoloy alone in detecting high-grade lesions and comparable to the co-testing; further, primary HPV testing requires less screening visits (T. C. Wright et al., 2015).

Most countries agree that cervical cancer screening with HPV-testing should begin around the age of 30, after the prevalence of HPV begins to decrease in the population, however the age of stopping screening varies greatly. Until the 2012 cervical cancer screening guidelines, there was no upper age limit for screening in the US and women were routinely screened well into their 80s. At present, cervical cancer screening should be stopped at age 65 for women with adequate screening history and no history of precancerous lesions. In other countries, the upper age for screening varies from 59 years to 70 years or older. Due to the limited understanding of the second HPV peak observed in older women and how that could affect a woman's risk of cervical cancer, the ideal age to stop screening has been based on expert opinion and mathematical models, rather than direct evidence. Findings from a recent study supports the US recommendation to halt screening at age 65, since women with three consecutive negative cytology screens between the ages of 50 and 64 years were at particularly low risk of a cervical

cancer diagnosis between the age of 65 and 83 years (Castañón et al., 2014). Overall, women with adequate negative screening history at 64 years have 84% less risk of being diagnosed with cervical cancer than unscreened women. However, the authors also found that the benefit of adequate negative screens at the age of 64, when a woman exits a screening program, begins to wane as time since last screen increases. Compared to 2 years after the last negative screening among adequately screening women, the odds of cervical cancer increased by nearly 20% in women who had their last negative screen 15 years ago (Castañón et al., 2014). Previously, it was believe the women who acquire new HPV infections later would not have time to develop cervical cancer. However, as the life expectancy increases in industrialized countries, HPV infections acquired later in life may have time to develop into cancer. It is also unknown if reactivated HPV in older women is more likely to persist or if it will clear at the same rate as newly acquired transient HPV infections. If reactivated HPV infections are more likely to persist and contribute to a higher proportion of the newly detected HPV in older women, this could affect the assumption that all older women are at low-risk for cervical cancer. Given that the risk of reactivated HPV may be associated with the number of previous sexual partners one has during adolescence and early adulthood, this becomes particularly relevant among the birth cohorts whose sexual debut was after the sexual revolution in the US. Women in these birth cohorts have reported higher number of lifetime partners and have higher HPV prevalence than women who initiated sexual activity before the sexual revolution (Gravitt et al., 2013). Understanding the dynamics of newly detected HPV in mid-adult and older women in more recent cohorts will play an important role in assessing cervical cancer risk and in reevaluating screening guidelines in older women, especially in the context of HPV-based screening programs, and therefore, it should be studied further.

4.0 MANUSCRIPT 1: DYNAMICS OF INCIDENT HPV INFECTION IN MIDDLE-AGED WOMEN

4.1 ABSTRACT

New detection and duration of HPV infection are not well studied in mid-adult and older women. Furthermore, more clarification is needed on the determinants of new HPV detection and HPV clearance in older ages. The purpose of this study is to estimate the new detection and clearance rates of HPV infections among a cohort of mid-adult women, aged 35-64 years old (N=736), and to determine factors associated with incident HPV and with HPV clearance/persistence. Women provided a cervical HPV specimen and completed health and sexual behavior questionnaires every 6-months over a 2-year period. Kaplan Meier methods and Cox shared frailty models were used to calculate new detection/clearance rates and risk factors for HPV. Two hundred and seventy new HPV infections were detected during the study period. Only 18% of the infections occurred among women who reported a new sexual partner. Sixty-nine percent of new detections occurred during periods of sexual abstinence or monogamy among women with ≥ 5 lifetime number of sexual partners (LTSP) and 11% of new detections occurred during periods of sexual abstinence or monogamy among women with < 5 LTSP. In addition to past and current sexual behavior, HPV detection increased with being unmarried and having an abnormal Pap result. Current hormone users were at increased risk of new HPV

detection among women with ≥ 5 LTSP. HPV persistence was more common with baseline HPV infection, while newly detected infections were transient. HPV persistence was more likely among women who were non-hormone users, unmarried, completed some post high-school education, and/or had a history of diabetes. Our findings among mid-adult women, practicing low-risk sexual behaviors, challenges the established paradigm that new HPV detected in this age group is primarily a result of new sexual exposure and could affect future cervical cancer prevention recommendations in older women.

4.2 INTRODUCTION

Dynamics of HPV infections are well studied in reproductive age women (15-45 years old), with a peak prevalence of HPV among adolescent and young women around the age of 25 years old and then decreasing with age (L Bruni, Barrionuevo-Rosas, Albero, et al., 2014; Laia Bruni et al., 2010, 2015; J. S. Smith et al., 2008; Tiggelaar et al., 2012). Newly detected HPV infections in this population are strongly associated with sexual risk behaviors (Althoff et al., 2009; Burk et al., 1996; Dondog et al., 2008; Moscicki et al., 1990; Vaccarella et al., 2006; Vinodhini et al., 2012).

Age-specific HPV prevalence curves have observed an increase in HPV among mid- and older adult women in various countries (Laia Bruni et al., 2010, 2015). There is a strong belief that most of the HPV detected in older women is driven by changing sexual behaviors due to new partnerships later in life. This explanation is predicated on the notion that type-specific HPV infections previously acquired clears, but the initial immune response does not confer complete protection against reinfection (reviewed in (Gravitt, 2011)). Alternatively, the newly

detected HPV among older women is due to a recurrent detection of a previously acquired infection that did not completely resolve, but instead became undetectable and latent (reviewed in (Gravitt, 2011)). Previously, we reported in a cohort of older women more likely to be engaged in current low-risk sexual behaviors that most of the incident HPV infection detected were attributed to past, not recent, sexual behavior (Rositch et al., 2012). Other studies have also shown that HPV detected in older women is explained by both new sexual partners and lifetime number of sexual partners (Gonzalez et al., 2010; Winer et al., 2016). These findings support a natural history model for mid-adult and older women that include both HPV latency and sexual acquisition.

Assuming HPV detected in this older population comprises of both redetection of prior HPV acquisition and newly acquired HPV, it is important to understand if these infections are mostly transient and become resolved or undetectable within 1-2 years of initial detection, similar to the natural dynamics in younger women, or if HPV clearance/persistence is different in older women. Characterizing HPV detected in mid- and older adult women could help to inform changes in cervical cancer screening policies in older ages and to understand the benefit of other cervical prevention strategies, such as HPV vaccination.

In the present longitudinal analysis, using the same population previously studied (Rositch et al., 2012), we aimed to estimate the incidence and clearance rates of HPV infections among a cohort of mid-adult women, estimate fraction of new HPV detection attributed to past and current sexual behavior, and to determine factors associated with incident HPV and with HPV clearance/persistence.

4.3 METHODS

4.3.1 Study population and data collection

Women were enrolled in the HPV in Perimenopause (HIP) Study, which is an outpatient obstetrics/gynecology clinic-based cohort in and surrounding areas of Baltimore, Maryland, if they were 35-64 years, had an intact cervix and were willing to provide informed consent. Women were excluded if they were currently pregnant, had plans to become pregnant within the next 2 years, or were considered immunocompromised due to history of organ transplantation or due to positive HIV status. Recruitment and enrollment of 951 eligible women attending clinics for routine exams was over a 3-year period, from March 2008 to March 2011. Women were followed every 6-months for 2-years. At baseline and every 6-month visit, a trained study physician or registered nurse conducted a speculum examine to collect an HPV DNA specimen using the Digene HPV cervical brush (Digene, United States). Cervical brushes were placed in standard transport medium and stored at 4°C. Within 24 hours of sample collection, cervical specimens were vortexed, aliquotted, and stored at -80°C. In addition to the gynecologic exam, detailed information on sociodemographic characteristics, medication use, reproductive health and sexual history, and recent sexual behaviors (previous six months) was collected with questionnaires at baseline (administered via telephone) and at each follow-up visit (administered face-to-face). All study protocols were approved by the Johns Hopkins Bloomberg School of Public Health Institutional Review Board. To conduct this analysis, additional approval was obtained from the University of Pittsburgh Institutional Review Board.

4.3.2 HPV genotyping

An 8-µl aliquot of DNA, which was extracted from the sample using the QIAmp DNA Blood Kit (Qiagen), was genotyped for HPV using the Roche HPV Linear Array PCR-based assay (Roche Diagnostics). The Roche HPV Linear Array uses the PGMY09/11 PCR primer system to detect 37 distinct high- and low-risk HPV types. Detection of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 was classified as high-risk (carcinogenic) HPV. The remaining HPV types detected by the system, classified as low-risk HPV in this analysis, are as follows: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108.

4.3.3 Statistical analysis

For this analysis, women were included if they had completed the enrollment questionnaire and had valid HPV DNA results at baseline and $\geq$ 1 follow-up visit. Descriptive statistics, frequencies and percentages, of the baseline characteristics of the analytical population were stratified by cumulative recent sexual behaviors over the 2-year follow-up period: women who reported never having sex, having sex but never with a new partner, and ever reporting a new sex partner during the follow-up period. Differences in proportions were tested using chi-square test.

In the incidence analysis, women were considered at risk for a maximum of 37 HPV types (e.g., no HPV presented at baseline); a woman would not be considered at risk for any HPV type detected at baseline. Time at risk was calculated from baseline to detection of first type-specific infection (using the mid-interval date between of the first positive HPV specimen and the collection date of the previous sample) or last study visit if negative for type-specific

infection. In the clearance analysis, HPV clearance was defined as two-consecutive type-specific negative results or if type-specific HPV was not detected at the last visit. Women were considered at risk for clearance for each type-specific HPV detected at baseline or during follow-up. Infections detected at the last study visit were excluded from the clearance analysis. Time at risk was calculated from date of first type-specific detection to loss of detection (clearance) of type-specific infection (using the mid-interval date between of the first negative HPV specimen and the collection date of the previous sample) or last study visit if type-specific infection remained detectable. If an HPV result was missing between 2 non-missing results, the prior non-missing HPV DNA result was carried forward.

Incidence and clearance rates were calculated for the total cohort, categories of recent sexual activity, and lifetime number of sexual partners by dividing the number newly detected infection or the number of infections no longer detected by the infection-months at risk, expressed as new HPV detected or loss of HPV detection per 1000 infection-months, respectively. Clearance rates were also compared between prevalent baseline infection and newly detected HPV infection. We also calculated woman-level incidence and clearance rates in addition to infection-level incidence and clearance used in the main analysis. In the woman-level analysis for incidence (or clearance), time origin for new detection (or clearance) of HPV DNA was defined as the first detection (or non-detection) of any HPV type, at which time the woman is censored, or the last study visit if no new HPV is detected (or no clearance event). Cumulative probability of new HPV detection or clearance (woman-level) was estimated using the Kaplan Meier method.

In an interim analysis of this cohort, we showed that both recent sexual activity and past sexual behavior contributed to incident HPV detection in this cohort (Rositch et al., 2012). With

the completion of the study, we again calculated the attributable risk and population attributable risk for lifetime number of sexual partners (past sexual behavior) and new sexual exposure (recent sexual activity) using unadjusted incidence rate ratios. Lifetime number of sexual partners (LTSP) was dichotomized as < 5 LTSP and ≥ 5 LTSP. Recent sexual activity was categorized as follows: no recent sexual activity, recent sexual activity with no new partner and recent sexual activity with new partner. To calculate the population attributable risk for recent sexual activity, a time-varying covariate, we used the cumulative recent sexual behavior.

Cox proportional models with shared frailty were used to determine risk factors for new HPV detection or HPV clearance. Given each woman's multiple visits and the 37 distinct HPV types, the frailty model accounts for normally distributed random effects due to individual susceptibility to type-specific infection and correlations among multiple events recorded for the same women. In addition to recent and past sexual behaviors, other variables considered as risk factors included the following: age (<50 or ≥50), race (white, black, or other), education (high school, post high school, or college/postgraduate), income (<$80,000, ≥$80,000, or unknown), marital status (married or not married), smoking (no/yes), obesity (<30 or ≥30), menopausal status (pre, post), hormone use (no/yes), recent sexually transmitted infection (no/yes), and abnormal pap (no/yes). Associations with history of diabetes, any non-cervical cancer, arthritis, and autoimmune disease were also explored. Age, current marital status, hormone use, and recent sexually transmitted infection were included in the model as time-variant variables. Variables tested in the multivariable models included recent and past sexual behaviors, factors that were hypothesized a priori as important confounders (age, race, education) and variables found to be statistically significant ($p<0.10$) in the univariate analysis. Using the premise that the risk of recurrent HPV can only be studied among people who are at greater risk, we also

tested an interaction term between lifetime number of sexual partners and recent sexual behavior in the incidence analysis. Statistical analyses were conducting using Stata 13.1 (StatCorp, College Station, Texas).

4.4 RESULTS

4.4.1 Study population characteristics

Out of the 951 women enrolled into the study cohort, 65 women were excluded due to incomplete baseline data. Of the remaining 885 women, we included 736 women in the incidence analysis who returned for at least 1 follow-up visit and had a valid HPV DNA result. Overall, the median follow-up time was 24.7 months (interquartile range: 22.2 – 26.2 months). Over half of the women completed all follow-up visits (56.1%) with a mean follow-up time of 26.3 months (standard deviation ± 4.2 months). The clearance analysis was restricted to 222 women who had at least one HPV type detected at baseline or anytime during the study follow-up period. We excluded 488 (66.3%) women who did not have any HPV detected at baseline or follow-up and 22 (3.0%) women who had their first HPV detected in their final visit.

The median age of the study population was 47 years old (interquartile range: 41 – 52) and the majority of the population was White/Caucasian (75.5%), college graduates (61.7%), and married at the time of enrollment (63.5%). Approximately 30% of the women were postmenopausal. Nearly three-fourths of the study population (73.2%) reported having sex with a single stable partner during the course of the study; only 11.7% of the women reported having sex with at least one new partner. Recent sexual behavior was correlated with age, race, income,

marital status, menopausal status, BMI, history of abnormal Pap, and lifetime number of partners (Table 4). The youngest women (age 35-39 years old) were more likely to report having sex with a new partner (21.3%), while the oldest women (age 55-60 years old) were more likely to report no sexual activity (25.5%). Black/African-American women and any women with ≥5 lifetime number of partners were more likely to report having sex with a new partner (20.0%, 15.8%, respectively). Postmenopausal women and women with a BMI ≥ 30 were more likely to report no sexual activity (24.5%, 22.7%, respectively). Unmarried women were equally likely to report sexual inactivity (30.7%) or recent sexual activity with a new partner (25.3%) (Table 4).

At baseline, 18.6% of the study population had a prevalent HPV infection. High-risk and low-risk HPV was detected in 8.4% and 13.3% of women, respectively. Forty-four percent of women with a baseline HPV infection had a persistent baseline infection with either a high- or low-risk type through their study follow-up.

The average number of HPV types detected during the study period, including baseline and follow-up, was higher in women reporting sex with at least one new partner compared to women not reporting a new partner, neither abstinent women nor women reporting sexual activity during the study period (Table 5). Within each cumulative recent sexual behavior strata, women with ≥ 5 LTSP had an approximate 2-fold increase in the number of HPV types detected compare to women with < 5 LTSP.

4.4.2 New detection of HPV infection

Out of the 736 women followed, 83 (11.2%) women had a new high-risk HPV detected and 121 (16.4%) women had a new low-risk HPV detected. Overall, 169 women had at least one incident infection detected (23.0%) with a women-level infection rate of 11.3 infections per 1000

women-months. Two hundred and seventy new type-specific infections were detected, for an infection-level incidence of 0.43 infections per 1000 infection-months (Table 6). New HPV detection rates differed by LTSP and recent sexual activity. Compared to women with < 5 LTSP, rate of new HPV detection was 3.5-fold higher among women with ≥ 5 LTSP. Reporting sexual activity with a new partner in the prior 6 months significantly increased the risk of incident detection (Incidence Rate (IR) = 7.46; 95% CI: 4.77, 11.76). Among women with a new sexual partner in the prior six months, 86.6% of new HPV detections could be attributed to new sexual partner. However, only 43.7% of the new infections in the study population were attributed to recent sex with a new partner since only 11.7% of the study cohort had a new partner. In contrast, 72.1% of new HPV detections among women with ≥ 5 LTSP were attributed to ≥ 5 LTSP, which translates to 60.5% of the new detections in the study population being attributed to high LTSP. Although the fraction of HPV detected among the different exposure categories (new sexual partner and ≥ 5 LTSP) was high, the population attributable fraction of ≥ 5 LTSP was higher because the prevalence of ≥ 5 LTSP (61.9%) was higher than the prevalence of having a new sexual partner.

4.4.3 Risk factors for new HPV detection

In unadjusted models with the full cohort, women who were unmarried, had ≥ 5 LTSP, reported recent sexual activity with same partner or with a new partner, were current hormone users, and had an abnormal Pap during the study period were associated with increased risk of new HPV detection (Table 7). Women who were ≥50 years old and reported a yearly income of $80,000 or more were associated with a decreased risk of new HPV detection. Risk factors for new HPV detection differed between women with < 5 LTSP and women with ≥ 5 LTSP (Table 7).

Although current hormone use was positively associated with new HPV detection in the full cohort, the stratified analysis shows the increased risk of new HPV detection was among women with ≥ 5 LTSP, not < 5 LTSP. Black women were at higher risk of new HPV detection (HR=3.06; 95% CI: 1.02, 9.11) but there was no statistically significant association with race and risk of new HPV detection among women with ≥ 5 LTSP. Recent sex with a new partner remained significant, irrespective of LTSP. After adjustment of confounders, there was a statistically significantly increased risk of new HPV detection with being unmarried, having a new sexual partner in the prior 6 months and/or having an abnormal Pap in women with < 5 LTSP (Table 8). In addition to the risk factors identified in women with < 5 LTSP, recent sexual activity with no new partner and current hormone use was statistically associated with new HPV detection among women with ≥ 5 LTSP. The strength of association for abnormal Pap (HR=5.82; 95% CI: 2.43, 13.93) and sexual activity with a new partner (HR=11.09; 95% CI: 3.63, 33.87) was stronger among women with < 5 LTSP compared to women with ≥ 5 LTSP ($HR_{Abnormal\ Pap}$ = 1.94; 95% CI: 1.15, 3.27; $HR_{Sexual\ activity,\ new\ partner}$ = 3.68; 95% CI: 2.11, 6.41; Table 5).

4.4.4 Combined effect of past and current sexual behavior

To explore the joint relationship of past and recent sexual behavior on new HPV detection, we compared new HPV detection rates between past and recent sexual behavior categories (Table 9). New HPV detection rates were nearly identical between non-sexually active women with < 5 LTSP (0.13 infections per 1,000 infection-months) and women reporting sex but with no new partner and < 5 LTSP (0.14 infections per 1,000 infection-months). Among sexually inactive women, women with ≥ 5 LTSP had an approximate 2-fold increase in new HPV detection

compared to women with < 5 LTSP. While, sexually monogamous women with ≥ 5 LTSP had a 4-fold increase in new HPV detection compared to < 5 LTSP. Lifetime number of sex partners does not appear to differentially increase rate of new HPV detection among women reporting sex with new partners (Incidence Rate $(IR)_{<5\ LTSP,\ Sex\ with\ new\ partner}$ = 2.14 infections per 1,000 infection-months vs. $IR_{\geq 5\ LTSP,\ Sex\ with\ new\ partner}$ = 1.90 infections per 1,000 infection-months). The relative rate of new HPV detection was highest among women with < 5 LTSP who reported sex with a new partner in the previous six months (Incidence Rate Ratio (IRR) = 17.04; 95% CI: 5.86, 52.76) and women with ≥ 5 LTSP who reported sex with a new partner in the previous six months (IRR = 15.13; 95% CI: 6.69, 40.09).

The combined effect of past and recent sexual behaviors on new HPV detection was significant after adjustment of selected variables (Table 9). The risk of incident HPV was 2.74 higher among sexually inactive women with ≥ 5 LTSP compared to sexually inactive women with < 5 LTSP. (p=0.02) Being sexually active with the same partner and having ≥ 5 LTSP, being sexually active with a new partner and having < 5 LTSP, and being sexually active with a new partner and having ≥ 5 LTSP were all significantly associated with increasing risk of new HPV detection (all p's <0.001).

4.4.5 HPV clearance and associated risk factors

Two hundred and twenty-two women with baseline or new HPV detection were followed to estimate HPV clearance rates. Proportion of women who cleared a high-risk HPV infection (72/104 or 69.2%) or a low-risk HPV infection (118/168 or 70.2%) was similar, as was the proportion of women who cleared a baseline HPV infection (99/137 or 72.3%) or a newly detected HPV infection (96/131 or 73.3%).

There were 242 infections that cleared during the course of the study, for a clearance rate of 55.4 infections per 1000 infection-months and infection-level clearance rates differed between baseline and newly detected HPV (Table 10). The median time of clearance of baseline HPV and newly detected HPV was 18 months and 6 months, respectively (Figure 2). HPV clearance rates did not vary significantly between sexually inactive women (46.5 infections per 1000 infection-months), sexually active women with no new partner in the prior 6 months (59.3 infections per 1000 infection-months), and sexually active women with a new partner in the prior 6 months (52.0 infections per 1000 infection-months).

In the unadjusted models, current hormone use was associated with HPV clearance, while HPV persistence was associated with initial detection at baseline, being unmarried, completed some post high-school education, being postmenopausal, and reporting history of diabetes (Table 11). Recent sexual behavior or number of lifetime sex partners was not associated with HPV persistence/clearance. After adjustment, the 40% increased risk of persistence among baseline HPV infections compared to newly detected infections remained unchanged from the unadjusted model (p=0.001). The increased risk of HPV clearance was strengthened for current hormone users after adjustment. Higher income was no longer associated with higher HPV clearance after adjustment for other socioeconomic indicators, such as race and education. On the other hand, the over 1.5-fold increased risk of persistence among women who completed some post high-school education, compared to high school educated women, remained statistically significant after adjustment of other socioeconomic indicators. Women with a history of diabetes had nearly a 50% increased risk of persistence (HR=0.52; 95% CI: 0.30, 0.91).

4.5 DISCUSSION

In a cohort of mid-adult women, we found that nearly a quarter of the women followed had a new HPV detection during the course of the study. The new HPV detection rate of 11.3 per 1000 women-months confirms what we observed in the interim analysis (Rositch et al., 2012). Our findings were within range of what has been reported in older women previously (Goodman et al., 2009; Trottier et al., 2010; Winer et al., 2016). Although the risk of new HPV detection associated with a new sexual exposure was strong, less than half of the HPV detected among the 35-60 year old women could be attributed to a recent sex with a new partner in this population. This fraction is higher than the 27% we reported previously (Rositch et al., 2012) and is most likely a result of increased reporting of new sexual partners during the additional months of follow-up included in this analysis. In contrast, 60.5% of the new HPV detections in the population were attributed to having ≥ 5 LTSP.

It is well established and understood that a recent sexual exposure, usually measured using self-reported indicators such as number of recent male sexual partners, new male partners, monogamous sexual partner, and marital status, is the primary risk factor for HPV acquisition. Given that the majority of new HPV detections could not be attributed to new sexual exposure, an alternative explanation needs to be explored. There is growing support that HPV detection in older ages is not just due to new acquisition, but is also a function of hormonal and age-related changes which reactivate HPV that is located deep in the basal layer at undetectable levels based on current detection methods (reviewed in (Gravitt, 2011)). Although mechanistically not well characterized, a previously acquired latent HPV infection may reactivate due to immunosuppression or immunosenescence (reviewed in (Gravitt, 2011). However, risk of HPV reactivation is conditioned on how many past HPV acquisitions a woman is harboring; therefore,

probability of past exposure plays an important role in reactivation. We measured past probability of HPV acquisition using lifetime number of sexual partners. High lifetime number of sexual partners is an indication of more exposure opportunities and associated with multiple HPV infections (Chaturvedi et al., 2011; Nielsen, Kjaer, Munk, & Iftner, 2008). The majority of the studies exploring associations of recent and past sexual behavior and HPV report independent associations of current number of sexual partners and/or lifetime number of sexual partners (Baldur-Felskov et al., 2014; Burk et al., 1996; Goodman et al., 2009; Sadate-Ngatchou et al., 2016; Trottier et al., 2010). We showed evidence of an interaction between LTSP and recent sexual activity on new HPV detection. Comparing women with similar recent sexual behavior, new detection rates were more than 2-fold higher for women with high number of LTSP among sexually inactive or sexually monogamous women; rates of new HPV detection were not different if women had a recent new sex partner.

The LTSP stratified models also indicate differences of race, income and education on risk of new HPV detection, which are markers of unmeasured past sexual activity (Kann et al., 2016), and therefore indicators of women with higher exposure who are at greater risk of reactivation. We may have underestimated the fraction of new HPV detection attributed to new sexual exposures since we do not have data on partners past sexual history or infidelity. However, since the new detection rates of HPV are similar among sexually abstinent women with < 5 LTSP (low probability of HPV exposure) and sexually active women with no new partner and a low probability of HPV exposure, it is unlikely that the excess risk of new sexual exposure from unmeasured partner behavior would change our findings. These findings strengthen the argument that although women remain at risk for new HPV acquisition at older ages, reactivation plays an important role.

Data on HPV clearance is limited in older women. New HPV detections were more likely to be transient, while HPV types present at baseline had a longer duration. Median time to clearance for baseline and new HPV detection was 18-months and 12-months. This is consistent with previous studies that estimate 25-50% of infections persist for more than 1 year in older women. (Goodman et al., 2009; Grainge et al., 2005; Molano et al., 2003; Sellors et al., 2003). Contrary to previous studies that showed increasing age was associated with HPV clearance/persistence (Goodman et al., 2009; Rosa et al., 2008), our study showed no association with age and clearance. We did observe a 2-fold increase risk of persistence among women with a history of diabetes. Elevated blood sugars cause negative effects on the immune system and diabetic patients are considered more immunosuppressed and more prone to lower genital tract infections (Donders, 2002), which could explain this finding. In addition, women with type 2 diabetes have a higher prevalence of cervical cancer (N. A. Jalil, Zin, & Othman, 2015), and may have more extensive genital warts, more recurrences, and may require more treatments (Yong, Parkinson, Goenka, & O'Mahony, 2010).

Unmarried women were associated with higher likelihood of new HPV detection and HPV persistence, even after accounting for recent sexual behaviors and socioeconomic factors. Currently unmarried women were more likely younger, Black, lower income, less educated (higher proportion of post high school among singles and higher proportion of post graduate among married), and having higher number of LTSP, which could explain this association. Marital status is considered a marker of sexual behavior and is usually not associated with new HPV infection, after adjustment of other sexual behavior variables. Residual confounding of unmeasured past and current sexual behavior could be a possible explanation for this finding. On the other hand, unmarried women may be a subpopulation who experience more "stressors",

such as depression and anxiety, which can have negative effects on health and has been shown to be related to HPV persistence (Moscicki, Ma, & Farhat, 2016; Saadat, Behboodi, & Saadat, 2015).

Some study limitations should be noted. We could not differentiate new HPV acquisitions from redetection or reactivation of a prior HPV infection that fluctuates at the lower detection limit of the assay. Availability of type-specific serology might provide additional, but incomplete information on previous exposure, since only 50-60% of women exposed have detectable serologic measurements (Stanley, Pinto, & Trimble, 2012; Tiggelaar et al., 2012). In addition, since there is no known serologic correlation with HPV immunity or minimum level of detection determined to be protective, we could not with certainty identify if an HPV infection truly cleared or was just undetectable. We used the conventional definition of clearance, which is two consecutive negative type-specific HPV results. However, this may be an underestimation, as women who have a negative HPV test between two positive HPV tests, maybe be representative of a woman who cleared her infection and then acquired a new infection of the previously detected type. Time at risk for clearance of new HPV infection was also limited since the median follow-up time for a new HPV detection was 9 months due to loss of follow-up or limited follow-up time among new infections detected half way through the study follow-up period. This underestimates the duration of infection and requires extended follow-up to address. Although the HIP Study is a prospective cohort design, it is a clinic-based population of middle-aged women who have positive reproductive health-seeking behaviors, such as routine cervical cancer screening. The women in this cohort are at relative low-risk for incident HPV detection; therefore, the findings from this study may not be representative of the general

population. However, the women are likely to represent the majority of older women who are receiving a HPV test as part of routine cervical cancer screening.

This study in mid-adult women adds to the scarce data on HPV natural history in this age group. In general, new HPV infection was mostly transient, similar to adolescent populations. Our findings among older screened women, with predominately low-risk current sexual behaviors, challenges the established paradigm that new HPV detected in this age group is primarily a result of new sexual exposure, as with younger populations. High number of lifetime sexual partners could explain more new HPV detection compared to recent new sexual partner. The clinical implications of these findings need to be evaluated, especially as cervical cancer prevention programs, such as HPV-FASTER (F Xavier Bosch et al., 2016), propose routine HPV vaccination be extended to older ages in combination with HPV-based screening. New infections detected due to previous exposure would not be prevented by HPV vaccination, consistent with previous recommendations that HPV vaccination in older women will likely provide little benefit (Westra et al., 2011). Furthermore, the presence of reactivated HPV in older women challenges current cervical cancer screening guidelines. If new HPV detected in older women followed only a sexual acquisition natural history model then these women would likely be of little risk of cervical disease because of mostly transient infection coupled with the decade-long period between persistent HPV infection and high-grade cervical disease. However, risk of cervical disease needs to be carefully evaluated in older women with new infections detected due to reactivation because the sexual debut of the currently aging women occurred after the sexual revolution. These women are more likely to have high number of past sexual partners and therefore more likely to get a recurrent HPV infection. Given the uncertainty of cause of new HPV detection, personalized prevention using a risk-based framework (Castle & Katki, 2016)

that assesses current and past sexual behavior and other risk factors to identify high-risk mid-adult or older women for HPV vaccination or cervical cancer screening may have a more meaningful public health impact.

4.6 TABLES AND FIGURES

Table 4. Baseline characteristics, stratified by cumulative recent sexual activity, HIP cohort

	No reported sex during study period (N=111)	Reported sex, no new partner (N=539)	Reported sex, at least 1 new partner (N=86)
Age (years)			
35-39	7 (5.1%)	100 (73.5%)	29 (21.3%)
40-44	23 (15.4%)	113 (75.8%)	13 (8.7%)
45-49	26 (14.0%)	140 (75.3%)	20 (10.8%)
50-54	28 (17.6%)	111 (69.8%)	20 (12.6%)
55-60	27 (25.5%)	75 (70.8%)	4 (3.8%)
Marital status			
Married	28 (6.0%)	421 (90.2%)	18 (3.9%)
Unmarried ǂ	83 (30.7%)	118 (43.9%)	68 (25.3%)
Race			
White	87 (15.9%)	409 (74.5%)	53 (9.6%)
Black	19 (14.1%)	89 (65.9%)	27 (20.0%)
Other	5 (11.6%)	33 (76.7%)	5 (11.6%)
Missing	--	8	1
Highest education completed			
High school	19 (16.2%)	81 (69.2%)	17 (14.5%)
Post high school	22 (13.3%)	120 (72.7%)	23 (13.9%)
College/Post graduate	70 (15.4%)	338 (74.5%)	46 (10.1%)
Yearly income (USD)			
≤80,000	54 (24.0%)	126 (56.0%)	45 (20.0%)
>80,000	44 (11.4%)	315 (81.4%)	28 (7.2%)
Unknown	13 (10.5%)	98 (79.0%)	13 (10.5%)
Smoking history			
Never	70 (13.7%)	384 (74.9%)	59 (11.5%)
Former	27 (18.1%)	106 (71.1%)	16 (10.7%)
Current	14 (18.9%)	49 (66.2%)	11 (14.9%)

Table 4 – Continued

	No reported sex during study period (N=111)	Reported sex, no new partner (N=539)	Reported sex, at least 1 new partner (N=86)
Menopausal status			
Premenopausal	61 (11.7%)	394 (75.6%)	66 (12.7%)
Postmenopausal	49 (24.5%)	133 (66.5%)	18 (9.0%)
Missing	1	12	2
Lifetime sexual partners			
<5 partners	44 (15.7%)	222 (79.3%)	14 (5.0%)
≥5 partners	67 (14.7%)	316 (69.5%)	72 (15.8%)
Missing	--	1	--
History of hormone use for any reason			
Never	14 (23.3%)	40 (66.7%)	6 (10.9%)
Former	78 (15.7%)	361 (72.5%)	59 (11.9%)
Current	19 (10.7%)	138 (77.5%)	21 (11.8%)
Ever STI, self-report			
No	104 (15.2%)	503 (73.3%)	79 (11.5%)
Yes	7 (14.0%)	36 (72.0%)	7 (14.0%)
Ever abnormal Pap, self-report			
No	62 (15.9%)	290 (74.6%)	37 (9.5%)
Yes	48 (14.2%)	242 (71.4%)	49 (14.4%)
Missing	1	7	--

Abbreviations: sexually transmitted infection (STI)
ǂ Includes never married, single, divorced, widowed at baseline

Table 5. Average number of HPV types detected during the study period, total and sexual behavior

	Total population			
	N	**Mean**	**SD**	**Range**
Total	736	0.63	1.16	0-7
Lifetime number of sexual partners*				
< 5 partners	111	0.36	0.68	0-3
≥ 5 partners	539	0.46	0.92	0-7
Recent sexual behavior†				
No sexual activity	111	0.36	0.68	0-3
Sexual activity, no new partner	539	0.46	0.92	0-7
Sex, with at least 1 new partner	86	2.06	1.76	0-6
Past and recent sexual behavior*†				
< 5 LTSP; No sexual activity	44	0.16	0.37	0-1
≥ 5 LTSP; No sexual activity	67	0.49	0.80	0-3
< 5 LTSP; Sexual activity, no new partner	222	0.25	0.68	0-5
≥ 5 LTSP; Sexual activity, no new partner	316	0.60	1.07	0-7
< 5 LTSP; Sex, with at least 1 new partner	14	1.29	1.27	0-4
≥ 5 LTSP; Sex, with at least 1 new partner	72	2.21	1.81	0-6

Abbreviations: standard deviation (SD), lifetime number of sexual partners (LTSP)

*1 observations missing LTSP information but reported sex with no new partner had 1 HPV type detected during study period.

†Recent sexual behavior defined as cumulative sex behavior (no sex over follow-up; sex but with no new partners; or sex with at least 1 new partner over follow-up).

Table 6. Incident infections to 37 HPV types for total cohort and by sexual behavior

	Incident infections	Infection-time *	Crude Incidence rate (95% CI)	Rate Ratio (95% CI)	$AR_{exposure}$	PAR†
Total	270	621.02	0.43 (0.39, 0.49)			
Lifetime sexual partners ‡						
< 5 partners	41	238.54	0.17 (0.13, 0.23)	Ref		
≥ 5 partners	228	381.54	0.60 (0.52, 0.69)	3.48 (2.48, 4.97)	71.2%	60.5%
Recent sexual behavior (past 6 months) ǂ						
No sexual activity	37	142.30170	0.26 (0.19, 0.36)	Ref		
Sexual activity, no new partner	182	445.10201	0.41 (0.35, 0.47)	1.57 (1.10, 2.30)	36.4%	29.5%
Sexual activity, new partner	49	25.256612	1.94 (1.47, 2.57)	7.46 (4.77, 11.76)	86.6%	43.7%

Abbreviations: attributable risk (AR); population attributable risk (PAR); prevalence (P); Rate Ratio (RR)

* Infection-time = 1000 infection-months

‡ Lifetime number of sexual partners (LTSP) was collected at baseline. One incident detection due to missing lifetime number of sexual partners information.

ǂ Recent sexual behavior is a time-varying variable and refers to the 6-month period before each study visit. Women can contribute infections and person-time to more than one category. One incident detection due to missing lifetime number of sexual partners and two incident detections due to missing recent sexual activity data are not included.

† Population attributable risk = Pexposure (RRexposure-1) / [1 + Pexposure (RRexposure-1)]. The exposure prevalence (Pexposure) was categorized cumulative sex behavior (no sex over follow-up, sex but with no new partners, or ever a new partner over follow-up).

Table 7. Risk factor for newly detected HPV, total and by lifetime number of sexual partners, unadjusted hazard ratios

	Total	<5 LTSP	≥5 LTSP
	Unadjusted HR (95% CI)	Unadjusted HR (95% CI)	Unadjusted HR (95% CI)
Demographics			
Age (years)*			
<50	Ref	Ref	Ref
≥50	0.75 (0.54, 1.05)††	0.78 (0.35 1.75)	0.81 (0.57, 1.16)
Race			
White	Ref	Ref	Ref
Black	1.31 (0.86, 1.99)	3.06 (1.02, 9.11)†	1.00 (0.65, 1.54)
Other	1.44 (0.74, 2.81)	0.99 (0.21, 4.74)	1.99 (0.93, 4.24)
Highest education completed			
High school	Ref	Ref	Ref
Post high school	1.49 (0.88, 2.53)	2.43 (0.67, 8.81)	1.15 (0.65, 2.02)
College/Post graduate	0.89 (0.56, 1.44)	0.95 (0.27, 3.34)	0.86 (0.49, 1.51)
Yearly income (USD)			
<80,000	Ref	Ref	Ref
≥80,000	0.71 (0.49, 1.01)††	0.20 (0.08, 0.52)†	0.89 (0.61, 1.30)
unknown	0.80 (0.51, 1.25)	0.35 (0.13, 1.02)	1.00 (0.62, 1.63)
Marital status*			
Married	Ref	Ref	Ref
Unmarried ǂ	3.87 (2.83, 5.30) †	7.03 (3.14, 15.77)†	2.8 (2.00, 3.95)†
Reported smoking in last 6 months*			
No	Ref	Ref	Ref
Yes	1.40 (0.84, 2.35)	0.37 (0.04, 3.65)	1.32 (0.79, 2.19)
BMI (baseline)			
<30	Ref	Ref	Ref
≥30	0.94 (0.65, 1.37)	0.79 (0.30, 2.05)	0.99 (0.67, 1.47)
Sexual Behaviors and Reproductive Health			
Lifetime sexual partners			
<5 partners	Ref		
≥5 partners	3.54 (2.39, 5.24)†		
Recent sexual activity (6 months)			
No sexual activity	Ref	Ref	Ref
Sexual activity, no new partner	1.83 (1.22, 2.75)†	1.14 (0.45, 2.87)	1.79 (1.15, 2.78)†
Sexual activity, new partner	5.66 (3.43, 9.34)†	11.81 (3.53, 39.43)†	4.25 (2.46, 7.37)†
Menopausal status			
Premenopausal	Ref	Ref	Ref
Postmenopausal	0.78 (0.53, 1.14)	0.58 (0.22, 1.51)	0.90 (0.58, 1.36)

Table 7 – Continued

	Total	<5 LTSP	≥5 LTSP
	Unadjusted HR (95% CI)	Unadjusted HR (95% CI)	Unadjusted HR (95% CI)
Current hormone use for any reason*			
No	Ref	Ref	Ref
Yes	1.55 (1.10, 2.17)†	0.73 (0.26, 2.06)	1.54 (1.08, 2.19)
Recent STI*			
No	Ref	Ref	Ref
Yes	0.69 (0.33, 1.43)	2.16 (0.54, 8.35)	0.96 (0.58, 1.56)
Ever abnormal Pap during study period			
No	Ref	Ref	Ref
Yes	3.20 (1.89, 5.42)†	8.89 (3.12, 25.27)†	2.49 (1.40, 4.40)†
Existing health conditions			
History of diabetes			
No	Ref	Ref	Ref
Yes	0.68 (0.40, 1.18)	0.31 (0.06, 1.59)	0.81 (0.46, 1.45)
History of any non-cervical cancer			
No	Ref	Ref	Ref
Yes	0.63 (0.33, 1.18)	0.21 (0.02, 1.98)	0.73 (0.38, 1.40)
History of arthritis			
No	Ref	Ref	Ref
Yes	0.87 (0.55, 1.36)	1.04 (0.34, 3.21)	0.82 (0.51, 1.32)
History of autoimmune disease			
No	Ref	Ref	Ref
Yes	1.04 (0.57, 1.90)	0.31 (0.03, 2.95)	1.15 (0.60, 2.06)

Abbreviations: hazard ratio (HR); body mass index (BMI); sexually transmitted infection (STI)
*Time-variant variable
ǂ Includes single, divorced, widowed
†Indicates $p<0.05$; ††Indicates $p<0.10$

Table 8. New HPV detection associated with selected risk factors stratified by lifetime number of sexual partners, adjusted hazard ratios

	< 5 LTSP ǂ HR (95% CI)	≥ 5 LTSP ǂ HR (95% CI)
Age (years)*		
<50	Ref	Ref
≥50	1.00 (0.47, 2.13)	1.09 (0.77, 1.55)
Race		
White	Ref	Ref
Black	1.91 (0.80, 4.54)	0.90 (0.60, 1.36)
Other	0.77 (0.19, 3.09)	1.61 (0.81, 3.22)
Yearly income (USD)		
<80,000	Ref	Ref
≥80,000	0.72 (0.28, 1.82)	1.32 (0.90, 1.93)
unknown	1.06 (0.40, 2.81)	1.04 (0.64, 1.67)
Marital status*		
Married	Ref	Ref
Unmarried ǂ	3.83 (1.41, 10.35) †	3.56 (2.46, 5.15) †
Recent sexual activity (6 months)*		
No sexual activity	Ref	Ref
Sexual activity, no new partner	2.08 (0.75, 573)	2.35 (1.50, 3.67) †
Sexual activity, new partner	11.09 (3.63, 33.87) †	3.68 (2.11, 6.41) †
Current hormone use for any reason*		
No	--	Ref
Yes		1.51 (1.08, 2.12) †
Ever abnormal Pap during study period		
No	Ref	Ref
Yes	5.82 (2.43, 13.93) †	1.94 (1.15, 3.27) †

Abbreviations: hazard ratio (HR)

*Time-variant variable

ǂ Hazard ratios are adjusted for all other variables presented in model

†Indicates $p<0.05$

Table 9. Combined effect of past and recent sexual behavior on new HPV detection

	Incident detections	Infection-time *	Crude Incidence rate (95% CI)	Rate Ratio (95% CI)	HR (95% CI) ǂ	P for interaction
Past and recent sexual behavior						
< 5 LTSP, No sex	7	55.87	0.13 (0.06, 0.26)	Ref	Ref	P=0.013
≥ 5 LTSP, No sex	30	86.43	0.34 (0.24, 0.50)	2.77 (1.19, 7.47)	2.74 (1.14, 6.54) †	
< 5 LTSP, Sex with no new partner	24	176.34	0.14 (0.09, 0.20)	1.09 (0.45, 2.99)	1.99 (0.81, 4.91)	
≥ 5 LTSP, Sex with no new partner	157	267.83	0.59 (0.50, 0.69)	4.68 (2.22, 11.83)	6.59 (2.95, 14.75) †	
< 5 LTSP, Sex with new partner	10	4.68	2.14 (1.15, 3.97)	17.04 (5.86, 52.76)	12.38 (4.25, 36.04) †	
≥ 5 LTSP, Sex with new partner	39	20.57	1.90 (1.38, 2.59)	15.13 (6.69, 40.09)	9.51 (3.98, 22.75) †	

Abbreviations: hazard ratio (HR)

*Infection-time = 1000 infection-months

ǂ Hazard ratios adjusted for age, race, income, marital status, current hormone use, Pap.

†Indicates $p<0.05$

Table 10. Clearance of baseline and newly detected HPV infections

	Clearance events ‡	Infection-time *	Crude Clearance Rate (95% CI)	Rate Ratio (95% CI)
Total	242	4.3689138	55.4 (48.4, 62.8)	
Type of HPV infections				
Baseline infection	120	2.7232401	44.1 (36.8, 52.7)	Ref
Newly detected	122	1.6456737	74.1 (62.1 – 88.5)	1.68 (1.30, 2.18)

‡ Clearance defined as 2 consecutive visits with negative type-specific HPV results. If last visit is negative, then defined as a clearance event
* Infection-time = 1000 infection-months

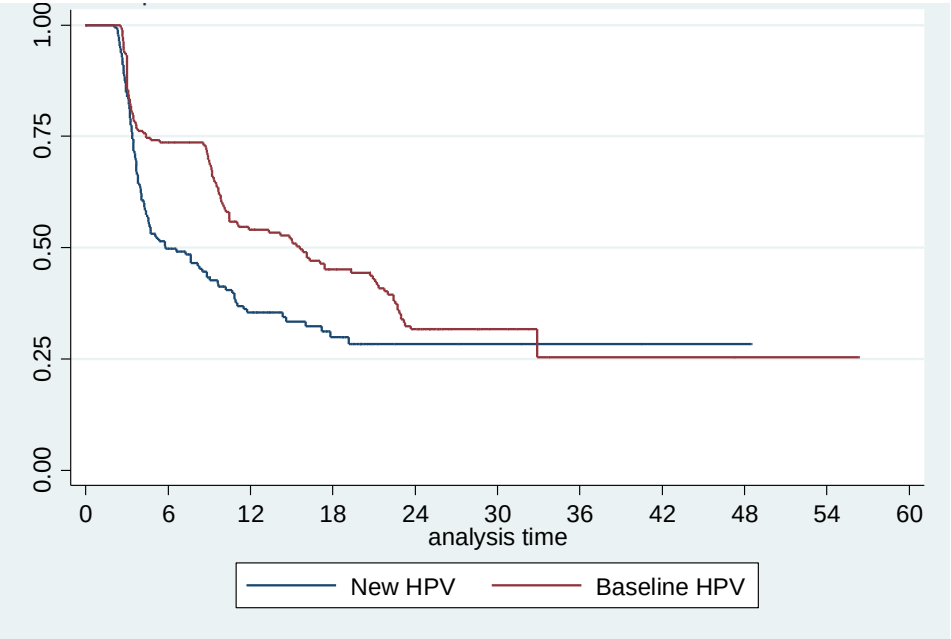


Figure 2. Comparison of HPV clearance between baseline and new HPV

Table 11. Risk factors of HPV clearance

	Unadjusted HR (95% CI)	Adjusted HR‡ (95% CI)
Time of initial detection		
Newly detected infection	Ref	Ref
Baseline infection	0.60 (0.46, 0.80) †	0.60 (0.45, 080) †
Demographics		
Age (years)*		
<50	Ref	Ref
≥50	1.05 (0.78, 1.42)	1.04 (0.76, 1.41)
Race		
White	Ref	Ref
Black	0.97 (0.69, 1.38)	1.17 (0.80, 1.70)
Other	0.83 (0.46, 1.50)	0.76 (0.42, 1.40)
Highest education completed		
High school	Ref	Ref
Post high school	0.56 (0.34, 0.97) †	0.58 (0.35, 0.94) †
College/Post graduate	0.79 (0.49, 1.30)	0.69 (0.43, 1.10)
Yearly income (USD)		
<80,000	Ref	Ref
≥80,000	1.48 (1.07, 2.03) †	1.17 (0.80, 1.71)
Unknown	1.30 (0.88, 1.92)	1.17 (0.77, 1.78)
Marital status*		
Married	Ref	Ref
Unmarried	0.72 (0.53, 0.96) †	0.77 (0.56, 1.06) ††
Reported smoking in last 6 months*		
No	Ref	
Yes	0.85 (0.55, 1.31)	
BMI (baseline)		
<30	Ref	
≥30	0.84 (0.61, 1.16)	
Sexual Behaviors and Reproductive Health		
Lifetime sexual partners		
<5 partners	Ref	
≥5 partners	1.06 (0.72, 1.56)	
Recent sexual activity		
No sex	Ref	
Sex, no new partner	1.06 (0.74, 1.53)	
Sex, new partners	0.95 (0.55, 1.63)	
Menopausal status (baseline)		
Premenopausal	Ref	
Postmenopausal	0.74 (0.53, 1.04)	
Current hormone use for any reason*		
No	Ref	Ref
Yes	1.36 (1.00, 1.84) ††	1.52 (1.10, 2.11) †

Table 11 – Continued

	Unadjusted HR (95% CI)	Adjusted HR‡ (95% CI)
Recent STI*		
No	Ref	
Yes	0.96 (0.60, 1.52)	
Ever abnormal Pap during study period		
No	Ref	
Yes	0.88 (0.60 1.29)	
Existing health conditions		
History of diabetes		
No	Ref	Ref
Yes	0.55 (0.32, 0.92) †	0.52 (0.30, 0.91) †
History of any cancer (not cervical)		
No	Ref	
Yes	0.98 (0.60, 1.61)	
History of arthritis		
No	Ref	
Yes	0.87 (0.57, 1.31)	
History of autoimmune disease		
No	Ref	
Yes	1.03 (0.64, 1.68)	

Abbreviations: hazard ratio (HR); body mass index (BMI); sexually transmitted infection (STI)
*Time-variant variable
‡ Hazard ratios (HR) are adjusted for all other variables presented in model.
†Indicates $p<0.05$; ††Indicates $p<0.10$

5.0 MANUSCRIPT 2: SEROPREVALENCE OF HPV IN YOUNG POSTPARTUM WOMEN

5.1 ABSTRACT

Substantial reduction in cervical cancer rates is anticipated with the introduction of the HPV vaccine in India. Although pilot vaccination programs have suggested high vaccination coverage is feasible among adolescent girls, some parental and provider attitudes are not supportive of targeting 9-12 year old girls for HPV vaccination. It may be important to identify other populations that are more culturally appropriate for India, such as young postpartum women. The objective of this study is to measure vaccine-specific HPV seroprevalence among one-month postpartum women, participating in a prospective cohort study exploring socioeconomic and environmental factors for maternal and child health in India, the LIFE (Longitudinal Indian Family hEalth) Study, to assess if this group is an appropriate target for HPV catch-up vaccination programs. Four hundred and eighty-eight women with no more than one previous live birth and had a postpartum serum specimen were selected. HPV seropositivity was tested with a Luminex-based multiple assay for the quadrivalent vaccine-specific HPV types (6, 11, 16, and 18). Descriptive statistics on demographic information and reproductive history for the population were described using data collected from interview-administered questionnaires. To determine if vaccinating young women after pregnancy is a viable option, a one-sided binomial

probability test was used to assess if the HPV 16/18 seroprevalence is no higher than 5%, similar to the seroprevalence in the adolescent vaccine target age range. Odds ratios were used to identify determinants for HPV seropositivity with 95% confidence intervals (CI). Twenty percent of the women were positive for any vaccine-specific HPV type. The population seroprevalence of HPV-6, HPV-11, HPV-16, and HPV-18 was 6.6%, 10.1%, 10.1%, and 3.9%, respectively. Among all postpartum women, HPV 16/18 seropositivity (11.6%) was significantly greater than 5%. Birth order, years of marriage, and being a homemaker were associated with increased seropositivity among postpartum women. Although HPV 16/18 seropositivity was greater than 5%, it was still low, suggesting a catch-up vaccination program targeting women after first or second delivery may be appropriate and have an impact to reduce cervical cancer burden. This older age of vaccination may be more appropriate in India given the cultural barriers to vaccinating adolescent girls.

5.2 INTRODUCTION

Even with a National Cancer Control Program and the establishment of cervical cancer screening guidelines in India, cervical cancer remains one the most common female cancers in the country (L Bruni, Barrionuevo-Rosas, Seranno, et al., 2014). A quarter of the global cervical cancer morbidity and mortality is shouldered in India (L Bruni, Barrionuevo-Rosas, Seranno, et al., 2014). It is estimated that 1 in 53 women will develop cervical cancer over her lifetime and 70% of these cancers present at an advance stage (L Bruni, Barrionuevo-Rosas, Seranno, et al., 2014), making successful treatment difficult. Although organized or population-based screening programs have effectively reduced the global burden of cervical disease, achieving high-levels of

participation and adherence remains a challenge in India. Human papillomavirus (HPV) vaccines, or cervical cancer vaccines, are thought to be able to significantly reduce cervical cancer rates, especially in settings like India where implementing sustainable secondary prevention efforts have been suboptimal (Krishnan, Madsen, Porterfield, & Varghese, 2013).

The quadrivalent Gardasil and the bivalent Cervix HPV vaccines have been licensed in India since 2008. Both cervical cancer vaccines target oncogenic types HPV-16 and 18, responsible for about three-fourths of cervical cancers in India and worldwide (L Bruni, Barrionuevo-Rosas, Seranno, et al., 2014). Data from clinical trials have demonstrated HPV vaccines to be more than 90% effective at preventing cervical precancers caused by these two oncogenic types (Harper & Demars, 2014; Schiller et al., 2012). The quadrivalent vaccine also protects against HPV-6 and 11, which is responsible for the majority of genital warts (*Gardasil [package insert]*, 2009).

HPV vaccination has the greatest impact when provided prior to HPV exposure (e.g., prior to sexual debut). Even though the quadrivalent vaccine is approved for females and males aged 9-26 years old, the WHO recommends HPV vaccination programs target girls between 9 and 13 years old to ensure girls are vaccinated before sexual debut (World Health Organization, 2014a). In India, the current recommendation for HPV vaccines is for 10-12 year old girls who can afford to purchase the vaccine through the private sector (Vashishtha et al., 2014). Although cervical cancer vaccines are not currently part of the routine immunization schedule provided by the public sector, a recent demonstration project exploring vaccine delivery strategies for HPV vaccines illustrated that high vaccine coverage among 10-12 year old girls was attainable through the government health system (LaMontagne et al., 2011). However large-scale introduction of HPV vaccines has been stalled due to public debate over whether the HPV

vaccine is an appropriate intervention to reduce the cervical cancer burden in India, which could greatly affect national vaccine coverage (Das, Hussain, Nasare, & Bharadwaj, 2008; Jayakrishnan, 2014; Krishnan et al., 2013).

Health and economic studies have shown that the greatest impact of the HPV vaccine is fully appreciated only when vaccine coverage is high. Catch-up populations for vaccinations play an important role in insuring that sufficient immunity is attained in the population. Countries have often identified 18-26 year olds girls as targets for catch-up programs. However, in India, this age group traditionally utilizes health care services only during pregnancy and through the first two years of their child's life. Therefore, one potential population for catch-up immunization programs may be targeting young postpartum women, particularly after their first pregnancy. Young postpartum women may still be naïve to vaccine-specific HPV types, therefore would benefit from vaccination. However, the success of an HPV vaccination programs targeting this population will depend on the level of previous exposure to HPV-16 and HPV-18 (Ryding et al., 2008).

Serology measures, a marker of previous HPV exposure, may provide valuable information for developing HPV immunization programs. In immunogenicity studies of the HPV vaccine in India, 4.6-12.5% and 4.1-19.6% women were HPV-16 and HPV-18 seropositive, respectively, prior to receiving the HPV vaccine (Bhatla et al., 2010; Rengaswamy Sankaranarayanan et al., 2015). To our knowledge, no data on HPV exposure exists among pregnant or postpartum women in India. The purpose of this study is to measure quadrivalent vaccine-specific HPV seroprevalence among married women aged 17-35 years old 1-month postpartum and to evaluate if the HPV-16 and HPV-18 seroprevalence is similar to seroprevalence observed among adolescent girls. To understand better if catch-up vaccination

programs during the postpartum period should focus on certain characteristics of a women, such as age or reproductive history, this study also determines the factors associated with HPV seropositivity.

5.3 METHODS

5.3.1 Study population

From October 2009 to March 2012, married women age 15-35 were enrolled into the LIFE (Longitudinal Indian Family hEalth) Study, a prospective cohort study exploring socioeconomic and environmental factors for maternal and child health (Kusneniwar et al., 2016). Women were eligible to participate if they were living in one of the Medchal Mandal villages served by MediCiti Institute of Medical Sciences (MIMS), not currently pregnant or less than 14 weeks' gestation. Women were excluded if they were not planning to have any more children or if they lived in one of the two villages with a highly transient population.

Consented women enrolled in the LIFE study provided urine, serum, stool and vaginal samples. After delivery women also consented to provide one-month postpartum specimens. Demographic information was collected using an interview-administered questionnaire at enrollment. Once pregnancy was confirmed, a more detailed reproductive history was collected.

As of May 2013, 668 women became pregnant and delivered at least one baby as part of the LIFE study. Of these 668 women, 180 women were excluded because of missing postpartum serum (n=64; 9.2%) or had two or more previous live births prior to entering the LIFE Study

(n=116; 17.7%). The remaining 488 (73.1%) women who were selected for the present study had no more than one previous live birth and had a postpartum serum specimen.

This study has been approved by the SHARE India Ethics committee at MIMS, Ghanpur, Telangana State, India and by the Institutional Review board at the University of Pittsburgh, Pittsburgh, PA, U.S.A.

5.3.2 HPV serologic measurement

Postpartum serum specimens were immediately processed, aliquotted into cryotubes and stored at -80°C until removed for batch HPV serology testing. A 50ul sub-aliquot for the selected women was sent to Rajiv Gandhi Centre for Biotechnology (RGCB, Kerala, India) for testing using the Luminex® assays (Dias et al., 2005; Waterboer et al., 2005). The Luminex® platform simultaneously assays antibodies to HPV 6, 11, 16 and 18. Type-specific antibodies were detected by competing with and blocking the binding of fluorescently tagged neutralizing monoclonal antibody to virus-like particles (VLPs) coated microspheres.

Serology assay results were dichotomized as “positive” and negative” based on setting cut-points using clinical sensitivity/specificity algorithm to make a distinction between "likely negative" and "likely positive" samples. A reference population of likely negatives from the LIFE cohort (63 women with serum specimens collected within a year of marriage) was used to set cut-points for each HPV serotype, by calculating the mean+2x Standard Deviation (SD) for each serotype, excluding any positive outliers. A total of 484 specimens had valid results.

5.3.3 Statistical analysis

Descriptive statistics on demographic information and reproductive history for the population were described. Mean and standard deviation was presented for the continuous variables and frequencies with percentages were presented for categorical variables.

To determine if vaccinating young women after pregnancy is a viable option, a one-sided binomial probability test was used to assess if the HPV 16/18 seroprevalence, or high-risk HPV seroprevalence, in the population was greater than 5%. We used a 5% threshold since it is the estimated HPV-16 and HPV-18 percent positivity in the vaccine target age range (adolescent girls aged 14-19) (Markowitz et al., 2009). Post hoc, we tested to determine if high-risk HPV seroprevalence was greater than 10%.

Associations of demographic and reproductive health variables were explored separately with high-risk, low-risk (HPV 6/11), and any quadrivalent vaccine-specific HPV (qHPV) using chi-square tests. Bacterial vaginosis was diagnosis by analyzing the presence of clue cells from vaginal samples that were collected at enrollment, Gram stained, and viewed at 400x magnification by trained laboratory microbiologists at MIMS. Odds ratios were used to assess independent determinants for HPV seropositivity with 95% confidence intervals (CI). All models were adjusted for serum collection time, which was defined as the number of months between delivery date and first postpartum visit date. Variables included in the multivariable model were selected through backward stepwise regression, maximum p-value for term to be removed was 0.2 and to be added was 0.1. All analyses were conducted in Stata 13.0 statistical package.

5.3.4 Uncertainty of cut-point value

To check the sensitivity of the chosen cut-point on the seroprevalence, we recalculated type-specific seroprevalence using a cut-point ± 1x SD.

5.4 RESULTS

5.4.1 Descriptive analysis

Table 12 shows the population characteristics (N=483). One seronegative woman was excluded from the analysis due to incomplete questionnaire responses. The mean age of postpartum women in the study was 22.5 years old (range 17 to 35 years) and approximately 50% of the women were married before the age of 20. The majority of the women were Hindu (88.2%) and from scheduled caste (57.3%). Only 11.8% were Muslim or Christian. The study population was educated, more than 79% completed secondary education or higher. More than three-fourths of the women did not work outside of the home. All women in the study were non-smokers and household exposure to tobacco smoke was reported by less than 20% of the participants.

Approximately 60% of the women in this study reported a prior pregnancy (Table 12). Among the 284 women previously pregnant prior to enrolling in the LIFE Study, 10.9% of the pregnancies did not result in a live birth. Age at marriage was unknown by nearly a quarter of the women; these women were less educated ($p<0.001$), had a previous live birth ($p=0.05$), and had clue cells present ($p=0.01$).

The mean collection time for the postpartum specimen was 2.7 ± 3.3 months (ranging from 21 days to 23 months), and did not vary by seropositivity (p=0.38).

5.4.2 Seroprevalence

HPV seroprevalence for all postpartum women, and stratified by previous number of pregnancies, is summarized in Table 13. One-fifth of the postpartum women were positive for any qHPV antibody and less than 1% of the women were positive for all four vaccine types. High-risk HPV seroprevalence (11.6%) among all postpartum women was significantly greater than 5% ($p<0.001$), but not significantly greater than 10% (p=0.12). Similar observation was observed among primipara women ($p_{(5\% \text{ threshold})}<0.001$, $p_{(10\% \text{ threshold})}=0.59$). However, the high-risk seroprevalence (13.4%) among women giving birth to their second child was significantly greater than 5% ($p<0.001$) and 10% (0=0.03).

HPV-16 and HPV-11 were the most common high-risk and low-risk vaccine-specific serotypes detected, respectively (Table 13). Generally, type-specific seropositivity was lower among primipara women compared to secundipara women, although the difference was only statistically significant for HPV-11 (p=0.01). The proportion of secundipara women with any qHPV serotype was 1.5-fold more than the seropositivity among primipara women.

5.4.3 Determinants of seropositivity

Single factors associated with any high-risk or low-risk HPV seropositivity were similar, but the final adjusted models were different (Table 14). Increasing number of previous pregnancies was associated with increased odds of HPV seropositivity in the univariable analysis. After

adjustment of other co-variates, the association of pregnancy history was no longer present; however, birth order remains associated with any HPV seropositivity and low-risk HPV seropositivity (Table 14). Working outside of the home was weakly inversely associated with any vaccine-specific HPV (p=0.09), high-risk HPV (p=0.06), and low-risk HPV (p=0.10). Although caste was not significantly associated with any HPV seropositivity, women identifying as backwards caste had 3 times higher odds of low-risk HPV compared to scheduled caste (p=0.03). High-risk HPV seropositivity was weakly associated with 1.8 greater odds among women who were married longer than 3 years than women who were married for less than three years (p=0.11). History of abnormal vaginal discharge and bacterial vaginosis were not associated with HPV seropositivity.

5.4.4 Influence of cut-point on seroprevalence estimates

The serotype specific prevalence of HPV was sensitive to the chosen assay cut-point, with the largest influence for HPV-11 and HPV-16 (Figure 3). Given this uncertainty, the seroprevalence estimate for any quadrivalent vaccine-specific HPV in this population ranged from 13.0% to 32.1%.

5.5 DISCUSSION

The seroprevalence of the quadrivalent vaccine-specific HPV types among postpartum Indian women in the present study was 6.6% for HPV-6, 10.1% for HPV-11, 10.1% for HPV-16, and 3.9% for HPV-18. The serotype-specific prevalence we observed was within range, but generally lower than that observed in pregnant and postpartum women in North American and European countries, which varied from 0-53% for HPV-6, 0-21% for HPV-11, 9-35% for HPV-16 and 6-27% for HPV-18 (K. Heim et al., 1995; Rama, Villa, Pagliusi, Andreoli, Costa, Thomann, Longatto-Filho, et al., 2010; Syrjänen et al., 2009). Premarital sex among Indian females is in the minority, ranging from 3-18% and is related to increasing age and socio-economic background (Anil Kumar, 2003; Jaya & Hindin, 2009; McManus & Dhar, 2008; K. G. Santhya, Acharya, Jejeebhoy, & Ram, 2011; K. Santhya & Jejeebhoy, 2012). For the majority of women in this population, sexual debut occurs within marriage unlike in industrialized countries where the average age of sexual debut is 5-7 years before marriage (Wellings et al., 2006). Younger age of sexual debut relates to multiple partners and more sexual contacts, which increase opportunities for HPV exposure. The lower seropositivity rate we see in our study may be due to the lower number of lifetime partners and fewer sexual contacts. More than 80% of the postpartum women in our study were negative for all of the quadrivalent vaccine types, which is similar to findings of another study that estimated the HPV seroprevalence among 301 postpartum women in Brazil (Rama, Villa, Pagliusi, Andreoli, Costa, Thomann, Longatto-Filho, et al., 2010).

Published reports on HPV seroprevalence in India are limited. There are two studies that report on HPV seroprevalence among Indian populations, specifically among female participants in two separate multicenter vaccine trials in India using serum specimens collected prior to

vaccination protocols (Bhatla et al., 2010; Rengaswamy Sankaranarayanan et al., 2015). In one study of nearly 2000 unmarried girls between the ages of 10 and 18 years old participating in an effectiveness trial of a 2-dose vaccine schedule compared to the standard 3-dose schedule for the quadrivalent HPV vaccine, the authors reported seroprevalence estimates for HPV-6 (5%), HPV-11 (5-6%), HPV-16 (5-6%), and HPV-18 (4-7%). These findings showed little type-specific variation in seroprevalence and the estimates were lower compared to our study (Rengaswamy Sankaranarayanan et al., 2015). The other study was an immunogenicity and safety vaccine study for the bivalent vaccine conducted in 354 women aged 18-35 years old (Bhatla et al., 2010). In this study, 29.2% of the vaccine group and 25.8% of the placebo group were HPV 16/18 seropositive, which is higher than the 12% of the women in our study who were positive for HPV-16 or HPV-18 serotypes. Even using the more conservative cut-point value in the present study, the HPV 16/18 seroprevalence in this population (20.5%) was still lower than that baseline seroprevalence reported in the bivalent vaccine trial. Since HPV seroprevalence is associated with age (Tiggelaar et al., 2012), the differences observed between these studies may be attributed to an older population in the bivalent vaccine trial population (mean age= 28.4 years) compared to the younger LIFE population (mean age = 22.5 years) and an even younger adolescent quadrivalent vaccine trial population.

In addition to population characteristics, such as age and sexual patterns, it is important to note that comparing serologic measurements across studies can be difficult because measurement accuracy is affected by assay type and the associated seropositive cutoff values used (Iftner & Villa, 2003). It is recognized that comparability of HPV serology would be aided by international standards for antibodies to specific HPV serotypes. Currently, the WHO HPV Laboratory Network (HPV LabNet) has created the first international standards for HPV-16 and

is developing international standards for HPV-18 and potentially other HPV serotypes (Faust, Eklund, Sukvirach, Ngamkham, & Dillner, 2016; Ferguson, Wilkinson, Heath, & Matejtschuk, 2011).

A recent meta-analysis on the benefits of HPV vaccination in older girls, 16 years and older, shows a protective effect against cervical carcinogenesis and other HPV-related high-grade diseases (Couto et al., 2014). Although the HPV16/18 seropositivity in the total study population was 11.6%, which was statistically greater than the hypothesized 5%, it was not statistically greater than 10%. Therefore, the majority of postpartum women in our study were not exposed previously to the two most common oncogenic HPV types by the time of the birth of their second child and this finding suggests that young postpartum women could still benefit from the HPV vaccine.

We found that women delivering their first child were at lower risk for any HPV compared to women who were having their second child. This suggests that assessing birth order may be important when assessing a strategy that involves HPV vaccination among postpartum women. Being married for three or more years was also a significant factor for high-risk seropositivity. This could either be an indication of an increased risk of extramarital relationships among married couples or more time for establishing an HPV infection. This observation aligns with the positive association observed with years of marriage and sexually transmitted infection risk in India (Prasad et al., 2005). Occupation was positively associated with HPV seropositivity, irrespective of high or low-risk HPV. The elevated odds of HPV detection among women who did not work outside of the home was surprising and contrary to previous studies that observed a decreased sexually transmitted infection risk among homemakers (Prasad et al., 2005).

Programmatically in India, including women after their first or second pregnancy for the HPV vaccine may be more culturally appropriate than an immunization program focused solely on adolescent girl. Acceptability studies among Indian populations suggest that HPV vaccination is not accepted by the majority of parents interviewed and should be delayed until "their daughters grew up" (Basu & Mittal, 2011; Krupp et al., 2010; Madhivanan et al., 2009, 2014; Paul et al., 2013). In a recent survey, only 20% of mothers think that the vaccine should be given before the age of sexual debut (Basu & Mittal, 2011). In addition, physicians, who are important facilitators for HPV vaccine acceptance, are often reluctant to recommend vaccination to the general population (Basu & Mittal, 2011; Krupp et al., 2010), may be more willing to recommend HPV vaccination after first pregnancy, when parental reactions and social stigma are not a concern. Recent studies in the United States offering adolescent and young adult women HPV vaccination after pregnancy support the notion that motivating postpartum women for HPV vaccination is feasible, convenient, and successfully increases vaccination initiation and completion rates among these women (Berenson, Rahman, Hirth, Rupp, & Sarpong, 2016; J. D. Wright et al., 2012).

The main limitation of our study was determining a serologic cut-point based on a population that may not be HPV naïve and may have underestimated the serologic HPV burden. Even so, the reference population was newly married women with serum specimens collected within a year of marriage. Given that that a recent report estimates that 5% of the females aged 15-24 engage in premarital sexual activity (K. Santhya & Jejeebhoy, 2012) and the median time to seroconversions for HPV infections is within one year (Carter et al., 2000), the assumption that the references populations is likely negative appears appropriate and the related seroprevalence estimates are reasonable.

Despite the limitation, this study addresses a gap in the HPV epidemiology of postpartum women in India, the majority of whom are age-eligible for catch-up HPV vaccination. Our results suggest that vaccinating young postpartum women, especially after first delivery, would be beneficial. These results support further research to examine impact on cervical cancer burden if vaccinating postpartum women is part of the national HPV vaccination strategy in India. Since screening strategies have not been successfully implemented and adolescent HPV vaccination is not nationally implemented, such an immunization program could reduce the cervical cancer burden and other HPV-related diseases.

5.6 TABLES AND FIGURES

Table 12. Demographic and reproductive history characteristics of study population

	Total (N=483)
Demographics	
Age (mean ± SD)	22.55 ± 2.75
Years of marriage	
< 3	155 (32.1)
3-4	126 (26.1)
5+	91 (18.8)
unknown	111 (23.0)
Religion	
Hindu	426 (88.2)
Muslim	31 (6.4)
Christian	26 (5.4)
Caste	
Scheduled Caste	277 (57.3)
Scheduled Tribe	78 (16.2)
Backward Caste	35 (7.3)
No caste reported	93 (19.2)
Education, highest level completed	
None	58 (12.0)
Primary	40 (8.3)
Secondary	263 (54.5)
Higher secondary or more	122 (25.3)
Currently employed	
No	373 (77.2)
Yes	110 (22.8)
Husband occupation	
Private	374 (77.4)
Agriculture	42 (8.7)
Semi-skilled/unskilled	21 (4.4)
Professional	25 (5.2)
Missing	21 (4.4)
Household tobacco smoke exposure	
No	401 (83.0)
Yes	82 (17.0)

Table 12 – Continued

	Total (N=483)
Reproductive history	
Previous pregnancy history	
None	199 (41.2)
One	239 (49.5)
Two	45 (9.3)
Birth order of child at delivery	
First	230 (47.6)
Second	253 (52.4)
History abnormal vaginal discharge	64 (13.3)
Bacterial vaginosis present	83 (17.2)

Table 13. HPV seroprevalence with 95% confidence intervals, total and stratified by birth order

Type	**Total** ***(N=483)***	**Birth order** ***First (N=230)***	***Second (N=253)***
Any qHPV type‡	20.3% (16.8 – 24.2)	15.2% (10.8 – 20.5)	24.9% (19.7 – 30.7)
All qHPV types‡	0.4% (0.1 – 1.5)	0.9% (0.01 – 3.1)	0.0% (0.0 – 1.4)*
High-risk HPV	11.6% (8.9 – 14.8)	9.6% (6.1 – 14.1)	13.4% (0.9 – 18.3)
HPV-16	10.1% (7.6 – 13.2)	7.8% (4.7 – 12.1)	12.2% (8.5 –16.9)
HPV-18	3.9% (2.4 – 6.1)	4.8% (2.4 –8.4)	3.2% (1.4 –6.2)
Low-risk HPV	12.6% (9.8 – 15.9)	9.1% (5.7 – 13.6)	15.8% (11.5 – 20.9)
HPV-6	6.6% (4.6 – 9.2)	5.2% (2.7 – 8.9)	7.9% (4.9 – 11.9)
HPV-11	10.1% (7.6 – 13.2)	6.5% (3.7 –10.5)	13.4% (9.5 –18.3)

Abbreviations: any quadrivalent vaccine-specific HPV type (qHPV)
‡ qHPV includes HPV 6/11/16/18
*One-sided 97.5% confidence interval

Table 14. Determinants of any HPV seropositivity, odds ratio (OR) with 95% confidence intervals

	Any seropositive		*High-risk seropositive*		*Low-risk seropositive*	
	*Unadjusted OR**	*Adjusted OR*[‡]	*Unadjusted OR**	*Adjusted OR*[‡]	*Unadjusted OR**	*Adjusted OR*[‡]
Demographics						
Age						
<25 years	Ref		Ref	Ref	Ref	
≥25 years	1.36 (0.81, 2.28)		1.73 (0.93, 3.20)[††]	1.60 (0.83, 3.09)	0.79 (0.39, 2.07)	
Years of marriage						
<3 years	Ref	Ref	Ref	Ref	Ref	
≥3 years	1.83 (1.07, 3.12)	1.51 (0.81, 2.78)[††]	1.87 (0.94, 3.70)[††]	1.81 (0.88, 3.70)	1.38 (0.65, 2.92)	
Unknown	1.22 (0.63, 2.34)	1.06 (0.52, 2.14)	1.36 (0.59, 3.10)	1.36 (0.59, 3.17)	2.15 (1.01, 4.56)[†]	
Religion						
Hindu	Ref		Ref		Ref	
Muslim	0.72 (0.27, 1.94)		0.51 (0.12, 2.21)		1.02 (0.34, 3.04)	
Christian	0.48 (0.14, 1.65)		0.28 (0.04, 2.10)		0.56 (0.13, 2.43)	
Caste						
Scheduled Caste	Ref		Ref	Ref	Ref	Ref
Scheduled Tribe	1.13 (0.60, 2.12)		1.06 (0.48. 2.33)	0.96 (0.43, 2.17)	1.29 (0.61, 2.70)	1.18 (0.56, 2.50)
Backward Caste	1.30 (0.56, 3.02)		0.76 (0.22, 2.62)	0.94 (0.27, 3.34)	2.32 (0.97, 5.56)	2.80 (1.14, 6.88)[†]
No caste reported	1.44 (0.82, 2.52)		1.43 (0.72, 2.83)	1.46 (0.72, 2.92)	1.05 (0.50, 2.19)	1.02 (0.49, 2.13)
Education, highest level completed						
< Secondary	Ref		Ref		Ref	
≥ Secondary	1.08 (0.62, 1.89)		0.92 (0.47, 1.82)		1.18 (0.59, 2.37)	
Currently employed outside of the home						
No	Ref	Ref	Ref	Ref	Ref	Ref
Yes	0.66 (0.37, 1.17)[††]	0.64 (0.36, 1.16)[††]	0.52 (0.24, 1.14)[††]	0.46 (0.21, 1.01)[††]	0.55 (0.26, 1.15)[††]	0.53 (0.25, 1.13)[††]

Table 14 – Continued

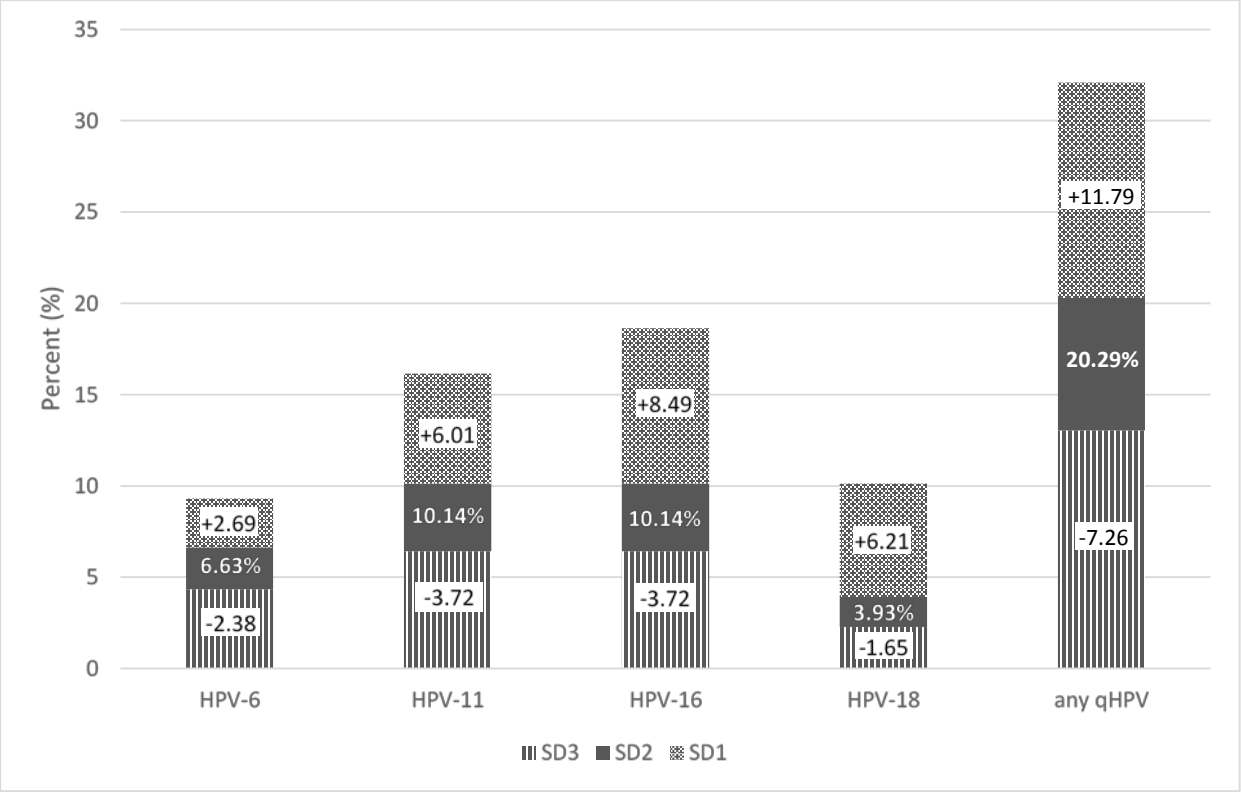
	Any seropositive		*High-risk seropositive*		*Low-risk seropositive*	
	*Unadjusted OR**	*Adjusted OR‡*	*Unadjusted OR**	*Adjusted OR‡*	*Unadjusted OR**	*Adjusted OR‡*
Husband occupation						
Private	Ref		Ref		Ref	
Agriculture	1.10 (0.50, 2.93)		1.37 (0.50, 3.45)		1.41 (0.59, 3.37)	
Semi-skilled/unskilled	1.28 (0.45, 3.60)		0.91 (0.20, 4.01)		1.20 (0.34, 4.25)	
Professional	1.34 (0.52, 3.51)		1.65 (0.54, 5.10)		1.01 (0.29, 3.53)	
Missing	0.95 (0.31, 2.91)		1.96 (0.63, 6.13)		0.75 (0.17, 3.31)	
Household tobacco smoke exposure						
No	Ref		Ref		Ref	
Yes	1.12 (0.63, 2.00)		0.92 (0.43, 1.96)		0.82 (0.39, 1.74)	
Reproductive history						
Number of previous pregnancies (0-2)	1.46 (1.04, 2.06)†		1.32 (0.87, 2.03)		1.34 (0.88, 2.02)	
Birth order of child at delivery						
First	Ref	Ref	Ref		Ref	Ref
Second	1.86 (1.18, 2.96)†	1.58 (0.93, 2.67)††	1.50 (0.85, 2.66)		1.89 (1.08, 3.32)†	1.96 (1.11, 3.47)†
History abnormal vaginal discharge						
No	Ref		Ref		Ref	
Yes	1.36 (0.74, 2.52)		1.29 (0.70, 1.12)		1.33 (0.64, 2.78)	
Bacterial vaginosis						
No	Ref		Ref		Ref	
Yes	1.10 (0.62, 1.97)		0.79 (0.36, 1.73)		1.52 (0.79, 2.92)	

Abbreviation: odds ratio (OR)

*Odd ratios adjusted for time of sample collection.

‡ Odds ratios adjusted for time of sample collection and all other variables presented in model.

†Indicates $p<0.05$; ††Indicates $p<0.1$



SD: Standard deviation

Figure 3. Percent difference in seroprevalence compared with the standard assay cutoff at two standard deviations from the mean in presumptive HPV naïve women, by HPV type

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6.0 MANUSCRIPT 3: EFFECTS OF DIETARY SOY AND TEA INTAKE ON CERVICAL CANCER RISK

6.1 ABSTRACT

Soy isoflavones and tea catechins have immunomodulating and chemopreventive properties relevant for cervical carcinogenesis; however, there are limited data from epidemiologic studies that have evaluated the relationship of soy and tea consumption with cervical cancer risk. The association between intake of soy and green tea drinking and risk of cervical cancer was investigated in a prospective, population-based cohort of 30,744 Chinese women in Singapore with an average 16.7 years of follow-up and 312 incident cases of cervical cancer. Multivariable proportional hazard regression models were used to estimate hazard ratio (HR) and 95% confidence interval (CI) of cervical cancer associated with intake levels of soy and tea. High intake of soy alone was associated with a statistically borderline significant 20% reduced risk of cervical cancer (HR=0.80, 95% CI: 0.61, 1.05) while green tea alone was not (HR=0.97, 95% CI: 0.76, 1.22). In stratified analysis, high intake of soy was associated with a statistically significant decrease in cervical cancer risk among green tea drinkers (HR=0.43; 95% CI: 0.28, 0.69) but not among non-drinkers of green tea. The difference in the soy-cervical cancer risk association between green tea drinkers and non-drinkers was statistically significant (*P* for interaction=0.004). This inverse association between soy intake and cervical cancer risk

remained after further adjustment for human papillomavirus serology status. These novel epidemiologic findings suggest that a protective effect of soy against cervical cancer development may depend on the constituents in green tea.

6.2 INTRODUCTION

A primary cause of cervical cancer is persistent infection with human papillomavirus (HPV) at the transformation zone, a region where columnar epithelium of the cervix changes into squamous epithelium (Schiffman et al., 2007; Vaccarella et al., 2013). Cervical cancer incidence has been declining in most developed countries since the 1960's due to successful efforts in screening for and vaccinating against oncogenic HPV types (i.e., 16 and 18) (Laara, Day, & Hakama, 1987; Vaccarella et al., 2013). Despite these efforts, cervical cancer remains one of the most common gynecological cancer worldwide (Gakidou et al., 2008; Newmann & Garner, 2005). In Singapore, cervical cancer is the third leading cause of cancer mortality among premenopausal women. Although cervical cancer incidence rates have been decreasing in Singapore, resulting from a nationwide screening program, a recent trend analysis showed that women born after 1960 may be at increased risk for cervical cancer compared to earlier birth cohorts (Vaccarella et al., 2013). Given the approximate decade-long latency period between the beginning of persistent infection with HPV and the clinical manifestation of pre-malignant lesions, only a small proportion of these early low-grade lesions will eventually progress to invasive cervical cancer in infected women (Castellsagué, 2008). Other factors, along with HPV, likely play an important role in the development of invasive cervical cancer.

Although cervical cancer is not considered a hormone-dependent malignancy, estrogen exposure in combination with persistent HPV infection is important in the development of cervical cancer. For example, epidemiological studies have identified multiparity and long-term oral contraceptive use as HPV cofactors associated with cervical cancer risk (Castellsague & Munoz, 2003) . Evidence from virology- and immunology-based experimental studies implicate estrogen in cervical carcinogenesis through mechanisms including the initiation of cell proliferation and differentiation in the cervical transformation zone, local cervical immune microenvironment and cytokine-dependent immune response changes, and HPV gene expression (Delvenne et al., 2007). Isoflavones are a plant-based estrogen (phytoestrogen) found most abundantly in soy products. As weak estrogens, soy isoflavones interact with estrogen receptors and have been shown to inhibit multiple estrogen-dependent and non-dependent cancers including breast, prostate, gastric and lung cancers (Ko et al., 2013; A H Wu, Yu, Tseng, & Pike, 2008; S. H. Wu & Liu, 2013; Yan & Spitznagel, 2009).

Green tea is a major source of epigallocatechin-3-gallate (EGCG) (Roy, Siddiqi, & Bhattacharya, 2001). EGCG inhibits cell growth of HPV infected cells by down-regulating E6 and E7 oncoproteins *in vitro*, and tumors regress when EGCG was combined with a DNA vaccine in tumor mouse model (Butler & Wu, 2011). Other major tea catechins in green tea are epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). Black tea contains only one-third to one-tenth catechins that green tea does, but has high levels of other tea polyphenols such as theaflavins, thearubigins, and gallic acid tannins (Y. Wang & Ho, 2009; Z. Y. Wang, Huang, Lou, & Reuhi, 1994). Given the different types of polyphenols and different concentrations of tea catechins, green and black tea may have different effects on risk of cervical

cancer. However, there have been no reports on prospective studies that have evaluated the relationship between green and black tea intake and cervical cancer risk.

The Singapore Chinese Health Study is a population-based, prospective cohort with detailed dietary intake information on a population with substantial intake of soy and green and black teas. The aim of this study is to investigate the direct and possible interactive effects of soy and tea intake on cervical cancer risk among Chinese women in Singapore. In a subanalysis for a nested case-control study of cervical cancer within the Singapore Chinese Health Study, we also evaluated whether HPV serological status modified the association between intake of soy and tea and risk of cervical cancer.

6.3 METHODS

6.3.1 Study participants

The details of the study design of the Singapore Chinese Health Study have been described previously in detail (Yuan, Stram, Arakawa, Lee, & Yu, 2003). Briefly, cohort participants were recruited from permanent residents or citizens of Singapore, aged 45-74 years, living in government housing and belonging to one of the two major dialect groups (Hokkien and Cantonese). The study enrolled 35,303 women and 27,954 men from April 1993 to December 1998. All cohort participants completed a baseline in-person interview, including questions on demographics, lifestyle factors, and medical history. Questions specific for women obtained information on reproductive and menstrual history, hormone use and history of cervical cancer screening (i.e., Pap-based test).

The baseline interview also included a validated 165-item food-frequency questionnaire (FFQ) to assess usual dietary intake (Hankin et al., 2001). The FFQ measured the intake of the seven most common nonfermented soy products in the Singapore Chinese diet in 8 predefined categories: never or hardly ever, once month, 2-3 times a week, 4-6 times a week, once a day, and 2 or more times a day. Soy intake was expressed based on grams of soy protein and soy isoflavones, as previously described (Anna H Wu, Stanczyk, Seow, Lee, & Yu, 2002; Anna H. Wu et al., 2008). Calculation of soy protein intake was based on the Singapore Food Composition Database (Hankin et al., 2001). Market samples of common soy foods in the Singapore diet were used to estimate the amount of genistein, daidzein, and glycitein soy isoflavones. The sum of these three isoflavones estimated the total soy isoflavone intake. Green tea and black tea drinking were assessed in the FFQ. Individuals indicated their drinking frequency for each tea separately based on the following categories: never or hardly ever, 1-3 times a month, once a week, 2-3 times a week, 4-6 times a week, once a day, 2-3 times a day, 4-5 times a day, or 6 or more times a day.

6.3.2 Identification of cancer cases

Cancer diagnosis and deaths among cohort participants were identified through linkages of with the Singapore Cancer Registry and the Singapore Registry of Births and Deaths, respectively. The national cancer registry has been in place since 1968 and is considered a comprehensive record of cancer cases in the country (Parkin, 2006). After excluding all men, and women with baseline prevalent invasive cancer or intraepithelial (non-invasive) cervical cancer (n = 1,276), and women who reported having a hysterectomy at baseline (n = 3,278), or who were missing cervical cancer screening history (n = 5), 30,744 women remained. During follow-up, 312

women developed incident cervical cancer including 120 carcinoma *in situ* (CIS) and 192 invasive cervical cancer.

6.3.3 Nested case-control study

A nested case-control study was conducted within the cohort to evaluate HPV status as a potential confounder in the assessment of soy and tea associations with cervical cancer risk. There were 75 CIS or invasive cervical cancer cases with serum samples available for the present study. For each case, three controls were randomly selected among all eligible women without history of cancer or hysterectomy who were matched with the index case on age at study enrollment (within 3 years), dialect group (Hokkien, Cantonese), date of study enrollment (within 2 years), date of biospecimen collection (within 6 months), and baseline menopausal status (pre-, post-menopausal). We successfully identified 3 controls for each of 52 cases, 2 controls for each of 20 cases, and 1 control for each of 3 cases, the total number of matched controls was 199.

6.3.4 Serologic testing

Antibody reactivities to HPV and herpes simplex-virus 2 (HSV-2) antigens were examined as previously described at the German Cancer Research Center, Heidelberg, Germany (Waterboer et al., 2005). Briefly, the Luminex® multiplex platform based on fluorescence-labeled polystyrene beads combined with glutathione S-transferase (GST) capture enzyme-linked immunosorbent assay (ELISA) was used to simultaneously test for 8 HPV types and HSV-2. The assay detected antibodies to the major capsid protein L1 of the high-risk mucosal HPV types

16, 18, 31, 33, 35, 45, 52, and 58. Serum antibodies against HSV-2 were also quantified. Reactivity of each HPV and HSV-2 antibody was quantified as Median Fluorescence Intensity (MFI) and antibody-specific cutoffs were defined to determine seropositivity for each type-specific capsid protein. Cut-offs were established based on a previously described study among 371 virgin Korean women with no evidence of genital HPV (Clifford et al., 2007). The cutoff was defined as 5 times the standard deviations above the mean of the final distribution of MFI values among these women after the exclusion of outliers. The case/control status of our test samples was blind to the laboratory personnel.

6.3.5 Statistical analyses

Proportional hazard regression models were used to estimate the cervical cancer risk, measured by hazard ratios (HR) and 95% confidence intervals (CI), associated with soy and tea intake. Person-years of follow-up were calculated for each person from the date of the baseline interview to the date of cervical cancer diagnosis, death, out migration, or December 31, 2013, whichever occurred first. Daily intakes of soy products and soy isoflavones (genistein, daidzein, glycitein, and total) were expressed as weight/1000 kcal to adjust for total energy and categorized by tertiles based on distribution among all women in the cohort. We categorized intake frequencies of green and black tea, separately, into nondrinker and drinker, and for drinkers, further grouped into monthly and weekly/daily drinkers. All multivariable regression models included following potential confounders: dialect group (Cantonese, Hokkien), age (years), education level (no formal/primary education, and secondary or higher), and total calorie intake (kcal/day). In addition, we used a stepwise regression approach to determine other potential confounders that were found to be associated with risk of cervical cancer in the study

population. According to an entry criterion of P <0.1 and a removal criterion of P < 0.2 for a given variable, the following variables were included in the multivariable models: parity (0-2, 3-4, 5 or more births), duration of oral contraceptive (OC) use (never use, <5 years, or ≥5 years), menopausal status (premenopausal, postmenopausal), and history of Pap-based screening. Adjustment for smoking status did not substantially alter the association between the exposure variables (i.e., soy and tea intake) and risk of cervical cancer, but smoking was retained in the regression models to account for potential residual confounding. For linear trend test, ordinal values of soy and tea intake were used for the assessment of cervical cancer risk. To evaluate potential interaction between soy and tea intake on risk of cervical cancer, a product term of these two was created and included in multivariable models in addition to stratified analyses.

In analysis of the nested case-control dataset, conditional logistic regression models were used to assess the association between intake of soy and tea and risk of cervical cancer with additional adjustment for serology status of HPV and HSV-2. The latter was a proxy for sexual behavior. A positive serology status of HPV was determined if antibodies against the L1 protein was detected for any high-risk mucosal HPV types. All analyses were performed in SAS 9.3 (SAS Institute, Inc.). All *P*-values are two-sided. P values <0.05 were considered statistically significant. Insert info on study population

6.4 RESULTS

The majority of the women were postmenopausal at baseline, with an average age of 56.2 years (SD=8.0). The mean years of follow-up was 16.7 (SD=4.3 years). The mean ages at diagnosis were 63.1, 60.4, and 64.8 years for total, CIS, and invasive cancer cases, respectively. Women with >5 years of OC use had a statistically significant 62% increased risk of cervical cancer compared to non-OC users; women with two or less children had a statistically significant 58% reduced risk of cervical cancer compared with those with five or more children (Table 15). A history of Pap test was also associated with a 30% reduced risk of cervical cancer. High level of education was associated with reduced risk of cervical cancer. We observed no association for cervical cancer risk with cigarette smoking or menopausal status. Similar patterns of the risk associations were observed for patients with CIS and invasive cancer (data not shown) with one exception. History of Pap-based screening was associated with lower risk of invasive cervical cancer (HR=0.52; 95% CI: 0.37, 0.74), but not with CIS (HR=1.01; 95% CI: 0.69, 1.18).

The distributions of baseline characteristics varied across different levels of soy and green tea intake (Table 16). Women who consumed higher amounts of soy or green tea at least monthly (drinkers) were younger, pre-menopausal, less likely to smoke cigarettes, had a higher level of education, and a history of Pap-based test. Compared with those who did not drink green tea, green tea drinkers were more likely to also drink black tea.

High consumption of soy was associated with a borderline statistically significant 20% reduced risk of cervical cancer in all women (*P*-value=0.10) after adjustment for level of education, duration of OC use, history of Pap test, parity, menopausal status and daily total calorie intake (Table 17). A weak inverse association for risk of cervical cancer was observed for total soy isoflavones (Table 17), as well intake of individual specific soy isoflavones such as

genistein, daidzein, or glycitein (data not shown). Similar associations for soy and isoflavones were observed with risk of invasive cancer and CIS (data not shown).

Overall green tea consumption was not associated with cervical cancer risk (Table 18). In contrast, more frequent intake of black tea was associated with a weak positive association with risk of cervical cancer overall, but a stronger association was observed with risk of CIS (HR=1.56; 95% CI: 1.04, 2.33, *P* for trend = 0.03). There was no association between intake of black tea and risk of invasive cancer (HR=1.08; 95% CI: 0.76, 1.54, *P* for trend = 0.72).

We evaluated the potential modifying effects of tea intake on a soy-cervical cancer association (Table 19). Among green tea drinkers, highest tertile of soy intake was associated with a statistically significant 57% reduced risk of cervical cancer among green tea drinkers who drank at least monthly (*P* for trend <0.001), whereas a null association among non-drinkers (*P* for trend = 0.52). The difference in the soy-cervical cancer risk association between green tea drinkers and non-drinkers was statistically significant (*P* for interaction = 0.004). Similarly, among green tea drinkers the HRs (95% CIs) of invasive cervical cancer and CIS for third versus first tertile of soy intake were 0.40 (0.22, 0.73) and 0.48 (0.23, 0.98), respectively. The significant inverse association between soy intake and cervical cancer risk among green tea drinkers remained after excluding cancer cases and person-years of first 3 years of follow up. Compared with the lowest tertile, the HRs (95% CIs) for the middle and highest tertiles of soy intake were 0.50 (0.29, 0.84) and 0.47 (0.27, 0.79), respectively (*P* for trend=0.005) among green tea drinkers only (*P* for interaction = 0.005). There was no evidence for effect modification by black tea on the soy-cervical cancer risk association (Table 19).

In the nested case-control analyses, women seropositive for high-risk HPV types had 2-fold higher odds of cervical cancer, compared to seronegative women (OR=2.07; 95% CI: 1.18,

3.62). A statistically significant inverse association with soy intake for cervical cancer remained after further adjustment for HPV and HSV serological status (Table 20). Similar to the findings from the cohort analysis, there was no association between green tea or black tea intake and cervical cancer in this case-control analysis, regardless of adjustment for serological status of HPV. In this case-control analysis, a stronger inverse association between soy intake and cervical cancer risk among green tea drinkers than non-drinkers (Table 20). The strength of the association for third versus first tertile soy intake did not meaningfully change after adjusting for HPV and HSV serology status.

6.5 DISCUSSION

Persistent infection of high-risk HPV types is a causal factor of cervical cancer (Vaccarella et al., 2013). Given that majority of women infected with HPV do not develop cervical cancer; infection with HPV alone is not a sufficient factor for the development of invasive cervical cancer. Other factors, such as diet, may play a significant role in the progression of HPV-initiated cervical cancer (Chih et al., 2013; Moore et al., 2003). The traditional Asian diet is characterized by relatively high soy and green tea. Isoflavones and catechins found in soy foods and green tea, respectively, have chemopreventive, anti-estrogenic and immune-modulating activities that may protect against infection-associated cancers including cervical cancer (Butler & Wu, 2011; Dhandayuthapani et al., 2013; Hussain et al., 2012; S.-H. Kim et al., 2009; Xiao et al., 2011). Using the data of a prospective cohort of Chinese women in Singapore, we tested the hypothesis that higher soy and green tea intake could be associated with lower risk of cervical cancer. We reported no statistically significant associations between either soy or green tea

alone and risk of cervical cancer. However, green tea intake modified the soy-cervical cancer association. A statistically significant inverse association for high soy intake with lower risk of cervical cancer was present among women who consumed green tea, but not among those who did not drink green tea.

Experimental evidence from HPV transgenic mice provides insight on the potential role of estrogen in cervical cancer carcinogenesis (reviewed in (Chung, Franceschi, & Lambert, 2010)), suggesting that estrogen contributes not only to the onset but to the growth and progression of cervical cancer (Brake & Lambert, 2005). As phytoestrogens, soy isoflavones may prevent the development of cervical cancer by blocking endogenous estrogen-stimulated pathways (J. Kim, 2008; Miller & Snyder, 2012). Women who regularly consume green tea may have lower circulating estrogens, compared with those who do not drink green tea (Fuhrman et al., 2013; Anna H Wu & Yu, 2006; Anna H. Wu et al., 2005). Thus, it is biologically plausible that the anti-estrogenic influence of soy and green tea are more apparent when both of them are examined jointly.

Few studies have examined the potential interactive effects of catechins and isoflavones on cancer development. *In vivo* experiments using the Nobel rat model showed a greater decrease in the number of precancerous lesions in the prostate treated with a combined dietary soy and green tea regimen than rats given soy or green tea alone (Hsu *et al*, 2011). In addition, the rats fed both soy and green tea catechins demonstrated suppression of nuclear factor-kappaB (NFκB) p50 binding activity and decreased expression of inflammatory cytokines. Not only is NFκB activity effected by E6 and E7, the primary oncoproteins for HPV induced carcinogenesis, but NFκB is constant and active during human cervical cancer progression.(Nair & Pillai, 2005). Both tea catechins and soy isoflavones are involved in pathways that block the activation of

NFκB, either directly or indirectly (Baeza & De la Fuente, 2013; Jin et al., 1999), thereby suppressing tumor progression and inducing apoptosis (reviewed in (Nair & Pillai, 2005)). The NFκB pathway may represent a potential target that soy and green tea act on in a synergistic manner to prevent cervical carcinogenesis.

A limited number of case-control studies have evaluated the potential relationship between soy and green tea intake on cervical cancer risk. In a study among Chinese women, no association was observed between increasing weekly soy food intake and cervical cancer or high-grade precancerous lesions (CIN2/3) (Jia et al., 2012). This study also reported a 45% statistically significant reduced odds of cervical cancer among green tea drinkers, compared to non-green tea drinkers, after adjusting for soy food intake and other dietary and lifestyle habits. The authors did not report whether there were interactive effects of soy and green tea, perhaps because the study size was relatively small (n=104 cases) (Jia et al., 2012). In a case-control study among Hawaiians, levels of soy and green tea intake were similar between cervical squamous intraepithelial lesions (SIL) cases (n=122) and controls (n=183) (Hernandez et al., 2004). Plasma levels of daidzein, glycitein, and genistein were also not associated with SIL, while a positive relationship with plasma equol was reported (Hernandez et al., 2004). This association with equol, an intestinal bacterial metabolite of the soy isoflavone daidzein (Miller & Snyder, 2012), was unexpected because equol and daidzein treatment inhibits cell growth in human cervical cancer HeLa cells and stimulate apoptotic cell death (Guo et al., 2004; E. Y. Kim et al., 2014). Our finding for an inverse association with soy intake among green tea drinkers was not consistent with the case-control findings from the Chinese (Jia et al., 2012) or US population (Hernandez et al., 2004). The use of a prospective study design and a validated FFQ limited the influence of bias due to differential misclassification of soy and green tea intake on

our findings (Hankin et al., 2001; Seow et al., 1998), but differential misclassification by disease status could have contributed to null and positive associations reported in the two previous case-control studies.

Black tea consumption was not associated with cervical cancer risk in our cohort analysis including both invasive and CIS cases. However, we did observe a statistically significant increase in risk of CIS for weekly/daily intake versus none. A recent review of black tea and gynecologic cancers found that an increased risk of endometrial cancer and black tea intake (OR=1.20; 95% CI: 1.05, 1.38) (Butler & Wu, 2011). Based on a recent meta-analysis of prospective cohort studies, an estimated 4% increase in breast cancer risk was reported per cup of black tea consumed (RR=1.04; 95% CI: 1.01, 1.08) (Zhang et al., 2014). In our Singapore cohort database, we reported an increase in prostate cancer risk for men who regularly drank black tea compared to nondrinkers (HR=1.41; 95% CI: 1.03, 1.92) (Montague et al., 2012). The potential adverse effect of black tea on hormone-related cancers may be related to higher levels of circulating estrogens associated with black tea intake (Anna H Wu & Yu, 2006). In addition, the levels of catechins, the chemopreventive compounds in tea, are 10-fold lower in black, compared with green tea (Y. Wang & Ho, 2009). Thus, it is not implausible that black and green tea have different or opposing associations with cancer risk. Our findings for a positive association between black tea and cervical CIS are certainly in line with results from studies of other hormone-related cancers.

The primary strength of our analysis was the prospective cohort design; we were able to ascertain soy and tea intake, as well as covariate exposure prior to cervical cancer diagnosis. Another strength of our study was the use of a validated FFQ that was designed for and validated in our study population (Hankin et al., 2001). Validation studies also demonstrated a strong

correlation between self-reported tea and soy with urinary biomarkers of catechins and isoflavones, respectively (Seow et al., 1998; Yuan, Gao, Yang, & Yu, 2007).

The lack of information on the history of sexual activity among cohort participants was a limitation of our analysis. Sexual behavior is a known risk factor for cervical cancer and we were unable to assess whether the observed associations were independent of sexual behavior. However, the nested case-control analysis allowed the testing of serological markers for HPV and HSV, which are good proxy markers of sexual activity. HPV serology is a good indicator of previous HPV exposure and a maker of persistent HPV infection, however there is potential misclassification of HPV infected women since not all women exposed to HPV will seroconvert (Stanley et al., 2012; Tiggelaar et al., 2012). Compared with women in the entire cohort, the controls were somewhat younger (mean age = 54 versus 56), were more likely to have had a Pap-based test (51% versus 39%), and consumed more soy (mean intake = 79 g/1000 kcal/day versus 75 g/1000 kcal/day). These differences may have contributed to the somewhat stronger, statistically significant inverse association between soy intake and cervical cancer risk in the case-control analysis, compared with the weak, nonsignificant inverse association in the cohort analysis. Another limitation was the single baseline measure measurements of HPV serostatus and dietary intake of soy and tea. Although these measurements were taken prior to cervical cancer development, we were unable to account for changes in diet or development of new HPV infection due to the single time point.

In conclusion, high soy intake was associated with a statistically significant reduction in cervical cancer risk among Singapore Chinese women who were drinkers of green tea. These novel findings add important information to the literature with limited number of retrospective case-control studies that so far reported inconsistent results of soy and green tea intake in relation

to cervical cancer risk. Future prospective epidemiologic studies are warranted to confirm our novel finding. In addition, experimental studies are needed to elucidate the mechanisms that underlie a potential combined effect of soy isoflavones and tea catechins on inhibiting cervical cancer progression. Even with current prevention strategies such as HPV vaccination and cervical cancer screening programs, further investigation of dietary factors is of public health interest.

6.6 TABLES AND FIGURES

Table 15. Selected baseline characteristics in relation to cervical cancer risk, the Singapore Chinese Health Study, 1993-2013

	Cases, n	HR (95% CI)*	*P-value*	*P* for trend
Characteristic				
Education level				
None/Primary	267	1.00 (reference)		
≥Secondary	45	0.59 (0.42, 0.82)	0.001	
Body mass index, kg/m^2				0.148
<24	209	1.00 (reference)		
24 - <28	72	1.08 (0.82, 1.41)	0.583	
≥28	31	1.34 (0.92, 1.95)	0.128	
Cigarette smoking				
Never	284	1.00 (reference)		
Ever	25	1.15 (0.78, 1.71)	0.480	
Parity				<0.001
≥5	101	1.00 (reference)		
3-4	143	0.87 (0.66, 1.16)	0.352	
0-2	68	0.42 (0.30, 0.60)	<0.001	
Oral contraceptive use				0.067
Never used	220	1.00 (reference)		
≤5 years	60	0.97 (0.73, 1.3)	0.854	
>5 years	32	1.62 (1.11, 2.35)	0.012	
Pap test history				
Never had a Pap	209	1.00 (reference)		
Had at least one Pap	103	0.70 (0.54, 0.90)	0.005	
Menopausal status				
Premenopausal	105	1.00 (reference)		
Postmenopausal	207	1.01 (0.73, 1.39)	0.974	

Table 16. Distribution of baseline demographic and lifestyle characteristics by levels of soy and green tea intake among women, the Singapore Chinese Health Study at baseline, 1993-2013

	Quartiles (Q) of Soy food intake				Green tea intake	
	Q1	Q2	Q3	Q4	Non-drinkers	Drinkers
Number of women	7,670	7,678	7,713	7,683	19,351	11,393
Mean age, year (SD)	57.8 (8.3)	56.3 (8.1)	55.6 (7.9)	55.6 (7.9)	56.6 (8.1)	55.7 (8.0)
Body mass index, kg/m^2 (%)						
<20	15.2	15.8	14.8	13.9	15.6	13.7
20-<24	55.0	56.2	54.8	54.2	56.3	53.1
24-<28	21.6	21.2	22.9	23.3	20.8	24.8
≥28	8.2	6.9	7.5	8.6	7.4	8.5
Education, ≥secondary level (%)	16.4	19.7	22.1	23.4	17.7	25.0
Smoking status, ever (%)	11.5	8.3	7.8	7.8	9.7	7.4
Parity, ≥5 births (%)	31.8	28.2	27.4	25.7	30.1	25.2
Pap-based test, never (%)	64.8	62.0	58.3	58.7	64.2	55.5
Menopausal status, postmenopausal (%)	74.3	68.7	66.8	65.3	70.5	65.9
Green tea, drinkers* (%)	29.8	36.2	39.9	42.4	----	----
Black tea, drinkers* (%)	22.5	28.9	32.2	32.5	23.6	38.9
Mean daily intake (SD)						
Total energy, kcal	1307 (435)	1380 (464)	1436 (478)	1472 (506)	1360 (458)	1465 (496)
Soy food, g/1000kcal	24.5 (10.9)	52.1 (7.0)	79.8 (9.5)	145.0 (53.1)	72.5 (53.3)	80.2 (51.1)
Soy protein, g/1000kcal	0.5 (0.2)	1.1 (0.2)	1.7 (0.3)	3.0 (1.0)	1.5 (1.1)	1.7 (1.0)
Soy isoflavones, mg/1000kcal	3.9 (1.9)	8.5 (1.8)	13.3 (2.5)	24.9 (10.7)	12.1 (9.8)	13.6 (9.4)

*Tea drinkers defined as those who drank tea at least monthly.

Table 17. Tertile levels of soy and soy isoflavone intake in relation to risk of cervical cancer, the Singapore Chinese Health Study, 1993-2013

	Tertiles (T)			*P* for trend
	T1	T2	T3	
Soy food				
Cases, n	115	102	95	
Median, g/1000kcal	31.29	64.70	115.86	
HR (95% CI)*	1.00 (ref)	0.86 (0.66, 1.13)	0.80 (0.61, 1.05)	0.111
Total soy isoflavone				
Cases, n	111	101	100	
Median, mg/1000kcal	4.85	10.60	19.63	
HR (95% CI)*	1.00 (ref)	0.88 (0.68, 1.16)	0.88 (0.67, 1.15)	0.347

Abbreviations: confidence interval (CI); hazard ratio (HR); tertile (T)

*Hazard ratios were adjusted for age, dialect group, year of interview, level of education, smoking status, duration of oral contraceptive use, history of Pap-based test, parity, menopausal status, and daily total calorie intake.

Table 18. Intake frequencies of green and black tea in relation to risk of cervical cancer, the Singapore Chinese Health Study, 1993-2013

	Green tea		Black tea	
	Cases, n	HR (95% CI)*	Cases, n	HR (95% CI)*
Non-tea drinkers	200	1.00 (reference)†	211	1.00 (reference)‡
Tea drinkers§	112	0.97 (0.76, 1.22)	101	1.19 (0.93, 1.51)
Non-tea drinkers	200	1.00 (reference)†	211	1.00 (reference)‡
Monthly	34	0.92 (0.64, 1.32)	22	1.00 (0.64, 1.55)
Weekly/daily	78	0.99 (0.76, 1.29)	79	1.26 (0.96, 1.64)
P for trend		0.868		0.102

Abbreviations: confidence interval (CI); hazard ratio (HR); tertile (T)

*Hazard ratios were adjusted for age, dialect group, year of interview, level of education, smoking status, duration of oral contraceptive use, history of Pap-based test, parity, menopausal status, and daily total calorie intake.

†The reference group included only non-green tea drinkers.

‡The reference group included only non-black tea drinkers.

§Tea drinkers defined as those who drank tea at least monthly

Table 19. Tertile levels of soy intake in relation to risk of cervical cancer stratified by intake of green or black tea, the Singapore Chinese Health Study 1993-2013

	Tertiles (T) of Soy Intake (g/1000kcal)			*P* for trend	*P* for interaction
	T1	T2	T3		
Non-green tea drinkers					
Cases, n	68	68	64		
HR (95% CI)*	1.00 (reference)	1.12 (0.8, 1.57)	1.12 (0.79, 1.58)	0.515	
Green tea drinkers†					
Cases, n	47	34	31		
HR (95% CI)*	1.00 (reference)	0.53 (0.34, 0.83)	0.43 (0.28, 0.69)	<0.001	0.004
Non-black tea drinkers					
Cases, n	83	64	64		
HR (95% CI)*	1.00 (reference)	0.83 (0.60, 1.15)	0.86 (0.62, 1.20)	0.369	
Black tea drinkers†					
Cases, n	32	38	31		
HR (95% CI)*	1.00 (reference)	0.90 (0.56, 1.45)	0.67 (0.41, 1.11)	0.111	0.528

Abbreviations: confidence interval (CI); hazard ratio (HR); tertile (T)

*Hazard ratios were adjusted for age, dialect group, year of interview, level of education, smoking status, duration of oral contraceptive use, history of Pap-based test, parity, menopausal status, and daily total calorie intake.

†Tea drinkers defined as those who drank tea at least monthly.

Table 20. Nested case-control analysis of soy and green tea intake in relation to cervical cancer with and without adjustment for human papillomavirus (HPV) serology status

	Cases (n=75)	Controls (n=199)	Multivariable-adjusted OR (95% CI)	HPV-adjusted OR (95% CI)[†]
Soy food				
1st Tertile (T1)	33	64	1.00 (reference)*	1.00 (reference)[†]
2nd Tertile (T2)	25	65	0.69 (0.35, 1.34)	0.72 (0.37, 1.42)
3rd Tertile (T3)	17	70	0.43 (0.21, 0.90)	0.43 (0.20, 0.92)
P for trend			0.025	0.030
Green tea				
Nondrinker	41	112	1.00 (reference)*	1.00 (reference)[†]
Drinker	34	87	0.96 (0.53, 1.74)	0.96 (0.52, 1.78)
Non-green tea drinker				
Soy food				
Low (T1+T2)	31	76	1.00 (reference)[‡]	1.00 (reference)[§]
High (T3)	10	36	0.66 (0.28, 1.58)	0.61 (0.25, 1.50)
Green tea drinker				
Soy food				
Low (T1+T2)	27	53	1.00 (reference)[‡]	1.00 (reference)[§]
High (T3)	7	34	0.20 (0.06, 0.65)	0.19 (0.06, 0.65)[#]

Abbreviations: confidence interval (CI); herpes simplex virus (HSV); human papillomavirus (HPV); odds ratio (OR); tertile (T).

*Conditional odds ratios were adjusted for education level, smoking status, duration of oral contraceptive use, Pap-based test history, parity, and daily calorie intake.

[†]Conditional odds ratios were adjusted for covariates listed in footnote (*), and HSV-2 and HPV seropositive status. HPV status was defined as seropositivity to L1 for any high-risk HPV (16, 18, 31, 33, 35, 45, 52 and 58).

[‡]Unconditional odds ratios were adjusted for matching factors (i.e., age, dialect group, interview year, time between baseline interview and biospecimen collection) and education level, smoking status, duration of oral contraceptive use, Pap test history, parity, menopausal status, and daily calorie intake.

[§]Unconditional odds ratios were adjusted for covariates listed in footnote ([‡]) and HSV-2 and HPV seropositive status. HPV status was defined as seropositivity to L1 for any high-risk HPV (16, 18, 31, 33, 35, 45, 52 and 58).

[#]*P* for interaction between green tea and soy intake = 0.316.

7.0 CONCLUSIONS AND PUBLIC HEALTH SIGNIFICANCE

This dissertation addresses key gaps in understanding HPV and cervical cancer natural history and associated risk factors by using populations with limited epidemiologic data available, such as older women, postpartum populations, and women exposed to high soy and high tea diets. By using these three different populations, we were able to provide new and important insight on HPV and cervical cancer disease that could translate to potential interventions at different points across the cancer continuum.

First, we were specifically interested in understanding HPV detection and clearance in mid-adult and older women to inform prevention recommendations in this age group. Using a clinic-based cohort of women aged 35-60 years old, the HIP Study, we were able to estimate incidence and clearance rates in a mid-adult population and to explore if new HPV detection in mid-adult women is primarily attributed to current sexual exposure. We observed that new detection rates were highest among women who reported a new recent sex partner. However, only 12% of women reported a new recent sex partner. Notably, 80% of new detections occurred during periods of sexual abstinence or monogamy, especially among women with higher number of lifetime sexual partners (LTSP). This effect modification of LTSP contradicts the conventional natural history paradigm that most of the HPV detected in older women is attributed to new sexual exposures and strengthens the argument that reactivation of a latent HPV infection explains some of the new HPV detected in this age group. Reactivation depends

on previous HPV exposure and assumes that a previously acquired infection does not completely resolve. Having a high number of LTSP increases the likelihood of exposure opportunity, as well as multiple types. Clearance rates were not associated with current or past sexual behavior, but persistence was more common with baseline HPV infection and newly detected infections were more transient. Our findings among older screened women, with predominately low-risk current sexual behaviors, challenges the established paradigm that new HPV detected in this age group is primarily a result of new sexual exposure, as with younger populations. New infections detected due to previous exposure would not be prevented by HPV vaccination. Therefore, our findings are particularly important in light of new cervical cancer prevention programs that propose the combination of cervical cancer screening in mid-adult women with HPV vaccination, and such interventions should be carefully evaluated.

Secondly, seroprevalence data in India and among postpartum populations is limited and is important as India continues to discuss appropriate prevention strategies against cervical cancer for their country. Additionally, the availability of serologic HPV data becomes increasingly important for identifying subgroups for catch-up vaccination in populations with low vaccine coverage among the target age group of 10-12 year old girls. The population-based LIFE cohort allowed us to estimate the seroprevalence of HPV in postpartum women in India. Among 15-35 year postpartum women, the seroprevalence of quadrivalent vaccine types was low and ranged from 3.9% for HPV-18 to 10.1% for HPV-16 and HPV-11. More than 80% of the postpartum women in our study were negative for all of the quadrivalent vaccine types; only 11.6% of the population had a previous exposure to oncogenic HPV16/18. Increasing birth order and being a homemaker were associated with any HPV seropositivity. High-risk HPV seropositivity may also be associated with longer marriage length. The low seroprevalence of

HPV in this population suggests a catch-up vaccination program targeting young postpartum women may be beneficial, especially since HPV exposure was associated with length of marriage.

Finally, because of the high soy and tea diet in the participants of the Singapore Chinese Health Study, we were able to evaluate the relationship between cervical cancer and soy and tea intake, which have a variety of chemopreventive properties but not well studied in cervical cancer. We found high soy intake was associated with a lower cervical cancer risk among green tea drinkers, but not among non-drinkers of green tea. In models adjusting for human papillomavirus serology, the inverse association between soy intake and cervical cancer risk remained. Frequent black tea intake increased carcinoma in-situ risk, but not risk for invasive cancer. Our novel findings suggest that a protective effect of soy against cervical cancer development may depend on the constituents in green tea. Given the potential disruption to cervical carcinogenesis, patients with pre-invasive disease or cervical cancer could benefit with interventions that incorporate dietary modifications or nutritional supplementation utilizing soy and tea components and should be developed and further tested for benefit.

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