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MOLECULAR EPIDEMIOLOGY AND GENOTYPE DISTRIBUTION OF HUMAN PAPILLOMAVIRUS AMONG ARAB WOMEN IN STATE OF QATAR

A Thesis in

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By

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Abstract

Background

Human papillomavirus (HPV) are the most commonly known sexually transmitted agents. To date, few reports are available on distribution of most prevalent and variants types of HPV in Arab women. Therefore, the aim of this study was to determine the age specific distribution of HPV types among Arab women being subjected to routine Pap smear test in State of Qatar.

Methods

Total 3008 pap smears have been collected in ThinPrep vials from the Arab women seeking routine gynecological care visiting Women's Hospital, Hamad Medical Corporation (HMC) in Qatar. Viral DNA from ThinPrep samples was extracted and was screened for HPV DNA by real-time PCR using L1 HPV specific (GP5+/6+) primers. The types specific distribution of the viruses was determined by HPV high and low risk typing RT-PCR kits and PCR-based sequencing (Genewiz, Inc, USA).

Results

Based on the collected data, HPV DNA was detected in 182 women (6%), and 17 different HPV genotypes were detected, comprising high-risk, intermediate and low-risk genotypes. The prevalence of HPV infection was seen in 6.2% Qatari and 5.9% non-Qatari women. With regard to age, 5.1% HPV infection were found in women 16-24 years of age, 5.8% in women 25-34 years of age, 5.5% in women 35-44 years of age, 6.5% in women 45-54 years and 7.2% in women more than 55 years old.

Conclusion

At the end, our study showed a relatively low prevalence (6%) of HPV infection and presence of a varied genotypic profile of HPV with a high prevalence of low risk HPV genotype 81 among the Arab women residing in State of Qatar when compared to the Middle Eastern and North African (MENA) countries.

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List of Abbreviations

HPV: Human Papillomavirus

LR-HPV: Low Risk Human Papillomavirus

HR-HPV: High Risk Human Papillomavirus

HMC: Hamad Medical Corporation

PHCC: Primary Health Care Corporation

STD: Sexual Transmitted Disease

ORF: Open Reading Frame

OR: Odd Ratio

CI: Confident Interval

PCR: Polymerase chain Reaction

+Ve: Positive Control

-Ve: Negative Control

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Chapter 1-Introduction

Human Papillomavirus (HPV) is the most common sexually transmitted infection and major causal agent for cervical cancer [Walboomers et al., 1999]. Worldwide, cervical cancer is the fourth most common cancer among women, with an estimated 83,195 new cases and 35,673 deaths in 2012 [Ferlay et al., 2013]. More than 85% of these cases and deaths occur in developing countries [Jemal et al., 2011]. Currently, more than 200 HPV genotypes have been identified and characterized based on nucleotide sequence relatedness in L1 gene, which codes for the major HPV capsid protein [Clifford, Smith, Plummer, Munoz, & Franceschi, 2003]. Among these, based on their oncogenic potential via associations with cervical cancer and precancerous lesions, about 30 to 40 genotypes are divided into high-risk (HR), causing cervical neoplasia and low-risk (LR) HPV genotypes, which causes benign signs and symptoms [Clifford et al., 2003]. Furthermore, these genomic variants can be considered as markers of specific HPV genomes and accordingly can be used in epidemiological and etiological studies in order to investigate the transformation of HPV within and among populations.

The prevalence of HPV infections in women within the general population differs considerably between countries and regions, as well as within regions, ranging from 1.6-41.9% [Seoud, 2012].In Extended Middle Eastern and North African (EMENA) countries, HPV prevalence reported between 0% and 25% in women with normal cytology and up to 98% in women with abnormal cytology [Khan, 2009; Seoud, 2012].Very limited data is available on the prevalence of HPV infection in the Arab countries where social, cultural and sexual behaviors differ greatly from the more-well reported Western countries [Wellings et al., 2006].

Presently, two FDA approved HPV vaccines (Gardasil and Cervarix) have been used to reduce the incidence of cervical cancer associated with HPV infections. These vaccines have been prepared from DNA free viral particles combined with an adjuvant by recombinant technology. Gardasil is a quadrivalent (types 6, 11, 16 and 18) HPV vaccine that prevents around 100% of precancerous lesions associated with HPV 16 and HPV 18. While, Cervarix is a bivalent (types 16 and 18) vaccine [Cutt et al., 2007].

Moreover, it has been revealed that the distribution of different HPV genotypes varies from one region to another, which influences the effectiveness of the HPV vaccines. In addition, the lack of studies on HPV prevalence and HPV genotype distribution affect the designing of effective vaccine used in different population [Al-Thani et al., 2010]. The data of HPV prevalence and genotypes distribution among Arab women is limited. Therefore, in the present study, we aimed to determine the HPV prevalence and distribution of HPV genotypes among Arab women residing in State of Qatar with normal and abnormal cytology.

Chapter 2-Literature of review

HPV Genome:

Human papillomaviruses (HPV) are group of small, non-enveloped circular double-stranded DNA viruses within an icosahedral capsid that infect the human epithelium. There are more than 200 different genotypes of HPV have been identified. Among these, about 30 to 40 infect human genital tract and divided into high-risk (HR)genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, and 58) and low risk (LR)genotypes (6, 11, 40, 42, 43, 44, 54, 81 and 90) [Muñoz et al., 2003]. Among the high-risk (HR) genotypes, HPV 16 is the most common oncogenic type that is responsible for almost half of all cervical cancers, and HPV 16 and 18 together are responsible for almost 70% of cervical cancers [Clifford et al., 2003].Among the low-risk (LR) genotypes, HPV 6 and 11 are the most common types associated with genital warts and responsible for almost 90% of these lesions.

HPV genome is circular and approximately 8000 bp, which encode eight major proteins, 6 located in the "early" region and 2 in the "late" region. The "early" proteins (E6, E7, E1, E2, E4, and E5) have regulatory function. They play roles in HPV genome replication, transcription, cell cycle, and structural modification of infected cell, immune alteration and apoptosis control [Doorbar et al., 1997] While L1 and L2, are late proteins that encode the viral capsid protein, which is required for virus transmission, and survival in the environment [Graham, 2010].

Establishment and virulence of HPV infection

Establishment of papillomaviruses infection requires that virus particles to get the access to the basal layer of epithelial cells [Doorbar, 2006]. Once they are accessed the basal layer of the epithelial cells, the viral genome stabilized as episome (without integration into the genome of the host cell). Expression of viral replication proteins, E1 and E2 is required in this phase of HPV infection. The E2 protein is required for initiation of viral DNA replication. Also, E2 protein is a DNA protein that is required for recruitment of the E1 helicase to the viral origin, which binds to cellular proteins necessary for DNA replication [Dell et al., 2003]. It is also considered as transcription factor that regulates the expression of the viral early proteins "oncogenes" (E6 and E7).Lesions in the cervix caused by HPV, is related to the expression of the viral oncogenes (E6 and E7) that lead to increase proliferation of basal epithelial cells [Doorbar, 2006]. The activity of these genes (E6 and E7) during natural infection,

allows the small number of infected cells to expand that subsequently produce infectious virions [Madison, 2003]. The mechanism by which papillomaviruses stimulate the progression of cell cycle is well known and it is the same way that other tumor viruses decontrol cell growth. E7 disrupts the association between pRb and the E2F family of transcription factors by associating with Rb (retinoblastoma protein). As a result, E2F activates cellular proteins such as cyclins A and E that are required for viral DNA replication. E7 is also associated with other proteins required for cell proliferation such as histone deacetylases [Brehm et al., 1999], and cyclin-dependent kinase inhibitors p21 and p27 [Funk et al., 1997]. In the high-risk HPV types, the two proteins (E6 and E7) are expressed together from a single polycistronic mRNA [Stacey et al., 2000]. The main role of E6 is its association with p53 that results in p53 ubiquitination and degradation. This mechanism prevents apoptosis or growth arrest that mediates the cell cycle entry in the upper epithelial layers by E7. E6 is an anti-apoptotic protein that is associated with Bak [Thomas & Banks, 1998] and Bax [Li & Dou, 2000]. The anti-apoptotic function of E6 plays an important role in developing cervical cancers by allowing the accumulation of secondary mutations to go unchecked. In high-risk HPV types, E6 protein plays an important role in mediating cell proliferation independently of E7 through its C-terminal PDZ ligand domain: PSD-95 (a 95 kDa protein involved in signalling), Dlg (the Drosophiladiscs large protein), and ZO1 (the zonula occludens 1 protein involved in maintaining epithelial cell polarity) [Thomas et al., 2002]. PDZ binding to the C-terminal domain of E6 mediate the cell proliferation [Nguyen et al., 2003] and develop metastatic tumors by disrupting the adhesion of normal cell.

High-risk HPV genotypes are associated with cervical cancer that increases the thickness of epithelial layers based on the grade of neoplasia and decrease their differentiation. Lesion formation and the maintenance of viral episomes require cell proliferation, so all types of papillomavirus ultimately amplify and package their genomes for the infectious virions to be produced. This is managed by the up-regulation of the differentiation-dependent promoter, which is within E7 ORF (P670 in HPV16; P742 in HPV31), in many of HPV genotypes [Bodily & Meyers, 2005; Spink & Laimins, 2005]. The activation of the differentiation-dependent promoter leads to an increase the viral proteins (E1, E2, E4 and E5) [Bodily & Meyers, 2005; Spink & Laimins, 2005] required for replication.

The E1, E2, E4 and E5 proteins play an important role in viral genome amplification of the virus [Fehrmann, Klumpp, & Laimins, 2003]. The E1 expressed at low level, so it requires the presence of E2 protein to increase its binding to the targeted binding site. The E5 is a transmembrane protein that is located in the ER (endoplasmic reticulum). It delays the endosomal acidification process by its association with the vacuolar proton ATPase [Crusius, Rodriguez , & Alonso, 2000].This mechanism of E5 protein affects the recycling of growth factor receptors resulting to an increase in (epidermal growth factor)-mediated receptor signaling and maintenance of viral replication [Crusius et al., 2000], while the role of E4 is not well established in viral genome amplification. Virus assembly "packaging into infectious particles" is the last stage of the papillomavirus productive cycle. After the viral genome amplification, capsid proteins (L1 and L2) accumulate [Florin, Sapp, Streeck, & Sapp, 2002].E2 protein is required for the assembly of infectious virions in addition to (L1 and L2) capsid proteins in the upper layers of epithelium [Zhao et al., 2000].Finally, the release of the viral particles required effective escape from the envelope at the cell surface of the epithelium, which is facilitated by the E4 protein. This mechanism is managed by disturbing the keratin network by E4 protein [Wang et al., 2004].

Transmission of HPV

HPV is thought to gain its entry to the body through micro-traumas or abrasions to the mucosa exposing the epithelium basal cells. So the transmission of HPV is through direct skin-to-skin contact or through penetrative vaginal or anal intercourse [Winer et al., 2003].Most studies have shown and supported sexual route is the primary route of the genital HPV infection documenting genital warts between partners [Hernandez et al., 2008].The risk of HPV infection is related to the number of sexual partners [Ho et al., 1998].Using condoms decrease the risk of transmission of HPV infection among partners. However it is not 100% protective tools, since women having sexual contact with men using condoms are still at risk of acquiring HPV infection [Matt, Feltman, & Twiggs, 2008].Despite the fact that HPV is the common pathogen that infects adults, but it is also found to infect children causing various mucosal and cutaneous types of infections. There are different modes of transmission of HPV infection among children including, auto and hetero inoculation, perinatal transmission, sexual abuse and indirect transmission through fomites. Children abuse is considered as the most common mode of HPV transmission among children [Syrjänen & Puranen,

2000]. Also, HPV could infect virgins who have never encountered vaginal intercourse through horizontal transmission such as floors and seats of public toilets, bathing resorts, public and private swimming pools, schools and private homes of a family with an infected individual [Syrjänen, 2010].

Signs and symptoms of HPV

HPV infection causes a lot of complications start with mild to moderate symptoms such as genital warts, skin warts, and recurrent respiratory papillomatosis until reach to the severe and difficult cause, which is the cancer and there are about 600,000 cases of cervical cancer, vulva, vagina and oropharynx every year [Arbyn et al., 2012].HPV could infect basal epithelial cells of the skin "hands and feet" and is categorized as cutaneous types of HPV while mucosal HPV types infect the inner lining of tissues such as mouth, throat, respiratory tract and anogenital epithelium[Burd, 2003].

Many of HPV infections are asymptomatic, staying for a short while without showing any symptoms and they are self-limiting infection by which the patient immune system clears the HPV within approximately 1-2 years [Lalonde, 2007].

Sexually transmitted HPV infection could lead to one of the three possible effects based on the HPV type involved in the site of infection [Burd, 2003]:

1. Anogenital warts (condyloma acuminatum): appear on or around the genitals and anus in men and women. HPV 6 and 11 are most common types associated with anogenital warts, but do not lead to cancer. These warts are asymptomatic and HPV may be cleared in 3 to 4 months.
2. Latent or inactive HPV infection: obvious or clear symptoms are rarely produced and few people can recognize that they are infected.
3. Active HPV infection: strongly associated with high-risk HPV types that cause visible changes to the infected epithelial cells leading to vaginal, vulvar, penile, urethral, bladder, and cervical intraepithelial neoplasia. This infection is strongly associated with cervical cancer.

HPV infection can cause recurrent respiratory papillomatosis (RRP) which is a rare, benign disease that infect the upper aerodigestive tract with no known cure. In most of the RRP cases, pediatric patients do not show any symptoms immediately. Since larynx is the most common site of infection, hoarseness is the first symptom appeared [Kashima, Mounts, Leventhal, & Hruban, 1993].Other symptoms in RRP include

chronic cough, dyspnea, pneumonia, recurrent upper respiratory infections, acute respiratory distress, dysphagia and failure to thrive [Derkay & Wiatrak, 2008]. RRP could be potentially life threatening benign tumor of the respiratory tract [Zacharisen & Conley, 2006].HPV 11 is the most common type (50%-100%) in RRP cases, followed by HPV 6 [Gissmann et al., 1983].

Diagnosis of HPV infection

Several studies have confirmed that infection of cervix with high-risk genotypes is considered as a precursor step to cervical cancer. Cervical cancer is a disease that progress from mild cervical intraepithelial neoplasia (CIN1), which is a reversible stage, to severe cervical intraepithelial neoplasia (CIN2 or CIN3), which is irreversible, and last stage of this process is invasive cervical carcinoma [Holowaty, Miller, Rohan, & To, 1999].HPV from clinical specimens cannot be cultured in the laboratory and immunological tests are not sufficient to detect HPV infection. So the main diagnostic tools to detect HPV infection in clinical samples are cytology and histology. Lately, molecular methods have been emerged to detect HPV DNA in clinical specimens.

- **Conventional Cytology:** Papanicolaou-stained (Pap) smear is primary method for detection of HPV infection. It is named after the pathologist George Papanicolaou, who introduced the method in 1949 [Holowat et al., 1999]. It is considered as screening tool that detect changes, often caused by HPV, in cells of the transformation zone of cervix. Cervical cancer incidence and mortality rates has been decreased half to two-third since the introduction of Pap smear as screening tool [Kurman, Henson, Herbst, Noller, & Schiffman, 1994].The reporting system for the Pap smear that replaced the CIN system is Bethesda System. Bethesda System was hosted in 1988 and amended in 1991 [Border, 1992].The CIN system was hosted in 1973 and it was based on the architecture of the tissue and its progression from precursor lesions to invasive cancer. However, Bethesda System categorized the abnormalities of squamous cells into: ASC (Atypical Squamous Cells), LSIL (Low-Grade Squamous Intraepithelial lesions), HSIL (High-Grade Squamous Intraepithelial lesions), and squamous cell carcinoma.

  There are some limitations using Pap smear techniques to detect abnormalities in cervical samples. Samples inadequacy constitutes 8% of received

specimens. 20 to 30% is the false-negative rates which results from clumping, not spread evenly on the microscope slide, of the cells and presence of blood, bacteria, or yeast that prevent proper detection of abnormal cells. Cervical cells could be distorted if the slide exposed to the air too long before being fixed. Human error is considered as the main limitation to an accurate interpretation [Burd, 2003].

- **Histopathology:** Colposcopy and colposcopy-directed biopsy are methods to evaluate the cervical lesion for patients who have abnormal Pap smear results. Low-grade and high-grade dysplasia could be detected using colposcopy. Biopsy is considered as diagnostic method to detect pathologic features of HPV infection such as hyperplasia and degenerative vacuolization of cytoplasm with atypical nuclei. Another method to detect the presence of HPV in cervical specimens is using monoclonal and polyclonal antibodies that are directed to HPV common antigen, epitope of major capsid protein that is expressed in different HPV types [Burd, 2003].
- **HPV DNA detection:** There are two PCR methods: type-specific PCR and general primer PCR to detect HPV in cervical specimens. In type-specific PCR, method based on variations of the sequence present in E6 and E7 genes of HPV. There are fourteen type-specific PCRs for detection of high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) which amplify 100 bp in the E7 ORF [Walboomers et al., 1999]. In general primer PCR, method based on using consensus primers to amplify a broad range of HPV genotypes. The principle of these primers is that they target conserved regions of the HPV such as the L1 capsid gene. MY09/MY11 primers amplify specific target in L1 gene producing 450 bp size products [Bosch et al., 1995]while GP5+/GP6+ primers amplify specific target within the region targeted by MY09/MY11 primers producing 150 bp size products [Hutchinson et al., 1994].

Treatment of HPV infection

Most of HPV that induces cervical changes are transient. 90% of cervical changes regress within 12 to 24 months by the action of the immune system eliminating HPV virus [Chua & Hjerpe, 1996].Cryotherapy or laser therapy is superficial ablative procedures that are used to treat intraepithelial lesions. In cryotherapy procedure, super-cooled probe is used to freeze the abnormal tissue and its surrounding to induce

necrosis for the abnormal lesions. In laser therapy, a laser beam of carbon dioxide is applied to remove abnormal tissue. Laser therapy is as effective as cryotherapy, but it is more expensive. A preferred treatment for squamous lesions is loop electrosurgical method. An electrically charged wire to remove the transformed lesion conducts this method. It is less expensive compared to the laser therapy and preserves the removed tissue for further histological analysis. Excisional cone biopsy is an effective procedure to manage micro-invasive cancers less than 3 mm in size. Radical hysterectomy is a method to control early invasive cancers. And radiotherapy is methods of choice to control locally advance cancers [Burd, 2003].In addition to surgical procedures, different antiviral agents have been used as method to treat cervical lesions caused by HPV such as Cidofovir, Podophyllin and IFNs. Exposure of cells containing HPV to Cidofovir, inhibit cell proliferation [Andrei, Snoeck, Piette, Delvenne, & De Clercq, 1998]. Podophyllinin combination with vidarabine, a DNA polymerase inhibitor, arrests the cell growth and suppresses the expression of HPV gene [Okamoto et al., 1999].IFN-α is one of the approved treatment for genital warts.

Prevention

The main approaches for prevention against HPV infection is development of HPV vaccines. To date, two HPV vaccines are available that protect against cervical cancers. Cervarix is one of the preventative vaccines (bivalent recombinant vaccine) that protect against two of high-risk HPV genotypes (HPV 16, 18) [Keam & Harper, 2008]. Gardasil is another HPV vaccine (quadrivalent recombinant vaccine) that protect against four HPV genotypes (HPV 6, 11, 16, 18) [Siddiqui & Perry, 2006]. HPV vaccine is produced by the expression of major and minor capsid protein and assembled into virus-like particles (VLPs) lacking viral DNA. The capsid proteins are immunogenic to induce the immune response. Other methods for prevention against HPV infection are using condom and spermicide. Recent studies have shown that condoms can reduce the risk of transmitting of HPV, but they are not 100% preventative, since HPV virus could be got by contact with scrotum, labia, or anus which are not covered by condom [Burd, 2003].

There are two preventative tools to decrease the cervical cancer in low and middle income countries (LMIC) since cancer is a main cause of death in LMIC. The combination of HPV vaccination and cervical screening are effective preventative

tools against developing cervical cancer and such tools are authorized by international organizations [Steben et al., 2012].

Epidemiology and incidence of HPV infection

HPV is one of the most common causal agents for sexually transmitted (STD) disease among men and women worldwide. It is considered as most common viral STD in United State [Burd, 2003].The worldwide HPV prevalence among women with normal cytology is 11-12% with rate of 24% in sub-Saharan Africa, 21% in Eastern Europe and 16% in Latin America. Whereas, among women with abnormal cytology, prevalence of HPV infection reported significantly higher (up to 98%) and it is proportional to the severity of the cervical lesions. HPV prevalence reaches 90% in women with grade 3 cervical intraepithelial neoplasia (CIN3) and invasive cancer (Forman et al., 2012).Worldwide, cervical cancer is the fourth most common cancer among women, with an estimated 83,195 new cases and 35,673 deaths in 2012 [Ferlay et al., 2013]. More than 85% of these cases and deaths occur in developing countries [Jemal A et al., 2011]. HPV16 (3.2%) and HPV18 (1.4%) are the most prevalent HPV genotypes.

In Guinea, the HPV prevalence is around 51% (47.9% among women with normal cervical cytology and 78.5% among women with abnormal cervical lesions). The prevalence of high-risk genotypes was 32.1% and the most common high-risk types with normal cervical cytology among Guinean women were HPV16 (6.7%), 45 (4.7%), 52 (4.0%), and 18, 35 and 58 (3.2% each). On the other hand, the prevalence of low-risk genotypes with normal cervical cytology was 30.5% and the most common low-risk types were HPV66, 42 and 81. In the same study, they found that HPV16 was the most common high-risk genotypes (13.9%) among women with abnormal cytology [Keita et al., 2009].

On the other hand, in Europe, around 6.6% of women evaluate to develop cervical cancer each year and about 73.3 % of cervical cancer is due to HPV-16 and/or HPV-18.In Italy, the HPV prevalence among Italian women was 8.8% in which HPV 16 was the most common high-risk type (32.6% of all HPV positive women). Based on the age group of the Italian study, HPV prevalence was (13.5%) at ages 25-39 years, (11.5%) at age 40-44 years, and approximately (5%) among older women [Ronco et al., 2005]. In France, HPV infection among French women with abnormal cytology was significantly more prevalent (90%) than French women with

normal cytology (48.3%). Furthermore, the most common HPV genotypes among French women were HPV-16 (14.8%), HPV-53 (9.0%), HPV-31 (8.7%), and HPV-51 (7.5%), whereas HPV-18 (3.8%), HPV-6 (2.9%), and HPV-11 (0.4%) were less common [Casalegno et al., 2011]. The prevalence of HPV infection in the other European countries was (9.9%) Canada, (8.9%) England, (7.5%) Finland, (6.3%) Germany and (5.5%) Sweden [Casalegno et al., 2011].

In Arab world, cervical cancer is one of the most common type of cancer in Tunisia, but the rate differ depend on the region [KrennHrubec et al., 2011].So the prevalence of HPV is relatively higher in prostitutes (39%) than in married women (14%). Furthermore, HPV genotyping analysis in Tunisian study showed that HPV 16 was the most common type among prostitutes while HPV 6 was the common among married women [Hassen et al., 2003].Furthermore, in Algeria, the prevalence of HPV in the general Algerian population was 6.3%. HPV infection was significantly higher among women with polygamous marriages, divorced women and husband's extramarital sexual relationships. In the same study, authors showed that HPV infection among married women in Algeria is lower than in sub-Saharan Africa [Hammouda et al., 2011]. In Kuwait, the prevalence of HPV among women with normal cytology was 2.4% [Al-Awadhi et al., 2011], while prevalence of HPV among women with abnormal cytology was 51% [Al-Awadhi et al., 2013].

The prevalence of HPV infections in women within the general population differs considerably between countries and regions, as well as within regions, ranging from 1.6-41.9% [Vaccarella, Bruni, & Seoud, 2013].In Extended Middle Eastern and North African (EMENA) countries, HPV prevalence reported between 0% and 25% in women with normal cytology and up to 98% in women with abnormal cytology [Khan, 2009; Seoud, 2012].Very limited data is available on the prevalence of HPV infection in the Arab countries where social, cultural and sexual behaviors differ greatly from the more-well reported Western countries [Wellings et al., 2006].

Therefore, in the present study, for the first time identified the prevalence and genotypic distribution of HPV among general Arab women, with normal or abnormal cytology, living in the state of Qatar.

Chapter 3-Materials and Methods

Ethical consideration

This study was approved by the Institutional Review Board of the Weill Cornell Medical College in Qatar (WCMCQ) and Research Office of Hamad Medical Corporations (HMC) Research Office, Doha, Qatar (Appendix I).

Study Population:

The study population of this study includes 3008cervical samples of Qatari women and women with Arab nationality living in Qatar were collected from Women's Hospital and Primary Health Care Corporation in Doha, Qatar between March 2012-January 2013.

Collection and cytological analysis of the samples:

The cervical samples were collected in ThinPrep vials (BD SurePath™) and were transported to the laboratory in an icebox container for further investigations. ThinPrep cytological smears were screened at Cytology lab, HMC and reported according to the Bethesda system for reporting of cervical cytology [Solomon D, Davey D 2002]. Furthermore, the age, nationality, clinical history, and cytological diagnosis of all the subjects were also retrieved and recorded.

DNA Extraction:

Viral DNA from cervical samples was extracted by QIAamp Min Elute virus spin kit according to manufacturer's instructions. Briefly, add 25 of protease in to 1.5 ml centrifuge tubes and then add 200l of sample and 100lof AL buffer (contain 28g/ ml of carrier RNA). Furthermore, close the cap of tube and mix it by pulse vortexing for about 15 sec. Incubate at 65 for 15 min in heating block and centrifuge tube to remove the drops from the inside of the lid briefly. Then, add 250 l of ethanol to the sample, close the cap and mix it by pulse vortexing for 15 sec., incubate the lysate for 5 min at room temperature and then centrifuge it briefly. After that, applying all lysate onto QIAamp MinElute column carefully without wetting the rim of this column that contain filter to remove all the debris except the DNA of the virus. Centrifuge at 8000 rpm for 1 min and place the QIAamp MinElute column in a clean 2 ml collection tube, discard the collection tube containing the filtrate. Then, add 500l of AW1 buffer that prepare by adding 25 ml of ethanol (95-100%) to 19 ml of AW1 buffer and store it at room temperature, centrifuge it at 8000 rpm for 1 min in clean 2 ml collection

tube, discard collection tube containing filtrate and add 500L of AW2 buffer that prepare by adding 30 ml of ethanol (95-100%) to a 13 ml AW2 buffer and store it at room temperature, centrifuge at 8000 rpm for 1 min in clean 2 ml collection tube, discard collection tube containing filtrate. After that, add 500l of ethanol, centrifuge at 8000 rpm for 1 min in clean 2 ml collection tube, and discard collection tube containing filtrate. Place the column in a clean 2 ml collection tube and then centrifuge at full speed 14000 rpm for 3 min to dry the column. Next, place the column into a new 2 ml collection tubes, open the lid, and then incubate at 56^0C for 3 min to dry the membrane. Later, discard the collection tube with filtrate and place the column in a clean 1.5 ml microcentrifuge tube and carefully open the lid and apply 50*l* of buffer AVE or RNase-free water to the center of the membrane, then close the lid and incubate at room temperature for 1 min. Finally, centrifuge tubes at full speed 14000 rpm for 1 min.

Measure the DNA concentration by using nanodrop:

All the extracted DNA samples were subjected to nano-drop to measure the DNA concentration and 260/280 ratios were calculated.

Detection of HPV infection by Real time PCR:

To determine the presence of HPV-DNA, L1 consensus degenerated primer (GP5+/GP6+) (GP5+ 5'- TTT GTT ACT GTG GTA GAT ACT AC -3' and GP6+ 5'- GAA AAA TAA ACT GTA AAT CAT ATT C -3') that amplify a conserved 150 bp sequence of the L1 open reading frame (ORF) and PC03/PC04 primers (Human β-globin as internal control) (PCO3 5'- ACACAACTGTGTTCACTAGC-3' and PCO4 5'-CAACTTCATCCACGTTCACC-3') were used to ensure the DNA quality of the samples (Figure 1) [de Roda Husman et al., 1995]Real-time PCR amplification was carried out in ABI 7500 real-time PCR (Applied Biosystems).

To detect the HPV DNA in clinical samples, we have carried out the real time PCR assay as follows: 2µl extracted genomic DNA (5ng/µl) was combined with 12.5µl of 2X SyberGreen (Applied Biosystems, Foster City, CA) containing ROX as a passive reference, 10pmol (10 µM) of each forward and reverse GP5+/6+ HPV and/or PCO3/PCO4 primers and the mixture was made up to 25µl volume with nuclease free

water (Ambion, Austin, TX). The PCR amplification was initiated at 950C for 10 min and completed by 45 amplification cycles (denaturation at 950C for 20 sec, annealing at 500C for 30 sec and extension at 600C for 30 sec).

All the samples were analyzed in duplicate on a 96 optical well reaction plate (Applied Biosystems). A positive control (plasmid HPV DNA gifted by Dr. Elham Hassen, Tunisia) and negative control (nuclease free water) were included in each amplification reaction. Real-time PCR amplification was carried out in ABI 7500 real-time PCR (Applied Biosystems).

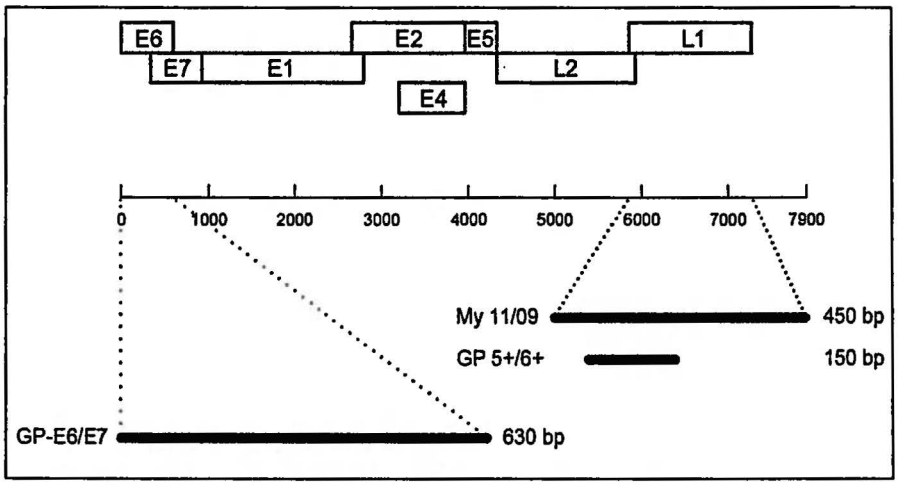

Figure 1: HPV genome showing the targeted region (L1 gene) by HPV PCR specific primers (GP5+/6+)

Data Analysis:

Fluorescence spectra were recorded during the elongation phase of each PCR cycle. Sequence Detection Software (SDS v1.7) of ABI 7500 real-time PCR was used to generate the amplification curve for each reaction. A DNA association curve was generated after each reaction to differentiate between specific amplicons from non-specific products. SyberGreen fluorescence was measured during each step and the first derivative of the fluorescence was plotted as a function of the temperature.

On the basis of the amplification curve, all samples with HPV amplification starting at and cycle and up to cycle number 40 (with a cut-off line of 0.2) were selected for the analysis (Figure 2a). HPV positive samples were detected by analyzing the amplicon dissociation curve and samples showing a melting Tm between 75 and 80^0C were considered as positive (Figure 2b). PCR products were also evaluated for β

globin and L1 bands with 2% agarose gel stained with ethidium bromide and visualized with UV light (Figure 2c).

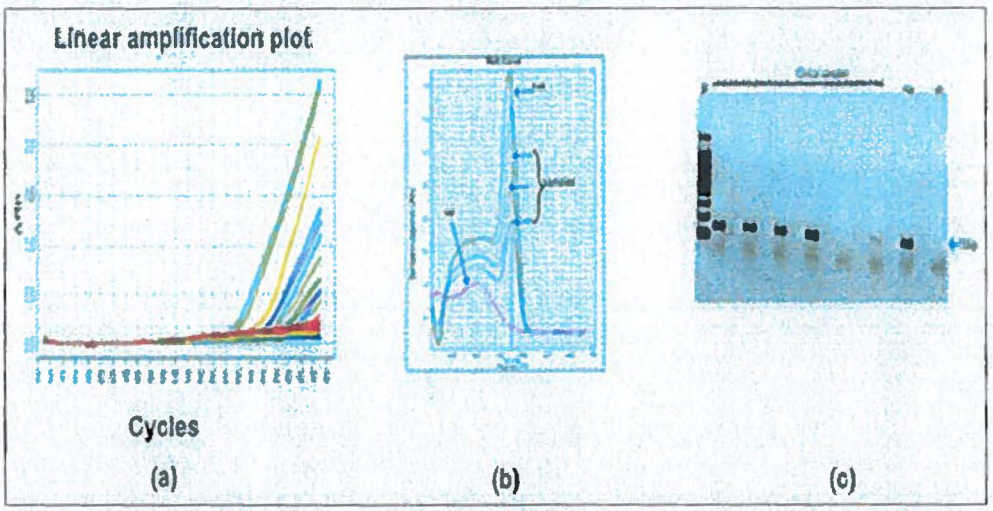

Figure 2 (a): Amplification plot showing Cts, fluorescence detected (Rn) in each well **(b)** melting curve analysis **(c)** Gel electrophoretic analysis of PCR products. Lane M: molecular marker; lanes 1,3,5,7,9 & 11 clinical samples; -ve: negative control; +ve: positive control

HPV Genotyping:

***HPV genotyping by RT-PCR kits and DNA sequencing*:** The genotype-specific distribution of HPV in the samples, which tested positive for HPV DNA was determined by RT-PCR based kits (Sacace Biotechnology, Italy), which tests for 12 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 2low-risk HPV genotypes (6,11).Briefly, for detecting low risk HPV genotypes (6, 11) add 10 µl of PCR mix-1 FRT, 5 µl of mix PCR buffer FRT containing TaqF DNA polymerase, and then add 10 µl of extracted DNA. For detecting high risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 57, 58, and 59), add 3.5 µl of PCR mix-1, 4.5 µl of PCR buffer FRT containing hot start DNA polymerase and 5 µl of extracted DNA. The reactions were carried out according to the manufacturer's instructions (Sacace Biotechnology, Italy).

All samples, which tested positive for HPV infection by RT-PCR, but where genotyping could not be assigned on the basis of the typing method described above, were subsequently subjected to sequencing (Genewiz Inc., NJ). PCR using

GP5+/GP6+ primer set and same reaction conditions as used during detection of HPV infection in cervical samples was used to generate amplicons. In cases where the primary PCR yields were low, an additional round of PCR using 2 ul of the primary PCR product and the same primers was done to ensure enough yields to perform direct sequencing. The PCR products were purified using MinElute PCR Purification Kit according to manufacturer's instructions (Qiagen, Valencia, CA). Purified products were sequenced using the Sanger method (Genewiz, South Plainfield, NJ) with GP6+ primer. The sequences were aligned using SeqMan Pro module of Laser gene 10.0 software (DNASTAR, Madison, WI). For genotyping, 35 bp sequences adjacent to the GP5+ primer-binding site was used for nucleotide-nucleotide BLAST analysis (blastn) against known HPV genotype sequences stored in the GenBank database (www.ncbi.nlm.nih.gov/ BLAST/). This 35 bp hyper-variable region of the L1 gene has been shown to be sufficient for distinguishing between various HPV genotypes [Feoli-Fonseca et al., 1998]. Genotypes were assigned only when there was a 100% similarity between the 35bp query and the subject sequence.

Statistical analysis

Sample characteristics including age, cytology results, clinical findings and nationality were summarized using frequency distributions to generate the numbers and percentages (Table 1). The prevalence of HPV positivity by each of the above-mentioned characteristics was assessed using the chi-squared test (Table 1). Unadjusted and adjusted odds ratios (ORs) for HPV positivity along with their 95% confidence intervals (CI) calculated according to the selected characteristics summarized in Table 1, using bivariate and multivariable logistic regressions respectively. The prevalence of HPV along with the 95% CI between Qatari and non-Qatari was plotted against age groups (Figure 1). Chi-squared test for trend was used to assess whether there is an increasing or decreasing trend in the proportions of HPV positivity by age, between Qatari and non-Qatari. The distribution of HPV genotypes was summarized using frequency distribution and stratified by cytology results (abnormal versus normal cytology) (Table 2). Statistical analysis was performed using IBM SPSS version 21.0. A p-value of less than 0.05 was considered significant.

Chapter 4- Results

Demographic, clinical and cytological characteristic of the study population

In this study, a total of 3008 cervical samples were collected from Qatari and non-Qatari Arab women visiting Women's Hospital, HMC and Primary Health Care Corporation, Doha, Qatar. These women came for either routine gynecological care and for different clinical conditions such as vaginal discharge, vaginal bleeding, genital warts, post-coital bleeding, cervical erosion, vulva itching, lower abdominal & pelvic pain, primary & secondary infertility and menorrhagia. The age range was 16-84 years with a mean age of 41.6 years (SD=10.9). The distribution of patients based on their age groups 16-24, 25-34, 35-44, 45-54, and ≥55 was 136 (4.5%), 756 (25.1%), 919 (30.6%), 835 (27.8%), and 362 (12%) respectively (Table 1). Total 46.7% of the women in the sample population were Qataris with the rest belonging to other Middle Eastern, North and East African countries in the League of Arab States (Table 1), which is reflective of the make-up of the general Arab society in Qatar.

Table 1 show clinical findings and majority of women underwent routine gynecological care (79.8%) and only 20.2% were symptomatic. Furthermore, based on cytological diagnosis, 98.4 % of the women (n=2959) had normal cytology without any lesions. A minority of women (n=49, 1.6%) had abnormal cytology with either low-grade squamous intraepithelial lesion (LGSIL) and/or high-grade squamous intraepithelial lesion (HGSIL).

HPV prevalence in study population (Qatari and Arab national) women

The overall HPV prevalence in Arab women was 6.0% (6.2% in Qatari and 5.9% non-Qatari) (Table 1). Of the non-Qatari Arab women, HPV prevalence was as follows: 5.6% in Egyptian, 4.5% in Arabian Peninsula, 6.5% in Fertile Crescent, 7.4% in North Africa and 6.5% in East Africa (Table 1). Furthermore, 5.8% HPV prevalence was found among women with normal cytology and 18.4% among women with abnormal cytology. Based on cytological findings, 36 samples were low-grade squamous intraepithelial lesions of which, 8 were HPV positive and 13 high-grade squamous intraepithelial lesions of which, 1 was positive for HPV. The odds of HPV positivity among participants with abnormal cytology were almost four times the odds of HPV positivity among participants with normal cytology (OR: 3.68, 95%CI: 1.75; 7.75) (Table 1).

The prevalence of HPV infection based on age groups among Qatari and non-Qatari Arab women

HPV prevalence overall was found higher in the age group >55 (Table 1). HPV prevalence increased with age in non-Qatari women, while no such trend was seen in Qatari women (Figure 1).Furthermore, among Qatari women, HPV prevalence was higher (7.1%) in 25-34 age group and 8.5% in >55 age group of non-Qatari women (Figure 1). However, by chi-square analyses, no significant difference was found in HPV prevalence between the Qatari and non-Qatari Arab nationals across the different continuous age groups used in this study.

Distribution of HPV genotypes in Arab women

The distribution of HPV genotypes among the 182 HPV positive DNA samples of Arab women with normal and abnormal cytology was evaluated and examined based on the number of HPV genotypes in a single sample. Results in table 2 show that women were divided into two groups based on their cytological diagnosis into women with normal cytology (N= 2959) and women with abnormal cytology (N= 49). Among HPV positive women with normal cytology, 144 (83.2%) diagnosed with single HPV genotype, 19 (10.9%) with two HPV genotypes, 5 (2.9%) with multiple HPV genotypes and 5 (2.9%) remained uncharacterized and have been listed in Table 2 as unknown. On the other hand, among HPV positive women with abnormal cytology 5 (55.6%) had single HPV genotype, 3 (33.3%) two HPV genotypes and 1(11.1%) had multiple HPV genotypes.

HPV 81 was the most frequent low-risk genotype among women with both normal (74.0%) and abnormal (33.3%) cytology. Women with HPV 81 genotype was significantly less (86.0%) at risk of showing abnormal cytology than women with high-risk genotypes (OR: 0.14, 95%CI: 0.03-0.58), which further enforces the low risk classification of this HPV genotype, as described in Table 3.The only intermediate risk HPV genotype identified was HPV 67, which had 3.5% prevalence in women with normal cytology. While, HPV16 (4.6%) was identified as the predominant high-risk HPV genotype, followed by HPV 59 (3.5%) and 56 (2.9%) in women with normal cytology whereas among women with abnormal cytology, the most common identified HR genotypes were HPV 16, 18 and 56 (22.2% each) (Table 2).

Table 1: Unadjusted and adjusted odd ratios (ORs) for HPV positivity and their corresponding 95% confidence intervals (CIs) according to age groups, cytology results, clinical findings and nationality among 3008 women in Qatar

	Total No. Women	HPV positive, N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age groups				
16-24	136 (4.5)	7 (5.1)	1	1
25-34	756 (25.1)	44 (5.8)	1.14 (0.50-2.58)	1.20 (0.53-2.73)
35-44	919 (30.6)	51 (5.5)	1.08 (0.48-2.44)	1.15 (0.51-2.59)
45-54	835 (27.8)	54 (6.5)	1.27 (0.57-2.86)	1.36 (0.60-3.07)
≥55	362 (12.0)	26 (7.2)	1.43 (0.60-3.37)	1.49 (0.63-3.54)
Cytology Results				
Normal Cytology	2959 (98.4)	173 (5.8)	1	1
Abnormal Cytology	49 (1.6)	9 (18.4)	3.62 (1.73-7.59)*	3.68 (1.75-7.75)
Clinical Findings				
Routine smear test	2401 (79.8)	142 (5.9)	1	1
Symptomatic results[ff]	607 (20.2)	40 (6.6)	1.12 (0.78-1.61)	1.15 (0.80-1.65)
Nationality				
Qatari	1404 (46.7)	87 (6.2)	1	1
Non Qatari	1604 (53.3)	95 (5.9)	0.95 (0.71-1.29)	1.00 (0.73-1.36)
Nationality				
Qatari	1404 (46.7)	87 (6.2)	1	--
Egyptian	465 (15.5)	26 (5.6)	0.90 (0.57-1.41)	
Arabian peninsula[$]	310 (10.3)	14 (4.5)	0.72 (0.40-1.28)	
Fertile crescent[£]	566 (18.8)	37 (6.5)	1.06 (0.71-1.58)	
North Africa[#]	95 (3.2)	7 (7.4)	1.20 (0.54-2.68)	
East Africa[^]	168 (5.6)	11 (6.5)	1.06 (0.55-2.03)	

*p-value<0.05

[$] Arabian Peninsula (KSA, Kuwait, Bahrain, UAE, Oman, Yemen)

[£] Fertile Crescent (Syria, Lebanon, Jordan, Palestine, Iraq, **excluding** Egypt)

[#] North Africa (Morocco, Algeria, Tunisia, Libya, Mauritania)

[^] East Africa (Somalia, Djibouti, Sudan, Comoros)

[ff] Symptomatic results include: cervical erosion, genital warts, lower abdominal pain, menorrhagia, pelvic pain, post coital bleeding, primary infertility, secondary infertility, vaginal bleeding, vaginal discharge, vaginal spotting and vulva itching

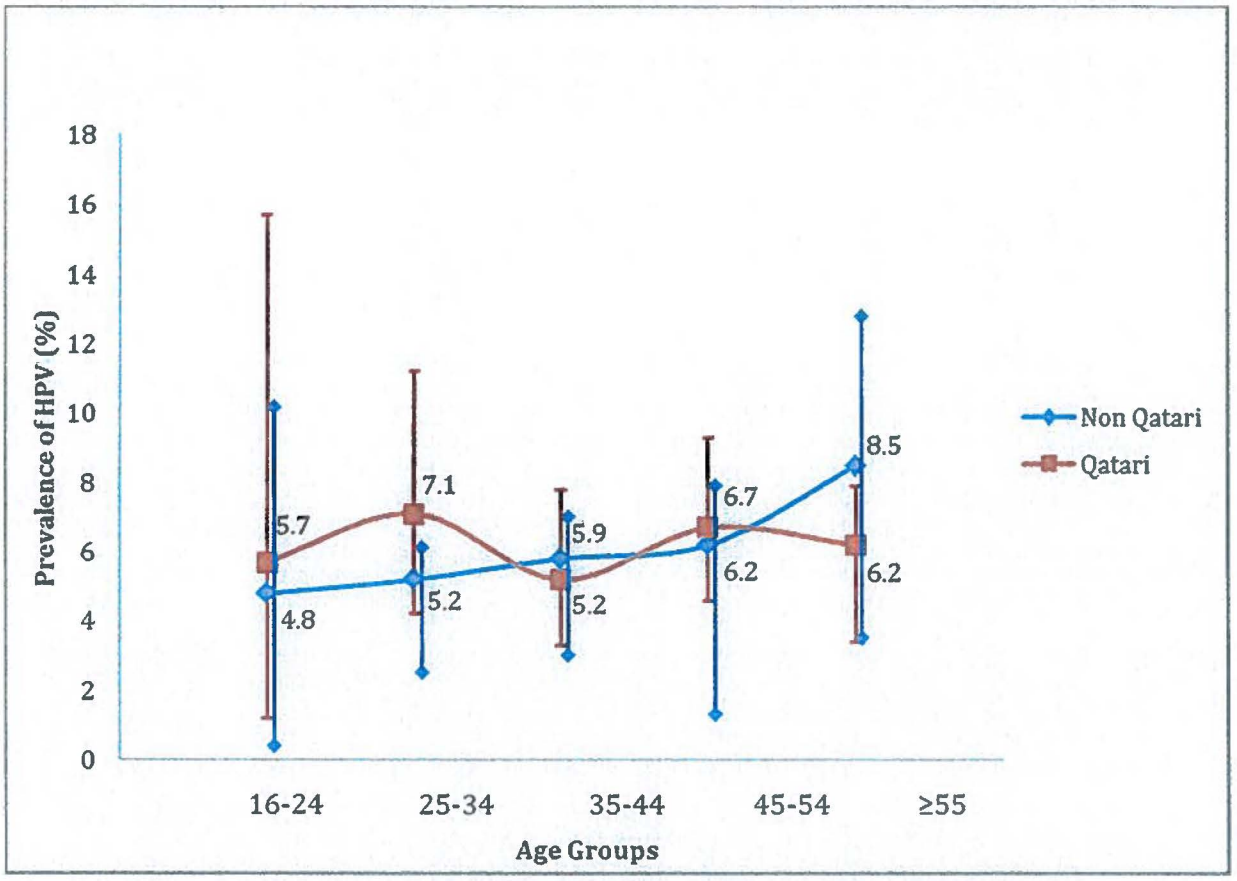

Figure 3: Prevalence of HPV by age groups and nationality and their corresponding 95% confidence interval (CI) among Qatari and non-Qatari Arab women

Table 2: The distribution of HPV types in 3008 Arab women in Qatar

	General Arab Population in Qatar													
	Normal Cytology (N=2959)						Abnormal Cytology (49)						All (N=3008)	
PV Type	Single	Double	Multiple	Unknown	Total	Percent	Single	Double	Multiple	Unknown	Total	Percent	Total	Percen
HPV sitive	144	19	5	5	173	5.8	5	3	1	0	9	18.4	182	6.1
High-risk														
	4	2	2	--	8	4.62	0	1	1	--	2	22.2	10	5.49
	0	0	3	--	3	1.73	0	2	0	--	2	22.2	5	2.75
	1	2	1	--	4	2.31	0	1	0	--	1	11.1	5	2.75
	1	2	1	--	4	2.31	0	0	0	--	0	0.00	4	2.20
35	0	1	1	--	2	1.16	0	0	0	--	0	0.00	2	1.10
	0	1	1	--	2	1.16	0	0	0	--	0	0.00	2	1.10
45	2	3	0	--	5	2.89	0	0	0	--	0	0.00	5	2.75
	0	1	1	--	2	1.16	0	0	1	--	1	11.1	3	1.65
52	1	0	0	--	1	0.58	0	0	0	--	0	0.00	1	0.55
	0	4	1	--	5	2.89	1	1	0	--	2	22.2	7	3.85
58	1	0	1	--	2	1.16	1	0	0	--	1	22.2	3	1.65
	4	1	1	--	6	3.47	0	0	0	--	0	0.00	6	3.30
Intermediate Risk														
67	4	1	1	--	6	3.47	0	0	0	--	0	0.00	6	3.30
Low risk														
	1	3	0	--	4	2.31	0	0	0	--	0	0.00	4	2.20
11	4	5	2	--	11	6.36	0	1	1	--	2	22.2	13	7.14
	119	9	0	--	128	73.99	3	0	0	--	3	33.3	131	72.0
90	2	3	0	--	5	2.89	0	0	0	--	0	0.00	5	2.75

Table 3: The distribution and prevalence of HPV types among women with normal and abnormal cytology

HPV Type	Normal Cytology	Abnormal Cytology	Total	Percentage
High Risk	44	9	53	29.1%
Intermediate Risk	6	0	6	3.3%
Low Risk	148	5	153	84.1%

Chapter 5- Discussion

HPV is one of most commonly occurring sexually transmitted disease. There are limited studies available on the prevalence of HPV infection and the distribution of different HPV genotypes in Arab countries. This may be due to the presence of a closed society, conservative socio-sexual norms in the Middle Eastern countries and sexual transmission nature of the disease; it has often been ignored in this region. This is the first study describing the prevalence of HPV infection and distribution of HPV genotypes in a large cohort of Arab women with normal and abnormal cytology in the State of Qatar. Currently, more than 200 HPV genotypes have been identified and based on their oncogenic potential via associations with cervical cancer and precancerous lesions, about 30 to 40 genotypes, cause genital tract infection in human, are divided into high-risk (HR), causing cervical neoplasia and low-risk (LR) HPV genotypes, which causes benign signs and symptoms[Clifford, Smith, Plummer, Munoz, & Franceschi, 2003].

In this study, the prevalence of HPV infection among Arab women with normal and abnormal cytology is very low (5.8% and 18.4% respectively) compared with previous reports from EMENA countries except Kuwait and Pakistan where the HPV prevalence among women with normal cytology was lower (2.4% and 2.2% respectively) [Al-Awadhi et al., 2011; Franceschi et al., 2010] than our results in Qatar. Whereas, HPV prevalence among women with abnormal cytology was higher in Kuwait and Pakistan (51% and 27.3% respectively) [Al-Awadhi et al., 2013;Franceschi et al., 2010]than our study (18.4%). The reason for these differences in prevalence rates could be partly due to the differences in the methodologies which were used to detect HPV infection especially since no significant differences in the HPV prevalence among Qatari and non-Qatari Arab women living in Qatar was identified in this study. The HPV prevalence of 18.4% in women with cervical abnormalities in our study is to be expected since virtually all studies from all over the world have reported the same finding [Muñoz et al., 2000].

The Age-specific distribution of HPV prevalence in this study shows a bell shaped curve in Qatari women, with a peak between 25 and 34 years of age, and dropping down at > 55 years of age, whereas HPV prevalence increased with age in non-Qatari women with highest HPV infection prevalence in age ≥ 55 (8.5%). The peak at early

age (25-34 years) in Qatari women was similar to the findings in Latin America, Italy, Netherlands, Spain, Argentina, Korea and Canada who also show increased HPV prevalence at early age (<25 years) [Franceschi et al., 2006; Bruni et al., 2010]. The highest peak at older menopausal age (≥ 55) of HPV prevalence in non- Qatari women is similar to studies done in North America, Africa, Chile, Colombia and Mexico [Franceschi et al., 2006], where increased HPV prevalence was found high in postmenopausal age groups [Bosch, Qiao, & Castelleague, 2006]. On the other hand, China, India and in Nigeria, the prevalence of HPV infection was reported high in all the age groups. Although, the reason for the higher prevalence of HPV in >55 years age group in non-Qatari were not clear, but this could be due to poor or the lack of effective cervical cancer screening method and lack of using the most sensitive HPV detection methods such as molecular tools [Richter, Becker, Horton, & Dreyer, 2013]. The detection of newly HPV infection in older women could be more likely due to reactivation of latent HPV compared to younger women [Velicer, Zhu, Vuocolo, Liaw , & Saah, 2009].

In our study we found a heterogeneous distribution of HPV genotypes, possibly due to broad geographical and cultural range of the nationalities included in this cohort. In the present study, HPV 16 was the most common genotype followed by HPV 56,59, 45,31, 33, 18, 35, 39, 58, 51, and 52 among the high risk HPV group and HPV 67 in intermediate risk group. This is in agreement with global studies where HPV 16 was identified as the most common HPV genotype [Clifford, Smith, Aguado, & Aguadoi, 2003], except in western Africa, where HPV 31 was the most prevalent [Bruni et al., 2010]. Similarly, in women with normal cytology, HPV 6, 11, 16 and 18 were the most commonly seen HPV genotypes in extended Middle East and North Africa region [Oakeshott et al., 2012]. In addition, several studies show that HPV 16, 18, 45, 31, 33, 52, 58, and35 are accountable for 95% of cervical cancers [Walboomers et al., 1999; Khan et al., 2009]. In our study, high-risk HPV genotypes were found in both women with normal and abnormal cytology. HPV16 was the most common high-risk genotype (4.6%), followed by HPV 59 (3.5%), 45 (2.9%) and 56 (2.9%) in normal cytology, whereas, prevalence of HPV 16, 18 and 56 was high among abnormal cytology (22.2% each).

Moreover, the HPV genotype distribution varies based on the geographical variations and that could be based on samples size and geographical distribution [Haghshenas et al., 2013]. Study done in South Africa showed that HPV 52 was the predominant type (12.2%) in ASCUS women, followed by HPV 45 (6.1%), while in LGSIL women, HPV 52 was the most common type (17.5%), followed by HPV 53 (15.8%) and HPV 16 and HPV 35 (both 12.3%). In HGSIL women, HPV 16 and HPV 35 were the most predominant types (both 18.9%) and HPV 18 was found at (7.6%) of women [Allan, Marais, Hoffman, Shapiro, & Williamson, 2008].

When taken into consideration the fact that HPV 16 and 18, taken together are responsible for 70% of the cervical cancer cases [Walboomers et al., 1999] worldwide, these results clearly highlight the need for HPV screening for cervical cancer even in the absence of cytological abnormalities and demonstrate the inadequacy of cytology based assays for cancer screening.

When a comparison was made of women with cytological findings and HPV genotype distribution, in this study, HPV 81 was the most frequent low-risk genotype among women with both normal (74%) and abnormal (33.3%) cytology. The unexpectedly much higher HPV genotype 81 prevalence than HPV 16 in our study was also seen in a previous study done on Southern Chinese women [Ngami Na Chloe Co et al., 2013] and can be attributed to variations in the biological distribution of HPV genotypes and the sensitivities of different methods used for HPV detection [Ngami Na Chloe Co et al., 2013]. HPV 81 is classified as a low risk genotype and has not been reported in high numbers in worldwide population studies however it is one of the most frequently observed HPV genotypes in HIV-positive patients and is also reported to be associated with precancerous or cancerous lesions [Tornesello et al., 2008; Choi et al., 2009; Garbuglia AR, et al., 2012; Ngai Na Chloe Co et al., 2013; Al-Awadhi R et al., 2013]. Cerqueira et al. (2007) has reported a high prevalence of HPV 81 (14%) in HIV-positive Brazilian women [Cerqueira et al., 2007]. In our study, we also found an intratypic variant of HPV 81, which matched an unique isolate identified in an HIV-1 positive patient in India [Mullick et al., 2011 (Accession: JQ353697)]. Although, our study indicate a higher association of HPV genotype 81 with women with normal cytology as opposed to abnormal cytology indicating a low oncogenic potential of this genotype. However given its occurrence in multiple infections and precancerous lesions, it is a possibility that infection with

HPV genotype 81 may make the environment more conducive for co-infection/prime the environment for cancerous conversion in case of infection with other high risk HPV genotypes.

Infections with numerous HPV types are generally found in women who are sexually active, especially in HIV-infected women [Marais et al., 2008; Plummer et al., 2011],but less frequently in cervical carcinoma than in normal cytology [An HJ et al., 2003]. However, other studies have found multiple HPV infection to be associated with a significantly increased risk of high-grade squamous intraepithelial lesion compared to infection with a single HPV type [Spinillo et al., 2009]. In the present study, mostly women with normal cytology had a single HPV type infection (81.8%) but infection with double and multiple HPV types has also been observed frequently (15.38%) in patients with cytological abnormalities, which is similar with previous reports [Ho GY et al., 1998; Herrero R et al., 2000]. Furthermore, we found HPV genotype 81 (61.7%), 11 (6.1%), 16 (4.7%),56 (3.3%) and 59 (2.8%) were most prevalent as single infections in all age groups. However, in an infection with more than one genotype, the most common HPV types were HPV16/18 and 11/81 (13.6%) and HPV11/16/18 and 16/18/59 (16.6%).

In conclusion, our study showed a relatively low prevalence (6%) of HPV infection and presence of a varied genotypic profile of HPV with a high prevalence of low risk HPV genotype 81 among the Arab women residing in State of Qatar when compared to the Middle Eastern and North African (MENA) countries. When considering a prophylactic vaccine, the data from this study show that the quadrivalent vaccine has the potential to prevent HPV infections in Arab women. However, as remarkable differences were noted in our study population with a high prevalence of HPV 81 and other types such as HPV 16, 56, 59 and 67, the potential for cancer prevention in this region would rise if these frequent high and intermediate risk HPV genotypes and possibly HPV 81 are also included in future HPV vaccines targeting this specific population.

Chapter 6- Conclusion

Human papillomavirus (HPV) are the most commonly known sexually transmitted agents. To date, few reports are available on distribution of most prevalent and variants types of HPV in Arab women. Therefore, the aim of this study was to determine the age specific distribution of HPV types among Arab women being subjected to routine Pap smear test in State of Qatar. Based on the collected data in this study, out of 3008 collected Pap smear samples, HPV DNA was detected in 182 women (6%), and 17 different HPV genotypes were detected, comprising high-risk, intermediate and low-risk genotypes. The prevalence of HPV infection was seen in 6.2% Qatari and 5.9% non-Qatari women. With regard to age, 5.1% HPV infection were found in women 16-24 years of age, 5.8% in women 25-34 years of age, 5.5% in women 35-44 years of age, 6.5% in women 45-54 years and 7.2% in women more than 55 years old.

Appendix I (Joint Institutional Review Board IRB Approval)

In Partnership with Cornell University
كلية طب وايل كورنيل في قطر
Weill Cornell Medical College in Qatar
Member of Qatar Foundation

Hamad Medical Corporation/Weill Cornell Medical College in Qatar
Joint Institutional Review Board

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JIRB SCH Registration:	SCH-Joint-111
JIRB DHHS Registration:	IRB00009413
HMC SCH Assurance:	SCH-A-HMC-020
WCMC-Q SCH Assurance:	SCH-WCMC-Q-002
WCMC DHHS Assurance:	FWA00000093

APPROVAL NOTICE

Date:	January 26, 2014
PI:	Ali Sultan
IRB Number:	13-00078
Protocol Title:	Human Papillomavirus in Arab Women: Molecular Epidemiologic, Clinico-pathological, Serological, and Vaccine Impact Studies in Select Arab Countries.
Submission Type:	Initial Application
Review Type:	Expedited Review
Approval Period:	January 20, 2014- January 19, 2015

The Joint Institutional Review Board (JIRB) has reviewed and approved the above-referenced protocol, including the following documents:

Type	Name	Uploaded Date
Consent/Assent Document	HPV Consent 2013-12-01-revised	01/12/2014
Questionnaire/Survey	Annexure 1 (HPV questionnaire)	11/13/2013

As the Principal Investigator of this research project, you are ultimately responsible for:

- Protecting the rights, safety and welfare of research subjects.
- Following the JIRB-approved protocol (i.e., the application and any materials submitted with it).
- Following the requirements in the Investigator Manual, especially with regards to obtaining prior approval of changes to the research, reporting events or new information, and submitting continuing review or final reports.
- The conduct of the study team with regards to all of the above.

If you have questions or need additional information, please contact the JIRB Office at the above e-mail address or telephone number.

Sincerely,

Lama Jamhawi, MSc
Manager, HMC/WCMC-Q JIRB

Appendix II (Poster)

كلية الآداب والعلوم
College of Arts and Sciences

Molecular Epidemiology and Genotype distribution of Human papillomavirus in Arab Women in State of Qatar

Acha Elmi[1,2], Devendra Bansal[2], Bini Ekuriah[2], Aicha Al Hamadi, Afaf Al Ansari[1], Nady Mohamed[2], Ali Sultan[2], Asma Al Thani[1]

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ABSTRACT

Molecular epidemiology studies have shown that Human Papillomavirus (HPV) infection represents the major etiological factor of cervical cancer. The data on population prevalence of HPV infection in the Middle Eastern region and Arab world, where social behavior is different from many other world populations, are very broad. The present, first ever study was designed to determine the prevalence of HPV infection in Arab women in Qatar. Between March 2008 and March 2009, 200 women were collected in Doha, Qatar. Out of 200 participants, 100 Qatari women (50 Qatari and 50 non-Qatari) from Women's Hospital, Hamad Medical Corporation and Primary Health Care Corporation in Doha, Qatar. The remaining 100 samples were collected in Doha, Qatar. The age of the participants was ranged from 18 to 65 years old. Cervical cytology was performed on the collected samples. DNA was extracted by QIAamp Viral kit (Qiagen, Germany) from cervical swab samples and HPV infection was detected by nested-PCR using L1 consensus primers MY09/MY11 and GP5+/GP6+ primers. The type specific distribution was determined by HPV high risk (HR) and low risk (LR) type specific probes kits (Abbott Diagnostics, USA) and DNA sequencing (Genome Inc.). Real-time PCR amplification was carried out in ABI 7500 real-time PCR. The result were based on the collected data. HPV DNA was detected in 18 (9.0%) participants where HPV-DNA detection rate in Qatari women was higher in age group 25-30 (7.1%) and 61-65 (8.3%) than age group of non-Qatari women. Among HPV DNA positive participants, 87 (6.2%) were Qatari and 13 (2.6%) non-Qatari. The LR HPV types were more predominant and the age peaks of low- intermediate- and high-risk types were 25-30 (4.2%), 31-35 (4.2%) and 36-40 (4.2%), respectively. The study shows that the prevalence of HPV infection is less common in Qatar in among the highest compared with previous reports. However, more extensive population-based studies should be undertaken before implementing HPV vaccination.

OBJECTIVES

The aim of the study is to examine the molecular epidemiology of HPV and distribution of HPV genotypes among women attending gynecological clinics in the State of Qatar.

LITERATURE REVIEW

METHODOLOGY

Study population and sample collection: The study designed in partnership between Weill Cornell Medical College in Qatar (WCMCQ) and Research Office of Hamad Medical Corporation (HMC) Doha, Qatar. During 2008, Gynaecologist Obstetricians and women's health nurses visiting in Qatar was collected from Women's Hospital, HMC and Primary Health Care Corporation in Doha, Qatar. The Pap smear samples collected in ThinPrep Vial and transported to the laboratory for cytology investigations. The age, ethnicity, clinical history and previous diagnosis of cervical cancer was recorded. ThinPrep cytology smear stained and reading is carried out by registered cytologists.

DNA extraction and HPV detection by Real-time PCR: The DNA extracted by QIAamp Viral kit (Qiagen, Germany) according to manufacturer's instructions. The quantity of DNA in all the HPV DNA positive samples was determined by TaqMan HPV-HR type and 2 HPV-common type assay. Nested-PCR was carried out by MY09/MY11 and GP5+/GP6+ primers and also by PCR based sequencing (Genome Inc.).

Statistical analysis: Statistical analysis was performed using appropriate statistical tests in the SPSS statistical package (SPSS Science Inc.). Proportions of categorical data was calculated. Difference between groups was calculated by chi square test and p-value was considered significant.

RESULTS

The highest positive rate was in 25-30 (7.1%), 31-35 (4.2%) and 36-40 (4.2%) age groups (Fig. 1). The age-group with mean age of 46 years (SD=10). The majority of women were married (83%) belonging to the Middle Eastern, Near and East Asian countries in the region of Arab States (Fig. 2).

Overall HPV prevalence in Arab women was 9.0% (18/200) in Qatar and 2.6% (13/500) in non-Qatar. Fig 3a. According to age group, HPV prevalence was highest in age group 25-30 (7.1%) and 61-65 (8.3%) in Qatari women (Fig 3b). The prevalence was found high in non-Qatari women in age group 36-40 (4.2%) and 41-45 (4.2%). Fig 3c. The prevalence of HPV infection was found to be 4.2% in Qatar and 2.6% in non-Qatar. Fig 3d. According to age group, HPV prevalence was highest in age group 25-30 (7.1%) and 61-65 (8.3%) in Qatari women (Fig 3b). The prevalence was found high in non-Qatari women in age group 36-40 (4.2%) and 41-45 (4.2%). Fig 3c. The prevalence of HPV infection was found to be 4.2% in Qatar and 2.6% in non-Qatar. Fig 3d.

DISCUSSION

CONCLUSION

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