HPV Infection and its Serotypes in Women with CIN and Cervical Cancer

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Abstract

Objective: To analyze detection of HPV and its subtypes in patients with CIN and Cervical cancer in tertiary care hospitals in Lahore.

Material and Methods: It was descriptive, Cross sectional study conducted in Jinnah, Mian Munshi and INMOL Hospital, Lahore. The study was completed in a duration of 48 months. All patients presenting with symptoms associated with cervical pathology, abnormal cytology on pap smear and confirmed cases of cervical cancer on biopsy were included in this study. Cervical biopsies were taken both for histopathology and HPV testing in patients showing dyskariosis on cytology or visible cervical growth. Confirmed case of carcinoma cervix on biopsy had their sampling done for HPV testing.

Results: There were total 89(81.7%) positive samples for HPV. HPV 16 is the commonest serotype detected (31.3%). HPV 66 was the second commonest variant detected (13.1%). HPV 31, HPV 18, HPV 59 and HPV 45 were the other common serotypes.

Conclusion: Different HPV vaccines available in market provide immunity against 6,11,16,18,31,33,45,52 & 58. However these vaccines are not effective against HPV 66,59,32,61, and 86, which are quite prevalent in our population. So, vaccination and screening should go side by side in Pakistan to cover the disease caused by these non-vaccine type variants.

Keywords: Human papilloma virus, prevalence, distribution of HPV serotypes, invasive cervical cancer, risk factors.

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Introduction

Cervical cancer is the fourth most frequently diagnosed cancer among women worldwide, with an estimated 604,127 new cases and 341,831 deaths in 2020.[1] The highest rates of cervical cancer incidence and mortality are in low- and middle-income countries due to major inequities driven by lack of access to national HPV vaccination, cervical screening and treatment services and social and economic determinants.[2]

Cervical cancer is caused by persistent infection with the human papillomavirus (HPV). Women living with HIV are 6 times more likely to develop cervical cancer compared to women without HIV.[3] Human papillomavirus is a double stranded DNA virus which is sexually transmitted. HPV is now a well-established cause of cervical cancer. HPV types 16 and 18 are responsible for about 70% of all cervical cancer cases worldwide.[4] However, the distribution of HPV subtypes varies in different parts of the world. More than 200 subtypes of HPV are known to exist according to oncogenic potential, HPV can be classified as low risk or high risk. The low risk

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subtypes include 6,11,40,42,43,44,54.61, 70,72,81 and the high risk include 16,18,31,33,35,45,51, 52,58,59,68,73,82.[5] Subtype 58 has been isolated in women with pre-invasive cervical cancer in Thailand, Uganda, Zambia, and Cameroon.[6] Different countries of Europe and America noted that subtypes 31,33,45,52 accounts for most cases of CIN3.[7]

Diagnosing cervical cancer at early stages and thus preventing it via screening measures is very important. During the past 30 to 40 years tremendous efforts have resulted in decrease in the prevalence and resulting deaths due to cervical cancer via introduction of many screening modalities (cervical cytology, HPV DNA detection and VIA) either singly or combined. It has resulted in marked reduction in incidence and mortality in developed countries.[8] In 2019/20 UK NHS has introduced HPV primary screening and cytology side by side with HPV prophylactic vaccination.[9] Non-vaccine types 35,39,51,56,59 are responsible for only 2-11% of CIN2/3, hence a monovalent HPV vaccine can probably prevent the majority of CIN3 irrespective of geographical variations.[10]

In Pakistan 62.8 million woman 15 years and older are susceptible of developing cervical cancer. Cervical cancer ranks as the 5th leading cause of cancer deaths of female cancer deaths in Pakistan. [1]

Annually around 5008 new cases and 3197 deaths occur due to cervical cancer. Prevalence of HPV 16 and 18 in Pakistani women with normal cytology is 0.5%, LSIL is 21.2%, HSIL CIN2 CIN3 CIS is 42.1% and Cervical cancer is 88.1%.[11] In Pakistan, screening of women aged 25-64 years is only 2.3%. The screening program is not very well developed besides vaccines are available to a limited extent.[12]

WHO fact sheet confirms that human papillomavirus subtype 16 is most common type detected in Pakistani women.[13] A study from Karachi reported that 24% of asymptomatic women are HPV 16 and 18 positive, however 88% of women with cervical cancer were screen positive.[14] Another study conducted in twin cities of Punjab (Islamabad and Rawalpindi) showed that HPV 16 is detected in 24.64% among total of 94.81% positive for HPV samples. HPV 18 single is detected in 25.97% and HPV 16 and 18 co-infection found in 40.26%.[15]Our population has different demographic risk factors like being a predominantly Muslim country with regular penile circumcision but poor socio-economic status and poor knowledge of screening results in a very great risk of HPV associated carcinoma and consequent mortality. More than 70% of patients have advanced stages of malignancy in Pakistan on presentation.[16] Other risk factors include unprotected early sex, early marriages, early reproductive cycles and multiparity, smoking and hormonal altered immune system i.e. HIV.[17]

Very few studies have been conducted in Pakistan detecting high risk HPV variants associated with development of Carcinoma cervix and related pathology. No study has been reported about HPV subtype variants in Lahore and adjacent areas. We also need to conduct studies in different areas of Pakistan to know efficacy of vaccines in protecting against infections and cancer development. This study will help us to know the prevalence of HPV viruses in women with moderate and severe dysplasia and carcinoma cervix in our population. By knowing the different subtypes responsible for HSIL, we can use vaccines locally available with confidence or develop an effective one.

Material and Methods

It was descriptive cross-sectional study, conducted in the Department of Gynecology of Jinnah, Mian Munshi, Inmol Hospital Lahore. After the approval from ERB Committee No. 30-01/ERB/30th dated 07-05-2016. Duration of study was 48 months from 1st March 2018 to 28th February 2022 and calculated sample size was 245. Sample technique was non-probability, purposive. Patients presenting with symptoms associated with cervical pathology (post coital bleeding, inter-menstrual bleeding, post-menopausal bleeding, persistent vaginal discharge and pelvic pain), abnormal cytology on pap smear and confirmed cases of cervical cancer on biopsy were included in study. After taking informed consent, detailed history was taken. On speculum examination the cervix was visualized with naked eye and pap smear was taken and recalled after one week. The patient showing dyskariosis on cytology reports had VIA done and biopsies were taken both for histopathology and HPV testing. All biopsy specimens were sent to histopathology in 10% formalin whereas specimen for HPV DNA detection was taken in 0.9% normal saline. However, in cases of visible cervical growth, patients were admitted for EUA and Cervical biopsy and HPV sampling was done. Confirmed case of carcinoma cervix on biopsy

Cytology and histopathology mostly were done by expert pathologist of central lab of Allama Iqbal Medical College/ Jinnah hospital Lahore. HPV DNA detection and PCR were carried out by expert molecular biologist of UHS resource lab and Chugtai Lab Lahore. DNA extraction from samples were done and then case blocks and blank blockers were subjected to real time PCR with modified general primers. 18 different type specific beads for HPV genotyping were used and then sequencing was done. Negative HPV tests were repeated twice taking tissue biopsy as gold standard to avoid any error. So, for 71 tests 100 test kits were used. Bosphore HPV genotyping high risk kit was used, which tested the samples for 18 high risk HPV type 16, 18, 31, 32, 33, 39, 45, 51, 52, 56, 57, 58, 59, 61,66,68,86

Results

A total of 300 women were included in the study. Women who presented with symptoms of irregular bleeding were 224 and 76 had cervical growth on examination. Out of 224, 148 women were reported as Bethesda code 1, had routine follow up. Twenty six of these 224 patients were lost on follow-up. However, 50 women with abnormal cytology on smear were enrolled for biopsy and HPV screening. A total of 109 women with either cervical growth or invasive carcinoma underwent screening for HPV serotypes.

Table 1: *Association of HPV Result with Socio-demographic Variables*

Variables	HPV Result		Total	Chi^2	p-value
	Positive (%)	Negative (%)			
Age (Years)					
≤50	55(78.57%)	15 (21.4%)	70	**0.411**	**0.521**
>50	34 (87.17%)	05(12.8%)	39		
Districts					
Lahore	46 (80.8%)	11(19.29%)	57	**0.023**	**0.879[a]**
others	43 (82.7%)	09 (17.3%)	52		
Total	**89(81.7%)**	**20(18.3%)**	**109**		

[a] *Fisher's Exact test*

Mean age of these women who underwent HPV screening were 49.90±9.39 years with 70(64.23%) were ≤50 years and remaining 39(35.77%) were>50 years; the residence address showed that most of the respondents belong to Lahore 57 (52.29%) followed by Gujranwala 29 (26.6%), Sahiwal 16 (14.6%), Sargodha 08(7.3%), Faisalabad 7 (6.4%) and 01 (0.9%) each in Bahawalpur Division and Multan respectively. The above table showed that among 89 positive cases of HPV there were 61.79% were ≤ 50 years and 38.20% having positive HPV result belonged to >50 years age group. The two attributes were not statistically significant (p=0.521). Whereas, 51.68% with HPV positive results were living in Lahore district and 48.32% with HPV positive result

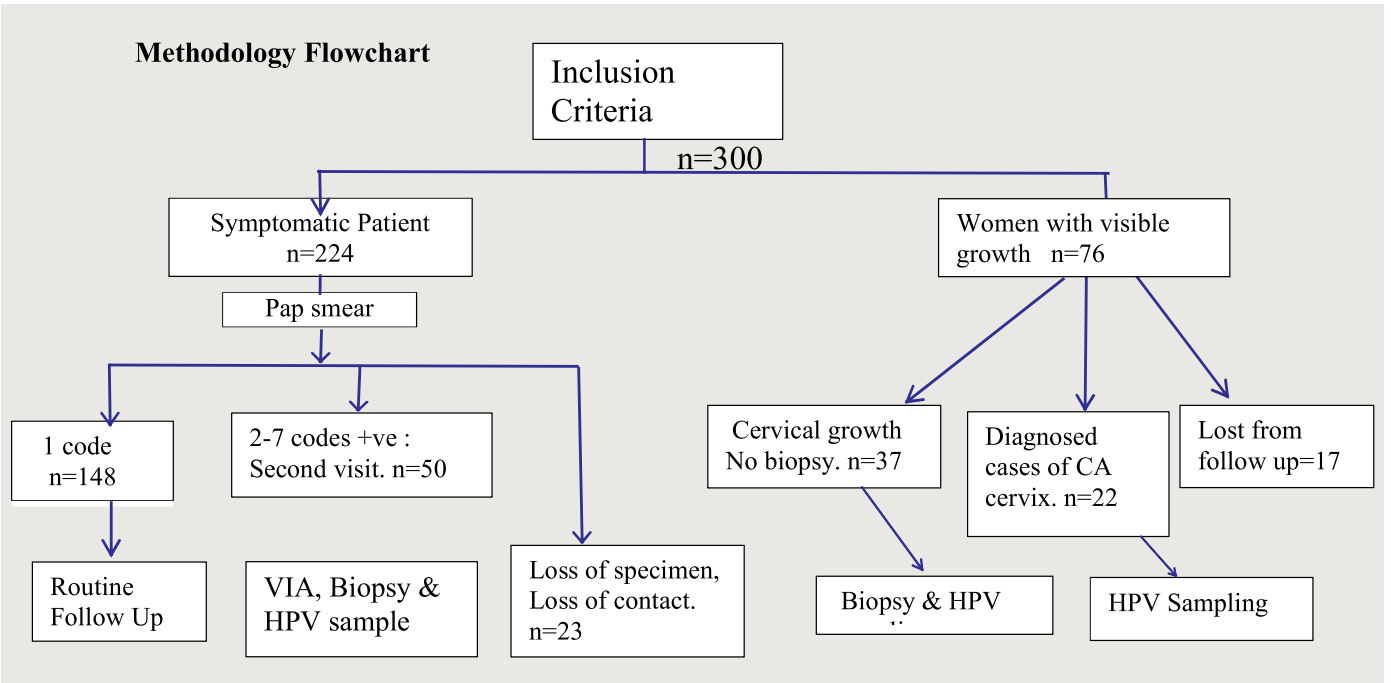

Fig-1: Study methodology.

was dwelling in other districts. The HPV result and districts were insignificant statistically (p=0.879). There were total 89(81.7%) positive samples for HPV

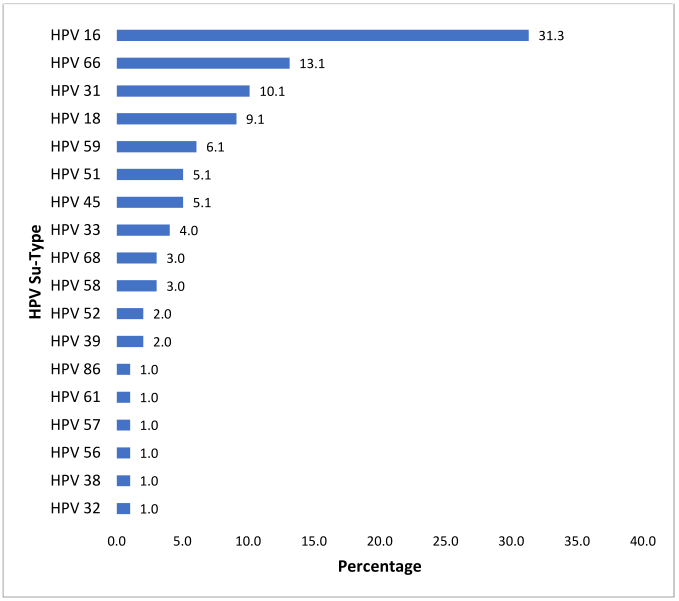

Fig-2: *Graphical Presentation of HPV Type (%) among Female Respondents*

type. HPV 16 is the commonest serotype detected (31.3%). HPV 16 and HPV 66 collectively account for 44.40% of the positive cases. Graphical presentation of percentages of various HPV subtypes found in our samples are as shown below.

Discussion

In 1983 HPV infection was recognized as first tumor virus. Since then over decades of research, it is still thought that HPV is a necessary cause of cervical cancer (99.7% 0f cervical cancer).[18] Regular screening with recall technique and cytology has reduced the disease incidence in developed countries. HPV vaccines solved a major health problem when in 2006 Gardasil a quadrivalent vaccine was first introduced and then in 2008 Cervarix bivalent. A nonvalent vaccine HPV is also available now. In 2009/20 NHS UK has introduced HPV primary screening and cytology side by side with HPV prophylactic immunization program.[19]

In Pakistan, though a preventable disease, the mortality rate is very high as there is no comprehensive national policy for screening and vaccination More than 70% of patients with cervical cancer patients report with very advanced stage of malignancy which results in high mortality rate, increased cost of treatment with poor survival rate.[20]The above-mentioned facts were confirmed in my study also.

In this study HPV 16 being the most common serotype is found in 31.3% of women with cervical pathology. Globally HPV 16 & 18 are found to be responsible for over 70% cases of invasive cancer of the cervix.[21] Similar studies conducted in Punjab and Karachi found HPV 16 to be the most common serotype in cases of carcinoma cervix.[11] In both these studies samples were tested only for HR-HPV 16 and 18 while in my study 17 HR-HPV subtypes have been tested.[22]

Globally HPV 16 is also the most common virus encountered having a proportion of 60.6%. All the vaccines available are effective against HPV 16 (bivalent, quadrivalent, nonvalent).[23]

The second most common HPV type detected in this study is 66 (13.1%). Although HPV 66 is a high-risk virus for cervical cancer but it is not included in first fifteen most common viruses. It may be due to geographical variations. Difference of the risk factors in different populations may account for it as well. No vaccine is yet available against HVP 66.[24]

HPV 31 has a frequency of 10.1% and is the third most common in my samples. Globally it has a frequency of 3.7%.[25] There is greater than average presence of subtype 31 in developing countries (Clifford et al 2005). HPV 31 was the second common (10.9%) in Nordic countries. Only pentavalent vaccines are effective against HPV 31.[26]

HPV 18 having a frequency of 9.1% in my study. However globally it is the second most common type having a frequency of 10.2%.[27] Together HPV 16, 18 and 45 are responsible for almost 90% of cases in most populations. In Pakistan, HPV 18 single had a frequency of 25.97%.[13]All available vaccines are effective against HPV 18. The changes in frequency noted in different areas of even Pakistan demarcates the different risk factors in different areas i.e. use of COCP, smoking, low socio-economic status and poor knowledge of screening.

HPV type specific prevalence varies in different countries and in my study 16, 66, 31, 18, 59, 51 are major five subtypes while other include 45, 33, 68, 58, 52, 39, 86, 61, 57, 56, 38 & 32 in decreasing order as high-risk HPV subtypes for cervical carcinoma and HSIL. HPV 66 has emerged as second common HPV subtype. Another polyvalent vaccine is required to prevent infection from all important subtypes of HPV

in Pakistan.[28]

HPV 61, 32, 86 and 57 found with a variable frequency in my study are not even considered in the first fifteen high risk HPV subtypes all over the world, which is a geographical variation. Further studies should be carried out in all parts of Pakistan before deciding on a national vaccination program.

Screening, knowledge and awareness is very important in developing countries like Pakistan to reduce the burden of late disease similarly vaccines should be developed according to HPV subtypes present in Pakistan.

Conclusion

Different HPV vaccines available in market provide immunity against 6,11,16,18,31,33,45,52 & 58. However available vaccines are not effective against HPV 66,59,32,61, and 86, which are quite prevalent in our population. So, vaccination and screening should go side by side in Pakistan to cover the disease caused by these non-vaccine type variants. Larger studies should be conducted in all parts of Pakistan before forming a vaccination policy.

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Authors Contribution

SY, ZNA, AZ: Conceptualization of Project
ZNA: Data Collection
SY, ZRM: Literature Search
AS, SS: Statistical Analysis
AS, SS: Drafting, Revision
SY, ZRM: Writing of Manuscript