Original Article

Retrospective study of correlation between high risk detection of HPV and liquid based cervical cytology for cervical cancer screening

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ABSTRACT

BACKGROUND

Cervical cancer is most common in Nepalese women. Human papilloma virus (HPV) plays a main role in pathogenesis of cervical cancer. Human papilloma virus serotype 16 is the most common type followed by HPV 18. Liquid based smear cytology (LBC) is a simple, safe, non-invasive and cost effective method for the detection of squamous intraepithelial lesion or squamous cell carcinoma. The aim of this study was to correlate HPV DNA testing result with the findings of Liquid based cytology.

METHODS

This was a retrospective study conducted for 2 years at Department of Laboratory Medicine Pathology, Nepal Mediciti hospital from 1st may 2021 to 30th april 2023. Total of 1456 cases were included in this study.

RESULTS

Out of 1456 cases, 100 cases were positive for HPV. The concordance between HPV DNA test and LBC findings is 57%. The highest HPV-positive cases were seen in the age group of 31-40years. The most common HPV genotype study was HPV 16 (28%) followed by HPV 18 (27%). HPV 16 was positive in 57% of ASCUS positive cases of LBC, is statistically significant (P value-0.017). HPV 18 was positive in 37.0% of ASCUS positive cases of LBC, is not statistically significant (P value-0.018). The concordance between HPV positive detection and LBC findings is 57% and discordance is 43%.

CONCLUSIONS

This study shows the prevalence of HPV is higher in middle to young age group. ASCUS was common finding in LBC in HPV positive cases followed by LSIL,HSIL and SCC. However 43% of HPV positive cases were reported as negative for intraepithelial lesion (NILM) as there was no abnormality in squamous epithelial cells. Both the HPV test and cervical LBC smears are valuable tools for early detection of cervical precursor lesion. However, neither test alone provides conclusive results for comprehensive screening. So, it is recommended to use both test in combination for more accurate and reliable findings in cervical cancer screening.

KEYWORDS

Human papilloma virus, Liquid based smear cytology, Cervical cancer screening

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INTRODUCTION

Cervical cancer is the most common cancers in Nepal. 1 According to the International Agency for Research on Cancer (IARC), the incidence of cervical cancer can be reduced by 90 % through periodic screening. 2

Human papilloma virus(HPV) plays a main role in carcinogenesis. HPV E7 stimulates the cell cycle by binding to RB and releasing E2F, E6 AND E7 are key gene that inhibit apoptosis to facilitate virus survival by blocking RB, P53 and P27, leading to carcinogenesis.3

The Bethesda system (TBS) for reporting cervical/vaginal cytological diagnosis is a uniform system for reporting & it is useful to provide effective communication among cytopathologists & referring physician.4 The squamous cellular abnormalities described in TBS are as ASC (Atypical Squamous Cells), LSIL, HSIL & Squamous cell carcinoma.The revised 2001 Bethesda System includes changes that are based on clinical input and advances in the understanding of the biology of cervical cancer and the latest revision version done in 2014. 5

In the 1990s, a new methodology was developed for the collection and preparation of cervical cytological samples for screening, a liquid-based cytology approved by United States Food and Drug Administration. 6,7

More than 150 different strains of HPV are present, 40 different types of HPV genotypes are known to affect the genital area. Based on the risk level, they are divided into three categories: high, low and intermediate risk types. The high-risk HPV types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, while low-risk HPV types are HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81 as they are only associated with benign warts and intermediate risk types are HPV 23, 53, 66. 8

HPV is associated with various kinds of clinical conditions that range from innocuous lesions to cancer. Most HPV infections are benign and generally resolve spontaneously within 1 to 5 years. 9 HPV was finally recognized as the important factor in causing cervical cancer in 1996, by the World Health Association, along with the European Research Organization on genital infection and Neoplasia. 9. Most importantly HPV 16 and 18 have been credited in causing nearly 70% of cervical cancer worldwide. 10. Many years of consistent studies have confirmed that the cervical infection which is caused by the high-risk HPV types is the starting point in causing cervical cancer. Therefore, early and timely detection of cervical cancer is a must in order to prevent and minimize the incidence of cervical cancer. Screening of cervical cancer usually starts at the age of 21 years. 10 Although HPV is necessary factor for the causation of cervical cancer, it alone is not responsible for the cancer, Various other factors like sexual orientation, number of sexual partner, long term use of oral contraceptives, immune compromise condition, co-infection with HIV or chlamydia leads to the progression of HPV-infected precursor lesions to cancer.

Apart from the preventive measures, recent development in the field of HPV vaccines are also rapidly increasing.These vaccines are design for targeting various different genotypes of HPV. The HPV vaccines are most effective when administered before individuals becomes sexually active and are potentially exposed to the virus. Gardasil is an effective HPV vaccine which targets nine different types of HPV (6, 11, 16, 18, 31, 33, 45, 52 and 58).It is usually administered in a series of two or three doses over a period of several months. Early diagnosis and detection of HPV is one of the major treatment for HPV infection and subsequent cervical cancer, since HPV cannot be cultivated in laboratory and immunologic assays are also inadequate for the diagnosis of HPV, cytology has become the basic tools for the diagnosis. With the recent development in the field of molecular science detection of HPV by PCR has gain worldwide recognition for its analytical sensitivity, rapid result and it's versatility. Currently various different commercial kits are available in the markets that caters to the clinicians and health professionals need for the detection of high-risk HPV genotypes. These kits are able to detect low-risk, high-risk and intermediate-risk HPV types depending upon the need and the type of sample from which it can detect the virus may also vary. 11

The aim of the study was to find out the different genotypes of HPV present by real-time PCR in the general female population visiting tertiary hospitals within in the Valley. We also collected the LBC result of these patients to look for changes in their cervical cells. This study helps in establishing the correlation of LBC(cytology) and HPV molecular test and aid in the screening and diagnosis of cervical cancer. This assessment can help doctors in better risk management and stratification of individuals with HPV detection and abnormal findings in LBC.

METHODS

This was a retrospective study conducted at Department of Laboratory Medicine and Pathology from 1st may 2021 to 16th may 2023. The proposal for the research was approved by IRC of NMC. Detection of high risk HPV was done by RT-PCR (REF-A28134, SN-272526621) and in sample collected for LBC test. Only HPV positive cases were taken and correlated with its cytological findings. Bethseda system 2014 used to report the LBC. Specimen (Ethanol fixed LBC slides) received from Department of GYN/OBS reported.

LBC were collected and prepared smears following standard protocol. All cases of HPV detection including high risks type (16, 18, 39, 45, 31, 33, 35, 51, 56, 59, 66, 67, 52, 58, 68) detection were included. The data analysis was done by version SPSS version 20.

RESULTS

Total of 1456 cases. Out of 1456 cases, 100 cases were HPV positive. Only HPV 100 positive cases, LBC report was traced and correlated simultaneously.

The table 1 and figure 1 presents age distribution of study patients. The findings indicated that the majority of participants were aged between 31 and 40 years, representing 38.0% of the total population. This was followed by the 20 to 30 age group, which accounted for 23.0% of the participants. The 41 to 50 age group made up 21.0% of the total, while 12.0% of participants were between 51 and 60 years old. The smallest proportion of participants, 6.0%, were aged over 60. Overall, the distribution showed that the highest concentration of participants fell within the younger to middle-aged categories, particularly those aged 31 to 40.

Table 1. Age distribution of study patients.

Age (in years)	Frequency	Percent (%)
20 to 30	23	23.0
31 to 40	38	38.0
41 to 50	21	21.0
51 to 60	12	12.0
>60	6	6.0
Total	**100**	**100.0**

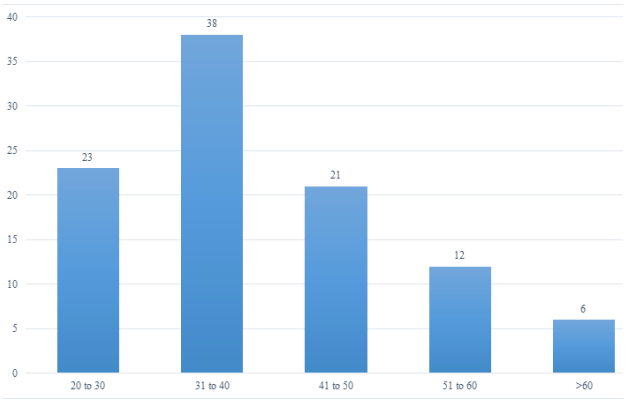

Figure 1. Age distribution of study patients.

Table 2 and figure 2 presents the status of positive and negative findings of LBC smears among 100 cases of high risk detection of HPV. Majority of the patients were diagnosed positive 57(57.0%) on LBC smears among HPV positive high risk detection while 43(43.0%) of the patients were detected with negative findings on LBC.

Table 2. Status of positive and negative cases of LBC smears among HPV positive high risk detections patients.

Cases	Frequency	Percent (%)
Positive	57	57.0
Negative	43	43.0

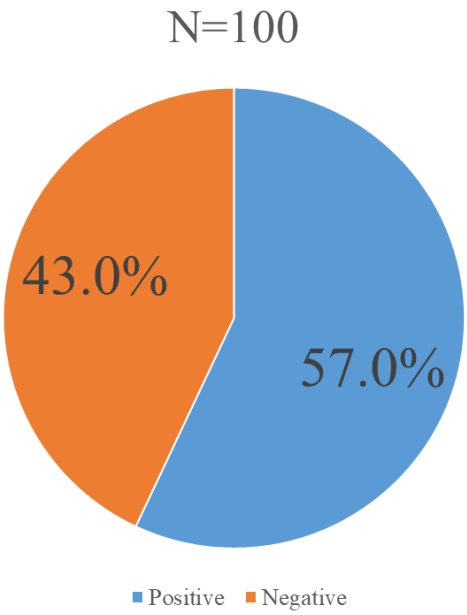

Figure 2. Status of positive and negative cases among patients.

Table 3 shows distribution of HPV variant positive cases with their respective LBC cytological findings. Overall, ASCUS was the most common finding across all variants, affecting 33.0% of the participants, followed by LSIL (16.0%), HSIL (7.0%), and SCC (1.0%), while 43.0% were negative for any cytological abnormality.

Variant	ASCUS n(%)	LSIL n(%)	HSIL n(%)	SCC n(%)	Negative n(%)	Total n(%)	P-value
HPV 16	16 (57.1%)	4 (14.3%)	3 (10.7%)	1 (3.6%)	4 (14.3%)	28 (100.0%)	0.017
HPV 18	10 (37.0%)	8 (29.6%)	2 (7.4%)	0 (0.0%)	7 (25.9%)	27 (100.0%)	0.118
HPV Others	7 (15.6%)	4 (8.9%)	2 (4.4%)	0 (0.0%)	32 (71.1%)	45 (100.0%)	<0.0001
Total	33 (33.0%)	16 (16.0%)	7 (7.0%)	1 (1.0%)	43 (43.0%)	100 (100.0%)	

Chi-Square Test

Table 4 shows the distribution of patient age group among different variant of HPV. So, HPV 16 is highest in age group of 20-30 and 31-40 age group. HPV 16 was not present in age group above 60. HPV 18 and HPV others than 16 and 18 was more common in age gorup of (31-40)years.Overall, 28.0% participants were positive for HPV16, 27.0% for HPV18, and 45.0% for other HPV variants across all age groups

Table 4. Distribution of patient's age among type of variant

AGE (in years)	HPV16 n(%)	HPV18 n(%)	HPV Others n(%)	Total n(%)	P-value
20 to 30	12 (52.2%)	3 (13.0%)	8 (34.8%)	23 (100.0%)	0.001
31 to 40	12 (31.6%)	9 (23.7%)	17 (44.7%)	38 (100.0%)	
41 to 50	2 (9.5%)	4 (19.0%)	15 (71.4%)	21 (100.0%)	
51 to 60	2 (16.7%)	8 (66.7%)	2 (16.7%)	12 (100.0%)	
>60	0 (0.0%)	3 (50.0%)	3 (50.0%)	6 (100.0%)	
Total	**28 (28.0%)**	**27 (27.0%)**	**45 (45.0%)**	**100 (100.0%)**	

DISCUSSION

This study was done in Nepal Mediciti Hospital. It shows highest HPV positive in age group of 31-40 shown in table 1. This finding was in agreement with study done by Findik S et al. where most common HPV-positive rates were found in the 30-40 age group. 13 Our results in contrast to a study done by Dunnel et al. which shows highest prevalence below 30 years of age13and a study done by Gupta et al. which showed most common in age group 41-50. 8

The most common HPV genotype in our study is HPV 16 (28%) followed by HPV 18 (27%). Others were 45% which included HPV 31, 33, 35, 39,45,51,52,56,58,59,66,67 and 68 shown in Table 3. Our results regarding HPV types were similar to a study done by Gupta et al. which shows HPV 16(60.4%).8 In agreement with our study, a study done by Shakya S et al. revealed (60.4%) HPV 16 and 12.5% were other high-risk HPV genotypes (18,45,59). 14 In contrast to this study, Ardhaoui M, et al. 15 showed that HPV 31 (1%), HPV 16 (0.9%), HPV 59 (0.7%) were the most prevalent genotypes.

Out of 100 cases in our study, total 28 cases were HPV 16. Among those 28 cases, 16(57.1%) shows ASCUS, 4(14.3%) shows LSIL, 3(10.7%) shows HSIL and least common was SCC with single cases. Only 4 (14.3%) out of 26 cases show negative for intraepithelial neoplasms on LBC. The association between HPV16 and these cytological abnormalities were statistically significant with a p-value of 0.017, indicating a strong correlation.

Out of 100 cases in our study, total 27 cases were HPV 18. Among those 27 cases, 10(37.0%) shows ASCUS, 8(29.6%) cases shows LSIL, 2(7.4%) shows HSIL and 7(25.9%) cases shows negative for cervical neoplasms in LBC findings. The relationship between HPV18 and the observed cytological outcomes were not statistically significant, with a p-value of 0.118.

Out of 100 cases in our study, total 45 cases were HPV other high risk. Among those 45 cases 7 (15.6%) shows ASCUS, 4 (8.9%) shows LSIL, 2(4.4%) cases shows HSIL and 32(71.1%) cases shows negative for cervical neoplasms in LBC findings. The association between HPV types other than 16 and 18 and cytological outcomes were highly significant, with a p-value of less than 0.0001.

Similar results were seen in studies done by HAQ NAWAZ F, et al 16, Chaudhary RD et al 17, G Yeoh GP, et al.18 and Bodal VK, et al.19 . In these studies, most common abnormal Pap smear finding was also ASCUS accounting for 47.08%, 50.0 % , 65.9 % and 25.67 % respectively. Whereas in studies by Jyothi R et al.20, BA.Jones et al.21, Vidhyadhar S et al.22, Verma I, et al.23, RC L, et al.24, Meenai FJ et al.25 and K Bhavani et al,26 most common epithelial cell abnormality was LSIL accounting for 45 %, 48.93%, 60%, 62.5%, 38.88%, 41.7%, 28.16%, 47.22%, 49.43%, 33.92% and 44.26% respectively among all pre invasive and invasive cases.

Contrast to all of these studies including our study, HSIL was the most common epithelial cell abnormality in study by Dhakal R et al.27.

A study by Pista A, et al.28found that the percentage of multiple infections was majorly negative for intraepithelial neoplasm or malignancy in cytology LBC smears findings and were different according to age, suggesting transient reinfection rather than a persistent infection

In our study concordance and discordance between LBC and HPV diagnosis is 57.0 % and 43.0% respectively. Similarly study done by Raj S, et al.29 showed concordance and discordance between LBC and HPV is 64.9% and negative results in 35.1%. In concordant to our study, a high correlation was found between HPV-DNA test and positive cytology by a study done by Kussaibi H.et al. 30

We analyzed the HPV DNA test and Liquid based cytology (Figure 2)and out of 100 positive cases of HPV high risk detection, 57% cases show positive for neoplasm of cervical lesion on LBC findings and 43.0% cases show negative for neoplasm of cervical lesion on LBC findings. Similarly study done by women Feldstein O, et al.31 showed women with HPV 16/18 infection were significantly more likely to have any concurrent abnormal cytology than women with other hrHPV infection. Mehta V,et al. 32 stated that since LBC smear relies upon the examination of cellular morphology, infection with HPV can cause visible changes in the cervical cells but these changes may not be apparent in microscopic examination. Macios A, et al..33 revealed there may be an absence of cervical cancer precursors lesions on the cervix at the time of sampling also the location may be deep in endocervical canal or under mucosa which impedes proper sampling. Delpero E, et al.34 revealed that current screening approaches acknowledge that LBC smear has high specificity (96.8%) but a low sensitivity (55.4%) which means that LBC smear screening may produce false-negative result.

Finally, in context of Nepal there are still many barriers present in cervical cancer screening one of the major problems are lack of clinical and laboratory setup as HPV detection by molecular method requires skill manpower, a well-established molecular lab. Due to this problem the overall cervical cancer screening in our country still relies on Pap smear. Whereas various countries around the world have started to introduce co-testing (HPV DNA test and Pap smear) as the primary screening tool. Therefore, one of the reason for this study was also to help understand and highlight the importance of HPV DNA testing as a parallel cervical screening tool with Pap smear.

Major strength of our present study included a large sample, the use of a standardized and well validated HPV DNA test.

CONCLUSION

This study shows the prevalence of HPV is higher in middle to young age group. ASCUS was common finding in LBC in HPV positive cases followed by LSIL,HSIL and SCC. However 43% of HPV positive cases were reported as negative for intraepithelial lesion (NILM) as there was no abnormality in squamous epithelial cells. Both the HPV test and cervical LBC smears are valuable tools for early detection of cervical precursor lesion. However, neither test alone provides conclusive results for comprehensive screening. So, it is recommended to use both test in combination for more accurate and reliable findings in cervical cancer screening.

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