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Molecular Diagnosis and Genotype Distribution of Human Papillomavirus among Women in Nepal

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ABSTRACT

Setting: Intrepid Nepal Pvt Ltd, Thapathali, Kathmandu, Nepal between August 2020 and August 2023

Objectives: To determine the prevalence of Human papillomavirus and their type specific distribution among women

Design: laboratory based, cross sectional study

Results: Among the 6474 samples received, 574 were identified as Human papillomavirus. Overall, the prevalence of the HPV isolates were found to be 9.4%.

Genotyping of 51 women sample were done. All infection with High-risk type. 66.6% single infection and 33.3% multiple infection. Most common HR HPV were HPV 16(33.3%), followed by HPV 56(17.6%), 66(15.6), 18(13.7%), 58(13.7%), 59(11.7%), 31(7.8%), 39(7.8%), 51(5.8), 52(5.8%), 68(5.8%). Higher HPV prevalence among young adult (20-39 years), followed by middle age adult and senior adult.

Conclusion: Our results highlight the current status of HPV prevalence and genotypes among women in Nepal. The highest prevalence was found among 20-30 years. HPV vaccine is strongly recommended for regular immunization before the onset of sexual activities 9-13 years to reduce the burden of cervical cancer.

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KEYWORDS:
Human papillomavirus, Genotyping, Prevalence, Nepali women

INTRODUCTION

Human papillomavirus (HPV) is one of the most common sexually transmitted disease around the world.[1] It mainly causes cervical cancer and other cancers including vulvovaginal, oropharyngeal, penile and anal cancers.[2]

In 2020, it was reported that cervical cancer is the fourth most frequent cancer among women globally, with an estimated 6,04, 000 new cases and 3,42, 000 deaths.[3] Approximately 90% of the new cases and death occur from cervical cancer globally in low and middle income countries (such as Nepal, India, Bangladesh, and Sri Lanka) compared to developed countries. [4]

The annual number of new cases of cervical cancer has been projected to increase from 570,000 to 700,000 between 2018 and 2030, with the annual number of deaths projected to increase from 311,000 to 400,000. [5]

In Nepal, cervical cancer continues to be the leading cancer among women, with an annual incidence of 2,942 new cases (21.5 per 100,000 women) and 1,928 deaths (14.3 per 100,000 women). [6]

In November 2020, the World Health Organization launched a Global Strategy to eliminate cervical cancer as a public health problem. The Strategy proposes an elimination threshold of 4 cases per 100,000 women. [5,7]

The national guideline for Cervical Cancer Screening and Prevention (CCSP) was introduced in Nepal in 2010 with the goal of screening at least 50% of women in the age group of

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30–60 years, which was revised to 70% in 2017. [8,9] By 2019, only 8.2% of women aged 30–49 years were screened. [10]

Over the past 30 years, the morbidity and mortality of cervical cancer have demonstrated a considerably decreasing tendency in developed countries due to enhanced prevention of cervical cancer and screening .[11] In contrast, Nepal, as a developing country, has experienced a rising trend in the morbidity and mortality of cervical cancer due to inadequate access to cervical cancer prevention, screening, and treatment.

More than 200 HPV genotypes have been identified and characterized based on nucleotide sequence relatedness of the L1 gene which codes for the major HPV capsid protein. [12] And they can be classified into high-risk (HR) and low-risk HPV (LR) genotypes based on their carcinogenicity.

Based on epidemiological and biological data, The International Agency for Research on Cancer (IARC) has classified twelve HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) as Group 1, carcinogenic to humans (often referred to as high-risk, HR).

Eight HPV types, (HPV-68) as Group 2A, probably carcinogenic to humans, HPV types (26, 53, 66, 67, 70, 73, and 82) as Group 2B, possibly carcinogenic to humans, due to low prevalence and lack of evidence of biological activity in tumour tissues.

While HPV types (6 and 11) as Group 3, often referred to as low-risk (LR), are not classifiable as to their carcinogenicity to humans. [13,14,15] HPV-16 and HPV-18 represent the most oncogenic types, and they are responsible for approximately 70% of cervical cancer cases worldwide.[16]

DNA detection and genotyping of HPV has emerged as an essential screening method for controlling and preventing HPV related diseases worldwide. HPV detection is included in various screening strategies for cervical cancer and precancerous lesions. It provides a more sensitive method of detection for high-grade lesions than cytology. [17] And it is recommended as the preferred screening method for cervical cancer screening by the latest WHO guidelines. [18]

This study findings will help to understand the burden of HPV related infection and type specific distribution of HPV isolates among women population. The study findings will also be used to raise awareness about the implementation of essential screening nationally and improve the infection prevention and control measures to predict change in risk of infection in future.

METHODS

Study design:

This was a laboratory based, cross sectional study using standard microbiological and molecular techniques between August 2020 to August 2023

Setting:

The study was conducted in Intrepid Nepal Pvt Ltd (INPL), Thapathali, Kathmandu, Nepal, an innovative biotechnology organization with base in Canada and Nepal. The division of Intrepid Diagnostic Center is the advance laboratory and molecular diagnostic services in Nepal since 2009. INPL is recognized by the the National Public Health Laboratory (NPHL).

Sample collection, processing, HPV detection and genotyping

Cervical swab samples from women patient sent to the Intrepid diagnostic center, were stored at -20°C when processing was delayed. While processing, sample vials were brought to room temperature and vortexed vigorously to detach the exfoliated cells. DNA (Deoxyribonucleic acid) is extracted using automated extraction kit (AIT extraction Kit) according to manufacturer's instructions. Extracted DNA samples were labelled and stored at -20°C when not processed immediately. The samples were analyzed using HPV High Risk Screen Real-TM Quant 2x in vitro Real Time amplification test for quantitative detection of Human Papillomavirus (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and HPV Genotypes 14 Real-TM Quant in vitro Real Time amplification test for quantitative or qualitative detection and genotyping of Human Papillomavirus (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) (Sacace Inc.). Jiangsu Mole Bioscience Co. Ltd Kit is used to qualitatively detect the DNA of 24 types of HPV in women's cervical shedding cell samples and identify the HPVs as Group 1 (6, 11, 42, 43, 44 or 81), Group 2 (16, 18, 31, 33, 35, 49, 51, 52, 56, 58, 58, 59, or 68) or Group 3 (26, 53, 66, 73 or 82). Briefly, PCR amplification were run on a Sacace Real Time PCR Cycler. Following the manufacturer's instructions, 5 µl of extracted DNA template were mixed in 8 µl HPV master mix solution. Positive and Negative control were also added. In order to avoid false negative results, the internal control (human beta-globine gene), were included. Results were interpreted as valid when both the positive and internal controls were positive. The result were invalid if internal control were negative or weakly positive, as recommended by the manufacturer.

Study population and duration

Study population for this study include patients of reproductive aged women. All biological samples of patients sent to Intrepid Diagnostic Center for molecular diagnosis of HPV from August 2020 to August 2023

Data collection

Demographic and biological sample characteristics of all patients with HPV isolates were recorded from Molecular laboratory registers and laboratory electronic records. Data included Patient identification (ID), Age, Sex, Hospitals, Sample type and outcome of the test. The dataset was counter checked by two independent microbiologists (MM and RM).

Data Analysis and Statistics

Collected data were entered in Excel Sheet where data were cleaned and checked for accuracy. Data were analysed using SPSS software (version 22.0). The rates of isolation of Human papillomavirus were presented as numbers and

proportions. We assessed the prevalence of HR or LR using chi-squared test; the level of significance was set using $p < 0.05$

Ethics

Ethical approval for the study was taken from National Health Research Council, Kathmandu, Nepal (ERB Protocol Registration No: 326-2024). As the study involved only the use of secondary data, no informed consent was necessary.

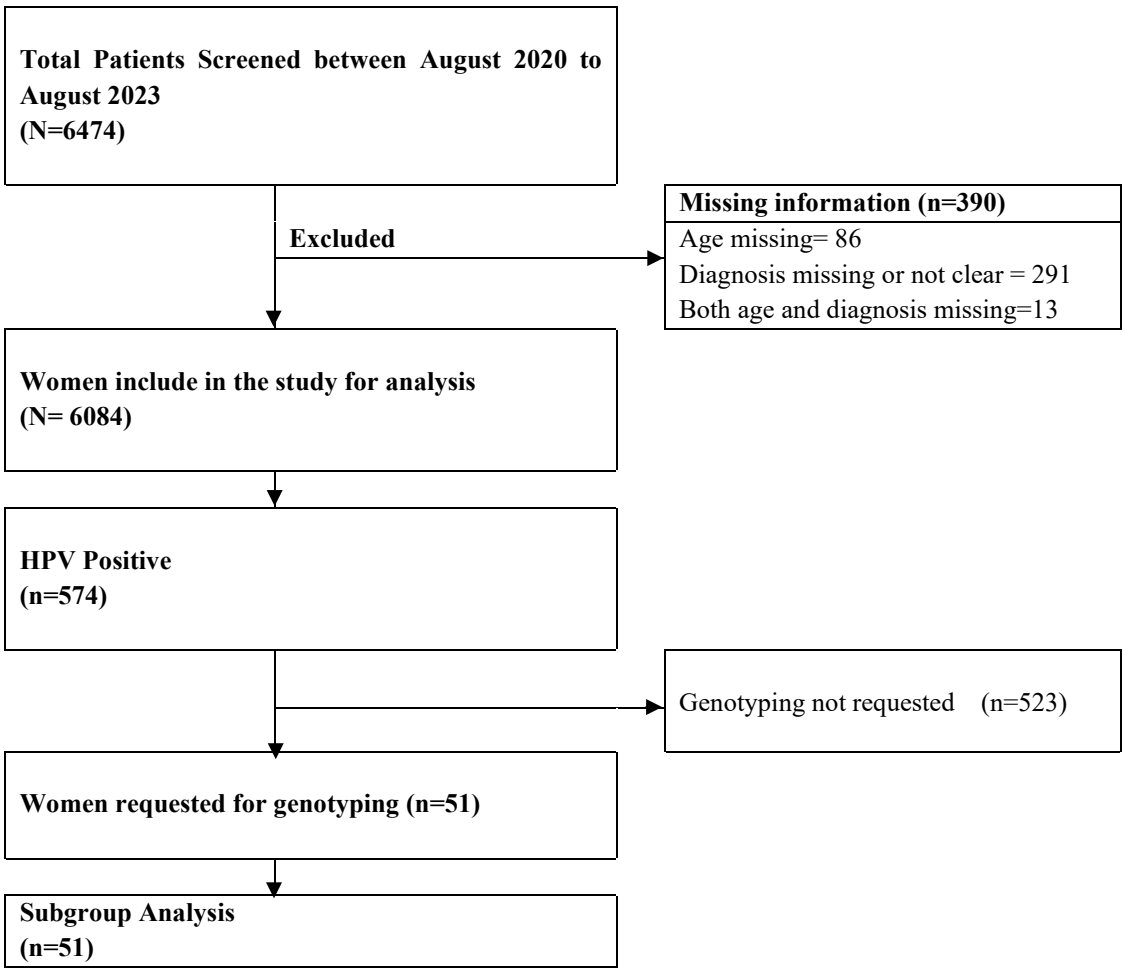

Figure-1: Flowchart for sample inclusion and analysis

RESULTS

During the study period of 3 years from August 2020 to August 2023, a total of 6474 specimens were collected. Out of these, 390 samples were missing information, resulting in 6084 specimens evaluated for HPV. Overall, 574 specimens were identified as positive for Human Papilloma Virus. Of those who tested positive, only 51 women underwent genotyping (Figure-1).

The prevalence of the HPV isolates among participants were found to be 9.4% (Figure-2).

The prevalence of HPV isolates were found to be higher among young adult of 20-39 years, followed by middle age adult and senior adult (Table 1).

Table 2 shows the distribution of Human papillomavirus types among women who underwent genotyping. Almost all had HPV infection with High-risk type. Of these, 34(66.6%) had single infection, whereas 17(33.3%) had multiple infection. Further, most commonly found HR HPV in either single or multiple type infections were HPV 16(33.3%), followed by HPV 56(17.6%), 66(15.6%), 18(13.7%), 58(13.7%), 59(11.7%), 31(7.8%), 39(7.8%), 51(5.8), 52(5.8%), and 68(5.8%).

Table 3 presents the association of human papillomavirus infection genotypes among women by age group. Overall, 66.7% of women reported having a single infection, while 31.3% reported multiple infection. While younger women tend to have multiple infection, older individuals prone to single infection, however, no statistically significant differences was found in genotype distribution across age groups (p=0.340).

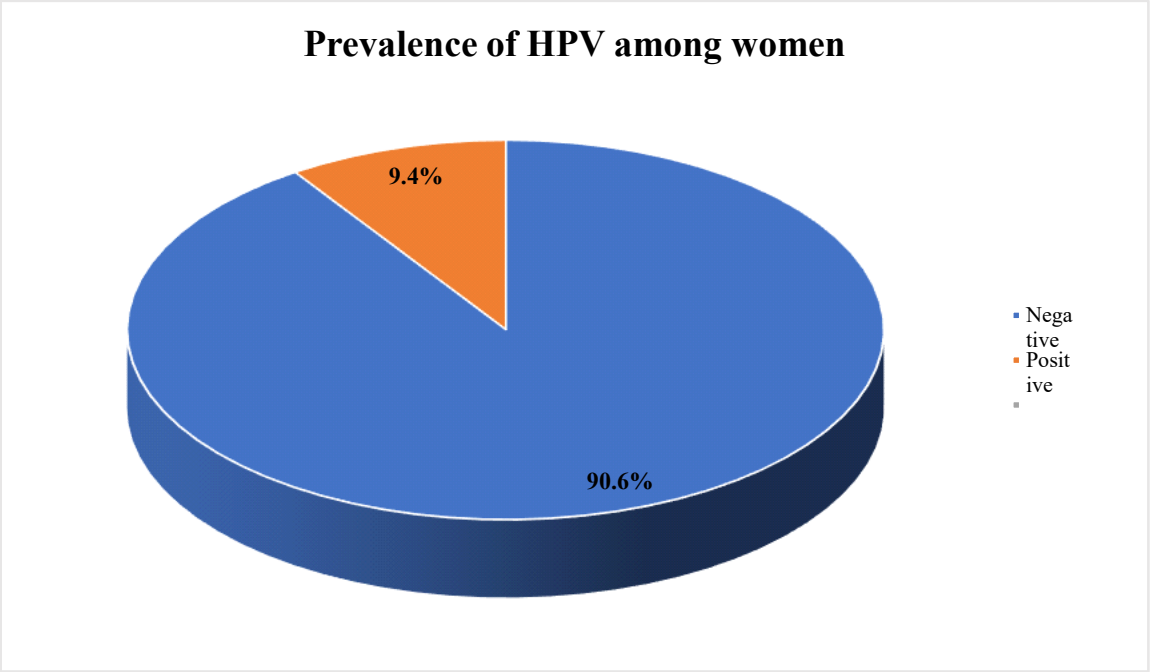

Figure 2: Overall prevalence of HPV among participants between August 2020 to August 2023 (n=6084).

DISCUSSION

This study was done to assess the prevalence of HPV types and their distribution among women in Nepal. The prevalence of the HPV isolates among participants were found to be 9.4%. About two-third of the patient had single infection with high-risk type and one-third had multiple infection with high risk type.

HPV prevalence in our study was found to be 9.4% (fig:2), similar to a study done by Johnson et al. [19] in Western Nepal (9.6%) , by Khoo et al.[20] in Malaysia (9.6%), and (9.9%) in neighboring Indian state of Uttar Pradesh by Shrivastava et al. [21] which is slightly higher, than found previously from urban Nepal (8.6%) by Sherpa et al. [22] and (8.9%) by Bhatta et al. [23] but lower than that found in a study done by Shakya et al. [24] (14.4%).

The reasons for this deviation are unclear and may be attributed to variations in the quality of the specimens tested and the sensitivity of the HPV detection assay used in the study.[25]

We found a diverse distribution of type-specific HPVs in our study, than previously reported. HPV 16 was the most common genotype followed by HPV 56, 66, 58, 18, 59, 31, 39, 51, 52, 68 among the high-risk HPV group. HPV 18 was in 4th position in the order. Diverse distribution of HPV genotypes, could be due to sample received in the laboratory are from wide geographical and cultural range of nationalities.

This study is in agreement with global studies where HPV 16 was identified as the most common high-risk HPV genotype[26,27,28,22,29] with the highest oncogenic risk.[30]

Sherpa et al. reported the most common high-risk types among women with normal cytology were HPV16, 58, 56, 18, and 52.[22] Meanwhile, Shakya et al. reported HPV-18, 51, 59, 31 and 16 as the five most common HPV. [24]

However, a study conducted by Thapa et al.[31] found HR HPV 16, 39, 58, 33 and 51 as the five most common HPV.

So, it is clear that there are variations in the prevalence of HPV infection frequency within Nepal.

Worldwide, the most common HPV types in women are 16, 18, 31, 58, and 52, which is about 50% of all HPV infections. [32] Besides HPV 16 and 18, other HR HPV types that are prevalent in Asian populations are 58, 33, 52, 45, 31, and 35 that accounted for additional 20% of cervical cancer cases. [33]

In our study, high-risk HPV genotypes were substantially more prevalent. None of the women in this study had infection with low-risk types. Most commonly found HR HPV in either single or multiple type infections were HPV 16(33.3%), followed by HPV 56(17.6%), 66(15.6), 18(13.7%), 58(13.7%), 59(11.7%), 31(7.8%), 39(7.8%), 51(5.8), 52(5.8%), 68(5.8%)

The Prevalence of single type infections was 34(66.6%) among HPV positive women. Among these women the most common HR HPV type were HPV16 (17.6%), HPV 18 (9.8%), HPV 56 (7.8%), HPV 58 (5.8%), HPV 66 (5.8%), HPV 31(3.9%), HPV 51(3.9%), HPV 59 (3.9%), HPV 68(3.9%), HPV 52(1.9%).

Multiple type infections were observed in 17(33.3%) HPV positive women. Multiple infections were also seen to be caused by high-risk type rather than low risk types.

The prevalence of multiple infections was markedly higher among women age 20-29 years (80%) than in women of other age groups. The most common HPV types involved in multiple infections were HPV 16 (31.3%), HPV 56(9.8%), HPV 66 (9.8%), HPV 59 (7.8%), HPV 58 (7.8%), HPV 39

(5.8), HPV 31(3.9%), HPV 52 (3.9%), HPV 51 (1.9%), HPV 68(1.9%)

This study shows that the prevalence of HPV infection was significantly higher in younger aged (20-29 year) women compared to middle aged or senior adults, which is in accordance with the previous study done in Nepal by Thapa et al. [31]

The higher incidence of HPV in younger women may be attributed to the following risk factors such as early marriage, multiple pregnancies, greater number of sexual partners, more likely to engage in health risk behaviour, including smoking, sexual debut at an early age, abortion, husband's extramarital affairs.

Globally, there is a variation in age-specific HPV prevalence. The prevalence seems to decline with increasing age[34,31] especially in high-income countries[35,36]

In our study the prevalence of single infection is higher in middle aged (82.4%) or senior adult (75%) while multiple infection were more prevalent in younger aged (80%) and it decline with the increase age.

Younger women were significantly more likely to harbor multiple high-risk HPV infections, reflecting common sexual transmission of multiple high-risk HPV. [37,38]

A study done by Spinillo A et al.[39] reported, multiple HPV infection with high risk genotypes have found to be associated with a significantly increased risk of cervical intraepithelial neoplasia compared to infection with a single HPV type.

Thus, more attention should be paid to single infection especially with HPV 16 and HPV 18 followed by other HPV. Further study is needed to understand the aspects of multiple HPV infection. The investigation of multiple HPV infection is of great importance to study the prevalence of HPV and is also of great significance to develop a multivalent HPV vaccine.

Furthermore, these evidences pose a significant health risk to women of different age group. Hence HPV testing as primary cervical cancer screening is a good support for early detection of cancer development and their genotypic distribution is important to know, since data regarding the distribution of HPV genotypes is concerning with the vaccine development. Despite the Nepal government protocol of cervical cancer screening, mere number of women actually undergo cervical cancer screening each year. [40]

Hence, our study provides new data on the overall prevalence of HPV and genotypic distribution of HPV among women in Nepal. In addition, it highlights the need for standardization of screening practices in Nepal and implementation of rigid prophylactic vaccination programs to reduce the disease burden.

Table 1: Age wise prevalence of HPV among participants between August 2020 to August 2023 (n= 6084)

Age group	Total Case	Positive Case	Prevalence
20-29 year	927	99	10.7%
30-39 year	2521	241	9.6%
40-49 year	2103	183	8.7%
50 years and above	533	51	9.6%
Total	**6084**	**574**	**9.4%**

Table 2: Distribution of Human papillomavirus types among women who underwent genotyping (n=51)

HPV genotype	Number	(%)*
Single infection		
High risk		
16	9	17.6
18	5	9.8
31	2	3.9
33	0	0.0
35	0	0.0
39	1	1.9
45	0	0.0
51	2	3.9
52	1	1.9
56	4	7.8
58	3	5.8
59	2	3.9
66	3	5.8
68	2	3.9

Low risk		
6	0	0.0
11	0	0.0
42	0	0.0
43	0	0.0
44	0	0.0
Multiple infection		
16/31	1	1.9
51/58	1	1.9
16/68	1	1.9
16/56	1	1.9
16/39	1	1.9
16/59	1	1.9
18/39	1	1.9
18/58	1	1.9
39/58	1	1.9
56/66	4	7.8
16/31/59	1	1.9
16/33/66	1	1.9
16/52/58/59	1	1.9
35/52/59	1	1.9

*column percentage

Table 3: Human papillomavirus infection genotypes among women by age group (n=51)

Age Group (years)	**HPV Genotypes**					
	Single infection		**Multiple infection**		**Total**	
	N	%	N	%	N	%
20-29	1	20.0	4	80.0	5	100
30-39	14	82.4	3	17.6	17	100
40-49	10	58.8	7	41.2	17	100
50++	9	75.0	3	25.0	12	100
Total	34	66.7	17	33.3	51	100
Chi square for trend = 0.908; p=0.340; Not significant						

STRENGTH AND LIMITATION

This study provides new data on the prevalence of HPV and genotype distribution of HPV among women in Nepal over a period of 3 years. A wide spectrum of HPV genotypes were seen among different age group, focusing attention on devising the optimum strategy for cervical cancer prevention. The study was limited by genotypic detection of the HPV. Pathological or cytological changes were not determined. Also, as full clinical information was not available, further exploration and correlation with the relevant patient characteristic, including the behavioural and sociodemographic factors were not possible.

CONCLUSION

Our study demonstrated higher HPV prevalence among young adult. Furthermore It provides valuable insight into the HPV genotype distribution among women in Nepal, which may lead to cervical cancer and adverse clinical outcomes, including the mortality. This study emphasizes the need for the introduction and standardization of screening practices in Nepal. Moreover, surveillance studies are needed to enable the identification of HPV type distribution and to determine the potential impact of the introduction of HPV vaccines. These data are essential to health care decision and policymakers in prioritizing available public health interventions.

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CONFLICT OF INTEREST: None declared.

AUTHORS CONTRIBUTIONS

MM conceived the study; MM, RM designed the study; MM, SMP and SB collected the data; MM, AS and JKS analysed

the data. MM wrote the first draft of the paper; all author agreed the final text.

REFERENCES

1. Crosbie EJ, Einstein MH, Franceschi S and Kitchener HC: Human papillomavirus and cervical cancer. Lancet *382(9895)*: 889-899, 2013.
2. De Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. 2017;141(4):664–70.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021:71:209–49. doi:10.3322/caac.21660.
4. World Health Organization. Human Papillomavirus(HPV) and cervical cancer. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer. Accessed 22 Feb, 2022
5. Global strategy to accelerate the elimination of cervical cancer as a public health problem. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
6. GLOBOCAN 2020. Global Cancer Observatory: Cancer Today [Internet]. Lyon, France: International Agency for Research on Cancer; 2020 [cited 2021 December 17]. Available from: https://gco.iarc.fr/today
7. Shrestha AD, Andersen JG, Gyawali B, Shrestha A, Shrestha S, Neupane D, et al. Cervical cancer screening utilization, and associated factors, in Nepal: a systematic review and meta-analysis. Public Health. (2022) 210:16– 25. doi: 10.1016/j.puhe.2022.06.007
8. Family Health Division. National guidelines for cervical cancer screening and prevention in Nepal. In: Department of Health Services, Ministry of Health and Population, editor. Kathmandu: Family Health Division; 2010.
9. Family Health Services. Cervical cancer screening and prevention in Nepal implementation plan 2016-2020. In: Department of Health Services, Ministry of Health and Population, editor. Teku, Kathmandu: Family Health Services; 2017.
10. Dhimal M, Bista B, Bhattarai S, Dixit LP, Hyder MKA, Agrawal N, et al. Report of non communicable disease risk factors: STEPS survey Nepal 2019. Kathmandu: Nepal Health Research Council; 2020. https://www.who.int/docs/default-source/nepal-documents/ncds/ncd-steps-survey-2019
11. Zeng, H., Chen, W., Zheng, R., Zhang, S., Ji, J. S., Zou, X., et al. (2018). Changing cancer survival in China during 2003-15: a pooled analysis of 17 population-based cancer registries. *Lancet Glob. Health* 6, e555–e567. doi: 10.1016/ S2214-109X(18)30127-X
12. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003, 348:518–527.
13. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents. Volume 100 B. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum.* 2012; 100B: 1–441.
14. Cogliano VJ, Baan R, Straif K, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, et al. Preventable exposures associated with human cancers. JNCI J Natl Cancer Inst. 2011;103:1827–39.
15. Halec G, Alemany L, Lloveras B, Schmitt M, Alejo M, Bosch FX, Tous S, Klaustermeier JE, Guimerà N, Grabe N, Lahrmann B, Gissmann L, Quint W, Bosch FX, de Sanjose S, Pawlita M. Pathogenic role of the eight probably/ possibly carcinogenic HPV types 26, 53, 66, 67, 68, 70, 73 and 82 in cervi- cal cancer. J Pathol. 2014;234(4):441–51.
16. Gultekin M, Ramirez PT, Broutet N, Hutubessy R. World Health Organiza- tion call for action to eliminate cervical cancer globally. Int J Gynecol Cancer. 2020;30:426-7
17. G. Ronco, N. Segnan, P. Giorgi-Rossi et al., "Human papillo- mavirus testing and liquid-based cytology: results at recruit- ment from the new technologies for cervical cancer randomized controlled trial," Journal of the National Cancer Institute, vol. 98, no. 11, pp. 765–774, 2006.
18. WHO, WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, WHO Guide- lines Approved by the Guidelines Review Committee, Geneva, 2nd edition, 2021.
19. Johnson D, Bhatta MP, Smith JS, Kempf MC, Broker TR, Vermund SH, et al. Assessment of high-risk human papillomavirus infections using clinician- and self-collected cervical sampling methods in rural women from Far Western Nepal. PLoS One. 2014;9(6):e101255.
20. Khoo SP, Bhoo-Pathy N, Yap SH, et al (2018). Prevalence and sociodemographic correlates of cervicovaginal human papillomavirus (HPV) carriage in a cross-sectional, multiethnic, community-based female Asian population. Sex Transm Infect, 94, 277-83

21. Srivastava S, Gupta S, Roy JK. High prevalence of oncogenic HPV-16 in cervical smears of asymptomatic women of eastern Uttar Pradesh, India: a population-based study. J Biosci. 2012;37:63–72.
22. Sherpa AT, Clifford GM, Vaccarella S, Shrestha S, Nygard M, Karki BS, et al. Human papillomavirus infection in women with and without cervical cancer in Nepal. Cancer Causes Control. 2010;21:323–30.
23. Bhatta, M.P., Johnson, D.C., Lama, M. *et al.* High-risk human papillomavirus infection and abnormal cervical cytology among Nepali and Bhutanese refugee women living in eastern Nepal. *BMC Infect Dis* **17**, 73 (2017). https://doi.org/10.1186/s12879-017-2186-2
24. Shakya S, Syversen U, Åsvold BO, Bofin AM, Aune G, Nordbo SA, et al. Prevalence of human papillomavirus infection among women in rural Nepal. Acta Obstet Gynecol Scand 2017;96:29–38. DOI: 10.1111/aogs.13036
25. Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, Munoz N: Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 2008, 26(Suppl 10):K1–K16.
26. Jacobs MV, Walboomers JM, Snijders PJ, Voorhorst FJ, Verheijen RH, Fransen-Daalmeijer N, Meijer CJ: Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age- related patterns for high-risk and low-risk types. Int J Cancer 2000, 87:221–227.
27. Berumen J, Ordonez RM, Lazcano E, Salmeron J, Galvan SC, Estrada RA, Yunes E, Garcia-Carranca A, Gonzalez-Lira G, Madrigal-de la Campa A: Asian- American variants of human papillomavirus 16 and risk for cervical cancer: a case-control study. J Natl Cancer Inst 2001, 93:1325–1330.
28. Rolon PA, Smith JS, Munoz N, Klug SJ, Herrero R, Bosch X, Llamosas F, Meijer CJ, Walboomers JM: Human papillomavirus infection and invasive cervical cancer in Paraguay. Int J Cancer 2000, 85:486–491.
29. Wang, X., Song, Y., Wei, X. *et al.* Prevalence and distribution of human papillomavirus genotypes among women attending gynecology clinics in northe*rn Henan Province of China. Virol J* ***19****, 6 (2022). https://doi.org/10.1186/s12985-021-01732-8*
30. Bruno MT, Scalia G, Cassaro N, Boemi S. Multiple HPV16 infection with two strains: a possible marker of neoplastic progression. BMC Cancer. 2020;20(1):444.
31. Thapa N, Maharjan M , Shrestha G, et al. Prevalence and type-specific distribution of human papillomavirus infection among women in mid-western rural, Nepal- A population-based study. BMC Infec Diseas 18:338 (2018) https://doi.org/10.1186/s12879-018-3175-9
32. Sanjosé S, Díaz M, Castellsagué X, Clifford G, Bruni L. Worldwide prevalence and genotype distribution of cervical HPV in women with normal cytology. Lancet Infect. 2007;7:453–9. Available from:https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(07)70158-5/fulltext.
33. Bao Y-P, Li N, Smith JS, Qiao Y-L, ACCPAB members. Human papillomavirus type distribution in women from Asia: a meta-analysis. *Int J Gynecol Cancer* 2008;18:71–79.
34. Hamlin-Douglas LK, Coutlée F, Roger M, Franco EL, Brassard P. Prevalence and age distribution of human papillomavirus infection in a population of Inuit women in Nunavik, Quebec. Cancer Epidemiol Biomark Prev. 2008;17: 3141–9.
35. Franceschi S, Rajkumar R, Snijders P, Arslan A, Mahe C, Plummer M, et al. Papillomavirus infection in rural women in southern India. Br J Cancer. 2005;92:601–6
36. Franceschi S, Herrero R, Clifford GM, Snijders PJ, Arslan A, Anh PTH, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer. 2006;119:2677–84.
37. Cuschieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore C, et al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. J Clin Pathol. 2004;57(1):68–72. doi: 10.1136/jcp.57.1.68. [DOI] [PMC free article] [PubMed] [Google Scholar]
38. Trottier H, Mahmud S, Costa MC, Sobrinho JP, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, Franco EL: Human papillomavirus infections with multiple types and risk of cervical neoplasia. Cancer Epidemiol Biomark Prev 2006, 15:1274–1280.
39. Spinillo A, Dal Bello B, Gardella B, Roccio M, Dacco MD, Silini EM: Multiple human papillomavirus infection and high grade cervical intraepithelial neoplasia among women with cytological diagnosis of atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions. Gynecol Oncol 2009, 113:115–119.
40. Department of Health Services, Ministry of Health & Population (2010) National Guideline for Cervical Cancer Screening and Prevention in Nepal. http://whonepal.healthrepository.org/bitstream/123456789/498/1/National%20Guideline%20for%20CCSP.pdf.