PriMera Scientific
Medicine and Public Health
Volume 4 Issue 1 January 2024
DOI: 10.56831/PSMPH-04-108
ISSN: 2833-5627

PriMera Scientific Publications

To Study the Prevalence and Distribution of HPV High Risk Types in Rural Population of Odisha

Type: Case Study
Received: November 16, 2023
Published: December 26, 2023

Citation:
Sarjan Shah., et al. "To Study the Prevalence and Distribution of HPV High Risk Types in Rural Population of Odisha". PriMera Scientific Medicine and Public Health 4.1 (2024): 03-07.

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Abstract

The second most prevalent cancer in women globally and the primary disease in Indian women is cervical cancer, which is mostly caused by infection with the Human Papillomavirus (HPV). Although there are a number of ways to prevent cervical cancer, vaccination is now thought to be the most effective strategy due to the availability vaccinations in the market. The efficiency, immunogenicity, and safety of the vaccination have all been the subject of several research. Particularly in the Indian scenario, there are still concerns and disagreements over immunization requirements, the need for booster shots, and cost- effectiveness.

More than one-fourth of malignancies worldwide associated with infection are caused by the human papillomavirus (HPV). In this paper, the high risk types of HPV in India are summarized, with a particular emphasis on rural parts of Odisha.

The spread caused by human papillomavirus (HPV) is diverse and varies from region to region. Given the limited cross protection provided by the current HPV vaccinations, it is crucial to comprehend the distribution of HPV genotypes among the various populations in order to predict the effectiveness of the present vaccine and develop alternative vaccination strategies. The current study focused into the distribution of HPV genotypes in women in Orrisa, in rural parts.

Keywords: Human Papillomavirus (HPV); cervical cancer; vaccinations

Introduction

The human papillomavirus is a sexually transmitted infection that is extremely common and has a wide genotypic distribution [1]. In 2020, it was estimated that there were 604 000 new cases of cervical cancer and 342 000 deaths worldwide, making it the fourth most widespread cancer among females. Low- and middle-income countries accounted for approximately 90% of the global new cases and fatalities in 2020 [2].

HPV infection causing cervical cancer can be divided into high risk type and low risk type depending upon their potent risk. HPV low-risk types include; 6, 11, 42, 43, and 44 whereas HPV high-risk types include; 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 [3]. To reduce the burden of prevalence of cervical cancer it is the need of the hour to have effective immunization programs with point of care and affordable screening. A proper immunization program would require knowledge of the most prevalent genotype in population. Cervical cancer is still very much prevalent in rural parts of India as it compromises of 70% of Indian population living there as compared to the cosmopolitan parts of India [4].

The cervical cancer programs in India are not well executed due to lack of documentation of epidemiology of HPV infection and pattern of the positive strains in different parts. Literature shows reports from research which have examined small parts of particular subcontinent which demonstrate significant heterogeneity in the prevalence of HPV infection and genotype distribution, which is explained by the country's diverse socioeconomic and climatic conditions [5].

This calls for a comparable assessment of the same in other national geographic regions. Here, we identified the genotypes, prevalence, and risk factors among women in Odisha who had or hadn't developed cervical cancer.

Materials and Methodology

Study population and sample collection

Samleswari clinic of Odisha was primary sample collection center for this study and enrolling of subjects. This study was conducted from February 2023 to April 2023. Married women, above 25 years showing any symptoms like abnormal vaginal discharge, painful menses or lower abdominal pain and other common symptoms of HPV were included in the study under the observation of clinicians. Women who are unmarried, pregnant or undergoing treatment were excluded from the study.

Clinical data including patient history and age were collected by interviewing the patients by predesigned questionnaire. Sample collection was done by using cytobrush and stored inside standardized viral transport media and transported to Research division of Accurate Diagnostics Private limited.

DNA extraction and HPV DNA detection

Following the manufacturer's instructions, the QIA amp DNA Blood Mini Kit (Qiagen) was used to extract DNA from a 200µl aliquot collected sample. For confirming the adequacy of extracted DNA, amplification of the human beta-globin gene was checked.

PCR was conducted to identify HPV by directing PGMY09/PGMY11 primers (MY11: 50- GCMCAGGGWCATAAYAATGG-30; MY09: 50-CGTCCMARRGGAWACTGATC-30-) towards a 450-bp part of the HPV L1 gene. The products which showed positive bands on the gel were confirmed using 14 HPV high risk type primers. Type-specific primers were used to detect HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV, HPV 66, HPV 68. Primers were obtained by studying the published. The entire process required two primer sets, the first PCR was with the MY-GP5+ and GP6+ primers. The second set of primers were used for confirmation with Nested PCR of 14 high risk type specific primers mentioned above.

Genotyping of HPV by nested type specific multiplex E6/E7 PCR

The test was developed to identify a broad range of HPV genotypes, including all high-risk kinds. A nested PCR-based method was selected for improved sensitivity. First-round PCR using GP-E6/E7 consensus primers should allow for initial amplification of the ge-

nomic DNA of all known mucosal HPV genotypes and give sufficient sample for nested PCRs with type-specific primers.

The isolated DNA was screened for HPV using standard nested PCR with the MY09/11 primer set [6] and GP5+/6+ set obtained from literature [7]. For PCR cycle the 5ul of DNA was amplified using the MY-GP set of primers and Emerald GT Takara master mix. Cyclic conditions were 94° C for 3 minutes, then amplification cycle of following conditions for 40 cycles; 94° C for 45 seconds, annealing temperature for MY as 48° C and 55° C for GP, and final extension of 72° C. In similar way the product of PCR for the first round of PCR was subjected to type specific 14 primers mentioned above. The nested PCR had cyclic conditions as 94° C for 1 minute, amplification cycles of 30, for 94° C for 30 seconds., 70° C for 40 seconds, and 72° C for 1 minute followed by final extension of 72° C for 2 minutes.

The amplified PCR products were loaded on 2% gel to check the bands against the 100bp ladder with positive controls.

Genotyping by sequencing

High risk HPV type products which were positive were reconfirmed using Sanger sequencing. Sequencing was done using Big Dye Terminator sequencing kit (Applied Biosystems) and analyzed in silico on Genetic Analyzer 3500 (Applied Biosystem). The results were then compared with the sequences available in the Genebank database using the National Center for Biotechnology Information BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

Total 88 samples were taken for the study. Out of 88 samples we found 5 to be positive. From this set of data, prevalence as distribution were studied. Out of 5 patients who were found to be HPV positive, none were in age group of 20-30 age, 1 was in age group of 30-40 (1.13%), 1 was in age group of 40-50(1.13%), the highest positives were 2, in age group of 50-60 (2.27%) and 1 was in age group of 60-70 (1.13%) as seen in Figure 1.

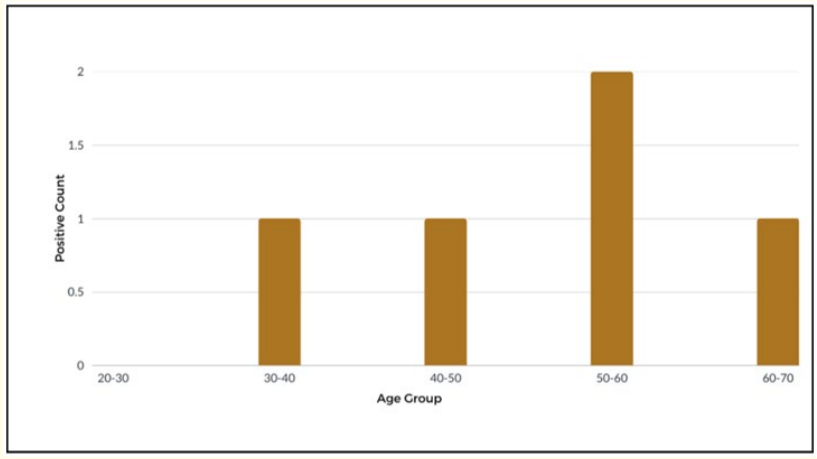

Figure 1: Age wise distribution of the positive genotypes of HPV high risk types.

The prevalence of any of the high-risk types in the study population was 5.68% (5/88). The most prevalent strain was that of HPV 33 type (50%), followed by HPV 16 (33%). The other high-risk HPV type comprised of 16.7% of total population.

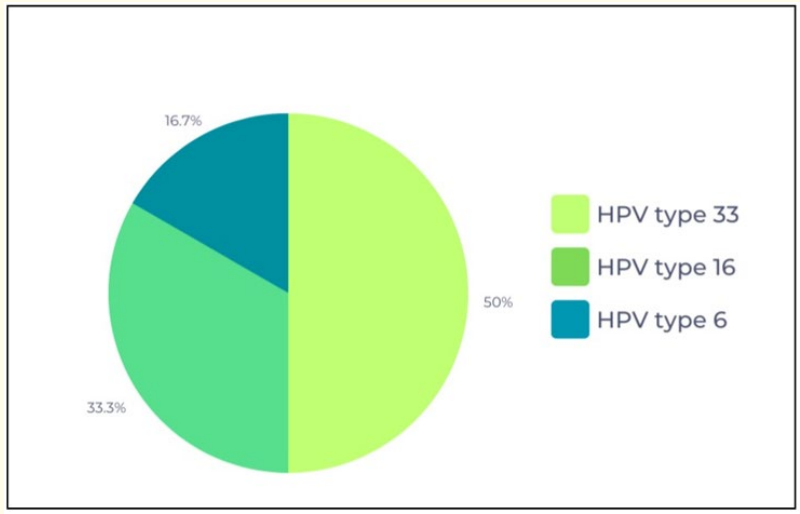

Figure 2: Prevalence of different types of HPV amongst the studied population.

Discussion

Worldwide 70% of cervical cancer is known to be caused by HPV 16 & HPV 18 [8]. The prevalence of different HPV types varies depending upon the region and ethnicity. Several studies show that the most common HPV high risk type is HPV 16 in Indian population [8].

A multivariate literature from Indian population showed that the distribution of HPV genotypes causing the cervical cancer were HPV 16 and HPV 18 followed by HPV 33. They have also shown that the most common genotypes with not much differences in all parts of India were 16, 18, 31, 33 and 45 [5].

Hence the immunization program available for HPV 16 & 18 should be also extended to the other HPV high risk types depending upon the findings in that particular area. A second generation HPV vaccine which would provide affordable solution for specific regions is needed [9].

In other studies, it is also shown that the incidence of HPV also varied with age and that there was a little or no increase in HPV for women who were above 45 years of age. These changes could be because of the hormonal and immunological health [10].

Our findings emphasize the need of conducting population-based HPV genotyping research prior to establishing an HPV immunization program.

Conclusion

In rural Odisha, the human papillomavirus type 16 and 33 genotype was discovered to be the most prevalent. We discovered that the advanced age was also major contributing factor in the distribution of the high-risk types. Our findings emphasize how crucial it is to incorporate these high-risk genes into vaccine development to make it more locally specific. In order to create effective novel HPV vaccines, it is essential to continuously analyses the geographic distribution of HPV genotypes in other parts of India.

Declarations

Conflict of interest: None.

Availability of Data: The raw data of this study will be available on demand.

Funding: None.

Acknowledgements: We would like to thank Samleswari Clinic for helping in sample collection.

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