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Original Research Article

HPV Genotypes and DNA Methylation Analysis in Patients with Anogenital Warts: A Cross-Sectional Study from Eastern India

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Abstract:

Background: Anogenital warts are predominantly attributed to human papillomavirus (HPV) infection, yet the influence of epigenetic factors, particularly DNA methylation, remains uncertain. Clarifying these relationships could enhance understanding of pathogenesis and potential oncogenic biomarkers.

Aim: This cross-sectional study aims to explore the prevalence of HPV genotypes and assess DNA methylation patterns in patients diagnosed with anogenital warts in eastern India.

Material and Methods: Patients aged 18 to 60 years, diagnosed with anogenital warts, were recruited from a tertiary care teaching hospital. Anogenital scrapings were subjected to RT-PCR for HPV genotyping and ELISA for DNA methylation analysis.

Results: Among participants, 93% of anogenital warts showed no association with HPV, while 2% were linked to HPV 16 and 1% to HPV 18, with an equal gender distribution. However, no significant differences in DNA methylation patterns were observed between patients with anogenital warts and healthy controls.

Conclusion: This cross-sectional investigation reveals a limited correlation between anogenital warts and HPV infection or DNA methylation in the studied population. Future longitudinal studies are warranted to explore additional factors influencing anogenital wart pathogenesis and identify potential biomarkers for oncogenic risk assessment.

Keywords: Anogenital warts, Human papillomavirus (HPV), HPV genotypes, DNA methylation.

Introduction

Human papillomavirus (HPV) infections are among the most prevalent viral infections worldwide, posing significant health risks, particularly concerning the development of anogenital warts and cervical cancer. HPV, a small non-enveloped virus belonging to the Papillomaviridae family, harbors highly conserved double-stranded DNA as its genetic material, typically measuring approximately 55 nm in size [1].

According to the World Health Organization (WHO), HPV infection ranks as one of the most common viral infections globally, with a notable prevalence among women with Normal Cervical Cytology (NCC), standing at 9.9% in the present scenario [1]. Cervical cancer, primarily caused by persistent HPV infections, constitutes a substantial global health burden, with an estimated 570,000 new cases diagnosed annually, representing 7.5% of all female cancer deaths worldwide [2]. Alarmingly, over 85% of these deaths occur in low- and middle-income countries, underscoring the urgent need for comprehensive preventive strategies [2]. In India, the burden of cervical cancer is staggering, with approximately 122,844 new cases diagnosed each year, leading to 67,477 fatalities [3]. The vulnerability of Indian women to cervical cancer is further highlighted by the vast population of 32.2 million women aged 15 years and older at risk of developing the disease [3].The oncogenic potential of HPV stems from its ability to undergo mutations following cellular infection, leading to viral genome integration and subsequent deregulation of critical proteins such as E2. E2, a repressor of oncogenic proteins, plays a pivotal role in maintaining cellular homeostasis. However, its disruption promotes carcinogenesis by facilitating the overexpression of E6 and E7 proteins, which, despite their lack of enzymatic activity, wield considerable influence over host cell activities through interactions with cellular proteins [4] .

Upon HPV infection, an incubation period averaging from three weeks to eight months ensues before symptomatic presentation, often in the form of warts. The virus can remain dormant within epithelial cells, manifesting as subclinical HPV infection, which is a significant concern given its

potential for transmission and oncogenicity. Studies suggest that the rate of subclinical HPV infection may be as high as 40%, emphasizing the need for vigilance in detection and management [5].

Clinical manifestations of HPV infection span a spectrum from benign lesions to malignancy, with HPV types classified as high-risk or low-risk based on their association with cervical cancer and precursor lesions. Notably, HPV types 16 and 18 are implicated in nearly 70% of cervical cancer cases, highlighting their oncogenic significance. Furthermore, HPV 16 has been implicated in over 90% of HPV-positive oropharyngeal malignancies [6]. HPV replicates within the basal cells of stratified squamous epithelium, exploiting breaches in the epithelial barrier for infection. The gradual onset of the infectious process necessitates 12-24 hours for transcription initiation, during which the host immune system plays a critical role in neutralizing the virus [7].

Among the most prevalent manifestations of HPV infection are anogenital warts (AGW), caused primarily by non-oncogenic strains such as HPV types 6 and 11. These strains are responsible for 90% of AGW cases, with occasional co-infections involving high-risk HPV types [8]. Notably, HPV types 6 and 11 have also been associated with warts in other anatomical locations, including the conjunctiva, nasal passages, oral cavity, and larynx. Despite the widespread prevalence of HPV, many infections remain asymptomatic, complicating efforts for early detection and intervention. Moreover, the high recurrence rate of AGW exacerbates the disease burden, underscoring the importance of comprehensive management strategies.

In addition to genetic mutations, epigenetic modifications such as DNA methylation play a crucial role in HPV-associated carcinogenesis. DNA methylation, a heritable epigenetic mark, involves the covalent transfer of a methyl group to the C5 position of the cytosine ring of DNA by DNA methyltransferases (DNMTs). Aberrant DNA methylation patterns, including promoter-specific hypermethylation and global hypomethylation, contribute to neoplasia and tumor growth, thereby influencing disease progression [9,10].This highlights the intricate interplay between HPV infection, anogenital warts pathology, and DNA methylation, underscoring the complexity of HPV-associated diseases and the urgent need for further research to elucidate underlying mechanisms and identify novel therapeutic targets.

Materials and Methods

The cross-sectional study received ethical approval from the Institutional Ethics Committee of SCB Medical College, Cuttack, conducted over a span of two years, from March 2021 to September 2022.Prior to participation, written informed consent was then obtained from each participant, affirming their voluntary agreement to take part in the research. The study comprised 180 participants selected through simple random sampling. Among these, 85 individuals were diagnosed with anogenital warts, while the remaining 95 participants served as age-, sex-, and socioeconomic status-matched controls. Eligible participants were individuals aged 18 to 60 of either gender, presenting to the Department of Skin and Venereal Diseases. The inclusion criteria for this study encompassed individuals aged 18 to 60 years, irrespective of gender, presenting at the Department of Skin and Venereal Diseases. Eligible participants were diagnosed with anogenital warts, forming the primary focus of the investigation. Exclusion criteria included individuals who declined to provide written informed consent, individuals with other sexually transmitted diseases and morbidly ill were excluded from the study to maintain homogeneity within the study population and minimize confounding factors during analysis. Anogenital scrappings of the subjects were collected in HPV Roche collection media and brush, which were further analysed for HPV genotypes by Real time Polymerase chain reaction (rt-PCR) in Cobas x 480 and Cobas z 480 according to manufacturer's protocol [11]. 2ml of venous blood was collected in EDTA vials for DNA extraction and stored at 2-8 degree celcius for DNA methylation analysis by Zymo Research 5-mc DNA ELISA kit protocol [12].

Results:

Table 1: Demographic Characteristics of Study Participants

Characteristic	Anogenital Warts (n=85)	No Anogenital Warts (n=95)
Gender		
- Male	30 (35.3%)	45 (47.4%)
- Female	55 (64.7%)	50 (52.6%)
Age Group		
- <20 years	9 (10.6%)	10 (10.5%)
- 21-40 years	57 (67.1%)	65 (68.4%)
- 41-60 years	15 (17.6%)	20 (21.1%)
Socioeconomic Status		
- Upper Class	8 (9.4%)	10 (10.5%)

- Middle Class	24 (28.2%)	30 (31.6%)
- Lower Class	53 (62.4%)	55 (57.9%)
Educational Background		
- Primary Education	15 (17.6%)	20 (21.1%)
- Secondary Education	45 (52.9%)	50 (52.6%)
- Higher Education	25 (29.4%)	25 (26.3%)

Table 2: Distribution of Anogenital Warts Patterns by Age Group and Origin

Age Group	Total (%)	New (%)	Recurrent (%)	Resistant (%)	Unknown (%)	External (%)	Internal (%)	Both (%)
<20 years	9	4	3	1	1	5	2	2
21-40 years	57	26	11	15	6	30	13	14
41-60 years	15	2	1	6	2	5	3	6
Total	85	32	15	22	9	40	18	22

Table 3: Distribution of Anogenital Warts by Origin

Types of Warts	Total (%)	Males (%)	Females (%)	Chi-square statistic	p-value*
External	38 (44.7%)	12 (14.1%)	26 (30.6%)	0.11	0.95
Internal	20 (23.5%)	7 (8.2%)	13 (15.3%)		
Both	27 (31.8%)	9 (10.6%)	18 (21.2%)		
Total	85	28	57		

Table 4: DNA Methylation Status of Study Participants (case) vs Healthy volunteers (control):

Group	Total	DNA methylation levels (%)	F statistic	p value
Case	85	0.8±0.1	5.29	3.9
Control	95	1.8±0.23		

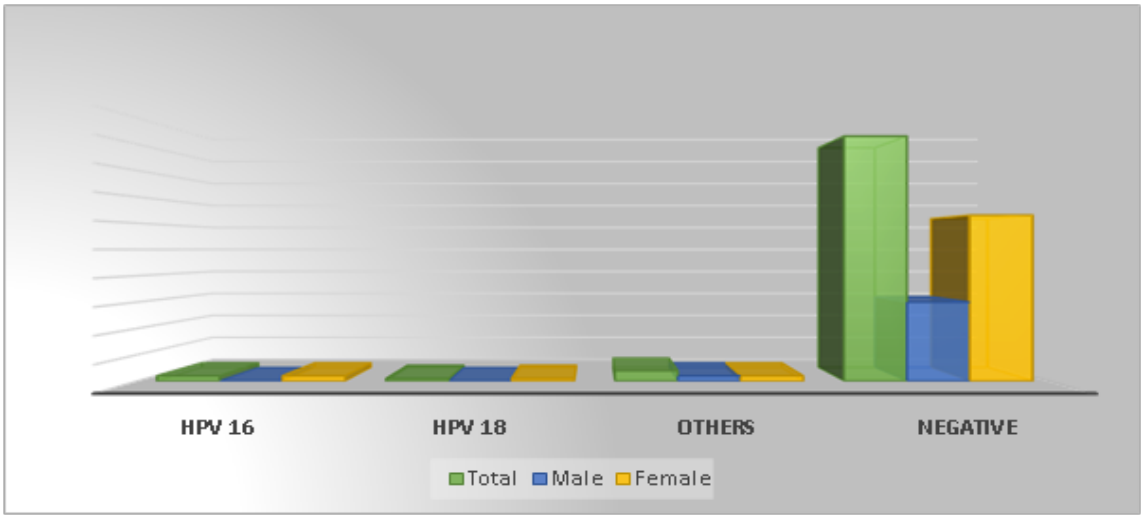

Figure 1: HPV Status in Study Group

A vast majority had negative association between HPV and the anogential warts. 93% had no association. 2% cases were associated with HPV 16, 1% case was associated with HPV 18 and 4% were associated with other strains. The 2 cases associated with HPV 16 and 1 case associated with HPV 18 were found in female subjects. Among the 4 cases associated with other strains, it was equally distributed among the either gender i.e. 2% in males and rest 2% in females.

Discussion

The present cross-sectional study aimed to investigate the incidence of Human Papillomavirus (HPV) infection and analyse HPV genotyping patterns among patients diagnosed with anogenital warts (AGW).

Our findings offer significant insights into the demographic distribution, HPV association, and AGW patterns observed in the study

population.HPV is a leading cause of cervical cancer and also causes benign condylomata acuminata, or genital warts. Condylomata acuminata (CA), commonly referred to as external genital warts (EGW), are among the most prevalent sexually transmitted diseases (STDs) affecting the general population [13]. The overall prevalence of genital warts in India is estimated to be 1.07% [14]. Genital warts (GW) or anogenital warts (AGW) are among the most common sexually transmitted diseases, characterized by high infectivity. They are primarily caused by specific strains of Human Papillomavirus (HPV), with 90% of cases attributed to non-oncogenic HPV types 6 and 11. Occasionally, other HPV types such as 16, 18, 31, 33, and 35 are found in anogenital warts, often in co-infection with HPV 6 or 11 [15]. Beyond anogenital warts, HPV types 6 and 11 are also associated with warts in the conjunctiva, nasal passages, oral cavity, and larynx.

Nearly all sexually active individuals are at risk of contracting at least one form of HPV at some point in their lives [16,17]. However, many infections remain asymptomatic. The high recurrence rate of anogenital warts exacerbates the disease burden, making effective management and prevention critical. Our results indicated a higher prevalence of AGW among females, comprising 64.7% of cases compared to 35.3% in males, consistent with previous research [18,19]. Male HPV infection is significant due to its potential for transmission to women and its association with anal cancer, as well as certain penile and oral cancers [20]. Studies have found that the prevalence of genital warts among STI clinic visitors in India ranges from 2% to 25%, with a higher prevalence in men compared to women and higher regional prevalence in northern areas like Delhi (2.17%) compared to southern regions like Bangalore (0.40%) [21,22]. Despite the female predominance in our study, it's essential to acknowledge that men are also at significant risk of HPV infection and genital warts [18]. This underscores the necessity for gender-neutral preventive measures and awareness initiatives.

The majority of AGW patients in our study belonged to the age group of 21 to 40 years, consistent with findings demonstrating a higher prevalence among young adults [18]. Another study found the highest AGW prevalence in patients aged 25 to 29 (1.42%) and reported that over 80% of infected individuals are between 17 and 33 years old [23]. A separate study reported a median patient age of 24 years, with the range spanning from 2 to 74 years, noting that women aged 21 to 25 were diagnosed of genital warts, followed by those aged 16 to 20 and 26 to 30 [24].

While genital warts are associated with sexually transmitted diseases, some studies suggest a negative correlation between genital herpes, hepatitis B, Chlamydia trachomatis, and Neisseria gonorrhoeae infections [25]. HIV infection and the number of sexual partners in the past year are significant risk factors for both prevalent and incident genital warts [26]. A noteworthy proportion of study participants belonged to the lower socioeconomic class with limited educational attainment, aligning with previous research [27]. Their study indicated that women below the poverty line had an HPV prevalence of 72.2%, which dropped to 42.3% for women of average socioeconomic status and was non-existent for those with a high socioeconomic status, demonstrating that HPV prevalence decreases with higher socioeconomic class ($p = 0.002$). However, other studies have shown no significant differences in socioeconomic factors such as education, occupation, or household income when comparing HPV cases and controls [28]. This highlights the impact of socioeconomic factors on HPV infection awareness, prevention, and management, as lower socioeconomic status has been associated with a higher prevalence of HPV.

Even after receiving complete treatment, genital warts can persist due to the ability of the virus to remain dormant within epithelial cells for extended periods, leading to recurrence. The recurrence of genital warts is common, with the majority reappearing within three months of initial infection, and long-term remission rates remain unknown. Factors such as host immune suppression, infection with high-risk HPV subtypes, and greater patient age contribute to the persistence of warts over time. Although genital warts are generally benign, leaving them untreated can result in various outcomes, including resolution, persistence, or an increase in size or number.

Anogenital warts are highly infectious. Patel et al., in a systematic review, reported that the annual incidence of any AGWs (new and recurrent) for all individuals ranged from 160 to 289 per 100,000, with a median of 194.5 per 100,000 [19]. New AGW incidence rates ranged from 103 to 168 per 100,000 males, with a median of 137 per 100,000, and from 76 to 191 per 100,000 females, with a median of 120.5 per 100,000 annually. Recurrent AGWs were observed to occur at rates up to 110 per 100,000 in females and 163 per 100,000 in males [27,29]. Another study revealed that there are 500,000 to one million new cases diagnosed annually in the United States alone [9].

In our study, 39% were newly diagnosed cases, 24% were recurrent cases, and 27% were resistant cases. This is similar to another study where newly diagnosed cases were the most numerous (74.07%), followed by recurrent cases (56.24%) and resistant cases (43.76%) [17]. A multicentric observational study found that 69% of the patients were newly diagnosed with genital warts, 15% had recurrent

genital warts, 6% had resistant genital warts, and for 10% the type of genital warts was not specified [23]. In a study of 70,000 Nordic women, the recurrence rate was 1.3% over the past 12 months. The virus can remain dormant within epithelial cells for extended periods, leading to recurrence. Most genital warts reappear within three months of infection despite treatment, and long-term remission rates are still unknown [21]. Significant risk factors for long-term wart persistence include host immune suppression, HPV infection with high-risk subtypes, and older patient age [22,30]. Although genital warts are common and benign, untreated genital warts may resolve, remain the same, or increase in size or number.

In our study, 45% of the warts were external and 23% were internal. Women with external lesions had them on the fourchette, labia minora and majora, clitoris, urethral meatus, perineum, anal region, vestibule, introitus (vaginal entrance), and vagina, while internal lesions were found on the ectocervix. Men's external lesions included the glans, coronal sulcus, fraenulum, foreskin, scrotum, perineum, anal region, penile shaft, and meatus, with internal lesions located in the urethra. This is consistent with another study where 74% of patients had external lesions, 21% had both internal and external lesions, and 5% had only internal lesions [23].

Genital warts are caused by specific strains of human papillomavirus (HPV), with over 40 strains capable of causing warts in the genital area. Although not all HPV strains cause genital warts, all genital warts are caused by some strain of HPV. In our study, we detected HPV strains 16, 18, and other unspecified strains. Previous research has identified up to 37 anogenital HPV DNA genotypes [6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108 [30-32]. Our study identified high-risk oncogenic strains HPV 16 and 18. Similarly, another study found that while low-risk strains were present in approximately 15% of cases, nearly 85% of the study population tested positive for either high-risk strains or a combination of high and low-risk strains [33].

DNA methylation is an inheritable epigenetic modification that involves the addition of a methyl group to the C5 position of the cytosine ring in DNA-by-DNA methyltransferases (DNMTs). This process is managed by a family of DNMTs, including DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L [34]. DNMTs exhibit a preference for methylating hemi-methylated DNA in vitro and are localized to replication foci during the S phase of the cell cycle [35]. Alterations in DNA methylation patterns, particularly promoter-specific hypermethylation and global (genome-wide) hypomethylation, are believed to play a role in neoplasia and tumour development [36].

The epigenetic characteristics of cancer often include global hypomethylation and locus-specific hypermethylation of CpG islands (CGIs) [37]. Hypomethylation typically results from the loss of methylation at normally heavily methylated repetitive elements, such as satellites (e.g., SAT2) and retrotransposons (e.g., LINES), leading to genomic instability and the activation of oncogenes [38]. Abnormal DNA methylation, particularly hypomethylation, disrupts replication and the cell cycle, contributing to tumour formation. During the progression of cervical cancer, there is an increase in rRNA levels, which is also associated with epigenetic changes, such as decondensation and hypomethylation of rDNA promoters. DNA methylation inhibits transcription by preventing proteins from binding to the methylated DNA [39].

HPV strains 16 and 18 are highly oncogenic. Cancer can result from factors affecting gene activity through genetic and epigenetic changes, such as DNA methylation. A study suggested a significant correlation between methylation status and HPV-induced oncogenesis, noting that the promoter regions of high-risk HPV-16 and 18 have minimal methylation compared to low-risk HPV-11 [7]. Since DNA methylation is a potential biomarker for oncogenesis, we analysed promoter region methylation patterns between patients with anogenital warts and healthy participants without warts. Our study found DNA hypomethylation in subjects with anogenital warts compared to healthy subjects.

The emerging role of specific methylation events in controlling the viral life cycle and their contribution to HPV-mediated neoplastic transformation suggests that treating HPV-transformed cells with demethylating drugs, such as the DNMT inhibitor 5-aza-2'-deoxycytidine, may reverse aberrant methylation patterns. Improved understanding of these epigenetic modifications in HPV-infected cells may enable truly tailored therapies for HPV-driven cancers. These novel treatments primarily target epigenetic alterations in HPV-induced pre-invasive and invasive tumours [40,41]. By understanding the functional significance of these epigenetic modifications, personalized therapies for HPV-driven cancers may become feasible, leading to improved patient outcomes and disease management.

Limitations of our study include its cross-sectional design and potential selection bias. Future research should focus on longitudinal studies to elucidate the natural history of AGW and explore the effectiveness of targeted interventions, including

HPV vaccination and educational programs, in reducing HPV-related disease burden.

In conclusion, our study provides valuable insights into the demographic distribution, HPV association, and patterns of AGW in the study population. These findings underscore the importance of comprehensive preventive strategies, including HPV vaccination programs and awareness campaigns, particularly among vulnerable populations.

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References

1. Monteiro JC, Tsutsumi MY, de Carvalho DO, da Silva Costa EDC, Feitosa RNM, Laurentino RV, de Souza Fonseca RR, Silvestre RVD, Oliveira-Filho AB, Machado LFA. Prevalence, diversity, and risk factors for cervical HPV infection in women screened for cervical cancer in Belém, Pará, and Northern Brazil. Pathogens. 2022; 11(9):960.
2. Zhu C, Wang Y, Mao W, Zhang H, Ma J. Prevalence and distribution of HPV types in genital warts in Xi'an, China: A prospective study. BMJ Open. 2019; 9(5):1–6.
3. Khopkar US, Rajagopalan M, Chauhan AR, Kothari-Talwar S, Singhal PK, Yee K, et al. Prevalence and burden related to genital warts in India. Viral Immunol. 2018; 31(5):346–51.
4. British Association for Sexual Health and HIV. United Kingdom National Guideline on the Management of Ano-genital Warts. 2007; 1:1–19.
5. Baken LA, Koutsky LA, Kuypers J, Kosorok MR, Lee SK, Kiviat NB, et al. Genital human papillomavirus infection among male and female sex partners: prevalence and type-specific concordance. J Infect Dis. 1995; 171(2):429–32.
6. Senapati R, Nayak B, Kar SK, Dwibedi B. HPV genotypes distribution in Indian women with and without cervical carcinoma: implication for HPV vaccination program in Odisha, Eastern India. BMC Infect Dis. 2017; 17(1):1–10.
7. Sherman SM, Nailer E, Minshall C, Coombes R, Cooper J, Redman CW. Awareness and knowledge of HPV and cervical cancer in female students: A survey (with a cautionary note). J Obstet Gynaecol. 2016; 36(1):76-80.
8. Centers for Disease Control and Prevention. Genital warts - 2010 STD treatment guidelines. [Internet]. 2018. Available from: www. cdc. gov.
9. Panesar K. Oropharyngeal squamous cell carcinoma. US Pharm. 2018; 43(8)–6.
10. Lakshminarasimhan R, Liang G. The role of DNA methylation in cancer. Adv Exp Med Biol. 2016; 945:151-172. doi: 10.1007/978-3-319-43624-1_7. PMID: 27826838; PMCID: PMC7409375.
11. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, Rush BB, Glass AG, Schiffman M. Elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 and 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst. 2005; 97(14):1072-1079.
12. Kumar BKP, Beaubiat S, Yadav CB, Eshed R, Arazi T, Sherman A, Bouché N. Genome wide inherited modifications of the tomato epigenome by trans-activated bacterial CG methyltransferase. bioRxiv. 2024. doi: 10.1101/2024.04.17.589930.
13. Yanofsky VR, Patel RV, Goldenberg G. Genital warts. Patient Care Health Information, Disease Conditions. 2012; 5(6):25–36.
14. Khopkar US, Rajagopalan M, Chauhan AR, Kothari-Talwar S, Singhal PK. Prevalence and burden related to genital warts in India. Viral Immunol. 2018; 31(5):346–51.
15. Chan CK, Aimagambetova G, Ukybassova T, Kongrtay K, Azizan A. Human papillomavirus infection and cervical cancer: Epidemiology, screening, and vaccination - review of current perspectives. J Oncol. 2019; 2019:1-11.
16. Yanofsky VR, Patel RV, Goldenberg G. Genital warts. Patient Care Health Information, Disease Conditions. 2012; 5(6):25–36.
17. Topazian HM, Kundu D, Peebles K, Ramos S, Morgan K, Kim CJ, Richter KL, Brewer NT, Peris M, Smith JS. HPV vaccination recommendation practices among adolescent health care providers in 5 countries. J Pediatr Adolesc Gynecol. 2018; 31(6):575-582.
18. Singh P, Zumpf KB, Liszewski W. Rates of genital warts after the age of 26: An analysis of the National Health and Nutrition Examination Survey. Int J Women's Dermatol. 2020; 6(5):429–30.
19. Patel H, Wagner M, Singhal P, Kothari S. Systematic review of the incidence and prevalence of genital warts. BMC Infect Dis. 2013; 13(1):39.
20. Palefsky JM. Human papillomavirus-related disease in men: Not just a women's issue. J Adolesc Health. 2010; 46(4)–S19.
21. Sharma VK, Khandpur S. Changing patterns of sexually transmitted infections in India. Natl Med J India. 2004; 17(6):310–9.
22. Mohta A, Jain SK, Kushwaha RK, Singh A, Gautam U, Nyati A, et al. Estimating the impact of extragenital warts versus genital warts on quality of life in immunocompetent Indian

adult patients: A comparative cross-sectional study. Indian J Dermatol. 2021; 66(1):44–8.
23. Delaney EK, Baguley S. Genital warts. BMJ. 2008; 337.
24. Widschwendter A, Böttcher B, Riedl D, Coban S, Mutz-Dehbalaie I, Matteucci Gothe R, et al. Recurrence of genital warts in pre-HPV vaccine era after laser treatment. Arch Gynecol Obstet. 2019; 300(3):661–8.
25. Wen LM, Estcourt CS, Simpson JM, Mindel A. Risk factors for the acquisition of genital warts: Are condoms protective? Sex Transm Infect. 1999; 75(5):312–6.
26. Dareng EO, Adebamowo SN, Famooto A, Olawande O, Odutola MK, Olaniyan Y, et al. Prevalence and incidence of genital warts and cervical human papillomavirus infections in Nigerian women. BMC Infect Dis. 2019; 19(1):1–10.
27. Kapoor CS, Sharma M. Prevalence of HPV infection in reproductive aged female in Delhi NCR region. Clin Epidemiol Glob Health. 2020; 8(2):612–5.
28. Liang X, Carroll X, Zhang W, Zhang W, Liu G, Li S, et al. Socioeconomic and lifestyle factors associated with HPV infection in pregnant women: A matched case-control study in Beijing, China. Reprod Health. 2018; 15(1):1–9.
29. Vora KS, Saiyed S, Joshi R, Natesan S. Prevalence of high-risk HPV among marginalized urban women in India and its implications on vaccination: A cross sectional study. Int J Gynecol Obstet. 2022; 6.
30. Kjær SK, Tran TN, Sparen P, Tryggvadottir L, Munk C, Dasbach E, et al. The burden of genital warts: A study of nearly 70,000 women from the general female population in the 4 Nordic countries. J Infect Dis. 2007; 196(10):1447–54.
31. Boda D, Neagu M, Constantin C, Voinescu RN, Caruntu C, Zurac S, et al. HPV strain distribution in patients with genital warts in a female population sample. Oncol Lett. 2016; 12(3):1779–82.
32. Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011; 128(4):927–35.
33. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. 1998; 338(7):423–8.
34. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature. 2009; 462(7271):315–22.
35. Cheng X, Blumenthal RM. Mammalian DNA methyltransferases: A structural perspective. Structure. 2008; 16(3):341–50.
36. Verlaat W, Van Leeuwen RW, Novianti PW, Schuuring E, Meijer CJLM, Van Der Zee AGJ, et al. Host-cell DNA methylation patterns during high-risk HPV-induced carcinogenesis reveal a heterogeneous nature of cervical pre-cancer. Epigenetics. 2018; 13(7):769–78.
37. Zhu C, Wang Y, Mao W, Zhang H, Ma J. Prevalence and distribution of HPV types in genital warts in Xi'an, China: A prospective study. BMJ Open. 2019; 9(5):1–6.
38. Clarke MA, Gradissimo A, Schiffman M, Lam J, Sollecito CC, Fetterman B, et al. Human papillomavirus DNA methylation as a biomarker for cervical precancer: Consistency across 12 genotypes and potential impact on management of HPV-positive women. Clin Cancer Res. 2018; 24(9):2194–202.
39. Kim YI, Giuliano A, Hatch KD, Schneider A, Nour MA, Dallal GE, et al. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. Cancer. 1994; 74(3):893–9.
40. Doeberitz MVK, Prigge E. Role of DNA methylation in HPV associated lesions. Papillomavirus Res. 2019; 7:180–3.
41. Mammas IN, Spandidos DA. Fighting against human papillomavirus: The 25-year old contribution of the University Of Crete School Of Medicine. J BUON. 2015; 20(1):17–21.