ORIGINAL ARTICLE

Polymerase chain reaction and deoxyribonucleic acid-sequencing based study on distribution of human papillomavirus 16/18 among histopathological types of cervical intra-epithelial neoplasia and primary invasive cervical carcinoma: A scenario in North Bengal, India

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ABSTRACT

Introduction: Human papillomavirus (HPV) types 16/18 are reportedly most common in cervical cancer (CaCx) with geographical variation of genotypes. HPV16 predominates both in squamous cell carcinoma (SCC) and adenocarcinoma in India, contrary to reported global predominance of HPV18 in the latter. Our study was aimed to determine the occurrence of HPV16/18 among histopathological types of cervical intra-epithelial neoplasia (CIN) and invasive CaCx from North Bengal, India and to identify any major deviation from the known Indian scenario of distribution of HPV16/18 genotypes in cases of SCC and adenocarcinoma.

Materials and Methods: This was a retrospective, cross-sectional, case-only type of study, in which 40 cases were histopathologically diagnosed as CIN/CaCx, on which polymerase chain reaction (PCR), deoxyribonucleic acid (DNA)-sequencing and bioinformatics by basic search local alignment tool were performed for HPV-genotyping.

Statistical Analysis: The distribution of HPV genotypes among cases of SCC and adenocarcinoma was compared by Fisher's exact-test.

Results: HPV was detected in 97.5% (39/40) cases. HPV16-infected cases (32/39; 82.05%) predominated over HPV18-infected ones (7/39; 17.95%). However, HPV18-only infection was significantly (P = 0.0045, one-sided Fisher's exact test) more among adenocarcinoma (3/4; 75%) than SCC (2/26; 7.69%) contrary to HPV16-only infection (SCC = 24/26, 92.31%; adenocarcinoma = 1/4; 25%) whereas both CIN3 cases were HPV16-positive.

Conclusion: Predominance of HPV18 over HPV16 in cases of adenocarcinoma in this region was contrasting to that of earlier Indian studies suggesting research on HPV18 related cervical carcinogenesis. PCR and DNA-sequencing could prove to be highly effective tools in HPV detection and genotyping. The study reported HPV16/18 infection in almost 98% of the cases, the knowledge about which might prove useful in future population based studies on HPV genotyping and designing of appropriate HPV-vaccines for this region.

Key Words: Cervical cancer, histopathology, human papillomavirus 16/18, polymerase chain reaction, North Bengal

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INTRODUCTION

Uterine cervical cancer (CaCx) is the third most common cancer among women world-wide with more than 85% of the global burden reported from the developing countries and India shares about one-fourth of the burden (http://globocan.iarc.fr/). The major etiological factor is persistent infection with oncogenic human papillomaviruses (HPVs), whereas early age of marriage, multiparty, smoking, etc., are probable cofactors.[1] HPVs are small, double-stranded deoxyribonucleic acid (DNA) viruses that belong to family papillomaviridae and they have been categorized into high-risk types HPV (HR-HPV), such as 16, 18, 31, 33, etc. and low-risk types HPV (LR-HPV), such as 6, 11, etc., with geographical variation in genotypic distribution.[1] The viral genome encodes for late (L1, L2) and early (oncogenic E6, E7, etc.) transcribing genes.[2]

Early precancerous cervical intra-epithelial neoplasias (CIN1/2) reportedly have higher chances of spontaneous regression compared with advanced CIN3, which progresses more frequently to invasive cancer.[3] Two major histopathological (H/P) types of CaCx are squamous cell carcinoma (SCC; 80-85%) and adenocarcinoma (10%). HPV16 is reportedly the most prevalent type both in cervical SCC and adenocarcinoma in India, while preferential occurrence of HPV18 has been reported in adenocarcinoma globally.[4] Classification systems such as the "World Health Organization classification of Epithelial Tumors of the Uterine Cervix", Modified Broder's, etc., have been used for H/P classification of cervical neoplasm.[5,6] Light microscopy can detect "koilocyte", the morphological marker of HPV infection.[6] More sensitive and specific molecular methods, like polymerase chain reaction (PCR), DNA-sequencing coupled with the application of bioinformatics like basic local alignment search tool (BLAST), have been used for HPV detection and genotyping.[7-9]

The currently available prophylactic HPV-vaccines reportedly provide about 80% protection, the remaining non-coverage being accounted by rarer HR-HPV types.[10] HPV genotyping is thus vital to procure knowledge about regional genotype distribution to help in development of a cost-effective, efficacious HPV-vaccine and can be used as an adjunct to Pap-smear screening, which has its own limitations.[11]

North Bengal covers a large area comprising of six districts namely Uttar Dinajpur, Dakshin Dinajpur, Coochbehar, Siliguri, Jalpaiguri and Darjeeling. To the best of our knowledge, previous published data on HPV genotyping and its distribution among H/P types of CIN/CaCx from North Bengal is unavailable. Thus, our study was an attempt to determine the same and to identify any major deviation in distribution of HPV genotypes 16/18 in cases of SCC and adenocarcinoma in this region.

MATERIALS AND METHODS

Study design

This was a North-Bengal based retrospective, cross-sectional, case-only type of study on distribution of HPV genotypes among H/P types of CIN/invasive CaCx, conducted over a period of 1 year (February, 2010 to January, 2011). Cervical biopsy tissue collection and H/P examination were performed respectively at Departments of Gynecology and Obstetrics and Pathology of a tertiary hospital, in North Bengal, while molecular methods for HPV genotyping, such as PCR and DNA-sequencing, were performed at the Human Genetics Unit (HGU) of Indian Statistical Institute, Kolkata with informed patient-consent and approval from the concerned ethical committees. Initially, elective cervical punch biopsy tissue collection was made from those patients at their first visit, presenting with clinically suspicious unhealthy cervix and H/P examination was carried out on those cases. Cases with an already previous H/P diagnosis of CIN/CaCx on or off treatment were excluded from the study. Out of initial 49 tissues, 40 were histopathologically confirmed as CIN/CaCx, while nine had chronic cervicitis and were excluded from the study. Thus, the study population finally comprised of 40 cases on which molecular methods like PCR, DNA sequencing coupled with BLAST were performed to determine the HPV genotype.

Types of specimen

The cervical biopsy tissues were divided into two parts in the operation theatre. One part was submitted in 10% buffered formalin and received at Department of Pathology for H/P examination and, the other part was collected in "RNAlater" (Ambion, Life Technologies Corporation, USA) and carried in ice-pack to HGU for HPV genotyping. HPV infection was screened by PCR with L1-specific primers MY09/11, which if negative, was reconfirmed by nested PCR with GP5/6 primers. Cases positive for MY09/11 or GP5/6 were typed for HPV16 and HPV18 by type-specific PCR (TS-PCR) with respective E6-specific primers. In the absence of both HPV16 and 18, the HPV-infected samples were subjected to DNA sequencing followed by bioinformatics-based genotyping using BLAST.[9] An overview of the study design has been depicted in Figure 1.

Histopathology of biopsy tissue

The formalin-fixed biopsy tissues were routinely processed, paraffin-embedded and stained with hematoxylin and eosin (H and E) and the slides were examined under light microscope (Olympus CH20i, Olympus Opto Systems, India).[12] The cases diagnosed as cervical adenocarcinoma with H and E staining, were further stained with Periodic Acid-Schiff stain (PAS) to demonstrate mucin.[12]

DNA isolation

Genomic DNA of both host and the virus was together isolated and purified from the samples by using QIAamp DNA mini kit (QIAGEN, Hilden, Germany) following manufacturer's protocol. The concentration and purity of DNA were estimated by spectrophotometry.

HPV genotyping using PCR, DNA-sequencing and BLAST

HPV detection

The reaction mixture (20 μl) for L1-specific conventional PCR contained ×10 PCR buffer (Roche Diagnostics, Switzerland), 1.5 mM $MgCl_2$, 200 μM dNTP, 60 ng primer (MY09/11), 1 U thermostable Fast Start Taq polymerase (Roche Diagnostics, Switzerland) and 100 ng DNA.[13] The PCR program consisted of initial denaturation (95°C; 5 min), 30 cycles of denaturation (95°C; 1 min), annealing (57°C; 2 min) and elongation (72°C; 1 min 30 s) and, final elongation (72°C; 5 min). The MY09/11-negative PCR-products were confirmed by nested PCR in a reaction mixture (20 μl) containing ×10 PCR buffer, 1 mM $MgCl_2$, 100 μM dNTP, 50 ng primer (GP5/6), 0.5 U FS Taq and 0.3 μl of MY09/11-product.[7] The PCR program consisted of initial denaturation (95°C; 5 min), 40 cycles of denaturation (95°C; 30 s), annealing (42°C; 1 min) and elongation (72°C; 30 s) and, final elongation (72°C; 5 min).

HPV TS-PCR

The HPV16-specific PCR mixture (20 μl) contained ×10 PCR buffer, 2 mM $MgCl_2$, 200 μM dNTP, 4 ng primers, 0.5 U FS Taq and 100 ng DNA. The touch-down PCR-program consisted of initial 14 cycles of denaturation (95°C; 30 s), annealing (61°C; 30 s; reduction by 0.5°C/cycle) and elongation (72°C; 30 s), followed by 23 cycles of denaturation (95°C; 30 s), annealing (55°C; 30 s) and elongation (72°C; 30 s), along with initial denaturation and final elongation as above. The HPV18-specific PCR mixture (20 μl) contained ×10 PCR buffer, 2 mM $MgCl_2$, 200 μM dNTP, 20 ng primers, 0.5 U FS Taq and 100 ng DNA. The PCR program consisted of initial denaturation (95°C; 5 min), 33 cycles of denaturation (95°C; 30 s), annealing (53°C; 30 s) and elongation (72°C; 30 s) and, final elongation (72°C; 3 min). HPV16-positive CaSki cell line DNA or HPV18-positive HeLa cell line DNA as a positive control, as applicable and HPV-negative genomic DNA and doubly distilled water as negative controls. The PCR-products were checked by gel-electrophoresis. The primer-sequences and amplicon sizes are provided in Table 1.

HPV-DNA sequencing for HPV16/18-negative cases

Following Sanger's method, the sequencing-PCR mixture (10 μl) consisted of ×5 sequencing buffer and 1 μl BidDye® Terminator (Applied Biosystems, Life Technologies

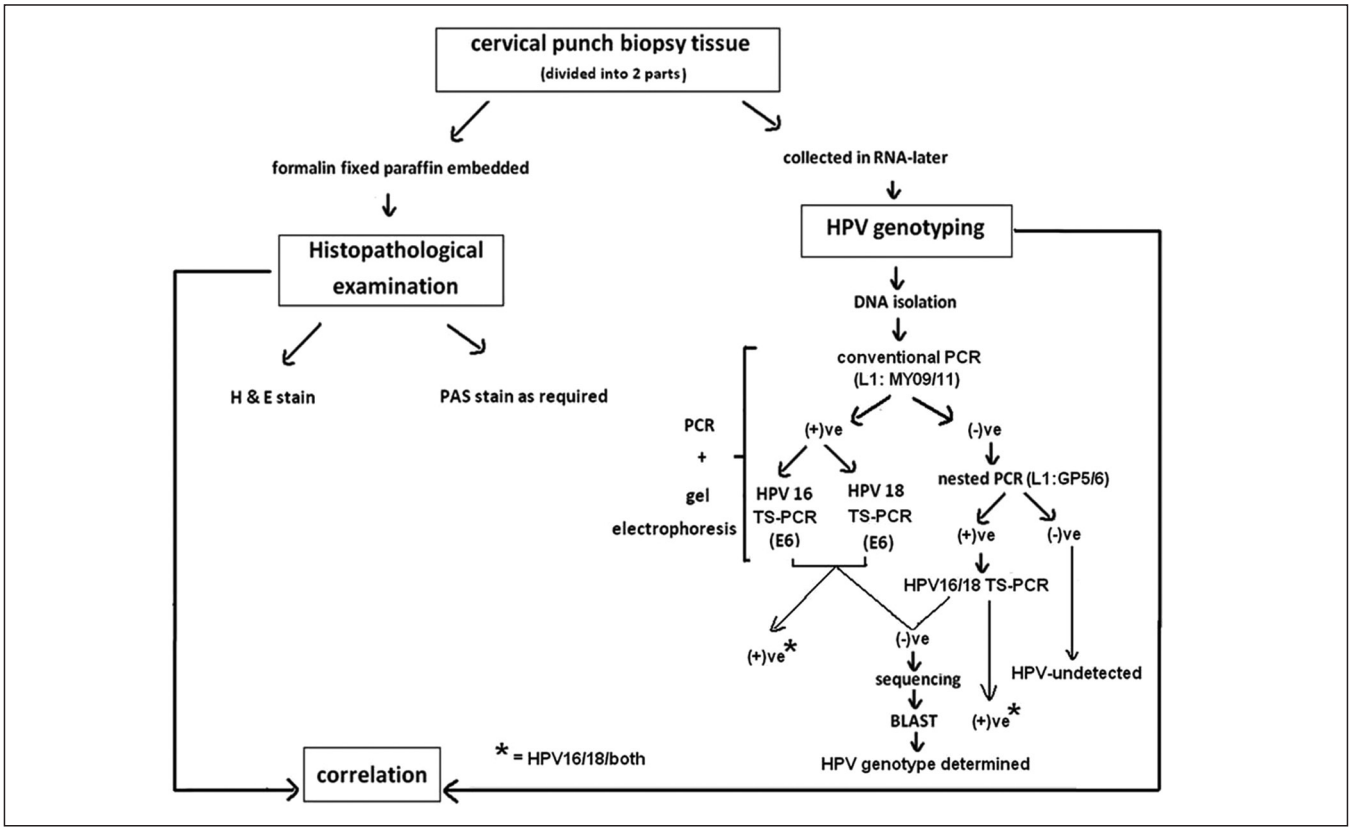

Figure 1: Flow chart depicting study design. TS-PCR: Type-specific polymerase chain reaction, HPV: Human papillomavirus, H and E: Hematoxylin and eosin, PAS: Periodic Acid-Schiff

Table 1: Primer sequences and product lengths for individual PCR

Primers	Primer sequences (5′-3′)	Amplicon size in bp
HPV16 type-specific primer	Forward: TCAAAAGCCACTGTGTCCTG	116 bp
	Reverse: CGTGTTCTTGATGATCTGCA	
HPV18 type-specific primer	Forward: ACCTTAATGAAAAACCACGA	100 bp
	Reverse: CGTCGTTGGAGTCGTTCCTG	
MY09/11	Forward: GCMCAGGGWCATAAYAATGG	450 bp
	Reverse: CGTCCMARRGGAWACTGATC	
GP5/6	Forward: TTT GTT ACT GTG GTA GAT ACT AC	150 bp
	Reverse: GAA AAA TAA ACT GTA AAT CAT ATTC	

*Amplicon: A stretch of DNA of specific length, which is a product of PCR, A: Adenine, T: Thymine, G: Guanine, C: Cytosine, M: A/C, W: A/T, Y: C/T, R: A/G, PCR: Polymerase chain reactions, HPV: Human papillomavirus, GP: General primer, bp: Base pair, DNA: Deoxyribonucleic acid

Corporation, USA), 25 ng primer and 2 μl diluted PCR-product (MY09/11 or GP5/6).[14] The PCR program consisted of initial denaturation (96°C; 6 min), 35 cycles of denaturation (96°C; 6 min), annealing (55°C; 30 s) and elongation (72°C; 30 s) and, final elongation (72°C; 7 min). The sequencing-PCR products were precipitated by applying 3 M sodium acetate (pH 5.2) and absolute alcohol and washed with 70% ethanol. The final precipitate was resuspended in Hi-Di™ formamide (Applied Biosystems) and subjected to capillary electrophoresis (3100 PRISM, Applied Biosystems). The electropherograms were studied using DNASTAR (www.dnastar.com).

BLAST analysis

The DNA-sequences were analyzed, using the bioinformatics application, called BLAST, to detect the matching HPV genotypes from the database (http://blast.ncbi.nlm.nih.gov/).[9,15] Chances of matching for each HPV genotype could be determined by a scoring system based on 'Expect-value' (E-value). Lower E-value signified higher probability of matching. The HPV genotypes matching with the query-sequence were displayed in increasing order of E-value by using BLAST.

Statistical analyses

Occurrence of HPV genotypes among different H/P types of intra-epithelial and invasive cervical neoplasia was compared by using Fisher's exact test. Other risk factors like smoking habit and patient's age were compared against different H/P types and HPV genotypes by *t*-test. The statistical analyzes were carried out by statistical software package SPSS v16.0 for windows (IBM, India). The level of significance (α) for every test was fixed at 0.05.

RESULTS

Data on demographic variables, clinical history and clinical findings

In this study, the mean age of cases, mean age of marriage, mean age of the first child birth (FCB) and the mean parity were respectively 49.22 years, 15.95 years, 18.35 years and 4.1 [Table 2]. Four cases were grand multipara (parity >6). Majority of the cases was Hindu (31/40; 77.5%) by religion. Eighty-five percent (34/40) of the cases was illiterate. Tobacco-users accounted for 27.5% (11/40) cases. Majority of them developed infiltrating SCC (10/11; 90.9%; relative risk [RR] = 1.146; 95% confidence interval [CI] = 0.88-1.49), whereas, only a single patient had infiltrating adenocarcinoma (1/11; 9.1%; RR = 0.66; 95% CI = 0.08-5.27). Age of detection for HPV infected cases belonged mostly to the range of 40-49 years (21/39; 53.85%), followed by 50-59 years and ≥60 years (7/39; 17.95% in each) and lastly, <40 years (4/39; 10.25%). The lowest age detected (35 years) was a case of HPV16+18 co-infection. There was no significant difference in mean age of detection between infiltrating SCC (mean age = 48.97 ± 8.77 years) and either infiltrating adenocarcinoma (mean age = 48.8 ± 9.49 years; $P = 0.99$; *t*-test) or CIN3 (mean = 54.5 ± 0.71 years; $P = 0.39$; *t*-test). The most common clinical symptom and sign were respectively serosanguinous discharge (29/40; 72.5%) and clinically evident cervical growth with contact-bleeding (27/40; 67.5%).

H/P types and subtypes

Out of the 40 cases, 33 (82.5%) were infiltrating SCC, 5 (12.5%) were infiltrating adenocarcinoma (mucinous) and 2 (5%) were CIN3. Twenty-two SCC cases (66.67%) were infiltrating non-keratinizing SCC (INKSCC) and 11 (33.33%) were infiltrating keratinizing SCC (IKSCC). Ten IKSCC cases (10/11; 90.91%) were moderately differentiated (IMDKSCC) and one (1/11; 9.09%) was well-differentiated. Four adenocarcinoma cases (4/5; 80%) were infiltrating moderately differentiated (IMD adenocarcinoma) and one (1/5; 20%) was infiltrating poorly differentiated (IPD adenocarcinoma). Figure 2a represents IKSCC showing characteristic keratin pearl formations. Figure 2b and c represents INKSCC and CIN3, respectively. Figure 3a and b represents H and E and corresponding PAS stained sections of adenocarcinoma.

HPV genotypes

HPV was detected in 39 (97.5%) out of 40 cases by PCR. There was a single HPV (−)ve case (1/40; 2.5%) undetected by PCR and sequencing. HPV genotypes were categorized into six groups, namely, (i) HPV16 only (27/39, 69.23%), (ii) HPV18 only (5/39, 12.82%), (iii) HPV16 + HPV18 (1/39, 2.56%), (iv) HPV16 + others (4/39, 10.26%), (v) HPV18 + others (1/39, 2.56%) and (vi) unidentified HPV type (1/39, 2.56%) [Figure 4]. Altogether, 82.05% HPV (+)ve cases carried HPV16 and 17.95% HPV (+)ve cases carried HPV18.

Table 2: Descriptive statistics of demographic variables

Variables	Range	Mean	Standard deviation
Age of detection (years)	35-70	49.22	8.77
Age of marriage (years)	12-21	15.95	2.19
FCB (years)	14-25	18.35	2.59
Parity	2-7	4.1	1.33

*FCB: Age at first child birth

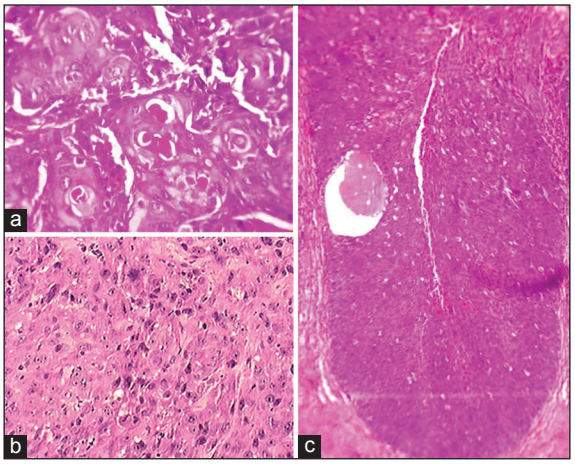

Figure 2: (a) Infiltrating keratinizing squamous cell carcinoma of cervix with keratin pearl formation (×100). (b) Infiltrating non-keratinizing squamous cell carcinoma of cervix (×100). (c) Cervical intraepithelial neoplasm, grade 3 (×400). All sections stained with H and E

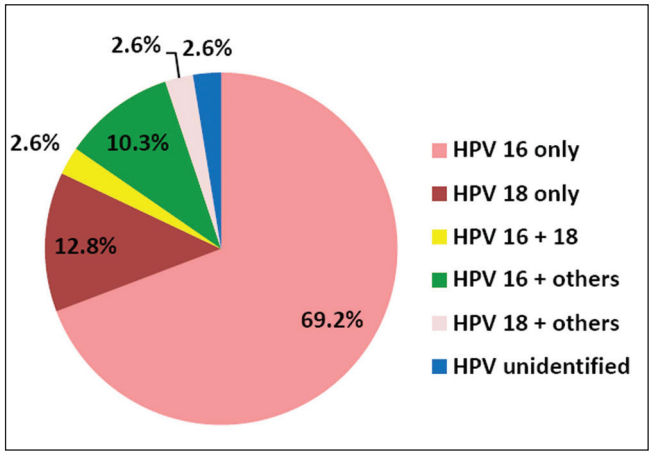

Figure 4: Percentage distribution of six different categories of human papillomavirus infection

Cases belonging to groups (i), (ii) and (iii) were positive for HPV16/18/both by TS-PCR. Cases belonging to groups (iv) and (v) were negative for HPV16/18 by TS-PCR, but were positive for the same along with other LR-HPV types (co-infection) by more sensitive DNA-sequencing coupled with BLAST, possibly due to low copies of HPV16/18.[9] Group (vi) failed to match with known HPV genotypes by BLAST.[9] Representative pictures of gel-electrophoreses for conventional PCR (L1-My09/11), nested PCR (GP5/6), HPV16/18 TS-PCR products are shown in Figures 5-8, respectively, whereas Figures 9 and 10 respectively depict DNA sequence-based electropherograms and BLAST-output.

Distribution of different H/P types and subtypes with respect to categories of HPV genotypes

The SCC cases harbored HPV groups (i) (24/33; 72.73%), (iv) (4/33; 12.12%), (ii) (2/33; 6.06%), (iii) (1/33; 3.03%) and (v) (1/33; 3.03%) in decreasing order of frequency. The HPV (−)ve case also belonged to SCC category. The adenocarcinomas harbored HPV groups (ii) (3/5; 60%), (i) (1/5; 20%) and (vi) (1/5; 20%) in decreasing order of frequency. CIN3 cases harbored group (i) only. The distributions are depicted in Figure 11.

Thus, HPV16 only group (24/26; 92.31%) was predominant over HPV18 only (2/26; 7.69%) in SCC cases. Contrarily, HPV18 only group (3/4; 75%) was predominant over

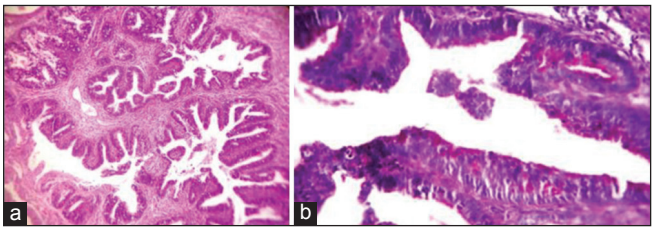

Figure 3: Infiltrating mucinous cervical adenocarcinoma (a) hematoxylin and eosin stain (×100). (b) Periodic acid-Schiff stain showing apical cytoplasmic positivity (×400)

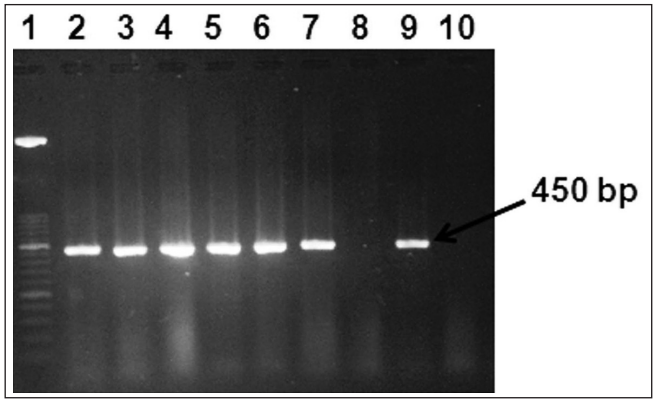

Figure 5: L1 (My09/11) polymerase chain reaction products. Lane 1: 50 bp deoxyribonucleic acid ladder; lanes 2-7: Samples showing specific band for L1 at 450 bp; lane 8: L1 (−)ve sample; lane 9: Positive control; lane 10: Negative control

HPV16 only (1/4; 25%) in adenocarcinoma. HPV18-only infection was significantly more among adenocarcinoma than SCC ($P = 0.0045$, one-sided Fisher's exact test). All the CIN3 cases were infected with HPV16 only. The distributions have been depicted in Figure 12.

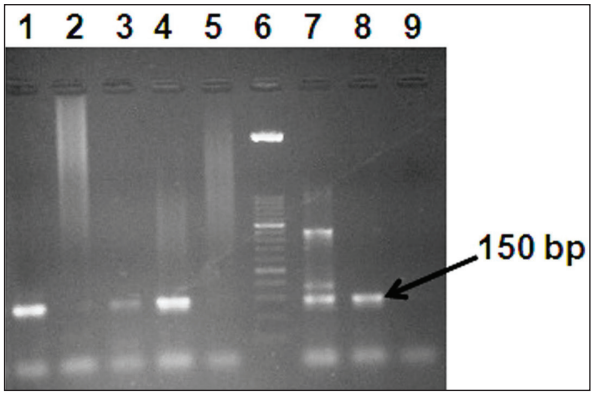

Figure 6: GP5/6 polymerase chain reaction products. Lane 6: 50 bp deoxyribonucleic acid ladder; lanes 3, 4, 7, 8: Samples showing specific band for GP5/6 at 150 bp; lane 2, 5: GP5/6 (–)ve sample; lane 1: Positive control; lane 9: Negative control

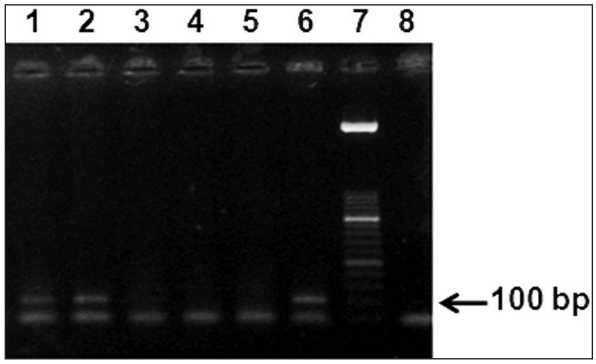

Figure 8: Human papillomavirus (HPV)18 type-specific polymerase chain reaction products. Lane 7: 50 bp ladder; lanes 1-2 : HPV18 (+)ve samples showing specific band size at 100 bp; lanes 3-5: HPV18 (–)ve sample; lane 6: Positive control; lane 8: Negative control

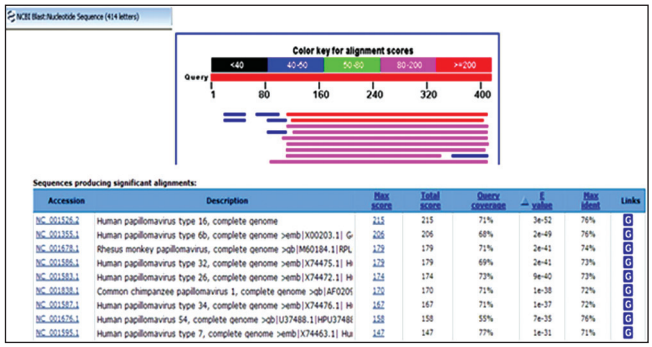

Figure 10: Representative basic local alignment search tool analysis. Human papillomavirus (HPV)-genotypes matching with the query-sequence are displayed in decreasing order of identity determined by a specific scoring system (E-value). The "color key" denotes "query coverage" (percentage of the query sequence overlapping with the matched sequence). In this figure, among all the above hits, probability of HPV16 and HPV6b are the highest

Out of the 22 INKSCCs and 11 IKSCCs, respectively 17 and 9 harbored HPV16/18-only infections. HPV16 only group was predominant in INKSCC cases (15/17; 88.23%) over HPV18 only (2/17; 11.77%). All the nine IKSCC cases harbored HPV16-only infection. There was no statistically

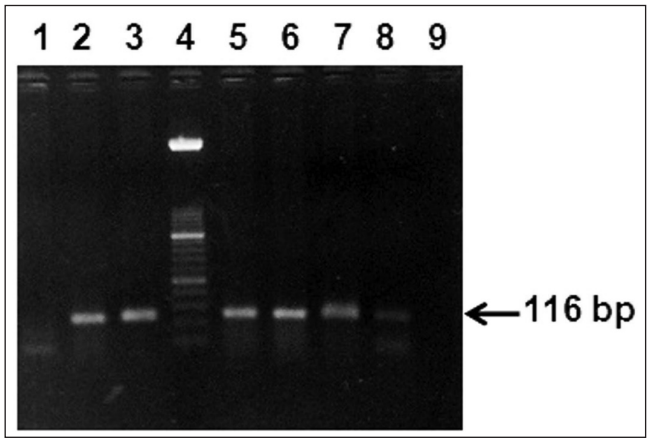

Figure 7: Human papillomavirus (HPV)16 type-specific polymerase chain reaction products. Lane 4: 50 bp ladder; lanes 3, 5-8: HPV16 (+)ve samples showing specific band size at 116 bp; lane 9: HPV16 (–)ve sample; lane 2: Positive control; lane 1: Negative control

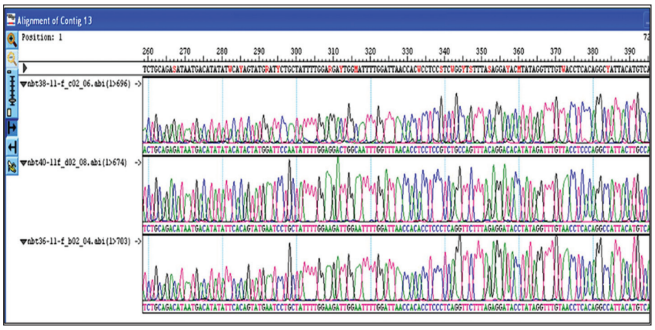

Figure 9: Representative electropherograms showing aligned sequences of L1 obtained by polymerase chain reaction with MY09/11 primers

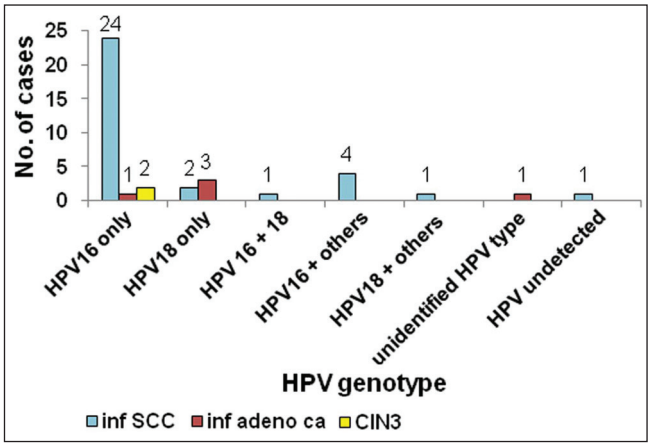

Figure 11: Distribution of different histopathological types of cervical neoplasm with respect to human papillomavirus genotypes. Inf: Infiltrating, adenoca: Adenocarcinoma, SCC: Squamous cell carcinoma, CIN: Cervical intraepithelial neoplasia

significant difference in HPV16-only infection between INKSCC and IKSCC in comparison to the HPV18-only infection (P = 0.42, Fisher's exact test). HPV18 only group (2/3; 66.67%) was predominant over HPV16-only (1/3; 33.33%) in IMD adenocarcinoma, while the single case of IPD adenocarcinoma harbored HPV18-only. The distributions have been depicted in Figure 13.

DISCUSSION

Our study detected HPV infection in 97.5% of cases of cervical neoplasia, which reiterates the pivotal role of HPV in cervical carcinogenesis. The study supported previous reports on role of co-factors such as early age of marriage (mean = 15.95 years), FCB (mean 18.35 years) and high parity (mean = 4.1) in CaCx.[1] The RR due to smoking was found to be higher for infiltrating SCC than infiltrating adenocarcinoma supporting earlier reports.[16] There was no significant difference (P = 0.99) between mean ages of infiltrating SCC (48.97 years) and adenocarcinoma (48.8 years) similar to many earlier reports.[11,17] However, contrasting reports exist on the increasing incidence of infiltrating adenocarcinoma among much younger women.[18] Supporting earlier reports, the present study has found, unlike the western scenario, a much later age of detection within 40-49 years, pointing toward the existing limitations of Pap-smear screening in developing countries.[11] Another peak of detection found at ≥60 years could possibly be due to a post-menopausal loss of immunity.[19] The lowest age of detection was found to be 35 years in a case of HPV16+18 co-infection. Previous studies have reported a lower age of detection in cases of co-infections due to a possible synergistic effect of multiple HR-HPV genotypes in CaCx.[20]

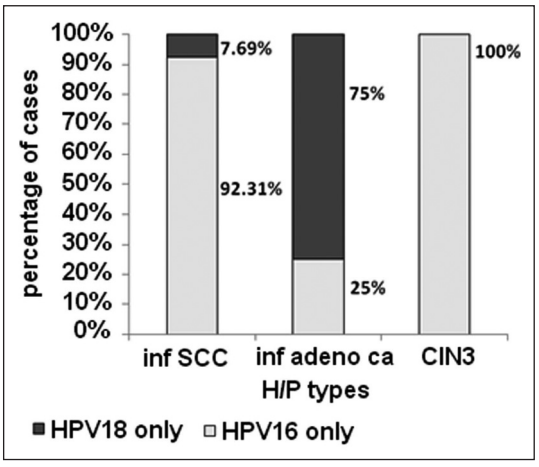

Figure 12: Distribution of human papillomavirus 16/18-only categories among histopathological types of cervical neoplasia. Inf: Infiltrating, adenoca: Adenocarcinoma, SCC: Squamous cell carcinoma, CIN: Cervical intraepithelial neoplasia

The present study found infiltrating SCC cases (82.5%) to predominate over adenocarcinomas (12.5%) supporting earlier reports from India and abroad.[1,21] However, it differed from certain previous reports on increasing incidence of adenocarcinoma.[22] CIN3 was detected in only 5% of the cases, indicating the need to strengthen Pap-screening programs. Cases of CIN1/2 were not found, possibly due to their higher chances of spontaneous regression.[3] Nevertheless, detection of HR-HPV types even in lower grade lesions would necessitate proper follow-up to monitor progression to invasive cancer.[23] No case of adenocarcinoma *in situ* (AIS) was found, possibly due to inadequate endocervical sampling.[24] However, progression to invasive cancer is reportedly much faster in AIS than squamous intra-epithelial lesion.[24] Cases of INKSCC (66.67%) were found to predominate over IKSCC (33.33%) and the moderately differentiated subtype predominated both amongst cases of IKSCCs (90.01%) and adenocarcinomas (80%), supporting earlier findings.[6]

The present study employing PCR, DNA-sequencing and BLAST with L1-specific MY09/11 and GP5/6 and, HPV16/18-specific primers, detected HPV in all but one case, reflecting high sensitivity of the methodology.[7] DNA-sequencing is reported as the gold-standard method for HPV genotyping.[25] The HPV-negative case was histopathologically IMDKSCC. HPV-negative CaCx can arise from transforming host genetic alterations.[26] However, HPV false-negatives can result from low viral load, disruption in HPV-genome during viral integration, less sensitive PCR conditions, poor quality of specimen, sampling error, absence of episomal forms in glandular lesions.[27] There was a single case of unidentified HPV-type histopathologically detected as infiltrating adenocarcinoma. Unidentified HPV types based on results of DNA-sequencing coupled with bioinformatics (BLAST) can be due to poor quality of sequence-data or represent novel HPV type.[27]

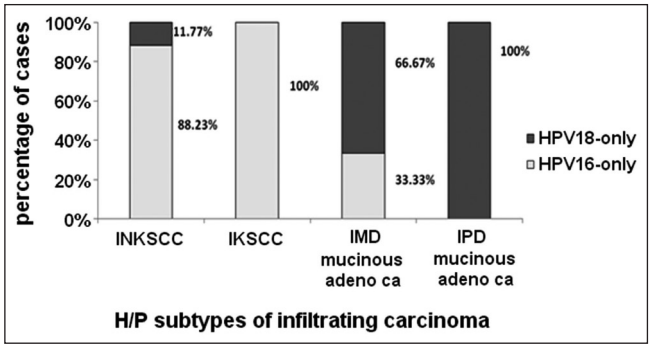

Figure 13: Distribution of human papillomavirus 16/18-only categories among histopathological subtypes of cervical neoplasia. INKSCC: Infiltrating non-keratinizing squamous cell carcinoma, IKSCC: Infiltrating keratinizing squamous cell carcinoma, IMD: Infiltrating moderately differentiated, IPD: Infiltrating poorly differentiated, adenoca: Adenocarcinoma

The present study found 97.44% (38/39) of the HPV-positive cases to be infected with HPV16/HPV18/ both with HPV16 (32/39; 82.05%) predominating over HPV18 (7/39; 17.95%) infection, supporting previous reports.[1,28] Other studies have found HR-HPV types, like 31, 33 besides HPV16 predominance.[29] However HPV52 predominance has been reported from several South East Asian studies, reflecting geographical variation of HPV genotypes.[30] There was a single case of HPV16+18 co-infection, while other co-infections harbored LR-HPV types 6b, 32, 26, etc., along with HR-HPV16/18 similar to previous reports.[31] However, many studies have failed to find a synergistic effect of LR - along with HR-HPV in increasing severity of lesions.[32]

Considering the HPV16/18-only cases, 92.31% HPV16-only and 7.69% HPV18-only infections were found among SCC cases, while 25% HPV16-only and 75% HPV18-only among adenocarcinoma cases. HPV18-only infection was significantly higher among adenocarcinomas than SCCs ($P = 0.0045$, one-sided Fisher's exact test) supporting some earlier studies.[33] In contrast, HPV16 has been earlier reported to be the most predominant type, irrespective of H/P types of CaCx in India.[21] HPV16-only infection (88.23%) predominated over HPV18-only (11.7%) in INKSCC cases and was the sole detected type in IKSCC cases supporting earlier reports.[34]

CONCLUSION

Our study has provided possibly the first report from North Bengal, India on HPV-genotyping in cases of CIN/CaCx. Predominance of HPV18 over HPV16 in cases of adenocarcinoma in this region was contrasting to that of earlier Indian studies. This finding was new and contrary to that of the Indian scenario and should encourage future studies on HPV-18 related cervical carcinogenesis to validate this finding. PCR and DNA-sequencing coupled with BLAST could prove to be highly effective tools in HPV detection and genotyping, which might encourage adoption of the same in future such studies. The study reported HPV16/18 infection in almost 98% of the cases of cervical neoplasia, knowledge about which might prove useful in future population based studies on HPV genotyping and designing of appropriate HPV-vaccines for this region.

Study limitations

A relatively short 1-year time period, lack of adequate funding, low turnover of operative cases in the hospital, in availability of colposcopic facilities causing a possible sampling error and consequent false-negative diagnosis, occasional per- or post-operative loss of samples due to incorrect handling of tissue required for molecular studies and last, but not the least, inadequate managerial and infrastructural facilities all were limitations of the study and contributed to reduction in sample size.

Suggestion

Well-funded, larger sample-sized, case-control population based studies, which was beyond the scope of our study, could further validate present findings and put more insight on HPV genotype distribution and to develop a cost-effective, efficacious HPV vaccine for this region.

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