ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 09 | September - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

E-CADHERIN IN PRECANCEROUS AND CANCEROUS LESIONS OF CERVIX AND CORRELATION WITH HUMAN PAPILLOMA VIRUS

Obstetrics & Gynaecology

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ABSTRACT

Introduction: Cervical Cancer is listed as the third commonest malignancy seen in women, worldwide. E-cadherin is a molecule with an important role in interaction of cell-cell and cell-extracellular matrix and its loss is important in tumor spread. This study was planned to evaluate the expression of E-Cadherin in precancerous and cancerous lesions of cervix. **Methodology:** In this study 60 biopsies of cervical lesions were included. Cervical biopsies were taken in women with CIN II, CIN III, Well- differentiated squamous cell carcinoma, Moderately differentiated carcinoma, Poorly differentiated carcinoma, Undifferentiated carcinoma and Lymphoepithelioma. Immunohistochemical staining for E cadherin was done on all paraffin blocks using monoclonal antibody. HPV DNA analysis was detected by PCR. Relevant personal and clinical data were documented. **Results:** E– cadherin expression showed significantly higher grade of expression in chronic cervicitis as compared to premalignant and malignant lesions. Out of the 60 cases screened for HPV DNA by PCR, 26.6 % (10 cases) tested positive for HPV infection. Further investigation for HPV type 16/18 showed that 3 (18.7%) out of 16 cases tested for HR- HPV type 16 and 7(43.7%) for type 18. **Conclusion:** Expression of E-cadherin was observed to be inversely proportional to loss of cell differentiation. Loss of E-cadherin staining was closely related to grade of dysplasia. It can be used as a prognostic marker for cervical squamous premalignant and malignant lesions of cervix.

KEYWORDS

E-cadherin, Immunohistochemistry, CIN, Squamous cell carcinoma cervix, HPV

INTRODUCTION

Gynecological malignancies are the most common cancers in females worldwide.[1] Of these, carcinoma of cervix is the most common.[2] Indian figures show that each year 132,000 cases of cervical carcinoma are diagnosed with morbidity of 74,000 per year. HPV Infection is found in 6.6% of women overall. It has been found that HPV serotypes 16 and 18 are the cause of 76.7% cervical cancers in India.[3,4]

For the last 50 years screening for cervical malignancy has been a very significant in early detection of cancer. It is a well-established fact that infection of cervical epithelium with human papillomavirus (HPV) leads to premalignant lesions in cervix with progression to Ca cervix.[5] It has been found that HPV-DNA testing is less specific but highly sensitive in identifying high grade cervical precancerous lesions with clinical symptoms although a huge variation is detected in frequency of HPV cases.[6]

Cadherins, are glycoproteins of 120 and 130kDa that are involved in cell adhesion. The intercellular adhesion which is firm due to adhesive interactions is responsible for formation of tissue. Its involvement consists of an important biomarker for development of tumor. [7] Large number of adhesion molecules cause the cells of cervical epithelium to strongly attach to each other and to the basement membrane. E-cadherin is one such important adhesion molecule that defines the architecture and differentiation of keratinocytes in the epithelium. A change in expression of these adhesion molecules results in intra-epithelial cancer.[8]

Many authors have concluded that decreased staining of E-cadherin in precancerous or cancerous lesions indicates its ability to invade the stroma of tissues and thus helps in metastasis. [9] From this it can be safely concluded that score of E- cadherin positivity is a useful parameter in evaluating the potential of malignancy in cervical lesions. Hence, the present study was done with the aim of determining E-cadherin positivity and its correlation with HPV in precancerous and cancerous lesions of cervix.

OBJECTIVE:

To evaluate the expression of E-cadherin in precancerous and cancerous lesions of cervix and to correlate the expression of E-cadherin with HPV status in these lesions.

METHODOLOGY

The present study was done in JSS Medical College, Mysore, Department of Pathology. It was a cross-sectional study of 2 years from August 2018 to August 2020. Study included all histopathologically proven cases of premalignant and malignant lesions of cervix. All benign lesions of cervix like cervical polyp, nabothian cyst, cervical fibroid, condyloma accuminata, squamous papilloma, adenosis, endometriosis and non-epithelial malignant tumors and post chemotherapy cases were excluded from the study. The sample size was calculated as 60, using the following formula:

$$n = \frac{Z^2_{\alpha/2}pq}{d^2}$$

where $Z_{\alpha/2}$ = 1.96 at 5% level of significance; p = prevalence of cervical cancer (5%= 0.05); q = 1-0.05= 0.95; and d = absolute precision (5% = 0.05).

All histopathologically confirmed cases of carcinoma cervix specimens and cervical biopsies were taken. Fixation of tissue was done in 10% formalin, grossed and processed according to standard procedure being followed in the department. 4-5 µm thickness was kept of sections. Hematoxylin and Eosin was used to stain them. After Histological diagnosis representative sections from each case were subjected to Immuno- histochemistry staining with E-Cadherin antibodies (Monoclonal rabbit- Ep6). E-cadherin immunostaining Intensity was scored as 0= negative, 1 = weakly positive, 2 = moderately positive and 3 = strongly positive. A score was now obtained with a maximum of 300.This was done by multiplying the number of cells stained in each category with intensity and taking the total. The final score was used to assess increased or impaired expression. <200 was considered as impaired expression and ≥200 as increased expression. [10]

After extracting the DNA, all the tissues were evaluated by PCR for the presence of Human Papillomavirus. MY09/MY11 primers previously described by Manos et al. [11] was commercially synthesized and used in the reaction. MY09/11 primers are consensus to the 450bp length of L1 region in the HPV genome. A brief master mix was prepared. 2 U/µL of Taq polymerase (Himedia), 2µM of dNTPs, 10X buffer with 25mM MgCl2 and 0.2µM were added for each of the forward and reverse primers. DNA samples were now added. Only those with concentrations between 50- 100ng/reaction was taken. Volume was corrected to 30µL using PCR grade water. Thermal Cycler (Eppendorf) was used for amplification. Initial denaturation was done for 5 mins. Now 32 cycles were done of 1 minute each at same temperature (denaturation), annealing was done for 45 seconds. Finally, extension of3 minutes was done at 72°C. GAPDH gene acted

as internal control to confirm proper PCR activity. HeLa cell line DNA acted as a control. The samples that tested positive for HPV were next examined for HR HPV-16 and HPV-18 using primers. Positive control was DNA from SiHa and HeLa cell lines. Lastly electrophoresis was done on PCR that was amplified on 2% agarose gel along using a 100bp ladder (Himedia).

Statistical Analysis:
Statistical Package for Social Sciences (SPSS) computer program 23 helped in final evaluation of data statistically. The correlation between membrane staining of E-Cadherin and HPV status was studied using the Chi square test. p values <0.05 were taken as significant.

RESULTS

In the present study, 60 cases of cervical premalignant and malignant lesions of cervix were evaluated.

The mean age was 50.72 years. The greatest number of patients were in the age group of 41-50 (n=25; 41.7%) years. None of the cases belonged to high socioeconomic status. Maximum number of cases had first sexual contact below 18 years with a mean of 19 years (SD ±3.0018). (Table 1)

Table 1: Distribution Of Study Population On The Basis Of Demographic Data

Variable	Frequency	Percentage (%)
Age Group		
30-40	12	20.0
41-50	25	41.7
51-60	15	25.0
60+	8	13.3
Socioeconomic status		
Low	21	35.0
Middle	39	65.0
Dietary habits		
Veg	23	38.3
Mixed	37	61.7
Age at first sexual contact		
Below 18	23	38.3
19-24	26	43.3
24+	11	18.3
Age at first conception		
below 18	6	10.0
19-24	38	63.3
24+	16	26.7
Parity		
1	9	15.0
2	8	13.3
3	25	41.7
4	12	20.0
5	3	5.0
6	3	5.0
Total	60	100.0

Table 2 shows age wise distribution of cases according to their histopathological diagnosis. Maximum cases were of moderately differentiated carcinoma (n=30 ;50 %) followed by poorly differentiated carcinoma (n=11;18 %). CIN I and CIN II were common in age group 51-60 years (N=2 ; 3%) while MD SCC was common in 41-50 years and poorly differentiated squamous cell carcinoma were also most common in the age group 41-50 years.

Table 2: Age-wise Distribution Of Cases According To Diagnosis

Type of lesion	21-30	31-40	41-50	51-60	61-70	>70 years	TOTAL
CIN II	00	00	01	00	00	00	01
CIN III	01	00	01	00	00	01	03
WDSCC	00	01	01	05	02	01	10
MD SCC	00	07	12	07	02	02	30
PD SCC	00	02	06	03	0	00	11
Undifferentiated	00	00	04	00	00	00	04
Lymphoepithelioma like carcinoma	00	01	00	00	00	00	01
Total	01	11	25	15	04	04	60

The intensity of staining was highest in CIN I and decreased as grade of tumor increased, being lowest in undifferentiated tumors. The higher the grade of the tumor, less was the score of E-cadherin. CIN II and CIN III both had a score of more than 200 whereas all undifferentiated tumors had a score of less than 200. (Table 3, Table 4)

Table 3: Comparison Of E-cadherin Expression In Cases

DIAGNOSIS	INTENSITY OF STAINING				Total
	0	1	2	3	
CIN II	00	00	00	01	01
CIN III	00	00	02	01	03
WDSCC	00	00	08	02	10
MDSCC	00	05	23	02	30
PDSCC	09	01	01	00	11
Undifferentiated	03	01	00	00	04
Lymphoepithelioma like Ca	01	00	00	00	01
Chronic cervicitis	00	00	06	24	30

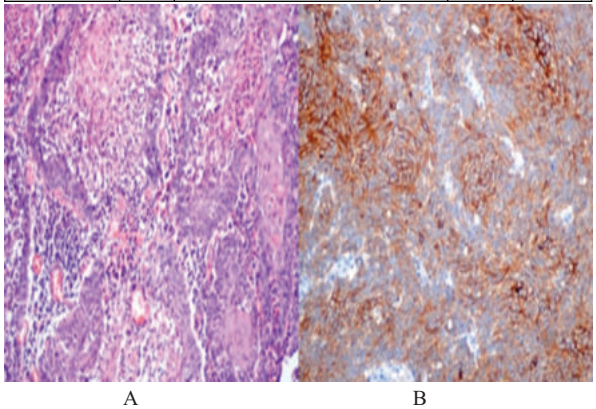
Table 4: E-cadherin- Score

DIAGNOSIS	<200 Decreased expression	>200 Increased expression
CIN-II	00	1 (100%
CIN -III	2 (66%)	1 (34%)
WDSCC	2 (20%)	8 (80%)
MDSCC	18 (60%)	12 (40%)
PDSCC	10 (91%)	1 (9%)
UNDIFFERENTIATED	4 (100%)	00
LYMPHOEPITHE-LIOMA LIKE CARCINOMA	1(100%)	00
Controls	00	30 (100%)

Table 5 shows the association between HPV and E cadherin. There is no significant relation between HPV and E Cadherin (p>0.05), HPV-16 and E Cadherin (p>0.05) and HPV-18 and E Cadherin (p>0.05).

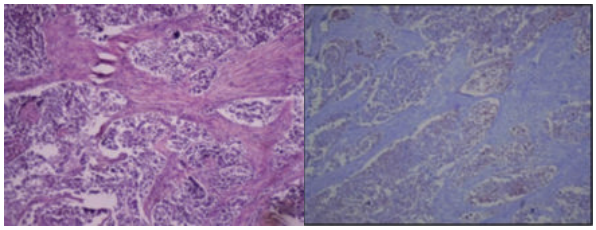
Table 5: Association Of HPV And E cadherin

			E cadherin		Total
			<200	>200	
HPV primer	Pos	Count	7	9	16
		% within HPV_primer	43.8%	56.2%	100.0%
	Neg	Count	23	21	44
		% within HPV primer	52.3%	47.7%	100.0%
Total		Count	30	30	60
		% within HPV primer	50.0%	50.0%	100.0%
HPV 16	Pos	Count	2	1	3
		% within HPV 16	66.7%	33.3%	100.0%
	Neg	Count	28	29	57
		% within HPV 16	49.1%	50.9%	100.0%
	Total	Count	30	30	60
		% within HPV 16	50.0%	50.0%	100.0%
HPV 18	Pos	Count	2	5	7
		% within HPV 18	28.6%	71.4%	100.0%
	Neg	Count	28	25	53
		% within HPV 18	52.8%	47.2%	100.0%
	Total	Count	30	30	60
		% within HPV 18	50.0%	50.0%	100.0%



A B

Figure 1 - A) Photomicrograph showing Moderately differentiated Squamous Cell Carcinoma (H&E, x100) B) Immunostaining for E-Cadherin showing a score of 150 (x100)



A B

Figure 2 - A) Photomicrograph showing Undifferentiated Carcinoma (H&E, x40) B) Immunostaining for E-Cadherin showing a score of 40 (x40)

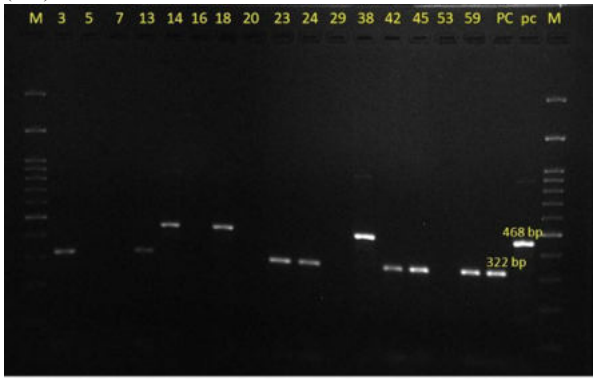


Figure 3- Electrophoretogram of PCR products of HPV positive samples on a 2% Agarose gel showing bands at 468 and 322 bp corresponding to HPV 16 and HPV 18 respectively. This type specific PCR was done using SiHa and HeLa cell lines as positive controls. ((— 100 bp marker, PC- positive control for HPV 18, pc- positive control for HPV 16)

Figure 4- Representative image of Nanodrop Spectrophotometer readings depicting DNA yield- Case 03

DISCUSSION

This study was done in the Department of Pathology, JSS Medical College, Mysuru. All cervical biopsies showing precancerous sand cancerous lesions were evaluated and correlation of expression of E-cadherin and HPV in these lesions was done.

Maximum patients were 41- 50 (n=25; 41.7%) years followed by 51-60 years (n=15; 25%), with p value=0.015. Most of the other studies showed the maximum number of cases in the 6th decade, however the mean age was almost same except in one study which showed a very low mean age of 36.5 years [12, 13]. Probably the reason may be the early consultation and detection of cancer due to very aggressive screening program. A correlation between low socioeconomic status and cervical cancer was found in the present study which could be due to poor hygiene, lack of awareness and lesser access to medical facilities for screening of cervical cancer. An evaluation of social status and the incidence of cervical carcinoma by Parikh et al, showed the risk in low socioeconomic class categories of incidence of cervical cancer was 100%.[12]

The present study showed that the maximum number of cases had first sexual contact below 18 years of age with an average age of 19 years (SD ±3.0018). A study by Plummer et al who studied adenocarcinoma and squamous cell carcinoma of cervix concluded that age at first sexual contact may be a factor responsible for development of carcinoma cervix.[14]

In the present study maximum cases 41.7% (n=25) were multiparous with parity of 3, followed by parity of 4 which was 20%(n=12). The increased risk (p value=0.035) of cervical premalignant and malignant lesions in females with high parity may be related to trauma caused during delivery per vaginum. Another cause may be cellular oxidative stress at cellular level resulting in DNA damage. Dhruva et al reported that majority of patients in their study(144; 36%) had 2 children and also reinforced that high parity was associated with higher occurrence of premalignant and malignant lesions of cervix.[15]

It has been seen that HR HPV infection is common in the ectocervix. Formation of precancerous or cancerous growth begins when the epithelial stem cell at the transformation zone or endocervix is infected. [16,17]

In the present study, the cervical biopsy was subjected to HPV DNA detection using PCR technique.

Table 6: Comparison Of HPV Infection Among Various Other Studies In India And Present Study

Authors	Sample size	HPV types detected	Prevalence of infection
Dutta et al [18]	Eastern India, (2,501)	16, 18	9.9% HPV-18 (1.4%) and HPV- 16 (0.6%)
Sowjanya et al [19]	Andhra Pradesh, 185	15,52	10.3%
Franceschi et al[20]	Tamil Nadu, 2000	44 subtypes(including16 &18)	HPV prevalence : 16.9%, high-risk HPV types :12.5%
Laikangbam et al[21]	Manipur (n=692) ;Sikkim (n=415) ;West Bengal (n=1,112)	16, 18	PV prevalence in Sikkim (12.5%), Manipur (7.4%), West Bengal (12.9%). HPV-18 was found in(0.2%) in Sikkim, Manipur (2.03%) and West Bengal (0.9%). HPV-16 showed just the opposite findings
Gupta et al [22]	Delhi, 769	16, 18	HR-HPV-16 was seen in 10.1% cases, whereas HPV-18 was found in 1% of cases
Arora et al [23]	Delhi, 160	16,18	High risk HPV by PCR – 10%
Srivastava et al [24]	Eastern Uttar Pradesh 2,424	15 cases of HR – HPV , 10 of LR-HPV , and 1 of intermediate-risk. 16, 31, 6, 81, 33 were found making a total of 26	9.9%
Barodawala et al[25]	Metropolis health care, Mumbai, 25563	16,18	10.34%
Current study	Mysuru , 60	16, 18	HPV 18 - 3 (5%) HPV 16 - 7(11.6%)

*HR – High Risk, LR – Low Risk

In this hospital based study, out of the 60 cases screened for HPV DNA by PCR, 26.6 % (16 cases) tested positive for HPV infection, further investigation for HPV type 16/18 showed that 3 (18.7%) out of 16 cases tested for HR- HPV type 16 and 7(43.7%) for type 18. This observation is comparable to the HPV prevalence, determined from various studies, seen in India i.e. 7.5% to 16.9% (Table 6).

A higher percentage of HPV infection, 60.33%, was noted in Sowjanya et al [19] in a study done in eastern India, however the authors evaluated in the presence of multiple strains of HPV and co-infection was also evaluated, in our study we only evaluated for two strains and the presence of co infection was not looked into.

The cadherin superfamily is a family of adhesion molecules that mediate cell-cell interactions. During metastatic progression in any tumor polarized epithelium cancer cells develop invasive migratory characteristics. This causes tumor cells to separate from primary tumor and spread to metastatic sites. This results in epithelial cells becoming transformed and developing properties of mesenchymal cells and acquiring a migratory nature. This phenomenon is known as the epithelial mesenchymal transition (EMT). [26] Loss of E-cadherin is a feature of EMT in epithelial tumors.[11]

Loss of E-cadherin in tumor tissues determines the ability to invade the collagen of tissues and thus in metastasis. [27] From this it can be safely concluded that that expression of E-cadherin is a useful parameter in evaluating the potential of malignancy of cervical cancer. This present study showed a decreased expression of E-cadherin staining from CIN II to Squamous cell carcinoma., with less staining in CIN and almost no expression in Squamous cell carcinoma. It clearly shows that precancerous lesions lose E-cadherin staining as they move towards a lesion of more severity, which is in concordance with previous studies. [28,29]

This study however could not verify that the adhesion molecule, E-cadherin ,showed an altered staining depending on the thickness of epithelium as seen in some other studies.[28]

The present study also observed that in precancerous lesions no differences in expression were there in cases that showed negative E-cadherin. These results are similar to those of Van de Putte G [29], E-cadherin disappeared in 40% of precancerous lesions in the membrane

The normal epithelium adjoining the precancerous areas had a greater positive E-cadherin staining than the diseased area. These results were expected, and this observation was similar to that in other studies.[30,31,32] In the present study no correlation was found between E-cadherin expression and HPV positivity. In another study it was also found that E- cadherin expression did not show any correlation to HR-HPV ($p = 0.982$), and there was no absence of HR-HPV after treatment of CIN.

A limitation of this study was the sample size and short duration of study which is not enough to comment on prognosis. Perhaps the association between HPV and E-Cadherin could not be established because of this.

However this study did show a decreased expression in E-cadherin staining from CIN II to Squamous cell carcinoma., with minor expression in CIN and almost no expression in Squamous cell carcinoma.

CONCLUSION

This study concluded that there are numerous risk factors involved in development of carcinoma of cervix. Amongst these are smoking, both active and passive, low socioeconomic status, early age at first sexual contact, early age at first childbirth and increased parity. In the present study the uninvolved epithelium adjoining the precancerous areas had a greater positive E-cadherin staining than the involved area. No correlation was found between E-cadherin expression and HPV positivity which was in concordance with other studies.

REFERENCES

1. Momenimovahed Z, Salehiniya H. Incidence, mortality and risk factors of cervical cancer in the world. Biomedical Research and Therapy. 2017 Jan 1;4(12):1795-811.
2. Ferlay JS, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Cancer incidence and mortality worldwide. Sources, methods and major patterns in GLOBOCAN 2012.International journal of cancer.2015;136.
3. Kaarthigeyan K. Cervical cancer in India and HPV vaccination. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology. 2012 Jan;33(1):7.
4. International Agency for Research on Cancer. GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012.
5. S.Altiok.Molecular markers in cervicalcytology.Clin Lab Med 23(2003) 709-728.
6. Beal CM, Salmerón J, Flores YN, Torres L, Granados-García V, Dugan E, Lazcano-Ponce E. Cost analysis of different cervical cancer screening strategies in Mexico. saludpública de méxico. 2014;56:429- 501.
7. Kaplanis K, Kiziridou A, Liberis V, Destouni C, Galazios G. E-cadherin expression during progression of squamous intraepithelial lesions in the uterine cervix. European journal of gynaecological oncology. 2005;26(6):608-10.
8. Bremnes RM, Veve R, Hirsch FR, Franklin WA. The E-cadherin cell– cell adhesion complex and lung cancer invasion, metastasis, and prognosis. Lung cancer. 2002 May 1;36(2):115-24.
9. Munhoz NG, Rodrigues DA, Takase Y, Pedregosa JF, Rodrigues JO, Miyazaki K, Junqueira MS, KamiyaYonamine PT, Pereira SF, Tokunaga O, Uezato S. The use of molecular markers (p16, Ki-67 and E-Cadherin) in uterine cervical biopsies. The Open Pathology Journal. 2009 Mar 13;3(1).
10. Fadare O, Reddy H, Wang J, Hileeto D, Schwartz PE, Zheng W. E- cadherin and β-catenin expression in early stage cervical carcinoma: a tissue microarray study of 147 cases. World journal of surgical oncology. 2005 Dec 1;3(1):38.
11. Behrens J, Mareel MM, Van Roy FM, Birchmeier W. Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. The Journal of cell biology. 1989 Jun;108(6):2435-47.
12. Sreedevi A, Javed R, Dinesh A. Epidemiology of cervical cancer with special focus on India. International journal of women's health. 2015;7:405.
13. Dutta S, Begum R, Mazumder D, Mandal SS, Mondal R, Biswas J, Dey B, Panda CK, Basu P. Prevalence of human papillomavirus in women without cervical cancer: a population-based study in Eastern India. International journal of gynecological pathology. 2012 Mar 1;31(2):178-83.
14. Plummer M, Peto J, Franceschi S, International Collaboration of Epidemiological Studies of Cervical Cancer. Time since first sexual intercourse and the risk of cervical cancer. International journal of cancer. 2012 Jun 1;130(11):2638-44.
15. Dhruva GA, Agravat AH, Bhojani KR. Analyses of cervical cancer in RAjkot population. Electronic Journal of Pharmacology and Therapy. 2011 Jan 1;4:15-20.
16. Kashyap N, Krishnan N, Kaur S, Ghai S. Risk factors of cervical Cancer: a case-control study. Asia-Pacific journal of oncology nursing. 2019 Jul;6(3):308.
17. Jensen KE, Schmiedel S, Norrild B, Frederiksen K, Iftner T, Kjaer SK. Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. British journal of cancer. 2013 Jan;108(1):234-9.
18. Dutta S, Begum R, Mazumder D, Mandal SS, Mondal R, Biswas J, Dey B, Panda CK, Basu P. Prevalence of human papillomavirus in women without cervical cancer: a population-based study in Eastern India. International journal of gynecological pathology. 2012 Mar 1;31(2):178-83.
19. Sowjanya AP, Jain M, Poli UR, Padma S, Das M, Shah KV, Rao BN, Devi RR, Gravitt PE, Ramakrishna G. Prevalence and distribution of high-risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India. BMC infectious diseases. 2005 Dec 1;5(1):116.
20. Franceschi S, Rajkumar T, Vaccarella S, Gajalakshmi V, Sharmila A, Snijders PJ, MUnoz N, Meijer CJ, Herrero R. Human papillomavirus and risk factors for cervical cancer in Chennai, India: A case-control study. International journal of cancer. 2003 Oct 20;107(1):127-33.
21. Laikangbam P, Sengupta S, Bhattacharya P, Duttagupta C, Singh TD, Verma Y, Roy S, Das R, Mukhopadhyay S. A comparative profile of the prevalence and age distribution of human papillomavirus type 16/18 infections among three states of India with focus on northeast India. International Journal of Gynecologic Cancer. 2007 Jan 1;17(1).
22. Gupta A, Arora R, Gupta S, Prusty BK, Kailash U, Batra S, Das BC. Human papillomavirus DNA in urine samples of women with or without cervical cancer and their male partners compared with simultaneously collected cervical/penile smear or biopsy specimens. Journal of clinical virology. 2006 Nov 1;37(3):190-4.
23. Arora R, Kumar A, Prusty BK, Kailash U, Batra S, Das BC. Prevalence of high-risk human papillomavirus (HR-HPV) types 16 and 18 in healthy women with cytologically negative Pap smear. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2005 Jul 1;121(1):104-9.
24. Srivastava S, Shahi UP, Dibya A, Gupta S, Roy JK. Distribution of HPV genotypes and involvement of risk factors in cervical lesions and invasive cervical cancer: a study in an Indian population. International journal of molecular and cellular medicine. 2014;3(2):61.
25. Barodawala SM, Chadha K, Kavishwar V, Murthy A, Shetye S. Cervical cancer screening by molecular Pap-transformation of gynecologic cytology. Diagnostic cytopathology. 2019 May;47(5):374- 81.
26. Jodele S, Blavier L, Yoon JM, DeClerck YA. Modifying the soil to affect the seed: role of stromal-derived matrix metalloproteinases in cancer progression. Cancer and Metastasis Reviews. 2006 Mar 1;25(1):35-43.
27. Crasta J, Ravikumar G, Koorse S, Siddharta P, Vallikad E. Expression of adhesion molecule epithelial cadherin and matrix metalloproteinase-9 in squamous neoplasia of the uterine cervix. Southern African Journal of Gynaecological Oncology. 2016 Apr 7;8(1):4-8.
28. Roura E, Travier N, Waterboer T, de Sanjosé S, Bosch FX, Pawlita M, Pala V, Weiderpass E, Margall N, Dillner J, Gram IT. The influence of hormonal factors on the risk of developing cervical cancer and pre- cancer: results from the EPIC cohort. PLoS One. 2016 Jan 25;11(1):e0147029.
29. Singh R, Shetty N, Naveed M, Pawar SB, Iska S, Alugubelli NR. Retrospective analysis of patients with carcinoma cervix in a rural/semi-urban setting in Western India. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology. 2016 Jan;37(1):25.
30. Han AC, Edelson MI, Peralta Soler A, Knudsen KA, Lifschitz-Mercer B, Czernobilsky B, Rosenblum NG, Salazar H. Cadherin expression in glandular tumors of the cervix: Aberrant P-cadherin expression as a possible marker of malignancy. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2000 Nov 15;89(10):2053-8.
31. de Leeuw WJ, Berx G, Vos CB, Peterse JL, Van de Vijver MJ, Litvinov S, Van Roy F, Cornelisse CJ, Cleton-Jansen AM. Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 1997 Dec;183(4):404-11.
32. Krishnadath KK, Tilanus HW, van Blankenstein M, Bosman FT. Decreased E-cadherin expression is associated with poor prognosis in patients with Barrett's adenocarcinomas. Gastroenterology. 1995 Apr 1;108(4):A493.