e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(5): 2158-2167

Original Research Article

Study of Expression of P-16 and Galectin-3 in Cervical Intraepithelial Neoplasia (CIN) and Invasive Squamous Cell Carcinoma of Uterine Cervix

Shubham Mishra[1], Shaila Mitra[2], Ankita Kumari[3], Harish Chandra Tiwari[4]

[1]Junior Resident, Department of Pathology, B.R.D. Medical College, Gorakhpur, Uttar Pradesh, India

[2]Professor & Head, Department of Pathology, B.R.D. Medical College, Gorakhpur, Uttar Pradesh, India

[3]Associate Professor, Department of Obstetrics & Gynaecology, B.R.D. Medical College, Gorakhpur, Uttar Pradesh, India

[4]Associate Professor, Department of Community Medicine, B.R.D. Medical College, Gorakhpur, Uttar Pradesh, India

Received: 17-03-2023 / Revised: 20-04-2023 / Accepted: 27-05-2023

Corresponding author: Dr Shubham Mishra

Conflict of interest: Nil

Abstract

Introduction: Cervical cancer is the second most common cancer in females worldwide and most frequent in India. Around 85% of the global burden occurs in the less-developed regions, where it accounts for almost 12% of all female cancers. In contrast to developed countries, cervical cancer is a public health problem in developing countries like India, so much so that India alone accounts for one-quarter of the worldwide burden of cervical cancers. The main cause of preneoplastic and neoplastic lesions of cervix is the persistent HPV infection. As p16 is considered as the surrogate marker of HPV infection, this study is expected to re-evaluate its role in progression of cervical SCC, especially the relative contribution of HPV in different morphological variants. Galectins are carbohydrate binding proteins having high affinity for beta–galactosides. Galectin-3 is directly and indirectly connected to cancer cell activity that can contribute to oncogenesis, angiogenesis, cancer progression, and metastasis.

Aims and Objective: Present study has been conducted with an aim to study the role of p-16 and Galectin-3 in cervical intraepithelial lesion and Squamous cell carcinoma of uterine cervix and to analyse the correlation between these two markers regarding their expression.

Results: In the study, 100 cases of uterine cervical lesion were included out of which 30 cases were of CIN and 70 cases were invasive carcinoma. Cases were evaluated by histopathology and by using p-16 and Galectin-3 marker. The age group of total cases ranged between 21 to 85 years. Maximum incidence of benign cases(46.67%) were in age group 41-50 years. Among invasive carcinoma majority of cases were observed in 5th and 6th decade. The study of expression of p-16 in CIN & SCC showed that p-16 expression was more pronounced in SCC and HSIL proportion of 3+ or 4+ was seen in >90% of cases as compared to LSIL. There was progressive increase in expression of p16 from LSIL to HSIL to Carcinoma. The expression of Galectin-3 in CIN & SCC showed that Galectin-3 expression was more pronounced in SCC and HSIL. Proportion of 3+ or 4+ was seen in >80% of cases as compared to LSIL. It was observed that as the lesion progress from LSIL to HSIL the expression of Galectin -3 increases with no difference between HSIL and SCC cases. There

was a statistical significance between the proportion of p-16 as well as Galectin-3 expression with the grade of cervical lesion. (P value <0.0001).

Conclusion: This study of p16 and galectin-3 expression with degree of dysplasia and SCC cervix can be used for screening and early detection of cervical lesions and thus aid their early treatment and increased survival.

Keywords: Cervical Cancer, Neoplastic Lesions, HPV Infection, Galectins, And, Cancer Progression.

Introduction

Cervical cancer is the second most common cancer in females worldwide and most frequent in India. Around 85% of the global burden occurs in the less-developed regions, where it accounts for almost 12% of all female cancers [1]. It is the one of the leading cause of cancer mortality, accounting for 17% of all cancer deaths among women aged between 30 and 69 years. It is estimated that cervical cancer will occur in approximately 1 in 53 Indian women during their lifetime compared with 1 in 100 women in more developed regions of the world [2]. The main cause of preneoplastic and neoplastic lesions of cervix is the persistent HPV infection [1]. Although association of positive p16 expression with high risk HPV related cases of squamous cell carcinoma of uterine cervix is now a well-established fact, there are instances of p16 negative cases also [3]. Approximately 82.5% of invasive cervical cancers are attributed to HPVs 16 or 18. [4,5]

p-16 a cyclin-dependent kinase inhibitor, plays an important role in cell cycle regulation by decelerating cells progression from G1 phase to S phase. As the detection of HPVs by hybridization or other molecular methods is quite cumbersome and pretty costly; p16 positivity in cervix gradually emerged as the surrogate marker of high risk HPV infection [6,7]. Galectins are carbohydrate binding proteins having high affinity for beta –galactosides. Galectin-3 is the lone member of the chimeric group having a single Carbohydrate Recognition Domain (CRD) with a unique N-terminal domain [8]. Galectin-3 is directly and indirectly connected to cancer cell activity that can contribute to oncogenesis, angiogenesis, cancer progression, and metastasis. In pre-neoplastic and neoplastic lesions of cervix, the intensity of galectin-3 expression increases as the cervical lesion progressed to invasive cancer [1].

Method

The study has been conducted in the department of Pathology, B.R.D medical College, Gorakhpur during a period from July 2021 to June 2022 for histopathological examination on the specimen of hysterectomy and cervical biopsies of premalignant and malignant lesions in uterine cervix. All samples of patients were selected on the basis of inclusion and exclusion criteria. All the specimens were fixed in 10% formal saline and subjected to histopathological examination using paraffin embedding technique. The sections stained by p-16 and Galectin-3 were also examined alongside H&E stained sections. Descriptive analysis was done. A P value <0.5 will be considered to be statistically significant.

Staining characteristics was noted based on Kim et al.(2011) [9]; in p16, a proportion of stained cells < 1%= 0, 1–4%= 1 +, 5–25% = 2 +, 25–75% = 3 +, and > 75% = 4 +.Internal Control for p16 were proliferating fibroblasts, endothelial cells

and some inflammatory cells. In galectin-3, the proportion of stained cells < 5% =0, 5–25% = 1 +, 25–50% =2 +, 51–75% =3+, and > 75%= 4+. Internal control for galectin-3 were macrophages and nerves.

Results

This study comprised of 100 untreated cases of CIN and SCC of uterine cervix. It was noted that the age of the patients ranged from 30 to 70 years with a mean age of 43.72±2.5 years. The majority of CIN cases were reported in premenopausal females whereas postmenopausal women formed 80% of the SCC cases. Out of 70 invasive carcinoma, SCC was most common histological type(92.86%) followed by adenocarcinoma(4.29%) and adenosquamous carcinoma(2.86%). LSIL comprised 10% (10 cases) and HSIL comprised 20% (20 cases) of all the cases. The most common presenting complaints in LSIL was white discharge per vagina (50%) while in HSIL and SCC vaginal bleeding in 50% and 85.72% cases respectively. The maximum incidence of CIN and SCC were noted in women who had 2 or more children. The expression of p16 in CIN and SCC showed that in patients of LSIL, the proportion ranged from 0 to 2+. In cases of CIN II and III (HSIL), the proportion was 2+ in 6 cases (30%), 3+ in 6 cases (30%) and 4+ in 8 cases (40%). There were no cases with 0 or 1+ response in CIN II and III. In carcinoma, the proportion was 3+ or 4+ in 64 cases (91.43%). Thus, as the lesion progressed from LSIL to HSIL to carcinoma the expression of p16 also increased. There was a statistical significance between the proportion of p16 expression and the grade of uterine cervical lesion (p-value < 0.00001) (Table 1).

Table 1: P-16 expression on the basis of percentage of cells in various premalignant and invasive carcinoma of cervix.

Grade	p-16 expression					Chi-square test	p-value
	00 <1% cells No.(%)	1+ 1-4% cells No.(%)	2+ 05-24% cells No.(%)	3+ 25-74% cells No.(%)	4+ >75% cells No.(%)		
LSIL(n =10)	02 (20%)	03 (30%)	05 (50%)	00	00	66.77	<0.000 01
HSIL (n=20)	00	00	06 (30%)	06 (30%)	08 (40%)		

Galectin-3 expression in CIN and cervical carcinoma cases showed that in patients of LSIL, proportion was 1+ and 2+ in 4 cases(40%) & 6 cases(60%) respectively. There were no cases with 0, 3+ or 4+ expression in LSIL cases. In cases of CIN II and III (HSIL) proportion of 2+ was observed in 5 cases (25%), 3+ in 6 cases (30%) and 4+ were observed in 9 cases (45%). There were no cases with 0 or 1+ expression in CIN II and III. In carcinoma, a proportion of galectin-3 expression was 3+ or 4+ in 58 cases (82.86%), 1+ in 3 cases (4.28%) and 9 cases (12.86%) showed 2+ expression. In the present study, as the lesion progressed from LSIL to HSIL, the expression of galectin-3 also increased, with no difference between HSIL and SCC cases. There was a statistical significance between the proportion of galectin-3 expression and the grade of cervical lesions like p16 (p-value< .05)(TABLE 2).

Table 2: Galectin-3 expression on the basis of percentage of cells in various premalignant & malignant lesion of cervix.

Grade	Galectin-3 expression					Chi square test	p-value
	00 <5% cells No.(%)	**1+** 5-25% cells No.(%)	**2+** 25-50% cells No.(%)	**3+** 51-75% cells No.(%)	**4+** >75% cells No.(%)		
LSIL(n=10)	00	04 (40%)	06(60%)	00	00	36.66	0.000002
HSIL (n=20)	00	00	05 (25%)	06 (30%)	09 (45%)		
SCC (n=70)	00	03(4.28 %)	09 (12.86%)	30 (42.86%)	28 (40%)		

Expression of p16 was correlated with galectin-3 expression. It was observed that in 2 cases with p16 proportion of 0, the galectin-3 proportion was 1+ & 2+ in each case(50%). In 3 cases with p16 proportion of 1+, the galectin-3 proportion was 1+ in 1 case(33.34%) & 2+ in 2 cases(66.66%) In cases of p16 proportion of 2+, galectin-3 proportion was 1+ in 1 case (5.88%), 2+ in 5 cases (29.41%),3+ in 6 cases (35.29%) and 4+ in 5 cases (29.41%) respectively. Cases with p16 proportion of 3+ showed galectin-3 proportion of 1+ in 3 cases (12%), 2+ in 6 case (24%) 3+ in 14 cases (56%) and 4+ in 2 cases (08%). Cases which expressed p16 proportion of 4+ showed a galectin-3 proportion of 1+ in 1 case (1.87%), 2+ in 6 cases (11.32%), 3+ in 16 cases (30.19%) and 4+ in 30 cases (56.60%). Thus, as the expression of p16 increased, an increase in galectin-3 expression was also observed. There was a statistical significance between the proportion of p16 and galectin-3 expression, with a p-value of < 0.05. (table 3).

Table 3: Correlation between p-16 expression and galectin-3 expression

p-16	Galectin-3				
	1(5-24%)	**2(25-49%)**	**3(50-74%)**	**4(>75%)**	**Total**
0(<1%)	01 (50)	01 (50)	00	00	**02**
1(1-4%)	01 (33.34)	02(66.66)	00	00	**03**
2(5-24%)	01 (5.88)	05 (29.41)	06 (35.29)	05 (29.41)	**17**
3(<25-74%)	03 (12)	06 (24)	14 (56)	02 (08)	**25**
4(>75%)	01 (1.87)	06 (11.32)	16 (30.19)	30 (56.60)	**53**
Total	**07**	**20**	**36**	**37**	**100**

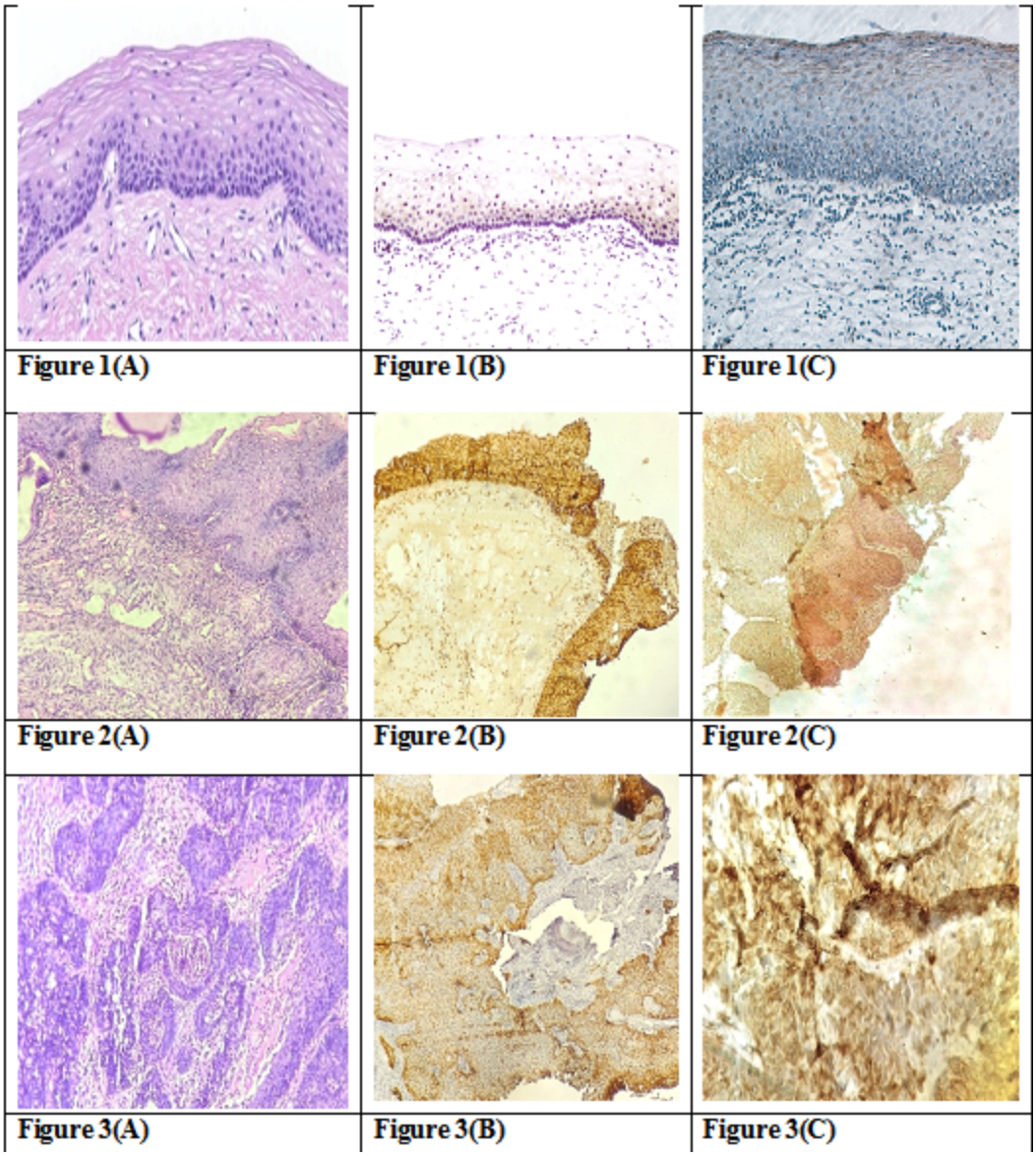

Figure 1(A). Normal Squamous epithelium of cervix. Figure 1(B). No expression of p-16 on normal squamous epithelium. Figure 1(C). No expression of galectin-3 on normal squamous epithelium. Figure 2(A). CIN III showing full thickness dysplasia. Figure 2(B). p-16 expression of 4+ in HSIL. Figure 2(C). Galectin-3 expression of 4+ in HSIL. Figure 3(A). SCC having pleomorphic atypical cells infiltrating the stroma. Figure 3(B). p-16 immunostaining showing 4+ positivity infiltrating the stroma. Figure 3(C). Galectin-3 immunostaining showing 4+ positivity infiltrating the stroma.

Discussion

Cervical cancer is the most prevalent cancer among women in India. The main cause of cervical cancer is persistent HPV infection and p16 expression is a surrogate biomarker of HPV infection. Galectin-3 is directly and indirectly connected to cancer cell activity and contribute to oncogenesis, angiogenesis, cancer progression and metastasis.

In the present study, the mean age group for LSIL lesions was 43 ± 11.68 years, for HSIL lesions 47 ± 8.89 years. This is in accordance with the studies done by Poste et al (2015) [10], Vincet M et al(2015) [11]. As the age progresses there was increase in severity of dysplasia from mild to moderate to severe dysplasia. This is in accordance with the study done by Gaikwad SL et al (2016) [12], who

observed the highest incidence of mild and moderate and severe dysplasia in the age group of 41-50 years.

Similarly Sheela et al (2016) [13] also observed the highest incidence of mild dysplasia and moderate in the age group of 41-50 years. Severe dysplasia was seen in the age group of 41-60 years. Gundrajakuppam L et al (2011) [14] also reported that with the progression of age, there was an increase incidence from mild to moderate and severe dysplasia. Hence forth, advanced age is considered as a significant risk factor for cervical carcinoma.

The mean age for malignant lesions of cervix was 56 ± 9.40 years. This is in accordance with Shanthi V et al (2014) [15], Parazzini F et al 1989 [16] and Poste et al (2011) [10]. They also reported maximum number of cases of carcinoma cervix in fifth and above fifth decade. Crasta J et al (2016) [24] also reported mean age of patients with CIN was 40.1 years and 50.9 years for those with SCC.

Many studies have also observed maximum number of cases of invasive carcinoma cervix in women beyond 40 yrs of age Misra et al. (2009) [17]; Kalyan et al. (2010) [18]; Paul S B et al. (2011) [19]; Gaikwad SL et al. (2016) [12]; Saini S et al. (2016) [20] and Sheela L et al. (2016) [13].

The significant lower incidence of carcinoma cervix in Muslim population may be due to circumcision in male (less HPV colonization in penis) Paul S B et al. (2011) [19], Castell et al. (2002) [21]; Wynder et al. (1954) [22].

In our study cases from rural areas predominated over urban population with a ratio of 1.44:1. This can be explained on the basis of fact that maximum number of patients reported belonged to rural areas however Parikh S et al (2003) [23] found that the risk of development of cancer of the cervix varies with lifestyle of an individual, social custom and geographical distribution.

In the present study, total 100 cases of premalignant and malignant lesions of uterine cervix were studied with 30 (30%) cases of premalignant lesions and 70 (70%) cases of malignant cervical lesions. This is in accordance with the studies done by Aijaz M et al (2019) [25], Gupta M et al (2018) [26], Agnihotri et al (2015) [27] and Crasta J et al (2016) [24], Kumar R et al (2020) [28] and Kim et al(2011) [9].

Out of the total 30 premalignant cases, 10 (33.33%) cases were LSIL (CIN1) and 20 (66.67%) were HSIL (CIN2+ CIN3). This is in accordance with the studies done by Kumar R. et al (2020) [28] and Kim et al (2011) [9], Aijaz M et al (2019) [25], Gupta M et al (2018) [26].

The highest incidence of CIN and invasive carcinoma were noted in women who had 2 or more children. The risk increases with multiple childbirth. Shanthi V et al. (2015) [15] showed that increased risk of various CIN lesions and carcinoma is directly proportional to parity. Similar finding was also reported by Satya B Paul (2011) [19] These finding suggests that multiparity is one of the important risk factors for the development of carcinoma cervix.

Among premalignant lesions, the commonest complaint with which the patients presented during our study was white discharge per vagina, (50%) in low grade CIN patients and irregular bleeding per vagina, (50%) in high grade CIN. Postmenopausal bleeding (64.29%) was the chief complaint in invasive carcinoma group.Our findings are in accordance with Shanthi V et al. (2015) [15] Hartmann D et al. (2016) [29]. Gaikwad SL et al. (2016) [12]. Gundrajakuppam L et al. (2011) [14]

Punch biopsy (78%) was the most common type of surgical specimen received for histopathological examination followed by simple hysterectomy (18%). This is correlated with the studies of Aoyama et al. (2005) [30].

Among invasive carcinoma, squamous cell carcinoma was the most common histological type accounting for (92.86%) of total invasive carcinoma followed by adenocarcinoma (4.29 %) and adenosquamous carcinoma (2.86%). Our findings are in accordance with Agnihotri et al. (2015) [27] and Poste et al. (2015) [10]. Squamous cell carcinoma cases were graded according to Broders grading system and moderately differentiated squamous cell carcinoma was the most common type (61.54%). Our results were in accordance with various studies conducted by Poste et al. (2015) [10]; Saini S et al. (2016) [20]; Misra et al. (2009) [17].

The study of the expression of p16 in CIN and SCC showed that p16 expression was more pronounced in SCC and HSIL (proportion of 3+ or 4+ expression was seen in >80% of cases) as compared to LSIL (where only a proportion of 1+ or 2+ expression was seen). There was progressive increase in expression of p16 from LSIL to HSIL to SCC. A statistically significant association of p16 with histological diagnosis was noted (p-value < .05). These findings were similar to Kumar R. et al. [28] , Kim et al. (2011) [9], who suggested that p16 was mainly expressed in HSILs and carcinoma (>25% expression in 100% of cases) as compared to LSIL (<25% expression was seen). Similarly, Klaes et al. (2001) [31] showed that p16 expression correlated with a degree of dysplasia and carcinoma. Volgareva et al. (2004) [32] and Wang et al. (2005) [33] also observed that the proportion of p16 positive samples increases in the following row: CIN I – CIN II – CIN III – carcinoma. Similarly, Kava et al. (2015) [34] demonstrated that p16 was positive in 20% of the CIN-1 cases, 80% of CIN-2 cases, and 66.66% of CIN-3 cases, and 100% of carcinoma cases.Wu et al. (2019) [36] conducted a comparison between the cytological screening results and the p16 immunostaining results,majority of cases were positive. In the LSIL and benign groups, very few lesions were positive for p16. These findings are like our findings of high p16 expression in HSIL and carcinoma.

The study of the expression of galectin-3 in CIN and SCC showed that galectin-3 expression was more pronounced in SCC and HSIL (proportion of 3+ or 4+ expression was seen in >70% of cases), as compared to LSIL (where only a proportion of 1+ or 2+ expression was seen). There was progressive increase in expression of galectin-3 from LSIL to HSIL, with no difference in HSIL and SCC cases. A statistically significant association of galectin-3 with histological diagno- sis was noted (p-value < .05). These findings were similar to those of Kumar R.et al [28] Li et al. (2017) [35]. Li et al. (2017) observed that normal cervix showed positivity of 13.3%, Cervicitis 14.3%, CIN-I 39.3%, CIN-II 54.8%, CIN-III 70% and cervical carcinoma 88.1%. Ma et al. reported that galectin-3 expression in CIN I was 30%, CIN-II 37.5% and CIN-III 63.3%. They concluded that galectin-3 expression in cervical tissue correlated with the degree of CIN and carcinoma. Kim et al. (2011) [9] suggested that galectin-3 protein was mainly expressed in HSILs (>50% expression in 80% of cases) and carcinoma (>50% expression in 75% of cases). In contrast, in LSIL the expression was 25–49% in 94% of cases. The intensity of galectin-3 expression increased as the cervical lesion progressed to carcinoma. In contrast, Lee et al. (2006) [37] observed that on real-time quantitative RT-PCR galectin-3 expression in tumour cells were significantly downregulated, compared with the corresponding normal tissue.

The expression of p16 was correlated with the expression of galectin-3. It was observed that cases with p16 proportion of 3+ or 4+ showed a galectin-3 proportion of 3+ or 4+ in >80% of cases. It was

observed that, as the expression of p16 increased from LSIL to HSIL to invasive SCC, the expression of galectin-3 also increased. A statistically significant

association was observed between p16 and galectin-3 expression (p-value of <0.05). Stiansy et al (2017) [38] observed that, in patients with cervical cancer with no or very low p16 expression, galectin-3 expression correlated with a poor prognosis in overall survival. That is galectin-3 expression level were negatively correlated with prognosis in p16 negative cases.

Conclusion

Expression of p16 & galectin-3 increases as the cervical lesion progresses to invasive cancers. This suggests that the using immunohistochemical stains is associated with the progression of cervical lesions. Therefore, immunohistochemical staining of p16 & Galectin-3 would be valuable for early detection of malignancy. But further research including research focusing on the association between these biomarkers and biopsy results is needed in the future.

References

1. Kurman R J, Carcangiu M L, Herrington C S, Young R.H.WHO classification of tumours of female reproductive organs, 4th edition. International agency for research on cancer. 2014.
2. Walboomers JM, Jacobs MV, Manos MM, Bosch F X, Kummer J A, Shah K V et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189(1): 12-49.
3. Moody CA: Human papillomavirus oncoproteins: Pathways to transformation, Nat Rev Cancer. 2010; 10(8): 550.
4. Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol. 1998;153(6): 1741-8.
5. Nakao Y, Yang X, Yokoyama M, Ferenczy A, Tang SC, Pater MM, et al. Induction of p16 during immortalization by HPV 16 and 18 and not during malignant transformation. Br J Cancer. 1997;75(10):1410-6.
6. Darragh TM, Colgan TJ, Cox T, et al. The lower anogenital squamous terminology standardization project for HPV-associated lesions: Background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol. 2013;32(1):76-115.
7. Klaes R, Benner A, Friedrich T, Ridder R, Herrington S, Jenkins D et al. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2002; 11: 1389-1399.
8. Ruvolo Peter P. Galectin 3 as the guardian of tumour microenvironment. Biochemica et Biophysica Acta. 2019; 1863(3): 427-37.
9. Kim S, Cho H, Kang S, Kim H, Park S. Is the expression of p16INK4A and galectin-3 correlated with disease progression of cervical neoplasia? Korean Journal of Obstetrics. 2011; 54: 192.
10. Poste P, Patil A, Andola SK. Incidence of nonneoplastic cervical pathologies recorded at a medical college. International Journal of applied science research and review, 2015;2(2):39-50.
11. Vincent M. Pap smear as a screening tool for evaluating cervical dysplasia and malignancy- a hospital-based study. J. Evolution Med. Dent. Sci. 2019;8(17):1368- 1371.
12. Gaikwad SL, Valand AG, Agarwal NU. Clinico-histopathological analysis of lesions of uterine cervix in

Ambejogai city of Maharashtra: A 2year study at tertiary level hospital. Journal of Diagnostic Pathology and Oncology, October-December 2016; 1(2):32-35.
13. 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 Metastasis Rev. 2006; 25:35-43.
14. Gundrajakuppam L, Shanthi V, Rao NM, Swathi S. Clinicopathological correlation of cervical carcinoma by pap smear; J Biosci Tech, 2011; 2(6): 439-445.
15. Shanthi V, Gandhi B, Rao N M, Rao B S S, carcinoma cervix screening- A Clinicopathological study, Int J Med Res Health Sci. 2015;4(2):287-293.
16. Parazzini F et al (1989); reproductive factors and the risk of invasive and intraepithelial cervical neoplasia. Br J Cancer. 1989; 59:805-809.
17. Misra JS, Srivastava S, Singh U. Risk-factors and strategies for control of carcinoma cervix in India: Hospital based cytological screening experience es of 35 years. Indian J Cancer, 2009; (46):155-9.
18. Kalyani R, Das D, Bindra Singh MS, Kumar H. Cancer profile in the department of pathology Sri Devraj Urs Medical College: a ten years study. Indian J Cancer, 2010;(47):160-5.
19. Satya B. Paul, Basant K. Tiwari, Arun Paul Choudhary, Department of Chemistry, Assam Uni, Silchar, Assam, Journal of sci and Tech, Biological and environmental science. 2011;7(1):36-42.
20. Saini S, Kanetkar RS. Histopath ological study of lesions of uterine cervix. J. Evid. Based Med. Healthc. 2016;3(103),5685-5694
21. Castellsague, X., Bosch, F.X., Munoz, N. International Agency for Research on Cancer Multicenter Study Cervical Cancer Study Group: Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Eng J Med, 2002; 346:1105-12.
22. Wynder, E.L., Cornfield, G., Schroff, P.D., Doraiswami, K.R. (1954). Study of environmental factors in carcinoma of the cervix. Am J Obstet Gynaeco. 1954; 68(4):1016-1047.
23. Parikh S**,** Oguntayo OA, Zayyan M, risk of development of cancer of the cervix varies with lifestyle E-cancer medical science. 2003 Jan 1; 5:219. 15
24. Julian crasta et al. Expression of adhesion molecule epithelial cadherin and matrixmetalloproteinase-9 in squamous neoplasia of the uterine cervix; Southern African Journal of Gynaecological Oncology. 2016; 8(1): 4–8
25. Aijaz M, Alam K, Maheshwari V, Hakim S, Kamal M. Clinicopathological study of role of CD34 expressions in the stroma of premalignant and malignant lesions of uterine cervix. Ann Diagn Pathol. 2019 Feb; 38:87-92.
26. Mamta gupta et al. Premalignant and malignant lesions of uterine cervix; national journal of laboratory medicine. 2018 Jan, 7(1):PO19-PO26
27. Pragati Agnihotri, Kafil Akhtar, Shahid A Siddiqui and Rana K Sherwani. Cervical metastasis: Significance of E-cadherin and vimentin. clin res open access. 2015;1(2).
28. Rabish Kumar, Shramana Mandal, Prerna Arora, Y. M. Mala & Nita Khurana. The expression of p16 and galectin-3 in cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC) uterine cervix, Journal of Obstetrics and Gynaecology, 2020.
29. Hartmann K, Hall SA, Nanda K, et al. systematic Evidence Review Number 25: Screening for Cervical cancer. Rockville, MD: US Department of Health and Human Services.

30. Aoyama, Kalyani R, Kai L J, Naryanaswamy M. Clinicopathological Correlation of cervical Carcinoma with use of punch biopsy. Asian Pac J Cancer Prev, 2005:1
31. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, et al. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. International Journal of Cancer. 2001; 92: 276–284.
32. Volgareva G, Zavalishina L, Andreeva Y, Frank G, Krutikova E, Golovina D, et al. Protein p16 as a marker of dysplastic and neoplastic alterations in cervical epithelial cells. BMC Cancer. 2004; 4: 58.
33. Wang JL, Zheng BY, Li XD, Nokelainen K, Angstr€om T, Lindstr€om MS, et al. p16INK4A and p14ARF expression pattern by immunohistochemistry in human papillomavirus-related cervical neoplasia. Modern Pathology. 2005; 18:629–637.
34. Kava S, Rajaram S, Arora V, Goel N, Aggarwal S, Mehta S. Conventional cytology, visual tests and evaluation of P16(INK4A) as a biomarker in cervical intraepithelial neoplasia. Indian Journal of Cancer. 2015; 52: 270–275.
35. Li M, Feng YM, Fang SQ. Overexpression of ezrin and galectin-3 as predictors of poor prognosis of cervical cancer. Brazilian Journal of Medical and Biological Research. 2017; 50: 5356.
36. Wu MZ, Wang S, Zheng M, Tian LX, Wu X, Guo KJ, et al. The diagnostic utility of p16 immunostaining in differentiating cancer and HSIL from LSIL and benign in cervical cells. Cell Transplantation. 2019; 28: 195–200.
37. Lee JW, Song SY, Choi JJ, Choi CH, Kim TJ, Kim J, et al. Decreased galectin-3 expression during the progression of cervical neoplasia. Journal of Cancer Research and Clinical Oncology. 2006; 132: 241–247.
38. Stiasny A, Freier CP, Kuhn C, Schulze S, Mayr D, Alexiou C, et al. The involvement of E6, p53, p16, MDM2 and Gal-3 in the clinical outcome of patients with cervical cancer. Oncology Letters. 2017; 14: 4467–4476.