Original Article

Association of P16, Ki-67, and CD44 expression in high-grade squamous intraepithelial neoplasia and squamous cell carcinoma of the cervix

Hajra K. Mehdi, Kalyani Raju, S. R. Sheela[1]

Departments of Pathology and [1]Obstetrics and Gynecology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India

For correspondence: Prof. Kalyani Raju, Department of Pathology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India. E-mail: drkalyanir@rediffmail.com

ABSTRACT

Background: Stem cells exist in niches in the cervical tissue at squamocolumnar junction, which when infected with HR-Human Papilloma Virus undergo malignant transformation to cancer stem cells and have a role in carcinogenesis and metastasis. The expression of CD44, P16, and Ki67 in high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) is assessed in this study.

Materials and Methods: Twenty-six cases each of normal cervix, HSIL, and SCC of cervix cases were subjected to immunohistochemistry markers; p16, Ki-67, and CD44. The association of expression of these markers between normal, HSIL, SCC cervix, and clinic-pathological parameters was statistically analyzed. $P < 0.05$ was considered significant.

Results: Of 26 cases of HSIL, 61.5%, 7.7%, and 30.8% cases were positive, ambiguous, and negative respectively for p16 expression. About 11.5%, 53.8%, and 34.6% of cases were strongly positive, positive, and weakly positive, respectively, for Ki-67 expression. About 42.3%, 42.3%, and 15.4% cases were strongly positive, positive, and weakly positive, respectively, for CD44 expression. Among 26 cases of SCC of the cervix 92.3% and 7.7% were positive and ambiguous respectively. About 73.1% and 26.9% of cases were strongly positive and positive, respectively, for Ki-67 expression. 65.4%, 30.8%, and 3.8% of cases were strongly positive, positive, and weakly positive, respectively, for CD44 expression. p16, Ki-67, and CD44 expression between the three groups were statistically significant. p16 expression versus FIGO stage including lymph node involvement and CD44 expression versus lymph node involvement in carcinoma cervix was statistically significant.

Conclusion: Expression of p16, Ki-67, and CD44 increases as the lesion progress from normal to HSIL to carcinoma cervix. p16 and CD44 expression increase with lymph node involvement. P16 expression was maximum in Stage II than Stage III.

KEY WORDS: Cancer stem cells, CD44, cervical cancer, Ki-67, p16

INTRODUCTION

Cervical cancer is the second-most common cancer in women worldwide and the most common cause of cancer-related death in women.[1] About 99.7% of these cervical cancers are associated with Human Papilloma Virus (HPV), particularly high-risk HPV (HR-HPV).[2] HR-HPV is associated with cervical intraepithelial neoplasia (CIN) and carcinoma. In India, 87.8% to 96.67% of cervical cancers are associated with HPV.[3-7] Infection by HR-HPV may be in the form of a transient or persist infection. The viral genome in persistent infection integrate with the host genome leading to increased risk of carcinogenesis.[8,9]

Stem cells exist in niches in the cervical tissue at squamocolumnar junction, which when infected with HR-HPV or affected by any risk factors, undergo malignant transformation to Cancer Stem Cells (CSC). These CSCs have the properties of multilineage differentiation, self-renewal, slow-cycling capacity, recurrence, and tumorigenicity.[10,11] CSCs are believed to be the starting point of carcinogenesis and play a role in cancer relapse and metastasis.[11-16]

Submitted: 08-Jan-2021
Accepted in revised form: 30-Apr-2021
Published: 05-Apr-2023

Access this article online
Website: https://journals.lww.com/cancerjournal/pages/default.aspx
DOI: 10.4103/jcrt.jcrt_43_21
Quick Response Code:

Cite this article as: Mehdi HK, Raju K, Sheela SR. Association of P16, Ki-67, and CD44 expression in high-grade squamous intraepithelial neoplasia and squamous cell carcinoma of the cervix. J Can Res Ther 2023;19:S260-7.

The present study aims to assess the association of p16 (HR-HPV gene integration with host genome marker), Ki-67 (proliferation marker), and CD44 (CSC marker) immunohistochemistry (IHC) expressions in normal, high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) of the cervix.

MATERIALS AND METHODS

This is a laboratory observation study. Ethical clearance was obtained from the institutional ethical committee. Inclusion criteria were the cases diagnosed as HSIL and SCC of the cervix. Normal cervical tissue was obtained from hysterectomy specimens diagnosed with disease other than the cervix. Exclusion criteria were SCC of the cervix with chemotherapy or radiotherapy and recurrent cases. The cervical biopsy and hysterectomy cases were retrieved from the records of the Department of Pathology and classified histologically into the normal cervix, HSIL (as per lower anogenital squamous terminology criteria (LAST)), and SCC of the cervix.[17] The sample size calculated was 78 cases. Hence 26 cases in each group were included in the study. Sociodemographic details of the patient as the age, presenting symptoms, colposcopic findings, FIGO staging, lymph node involvement, and size of the tumor were collected from the hospital record section. Cervical SCC cases were classified histologically as; Keratinising SCC (well-differentiated SCC [WDSCC]), Non-keratinizing large cell SCC (moderately differentiated SCC [MDSCC]), and Non-keratinizing small cell SCC (poorly differentiated SCC [PDSCC]).[17] Additional sections of 4um thickness were cut from the paraffin blocks of these cases ($26 \times 3 = 78$) and subjected to IHC staining with p16 (Biogenex, mouse monoclonal antibody), Ki-67 (Biogenex, mouse monoclonal antibody), and CD44 (Biogenex, mouse monoclonal antibody) according to the manufacturer's protocol using appropriate positive and negative controls.

Nuclear staining by P16 was considered positive [Figure 1]. Staining was interpreted based on four parameters such as intensity, extent, continuity, and location as per LAST criteria. The intensity was taken as either strong (dark brown) or weak (yellow). The extent was divided into diffuse (expression in more than 50% of the epithelium) and focal (expression in $<$50% of the epithelium). Continuity of staining was classified as either continuous (staining extends laterally over a significant distance) or discontinuous (alternating clusters stained cells). Location was divided into positive cells seen in the lower one-third, two-thirds, or entire thickness of the epithelium.[13,18] Based on these parameters, the lesions were then categorized into block-positive, negative, and ambiguous. The lesion was taken as block-positive when it fulfilled all criteria as described in LAST; showing strong and diffuse immunoreactivity extending upward from the basal layers, involving more than one-third of the epithelium and showing a continuous involvement. Negative immunostaining was defined as the complete absence of staining or weak, focal, and/or discontinuous staining. Cases that did not meet the criteria of block-positive and negative immunostaining were labeled as ambiguous.[13,18]

Cytoplasmic membrane staining by CD44 was considered positive [Figure 2]. Two features of immunohistochemical reactions were assessed separately on a semi-quantitative basis (H score). The extent of staining was expressed as the percentage of positively stained cells in 10 high-power fields in the hotspot areas in each case. The mean of the percentages was calculated and scored as 0% positive cells or positive cells located in the basal layer (score 0), 1-10% positive cells (score 1), 11%-40% positive cells (score 2), 41%-75% positive cells (score 3), and $\geq$ 76% positive cells (score 4). The staining intensity was scored subjectively as mild or weak (score 1), moderate (score 2), and strong (score 3). The final score was expressed as a product of the two scores and interpreted as 0-1 point– negative (-); 2-3 points-weakly positive (+); 4-7 points-positive (++); and $\geq$ 8 points-strongly positive (+++).[19]

Nuclear staining by Ki-67 was considered positive [Figure 3]. It was interpreted semi-quantitively by H-score similar to CD44. The final score was expressed as a product of the two scores and interpreted as 0-1 point– negative (-); 2-3 points-weakly positive (+); 4-7 points-positive (++); and $\geq$ 8 points-strongly positive (+++).[20]

Data were entered into Microsoft Excel datasheet and was analyzed using SPSS 22 version software. Categorical data were analyzed as frequencies and proportions. Chi-square test or Fischer's exact test (for 2×2 tables only) was used as test of significance for qualitative data. Yates correction was applied wherever the Chi-square test could not be applied (for 2×2 tables only). Continuous data were represented as mean and standard deviation. Independent *t*-test or Mann Whitney U test was used as test of significance to identify the mean difference between two quantitative variables and qualitative variables respectively. $P < 0.05$ was considered statistically significant. Paired *t*-test or Wilcoxon signed-rank test is the test of significance for paired data. Analysis of Variance or Kruskal–Wallis test was the test of significance to identify the mean difference between more than two groups for quantitative and qualitative data, respectively.

RESULTS

The mean age of subjects in the normal, HSIL and SCC Cervix group was 42.3 ± 9.3, 47.0 ± 13.4, and 50.4 ± 10.3 years, respectively. In the HSIL group, 42.3%, 38.5%, 11.5%, and 7.7% had white discharge per vagina, postmenopausal bleeding, bleeding per vagina, and abnormal uterine bleeding respectively. In SCC cervix group, 50%, 30.8%, 15.4%, and 3.8% had postmenopausal spotting, white discharge per vagina, bleeding per vagina, and abnormal uterine bleeding, respectively. In HSIL cases, 96.2% and 3.8% had cervical erosion and growth on colposcopic examination. In carcinoma

cases, all 100.0% had cervical growth. Among SCC cervix, 46.2% ($n = 12$), 30.8% ($n = 8$) and 23.0% ($n = 6$) were in IIB, IIA and IIIA stages respectively. In SCC cervix cases, 53.8% 30.8% and 15.4% were WDSCC, MDSCC and PDSCC respectively.

In the normal group, 100% showed negative expression for p16. In the HSIL group, 61.5%, 7.7%, and 30.8% showed block positive, ambiguous, and negative expression for p16, respectively. In the SCC cervix group, 92.3% and 7.7% showed block positive and ambiguous expression, respectively. There was statistical significant association of p16 expression between the three groups ($P < 0.001$) [Table 1].

In carcinoma cervix, among WDSCC, 85.7% and 14.3% cases showed positive and ambiguous p16 expression, respectively. Both MDSCC and PDSCC showed 100% p16 positive expression. However, there was no statistically significant association between the three groups with respect to P16 expression. Stage IIA and IIB SCC cases showed 100% P16 block positive expression. Stage IIIA SCC showed 66.7% and 33.3% block positive and ambiguous p16 expression, respectively. There was statistical significant association between p16 and FIGO stage in SCC of the cervix ($P = 0.027$).

P16 positive expression was 100.0% among SCC of cervix cases with lymph node involvement. Among SCC of cervix cases without lymph node involvement, p16 expression was block positive and ambiguous in 71.4% and 28.6% of cases, respectively. There was a statistical significant association between p16 expression and lymph node status in SCC of cervix ($P = 0.015$). Among SCC of cervix cases with tumor size <3 cm, there was 100.0% positive p16 expression. Among tumor size, more than 3 cm, 92.0% and 8.0% of cases showed block positive and ambiguous p16 expression, respectively. No significant association was found between p16 expression and the size of the tumor.

In normal cases, there was 100.0% negative Ki-67 expression. In HSIL cases, 11.5%, 53.8%, and 34.6% showed strong positive, positive, and weak positive Ki-67 expression, respectively. In SCC cervix cases, 73.1% and 26.9% showed strong positive and positive expression, respectively. There was statistical significant association of Ki-67 expression between the three groups ($P < 0.001$) [Table 2].

In carcinoma cervix, among WDSCC cases, 64.3% and 35.7% showed strong positive and positive Ki-67 expression respectively. Among MDSCC cases, 75.0% and 25.0% showed strong positive and positive expression, respectively. Among PDSCC cases, 100.0% showed strong positive expression. There was no statistical significant association between the three groups and Ki-67 expression. In Stage IIA SCC cases, 75.0% and 25% showed strong positive and positive Ki-67 expression, respectively. In Stage IIB SCC cases, 83.3% and 16.7% showed strong positive and positive expression respectively. In Stage IIIA cases, 50.0% each showed strong positive and positive expression. There was no statistical significant association was seen between Ki-67 expression and FIGO stage of carcinoma cervix.

Among SCC cervix cases with lymph node involvement, 68.4% and 31.6% showed strong positive and positive Ki-67 expression, respectively. Among SCC cervix cases without lymph node involvement, 85.7% and 14.3% showed strong positive and positive expression, respectively. There was no statistical significant association between Ki-67 expression and lymph node status in the SCC cervix. Among SCC cervix cases with tumor size <3 cm, there was 100.0% strong positive Ki-67 expression. Among SCC cervix cases with tumor size more than 3 cm, 72.0% and 28.0% showed strong positive and positive expression, respectively. There was no significant association between Ki-67 expression and size of the tumor in the SCC cervix.

In normal group, there was 100% negative CD44 expression. In the HSIL group, 42.3%, 42.3%, and 15.4% showed strong positive, positive, and weak positive CD44 expression, respectively. In the SCC cervix group, 65.4%, 30.8%, and 3.8% showed strong positive, positive, and weak positive expression respectively. There was statistical significant association of CD44 expression among three groups ($P < 0.001$) [Table 3].

Table 1: p16 expression comparison between normal, high grade squamous intraepithelial lesion and carcinoma in present study

p16 expression	Count (*n*=26), *n* (%)		
	Normal	HSIL	Carcinoma cervix
Negative	26 (100.0)	8 (30.8)	0
Ambiguous	0	2 (7.7)	2 (7.7)
Positive	0	16 (61.5)	24 (92.3)

χ^2=55.69, df=4, *P*<0.001. HSIL=High grade squamous intraepithelial lesion

Table 2: Ki-67 expression comparison between normal, high grade squamous intraepithelial lesion and carcinoma cervix cases

Ki-67 expression	Count (*n*=26), *n* (%)		
	Normal	HSIL	Carcinoma cervix
Negative (*n*=26)	26 (100.0)	0	0
Weak positive (*n*=9)	0	9 (34.6)	0
Positive (*n*=21)	0	14 (53.8)	7 (26.9)
Strong positive (*n*=22)	0	3 (11.5)	19 (73.1)

χ^2=112.45, df=6, *P*<0.001. HSIL=High grade squamous intraepithelial lesion

Table 3: CD44 expression comparison between normal, high grade squamous intraepithelial lesion and carcinoma cervix in present study

CD44 expression	Count (*n*=26), *n* (%)		
	Normal	HSIL	Carcinoma
Negative (*n*=26)	26 (100.0)	0	0
Weak positive (*n*=5)	0	4 (15.4)	1 (3.8)
Positive (*n*=19)	0	11 (42.3)	8 (30.8)
Strong positive (*n*=28)	0	11 (42.3)	17 (65.4)

χ^2=83.33, df=6, *P*<0.001. HSIL=High grade squamous intraepithelial lesion

In SCC cervix cases, among WDSCC, 50.0%, 42.9%, and 7.1% showed strong positive, positive, and weak positive CD44 expression, respectively. Among MDSCC cases, 75.0% and 25.0% showed strong positive and positive expression, respectively. Among PDSCC cases, there was 100.0% strongly positive CD44 expression. There was no statistical significant association. In Stage IIA SCC cervix cases, 87.5% and 12.5% showed strong positive and positive CD44 expression respectively. In Stage IIB SCC cases, 58.3% and 41.7% showed strong positive and positive expression respectively. In Stage IIIA cases, 50.0%, 33.3%, and 16.7% showed strong positive, positive, and weak positive expression, respectively. There was no statistically significant association between CD44 expression and the FIGO stage of carcinoma cervix.

Among SCC cervix cases with lymph node involvement, 78.9% and 21.1% showed strong positive and positive CD44 expression, respectively. Among SCC cervix cases without lymph node involvement, 28.6%, 57.2%, and 14.2% showed strong positive, positive, and weak positive expression, respectively. There was statistical significant association between CD44 expression and lymph node status in the SCC cervix ($P = 0.032$). Among SCC cervix cases with tumor size <3 cm, there was 100.0% strongly positive CD44 expression. Among SCC cervix cases with tumor size more than 3 cm, 64.0%, 32.0%, and 4.0% showed strong positive, positive, and weak positive expression, respectively. There was no significant association between CD44 expression and the size of the tumor in the SCC cervix.

There was statistical significant ($P < 0.001$) positive association between the expression of p16 versus Ki-67 among normal, HSIL, and SCC. There was statistical significant ($P < 0.001$) positive association between the expression of p16 versus CD44 among normal, HSIL and SCC. There was statistical significant ($P < 0.001$) positive association between the expression of Ki-67 versus CD44 among normal, HSIL, and SCC.

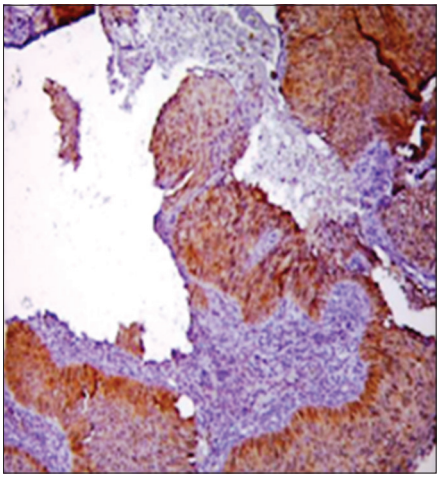

Figure 1: Microphotograph of squamous cell carcinoma of cervix showing nuclear stain of P16 marker (P16 IHC, ×100)

DISCUSSION

Cervical cancer is the second-most common cancer among women worldwide. It is the most commonly reported gynecological malignancy in India and is also one of the major cause of cancer-related morbidity.[1] In South India, the prevalence of cervical cancer reported is 17.55% among female cancer. The incidence of HR-HPV infection peaks around 25 years of age, which coincides with the peak age for sexual activity. More than 90% of HSIL and virtually all cases of cervical cancer are associated with HR-HPV infection.[2]

In the present study, all the normal cases showed negative p16 expression. Similar observations were made in the study done by Izadi-Mood *et al*. and Sarma *et al*.[21,22] In the present study, 61.5% and 30.8% of HSIL cases showed block positive and negative p16 expression respectively. These findings were similar to the findings of Izadi-Mood *et al*. and Sarma *et al*.[21,22] However, 7.7% of the HSIL cases in the present study showed ambiguous p16 expression. In a study done by Liu *et al*. in 2017 on 220 CIN 2 cases, 23% showed ambiguous p16 expression. p16 ambiguous cases were distinct form of HSIL that had an intermediate risk of progression [Table 4].[23] In the present study, 92.3% and 7.7% of cases of SCC of cervix showed block positive and ambiguous p16 expression, respectively. In a study done by Izadi-Mood *et al*., 75%, 15%, and 10% of the carcinoma cases showed block positive, ambiguous, and negative p16 expression, respectively. In a similar study done by Sarma *et al*., all the carcinoma cases showed strong positive p16 expression.[22] The two ambiguous positive cases in the present study probably may be due to technical issues [Table 5].[21,22] There was statistical significant association in the p16 expression between the normal, HSIL and SCC cervix groups ($P < 0.001$). The findings in the present study were in conjunction to the findings in the other studies as shown in Tables 4 and 5.

In the present study, there was statistical significant association between p16 expression and the stage of SCC of the cervix ($P = 0.027$). In a study by Weng *et al*. and Son *et al*., there was no statistical association in the expression of p16 and stage of carcinoma ($P > 0.05$).[24,25] The findings of the studies by Mahajan and Amaro-Filho *et al*. were similar to the present study, where P16 expression was maximum in Stage II than in Stage III.[26,27] In a study by Huangfu *et al*. serum P16 auto-antibody levels were maximum in Stage I of cervical cancer than in later stages and P16 was considered as one of the early prognostic parameters.[28] In the present study, there was no significant association between p16 expression and histological types. However, all cases of MDSCC and PDSCC showed block positive expression of p16. Similar findings were reported in a study by Huang *et al*., where there were no significant findings in p16 expression among the different grades of SCC.[11]

In the present study, there was significant association between p16 expression and lymph node involvement ($P = 0.015$).

However, in a study by Son *et al*. and Weng *et al*., there was no significant correlation in p16 expression with respect to lymph node involvement.[24,25] In the present study, there was no statistical association between p16 expression and size of the tumor ($P = 0.768$) similar to the by Weng et *al*.[24] However, in the present study, tumor of <3 cm showed 100% p16 expression.

In the present study, all the normal cases showed negative for Ki-67 expression. Similar findings were observed in a study by Hebbar and Murthy[29] Amaro-Filho *et al*. in their study found that weak Ki-67 positivity was localized in the basal layer in 34.9% of the normal cervix. This was explained by the presence of squamous metaplastic cells and regenerative cells which stain positive for Ki-67 immunostaining.[27] In the present study, 11.5%, 53.8%, and 34.6% of HSIL cases showed strong positive, positive, and weak positive Ki-67 expression respectively. These findings were similar to the findings of Hebbar and Murthy and Agoff *et al*. [Table 6].[29,30] In the present study, 73.1% and 26.9% of cases of SCC cases showed strong positive and positive Ki-67 expression, respectively. Similar findings were seen in the findings of Hebbar and Murthy, Amaro-Filho *et al*. and Agoff *et al*. [Table 7].[27,29,30] There was a significant association of Ki-67 expression between the normal, HSIL, and SCC cervix groups ($P < 0.001$). The rising trend of Ki-67 expression from normal to HSIL to SCC is reported in other studies as seen in the present study [Table 2].[27,29,30]

There was no statistical significant association between Ki-67 expression and the grade of SCC in the present study. This finding was similar to the findings of a study by Yu *et al*.[21] However, there was 100% Ki-67 expression in PDSCC in the present study. In the present study, there was no statistical significant association between the Ki-67 expression and stage of SCC. Similar findings are reported by various authors.[31-33] However, Amaro-Filho *et al*. reported a statistical significant association of Ki-67 immunostaining with the stage of carcinoma.[27]

In the present study, there was no statistical significant association between the Ki-67 expression and lymph node status in the SCC cervix ($P = 0.378$). This was in contrast to the findings in a study by Yu *et al*., which reported significant statistical association.[21] In the present study, there was no statistical significant association between the size of the tumor and the Ki-67 expression. Yu *et al*. reported significant association of Ki-67 expression (72.7% cases) with respect to the size of the tumor.[21]

In the present study, all the normal cases showed no expression or weak positive expression located in the basal layer which was interpreted as negative for CD44 expression. Similar findings were also reported in the study by Faleiro-Rodrigues and Lopes and Steidl *et al*.[34,35] In

Table 4: p16 expression in high grade squamous intraepithelial lesion cases in present study compared with other studies

P16 expression	Izadi-Mood *et al*., 2012,[21] *n* (%)	Sarma *et al*., 2017,[22] *n* (%)	Present study 2018, *n* (%)
Negative	2 (18.2)	10 (30.3)	8 (30.8)
Ambiguous	0	-	2 (7.7)
Positive	9 (81.8)	23 (69.7)	16 (61.5)
Number of cases	11	33	26

Table 5: p16 expression in carcinoma cervix cases in present study compared with other studies

P16 expression	Izadi-Mood *et al*., 2012,[21] *n* (%)	Sarma *et al*., 2017,[22] *n* (%)	Present study (2018), *n* (%)
Negative	2 (10.0)	0	
Ambiguous	3 (15.0)	-	2 (7.7)
Positive	15 (75.0)	26 (100.0)	24 (92.3)
Number of cases	20	26	26

Table 6: Ki-67 expression in high grade squamous intraepithelial lesion cases in present study compared with other studies

Ki-67 expression	Hebbar *et al*., 2017,[29] *n* (%)	Agoff *et al*., 2003,[30] *n* (%)	Present study 2018, *n* (%)
Negative	1 (5.0)	0	0
Weak positive	2 (10.0)	20 (11.3)	9 (34.6)
Positive	9 (45.0)	38 (21.3)	14 (53.8)
Strong positive	8 (40.0)	120 (67.4)	3 (11.5)
Number of cases	20	178	26

Table 7: Ki-67 expression in carcinoma cervix cases in present study compared with other studies

Ki-67 expression	Hebbar *et al*., 2017,[29] *n* (%)	Amaro-Filho *et al*., 2013,[27] *n* (%)	Agoff *et al*., 2003,[30] *n* (%)	Present study 2018
Negative	0	0	0	0
Weak positive	0	0	0	0
Positive	1 (17.0)	14 (17.1)	3 (6.7)	7 (26.9)
Strong positive	5 (83.0)	68 (82.9)	42 (93.3)	19 (73.1)
Number of cases	6	82	45	26

the present study, 42.3%, 42.3%, and 15.4% of HSIL cases showed strong positive, positive, and weak positive CD44 expression. These findings were similar to the findings of Faleiro-Rodrigues and Lopes and Callagy *et al*. [Table 8].[34,35] In the present study, 65.4%, 30.8%, and 3.8% of SCC of cervix cases showed strong positive, positive, and weak positive for CD44 expression. These findings were similar to the findings of Faleiro-Rodrigues and Lopes and Uhl-Steidl *et al*. [Table 9].[34,35] The cases in the HSIL group and SCC group that are weak positivity and negative for CD44 expression can be explained by the unstable expression of the CD44 gene or failure of the protein to translocate and/or attach to the cell membrane due to the absence of supporting proteins in cases of HSIL and SCC.[34] CD44 expression is reported to be high in cervical cancer than in normal tissues and CD44 positive cells has shown increased capacity for self-renewal in cervical cancer cell lines.[31]

In the present study, no significant association was established between the expression of CD44 and the grade of SCC. Similar findings are reported in different studies by various authors.[32-40] There was no statistical significant association between FIGO stage and CD44 expression in the present study. Similar findings were reported by Uhl-Steidl *et al*.[35]

In the present study, there was statistical significant association between CD44 expression and lymph node status ($P = 0.032$). This finding was in contrast to the study done by Ayhan *et al*., which did not report significant statistical association.[16] However, Dasari *et al*. in their study showed significant association between the serum levels of soluble CD44 and the lymph node involvement in carcinoma cervix. Serum soluble CD44 levels were higher in patients with lymph node involvement.[37] In the present study, there was no statistical significant association between the size of the tumor and the CD44 expression. Ayhan *et al*. and Bouda *et al*. found significant statistical correlation between the size of the tumor and CD44 expression.[16,40] However in the present study, there was 100% CD44 expression in tumor with <3 cm which indicate expression in the early phase of SCC of the cervix as stated in the study by Callagy *et al*.[32] CD44 shows high expression in radiation-resistant cervical cancer indicating epithelial-mesenchymal transition and predicts radiation-resistant patients in cervical cancer. CD44

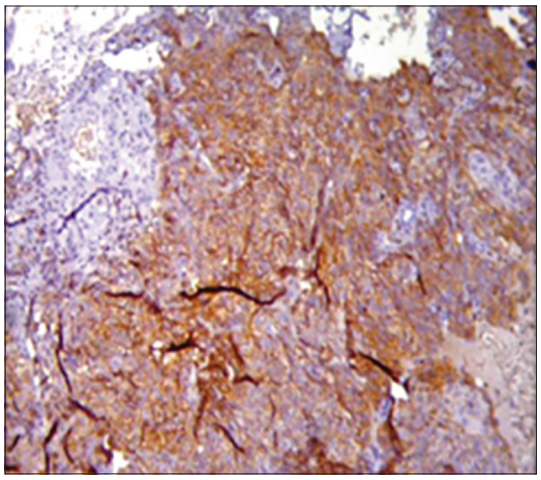

Figure 2: Microphotograph of squamous cell carcinoma of cervix showing cytoplasmic stain of CD44 marker (CD44 IHC, ×100)

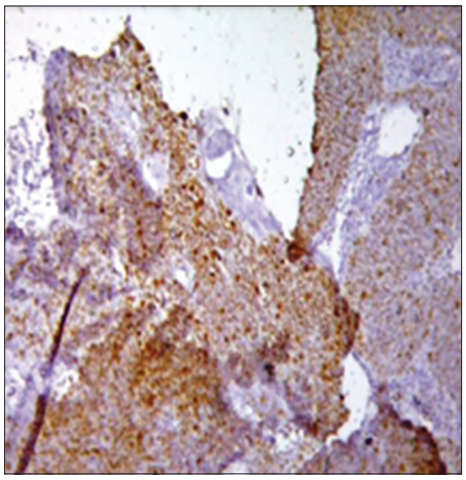

Figure 3: Microphotograph of squamous cell carcinoma of cervix showing nuclear stain of Ki67 marker (Ki67 IHC, ×100)

Table 8: CD44 expression in high grade squamous intraepithelial lesion cases in present study compared with other studies

CD44 expression	Callagy *et al*., 2000,[32] *n* (%)	Rodrigues *et al*., 2004,[34] *n* (%)	Present study 2018, *n* (%)
Negative	1 (4.2)	3 (5.6)	0
Weak positive	0	-	4 (15.4)
Positive	5 (20.8)	-	11 (42.3)
Strong positive	18 (75.0)	51 (94.4)	11 (42.3)
Number of cases	24	54	26

Table 9: CD44 expression in squamous cell carcinoma cervix cases in present case in comparison with other studies

CD44 expression	Faleiro- Rodrigues *et al*., 2004,[34] *n* (%)	Uhl- Steidl *et al*., 1998,[35] *n* (%)	Present study 2018, *n* (%)
Negative	5 (19.2)	0	0
Weak positive	-	14 (51.9)	1 (3.8)
Positive	-	11 (40.7)	8 (30.8)
Strong positive	21 (80.8)	2 (7.4)	17 (65.4)
Number of cases	26	27	26

exhibits stem cell characteristics.[31,38] CD44 is reported to independently predict reduced locoregional control in locally advanced cervical cancer.[36]

Limitations of this study are; small sample size, radiological lymph node status was considered with no lymph node sampling for histopathological evaluation, unavailability of FIGO Stage I and IV cases, and cases were not followed.

However, in the present study, all the three markers expression showed statistical significant positive association from normal to HSIL to SCC showing gradual increase of expression from normal to HSIL to SCC of cervix indicating the transformation of normal cells to HSIL and later to cancer cells with progressive expression of p16 marker (integration of HPV viral gene with host gene leading to immortalized cells) also gradual increase in expression of both Ki-67 (indicating proliferation of immortalized cells) and CD44 (indicating role of CSCs and its increased expression in SCC). All three markers showed 100% expression in PDSCC cases and the P16 marker also showed 100% expression in MDSCC indicating poor prognosis in cases showing expression of these three markers. Stage II SCC cases showed 100% expression of p16 and CD44 expression was maximum in Stage II compared to Stage III tumor indicating increase in expression of these markers in evolving tumor in initial stages. P16 and CD44 markers show maximum expression in SCC cases with lymph node involvement suggesting the role of these markers in lymph node metastasis and spread of cancer. All three markers showed maximum expression in SCC cases with <3 cm size indicating their role in the initial phase of the disease toward the progression of the disease. Hence, this information can be exploited for adjuvant targeted chemotherapy in SCC cases. However, further studies with molecular diagnosis and in larger sample size may help in reaching a consensus.

CONCLUSION

The stem cells in cervical tissue when integrates with the HPV viral genome (p16 marker) and acquires the property to proliferate (Ki-67 marker) transform itself into CSCs (CD44 marker) and responsible for carcinogenesis. The expression of P16 and CD44 increases proportionately with PDSCC, Stage II disease, lymph node involvement and in lesions < 3 cm. These findings can be used to assess the prognosis of SCC of the cervix and throw light in the development of adjuvant targeted therapy.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol 2010;117:S5-10.
2. Kalyani R, Das S, Singh MS, Kumar HM. Cancer profile in Kolar: A ten years study. Indian J Cancer 2010;47:160-5.
3. Sreedevi A, Javed R, Dinesh A. Epidemiology of cervical cancer with special focus on India. Int J Womens Health 2015;7:405-14.
4. Sowjanya AP, Jain M, Poli UR, Padma S, Das M, Shah KV, *et al.* 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 Infect Dis 2005;5:116.
5. Kulkarni SS, Kulkarni SS, Vastrad PP, Kulkarni BB, Markande AR, Kadakol GS, *et al.* Prevalence and distribution of high risk human papillomavirus (HPV) Types 16 and 18 in Carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. Asian Pac J Cancer Prev 2011;12:645-8.
6. Gheit T, Vaccarella S, Schmitt M, Pawlita M, Franceschi S, Sankaranarayanan R, *et al.* Prevalence of human papillomavirus types in cervical and oral cancers in central India. Vaccine 2009;27:636-9.
7. Basu P, Roychowdhury S, Bafna UD, Chaudhury S, Kothari S, Sekhon R, *et al.* Human papillomavirus genotype distribution in cervical cancer in India: Results from a multi-center study. Asian Pac J Cancer Prev 2009;10:27-34.
8. Kalyani R. Markers in cervical cancer screening and diagnosis. In: Recent Advances in Cervical Cancer. Ch. 2. Germany: Avid Publisher; 2016. p. 2-35.
9. Münger K. The role of human papillomaviruses in human cancers. Front Biosci 2002;7:641-9.
10. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell 2014;14:275-91.
11. Huang R, Rofstad EK. Cancer stem cells (CSCs), cervical CSCs and targeted therapies. Oncotarget 2016;10:1-7.
12. Feng D, Peng C, Li C, Zhou Y, Li M, Ling B, *et al.* Identification and characterization of cancer stem-like cells from primary carcinoma of the cervix uteri. Oncol Rep 2009;22:1129-34.
13. Liu H, Wang YJ, Bian L, Fang ZH, Zhang QY, Cheng J×. CD44+/CD24+cervical cancer cells resist radiotherapy and exhibit properties of cancer stem cells. Eur Rev Med Pharmacol Sci 2016;20:1745-54.
14. López J, Poitevin A, Mendoza-Martínez V, Pérez-Plasencia C, García-Carrancá A. Cancer-initiating cells derived from established cervical cell lines exhibit stem-cell markers and increased radioresistance. BMC Cancer 2012;12:48.
15. López J, Ruíz G, Organista-Nava J, Gariglio P, García-Carrancá A. Human papillomavirus infections and cancer stem cells of tumors from the uterine cervix. Open Virol J 2012;6:232-40.
16. Ayhan A, Baykal C, Al A, Ayhan A. Altered CD44 variant 6 expression in FIGO stage IB cervical carcinoma. Gynecol Oncol 2001;83:569-74.
17. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, *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. Arch Pathol Lab Med 2012;136:1266-97.
18. Lesnikova I, Lidang M, Hamilton-Dutoit S, Koch J. p16 as a diagnostic marker of cervical neoplasia: A tissue microarray study of 796 archival specimens. Diagn Pathol 2009;4:22.
19. Rozeik MS, Hammam OA, Ali AI, Magdy M, Khalil H, Anas A, *et al.* Evaluation of CD44 and CD133 as markers of liver cancer stem cells in Egyptian patients with HCV-induced chronic liver diseases versus hepatocellular carcinoma. Electron Physician 2017;9:4708-17.
20. Yu JQ, Zhou Q, Zheng YF, Bao Y. Expression of vimentin and Ki-67 proteins in cervical squamous cell carcinoma and their relationships with clinicopathological features. Asian Pac J Cancer Prev 2015;16:4271-5.
21. Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S, Ahmadi SA, Sani S, *et al.* Potential diagnostic value of P16 expression in premalignant

and malignant cervical lesions. J Res Med Sci 2012;17:428-33.
22. Sarma U, Biswas I, Das A, Das GC, Saikia C, Sarma B. p16INK4a expression in cervical lesions correlates with histologic grading – A tertiary level medical facility based retrospective study. Asian Pac J Cancer Prev 2017;18:2643-7.
23. Liu Y, Alqatari M, Sultan K, Ye F, Gao D, Sigel K, *et al.* Using p16 immunohistochemistry to classify morphologic cervical intraepithelial neoplasia 2: Correlation of ambiguous staining patterns with HPV subtypes and clinical outcome. Hum Pathol 2017;66:144-51.
24. Weng MY, Li L, Feng SY, Hong SJ. Expression of Bmi-1, P16, and CD44v6 in uterine cervical carcinoma and its clinical significance. Cancer Biol Med 2012;9:48-53.
25. Son SM, Noh K, Lee HC, Park YJ, Jeong EH, Kim HS, *et al.* Evaluation of p16INk4a, pRb, p53 and Ki-67 expression in cervical squamous neoplasia. J Biomed Res 2012;13:209-17.
26. Mahajan A. Practical issues in the application of p16 immunohistochemistry in diagnostic pathology. Hum Pathol 2016;51:64-74.
27. Amaro-Filho SM, Golub JE, Nuovo GJ, Cunha CB, Levi JE, Villa LL, *et al*. A comparative analysis of clinical and molecular factors with the stage of cervical cancer in a Brazilian cohort. PLoS One 2013;8:e57810.
28. Huangfu M, Liu L, Xu S, Li S, Jiang K, Sun B, *et al.* Detecting of p16 autoantibody as a potential early diagnostic serum biomarker in patients with cervical cancer. Clin Lab 2016;62:1117-20.
29. Hebbar A, Murthy VS. Role of p16/INK4a and Ki-67 as specific biomarkers for cervical intraepithelial neoplasia: An institutional study. J Lab Physicians 2017;9:104-10.
30. Agoff SN, Lin P, Morihara J, Mao C, Kiviat NB, Koutsky LA. p16(INK4a) expression correlates with degree of cervical neoplasia: A comparison with Ki-67 expression and detection of high-risk HPV types. Mod Pathol 2003;16:665-73.
31. Organista-Nava J, Gómez-Gómez Y, Garibay-Cerdenares OL, Leyva-Vázquez MA, Illades-Aguiar B. Cervical cancer stem cell-associated genes: Prognostic implications in cervical cancer. Oncol Lett 2019;18:7-14.
32. Callagy G, O'Grady A, Butler D, Leader M, Kay E. Expression of CD44 in uterine cervical squamous neoplasia: A predictor of microinvasion? Gynecol Oncol 2000;76:73-9.
33. Ancuţa E, Ancuţa C, Cozma LG, Iordache C, Anghelache-Lupaşcu I, Anton E, *et al.* Tumor biomarkers in cervical cancer: Focus on Ki-67 proliferation factor and E-cadherin expression. Rom J Morphol Embryol 2009;50:413-8.
34. Faleiro-Rodrigues C, Lopes C. E-cadherin, CD44 and CD44v6 in squamous intraepithelial lesions and invasive carcinomas of the uterine cervix: An immunohistochemical study. Pathobiology 2004;71:329-36.
35. Uhl-Steidl M, Huy VQ, Müller-Holzner E, Ruth N, Zeimet AG, Stauder R, *et al.* CD44 splice variant expression in normal and malignant uterine cervical epithelium. Int J Gynecol Cancer 1998;8:460-6.
36. Chopra S, Deodhar K, Pai V, Pant S, Rathod N, Goda JS, *et al.* Cancer stem cells, CD44, and outcomes following chemoradiation in locally advanced cervical cancer: Results from a prospective study. Int J Radiat Oncol Biol Phys 2019;103:161-8.
37. Dasari S, Rajendra W, Valluru L. Evaluation of soluble CD44 protein marker to distinguish the premalignant and malignant carcinoma cases in cervical cancer patients. Med Oncol 2014;31:139.
38. Zhang J, Chen X, Bian L, Wang Y, Liu H. CD44+/CD24+-Expressing cervical cancer cells and radioresistant cervical cancer cells exhibit cancer stem cell characteristics. Gynecol Obstet Invest 2019;84:174-82.
39. Costa S, Terzano P, Bovicelli A, Martoni A, Angelelli B, Santini D, *et al.* CD44 isoform 6 (CD44v6) is a prognostic indicator of the response to neoadjuvant chemotherapy in cervical carcinoma. Gynecol Oncol 2001;80:67-73.
40. Bouda J, Boudova L, Hes O, Havir M, Tempfer C, Kohlberger P, *et al.* CD44v6 as a prognostic factor in cervical carcinoma FIGO stage IB. Anticancer Res 2005;25:617-22.