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Original Contribution

Clinicopathological study of role of CD34 expressions in the stroma of premalignant and malignant lesions of uterine cervix

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ABSTRACT

CD34 is a transmembrane glycoprotein that is thought to be involved in the modulation of cell adhesion and signal transduction. The connective tissue stroma of virtually all human organs contain large amounts of resident CD34+ fibrocytes, which are involved in multiple functions such as wound healing, secretion of cytokines and also participate in stromal remodeling. It has been seen in various studies that absence of CD34+ fibrocytes within the stroma is associated with invasive carcinomas. In our study, we also investigated the presence and distribution of CD34+ fibrocytes in cervical intraepithelial neoplasia, invasive cervical carcinoma and adjacent normal cervical stroma. It was seen that normal cervical stroma and the stroma adjacent to cervical intra epithelial lesions harbours a dense meshwork of CD34+ fibrocytes, whereas the stroma of invasive carcinoma was nearly devoid of this cell population. Early stromal invasion by squamous carcinoma was characterized by a focal loss of CD34+ fibrocytes. This can be used as a sensitive tool in detecting tiny foci of stromal invasion in early cancer.

1. Introduction

During carcinogenesis, infiltration of epithelial cancer cells into the stroma induces new matrix formation by activating the peritumoral stromal cells. Thus reflecting a complex interaction between the neoplastic epithelial cells and its microenvironment.

Previous studies have shown that normal tissues harbor large amounts of CD34 fibrocytes in the stroma whereas corresponding stroma in case of invasive carcinomas is accompanied by lack of CD34+ cells. This type of strong reduction of CD34 staining in the stroma actually reflects a high grade of peritumoral stromal remodeling that is seen in a variety of squamous cell cancers, including cervical carcinomas. However, it has been observed that this loss of CD34 expression in the stromal tissue is partly associated with gain of α-smooth muscle actin (α-SMA) positive myofibroblasts. Such changes have been detected in stroma of colorectal adenocarcinoma [1], pancreatic adenocarcinoma [2] and invasive ductal breast cancer [3] and also at many other sites.

Studies have shown that the stroma of the uterine cervix is also partly composed of CD34+ fibrocytes [4,5]. There are two distinct subepithelial compartments, within the etocervix and the endocervix. It has been observed that endocervix has twice the population of stromal cells, compared to ectocervix, regardless of age. Cervical plasticity is partially attributed to these stromal cells and thus study of these cells help in better understanding of neoplastic processes related to these stromal cells [6]. The present study is, therefore, aimed to study CD34 expressions in the stroma of premalignant and malignant cervical lesions and discuss the role of CD34 in differentiation of premalignant and malignant diseases of the uterine cervix.

2. Material and methods

The present study was conducted in the Department of Pathology, JNMCH, Aligarh (U.P) from July 2014 to July 2016. A total of two hundred fifteen (215) patients suffering from premalignant and malignant lesions of cervix were evaluated by H&E–stained tissue sections.

Immunohistochemistry was performed using the standard avidin biotin complex (ABC)-peroxidase method and 3,3′-diaminobenzidine (DAB) as chromogen. CD34 antigen was detected by means of a monoclonal antibody (QBEND10, Dako, Hamburg, Germany, dilution 1:50) after microwave pretreatment.

3. Observations

All 215 cases collected were grouped into two broad categories, premalignant and malignant cervical lesions. A total of 72 (33.5%) cases of premalignant lesions and 143 (66.5%) cases of malignant lesions of cervix were reported. Out of the premalignant lesions, LSIL was

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Table A1
Distribution of lesions of Cervix on the basis of histopathological findings.

Type of lesions	No. of cases (%)
A) Premalignant	72 (33.5%)
LSIL	42 (58.3%)
HSIL including carcinoma in situ	30 (41.7%)
B) Malignant	143 (66.5%)
Squamous cell carcinoma	127 (88.8%)
Adenocarcinoma	12 (8.4%)
Adenosquamous carcinoma	03 (2.1%)
Spindle cell sarcoma	01 (0.7%)

found to be more common than HSIL. (Table A1).

The Age of patient with cervical lesions ranged from 20 to 85 years with majority of cases seen in between the 4th and 6th decade. Squamous Cell Carcinoma (88.8%) was found to be the most common malignant lesion. In the premalignant category, the mean age observed was 43.63 years with a SD of 11.30 years. Whereas the mean age of patients with malignant lesions was 51.14 years with a SD of 11.34 years

In malignant squamous lesions, Out of 127 cases of squamous cell carcinoma, most common histological type observed was of Large Cell Non Keratinizing Squamous Cell carcinoma (47.2%), followed by Large Cell Keratinizing Squamous Cell Carcinoma (31.5%) and then other rare variants (21.3%).(Fig. A1)

Majority of malignant cases presented with bleeding per vaginum, predominantly post-menopausal bleeding (56.64%) seen in elderly women, followed by post coital bleeding (39.86%) and abnormal menstruation (intermenstrual bleeding, menorrhagia).

Out of 143 malignant lesions of cervix, 42 cases (29.4%) showed lymph node metastasis.

On the basis of FIGO staging, majority of them i.e. 101 cases (70.60%), belonged to FIGO Stage I, followed by Stage II (16.10%). Stage III and Stage IV comprised of (8.40%) and (4.20%) respectively.

3.1. CD34 expression

A total of 105 cases were selected and subjected to immunohistochemical staining for CD34. The number of cases selected in each category is shown in Table A2.

Immunohistochemistry for CD34 was performed on paraffin embedded sections using anti CD34 antibody (QBEnd/10) and semi quantitative assessment of the staining was done using the following protocol:

Grading:

Table A2
Grading of CD34 in premalignant and malignant lesions.

Grading	LSIL	HSIL	Squamous cell carcinoma	Adenocarcinoma
0	0	0	50 (76.92%)	06 (60%)
+	0	03 (20%)	07 (10.76%)	03 (30%)
++	15 (100%)	12 (80%)	08 (12.32%)	01 (10%)
Total	15 (100%)	15 (100%)	65 (100%)	10 (100%)

0: upto 10% cells immunoreactive.
+1: > 10% and upto 50% cells immunoreactive.
+2: > 50% cells strongly immunoreactive.

Assuming that a high power microscopic field harbored 100 stromal cells.

When stained for CD34, endothelial cells normally show cytoplasmic staining with membranous accentuation.

3.1.1. CD34 expressions in normal ectocervix and endocervix

The ectocervix of an adult female has non keratinized stratified squamous epithelium lined surface, which shows cyclical change in response to hormones or menstrual activity, whereas endocervix which is lined by mucinous columnar cells shows minimal cytological change in response to these steroid hormones. Moreover, underlying stroma also differs in the two epithelial compartments. The endocervical stroma has twice the number of stromal cells, compared to ectocervical counterpart. Hence immunohistochemical features of CD34 staining appear to be more marked in endocervix, when compared to ectocervix, due to more density of stromal cells in the former. Although normal stroma contains a dense network of CD34+ fibrocytes, they are predominantly located in subepithelial and perivascular areas (Fig. B1 & B2).

3.1.2. CD34 expression in premalignant lesions

Out of 30 cases of premalignant lesions, all cases of LSIL showed diffuse (+2) immunoreactivity (Fig. B3 & B4) whereas 12 cases of HSIL showed diffuse (+2) immunoreactivity and 3 cases showed focal immunoreactivity (Fig. B5 & B6). Adjacent normal cervical stroma also showed dense reticular network of CD34+ fibrocytes (+2 positivity) predominantly in subepithelial and perivascular area (Fig. B2). None of the cases showed negative immunostaining. Hence no significant difference was seen in CD34 expression between LSIL and HSIL. (p-value > 0.05). (Table A2).

3.1.3. CD34 expression in malignant lesions of cervix

Out of 65 cases of squamous cell carcinoma, majority of cases (54

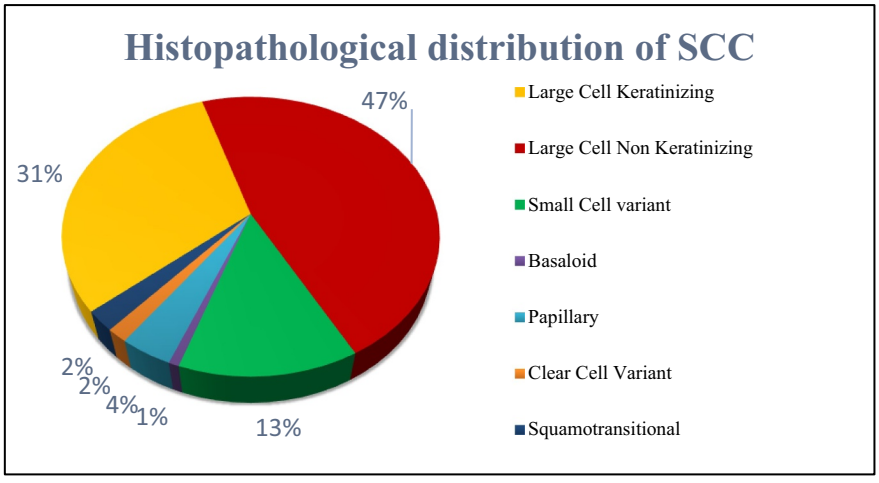

Fig. A1. Histopathological distribution of squamous cell carcinoma of cervix.

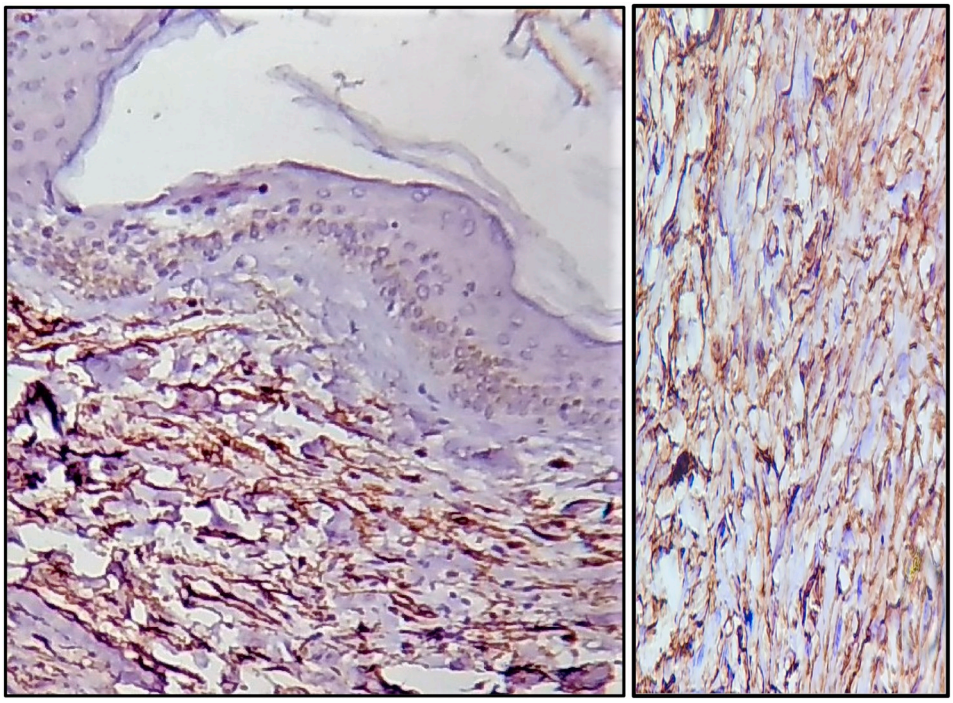

Fig. B1. Normal histology of ectocervix with CD34 immunostaining of stromal cells. (Intensity score 2+); 400X.

cases) showed negative immunostaining (Fig. B10). 8 cases showed focal loss of immunoexpression that also include 3 cases of micro-invasion (Fig. B8). Only 3 cases showed retained expression of CD34 and showed diffuse positivity in the stroma. Whereas in adenocarcinoma, out of 10 cases 6 cases showed negative immunoreactivity (Fig. B12), 3 cases showed focal loss of expression and only 1 case showed diffuse immunostaining. The difference in loss of CD34 expression between squamous cell carcinoma and adenocarcinoma was statistically non-significant. (p-value > 0.2) (Figs. B7 to B12).

4. Results

On comparison of CD34 expression in the stroma of premalignant and malignant lesions of cervix, the loss of CD34 expression was found

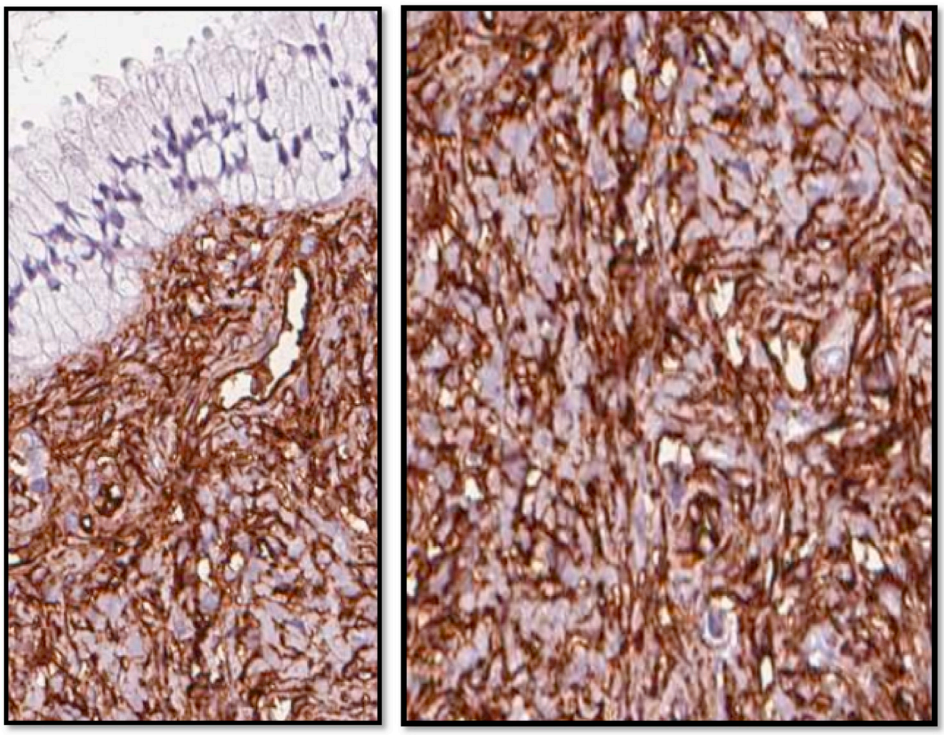

Fig. B2. Normal histology of endocervix with CD34 immunostaining of stromal cells. Almost 100% of cells showing membranous immunoreactivity (intensity score 2+). CD34 is a transmembrane protein which shows membranous positivity in fibroblasts of normal uterine cervix (400X).

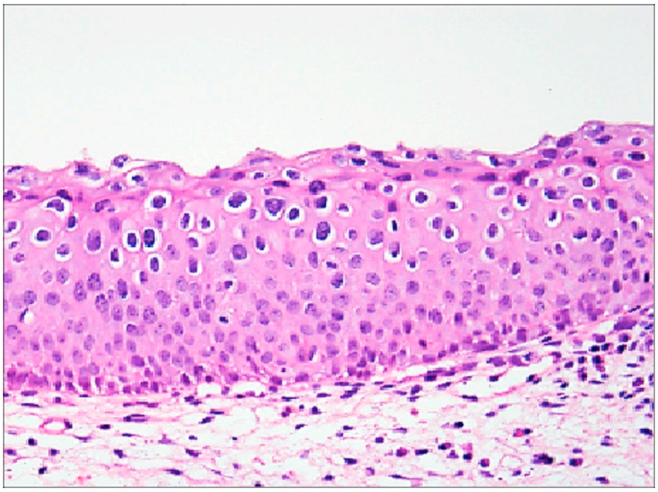

Fig. B3. Micrograph showing LSIL, with dysplastic changes in 1/3rd of epithelium. Note the presence of koilocytes, suggesting HPV infection (a cell with increased N/C ratio, irregular nuclear membrane, perinuclear halo with cytoplasmic condensation {H & E; 400X}).

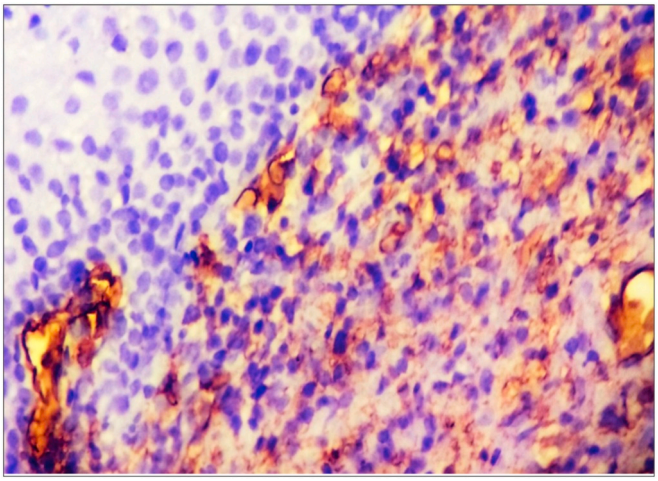

Fig. B4. Micrograph showing LSIL or CIN I, with CD34 immunostaining of stromal fibroblasts (+2 intensity score); 400X.

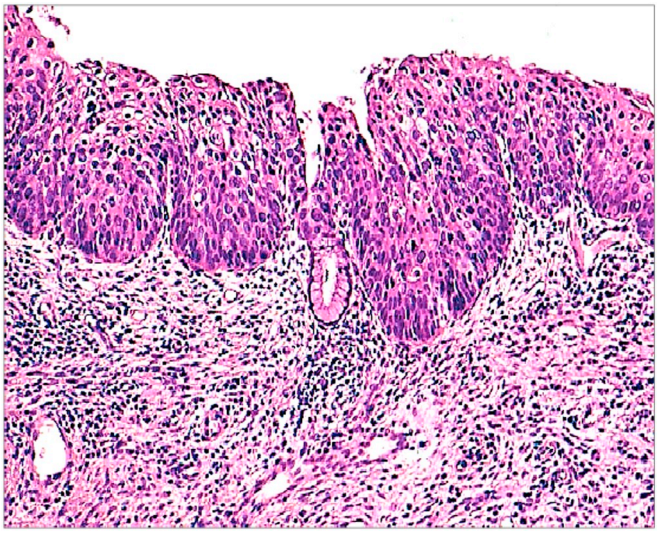

Fig. B5. Micrograph showing HSIL or CIN III with > 2/3 rd of the epithelium showing dysplastic features. Koilocytes are also seen here. (H & E; X400).

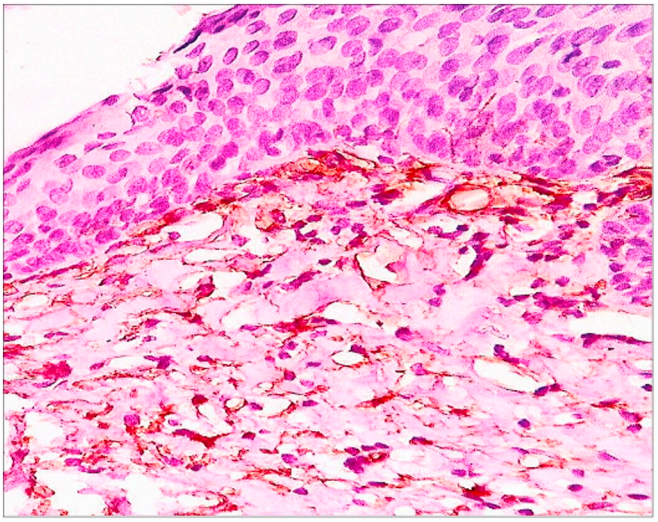

Fig. B6. Micrograph showing HSIL or CIN II; almost 90% of stromal fibroblasts are showing strong membranous positivity. (intensity score: 2+). X400.

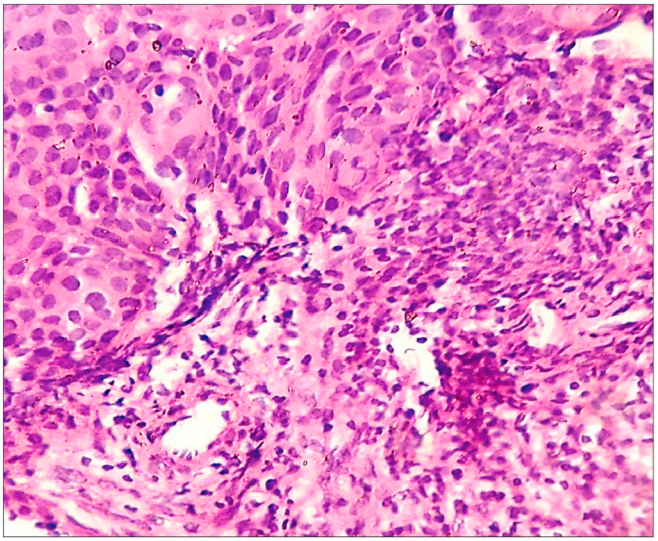

Fig. B7. Microinvasive carcinoma, showing focus of neoplastic cells invading into the stroma, surrounded by lymphocytes (H&E; X400).

to be statistically significant between LSIL and invasive carcinoma (p-value < 0.001) and similarly between HSIL and invasive carcinoma loss of CD34 was found to be statistically significant (p-value < 0.001).

5. Discussion

The cancer of uterine cervix is the second most prevalent malignancy of women in India and is a major cause of mortality and morbidity among women. According to the WHO report, globally, cervical cancer comprises 12% of all cancers in women and it is the leading gynaecological malignancy in the world.

Many studies have been published till now regarding the expression of CD34 in various malignancies. However, there have been varying data and slightly different results were obtained by different authors. Nonetheless, there is a general consensus that CD34 appears to be a new and upcoming marker which can be useful in assessing early diagnosis of invasive cervical cancer.

CD34, a 110-kDa transmembrane cell-surface glycoprotein, has been identified as a marker of human hematopoietic cells [7]. CD34-

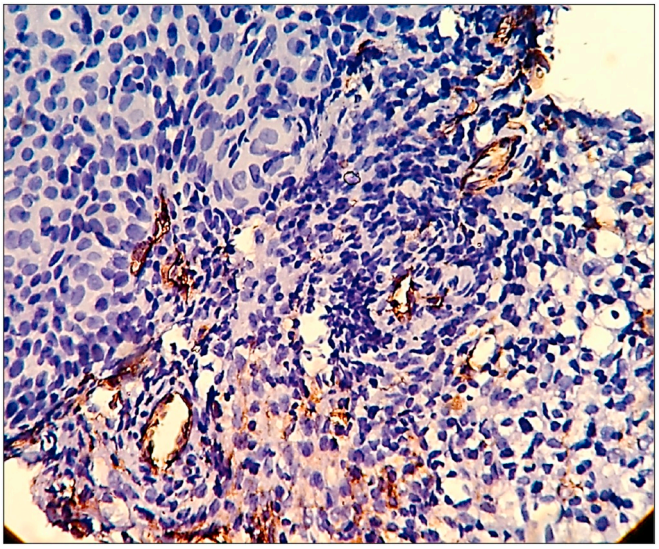

Fig. B8. Microinvasive carcinoma. (Same section as that of Fig. B7). Less than 10% of stromal cells showing positive immunostaining of CD34. (intensity score 0); X400.
Here blood vessels acting as positive control for CD34 immunostaining.

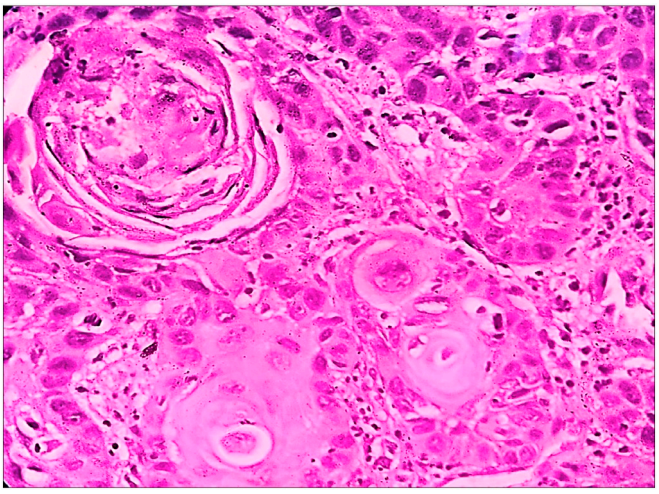

Fig. B9. Micrograph showing: Large cell keratinizing squamous cell carcinoma; well differentiated type. Nests of malignant squamous cells infiltrating into the stroma. The cells show marked pleomorphism, increased N/C ratio, with evidence of cytoplasmic keratinization in the form of keratin pearls. (H &E; 400X).

positive stromal cells are also distributed in various normal organs including salivary gland, thyroid gland, tonsil, stomach, colon, uterus, Fallopian tube and testis [8-10]. Several studies have been conducted to investigate the distribution of CD34-positive stromal cells in neoplasms of various organs including salivary gland, stomach, colorectal tissue, breast, pancreas and uterine cervix [1-4,11].

The present study was conducted to evaluate the presence of CD34 expression in lesions of uterine cervix. A total of 215 cases, comprising of premalignant and malignant lesions, were collected over the duration of 2 years. Thorough clinical history and examination of the patients was done and recorded in the proforma. CD34 immunostaining was done on 105 cases that include 30 cases of premalignant cervical lesions (15 cases of each HSIL and LSIL) and 75 cases of malignant cervical lesions (65 cases of squamous cell carcinoma and 10 cases of adenocarcinoma cervix). CD34 immunostaining was studied for its pattern of expression and its correlation with histological subtypes.

It was observed that all the premalignant cases showed positive

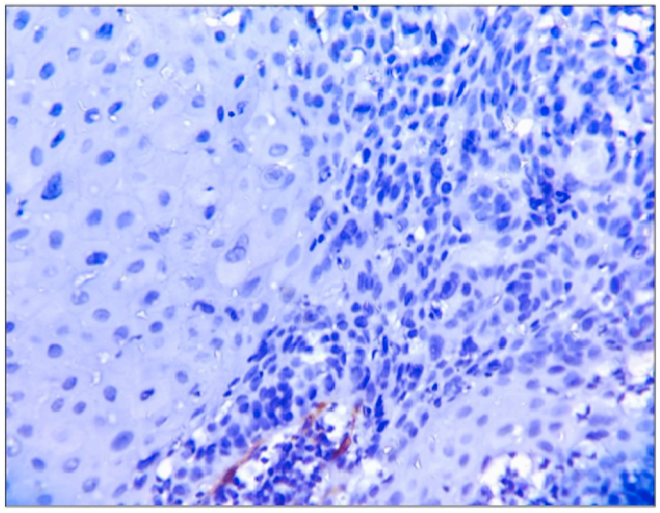

Fig. B10. Large cell keratinizing squamous cell carcinoma; well differentiated type. CD34 immunostaining (intensity score 0: total negativity in stroma). only blood vessels are positive; X400.

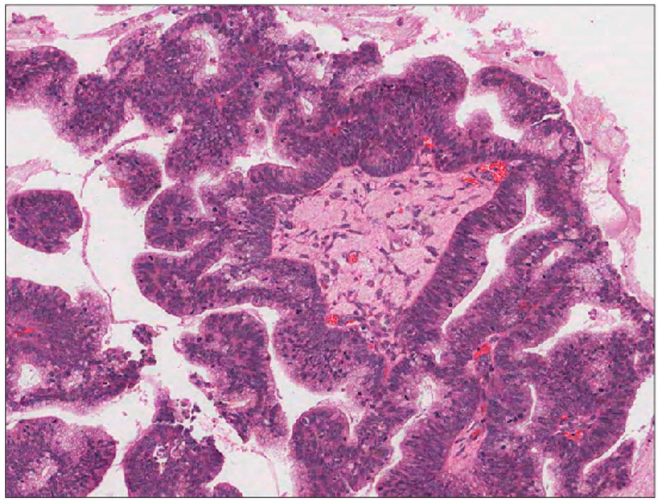

Fig. B11. Micrograph showing cervical adenocarcinoma, with neoplastic glands showing cellular proliferation, stratification, and numerous mitoses. (H& E; 400X).

CD34 immunostaining. Adjacent normal cervical stroma also showed dense network of CD34 fibrocyte in the stroma (2+ score). However, there was some difference in intensity score between LSIL and HSIL. Considering LSIL and HSIL cases separately, all the 15 cases of LSIL (100%) showed 2+ intensity score and none of the cases showed 0 or 1+ intensity. On the other hand, 12 cases of HSIL (80.0%) showed 2+ intensity score, only 3 cases (20.0%) showed 1+ intensity score. Out of these 3 cases, 2 cases were of carcinoma in situ. None of the HSIL cases showed total negativity. It was evident that with increased grade of disease, intensity of CD34 expression decreased, but this was not statistically significant in the absence of invasion (p value > 0.05). In a study by Barth et al., 2002, overexpression of CD34 was observed in 100% cases of CIN III. Similarly Li et al., 2009, in their study showed CD34 positivity in 100% of both low grade CIN and high grade CIN cases and the intensity was also 2+. [12]

In our study, immunohistochemistry for CD34 was done on 75 malignant cases which included 65 cases of squamous cell carcinoma and 10 of adenocarcinoma cervix. Out of these 65 cases, majority of cases (54 cases, 83.1%) showed negative staining whereas 11 cases showed positivity. Among these positive cases, 08 cases (12.3%)

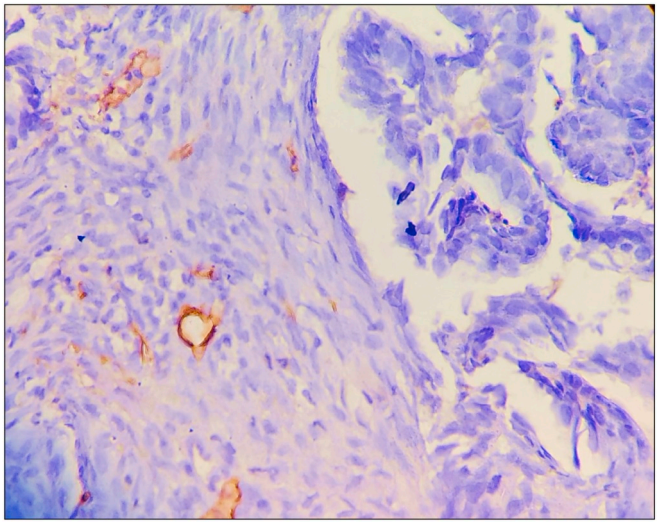

Fig. B12. Cervical adenocarcinoma, with malignant glands invading into the stroma. Only < 10% of cells showing CD34 membranous positivity (intensity score 0). 400X.
Blood vessels acting as positive control, showing endothelial cells immunoreactivity.

showed intensity score of 1+ or focal positivity, 03 cases (4.6%) showed 2+ intensity score. These findings are similar to the study of Li et al., 2009, who reported CD34 negativity in 89.5% of squamous cell carcinoma, 1+ positivity and 2+ positivity in 5% cases each. However lower negativity was reported by Barth et al in 2002, where CD34 negativity was seen in 55.5% (10 out of 18) cases, and 1+ positivity in 38.8%(7 cases). 1 case (5.5%) showed 2+ positivity.

Horn et al., 2013 in their study of 97 cases of squamous cell carcinoma found a decreased expression in 78.3% cases (i.e. < 5% stromal positivity), similar to our study [13].

To the best of our knowledge, we are the first to describe pattern of CD34 expression in adenocarcinoma of cervix. In our study out of 10 cases of Adenocarcinoma cervix on which immunostaining was done, 6 cases (60%) showed negative staining whereas 4 cases (40%) showed positive staining. Among all positive cases, 3 case showed 1+ intensity score and only one case showed 2+ intensity score. Loss of CD34 in cases of squamous cell carcinoma and adenocarcinoma showed some variation. We observed that 90% of squamous cell carcinoma showed negative CD34 immunostaining whereas only 60% of adenocarcinoma showed negative immunostaining. The reason for this variation may be due to smaller sample size of adenocarcinoma or may be due to some other reason that has not been studied so far. However CD34 expression when compared between squamous cell carcinoma and adenocarcinoma was statistically non-significant. (p value > 0.05).

In the present study, we observed that CD34 was consistently lost in most of the malignant tumors in our study, irrespective of the histological type. This was similar to the findings of various researchers earlier. When both malignant and premalignant lesions were compared for loss of CD34, it was observed that almost all of the malignant lesions showed loss of CD34 immunostain in the stroma of invasive carcinoma whereas in premalignant lesions (CIN I, CIN II, CIN III or CIS) retention of CD34 immunostain was there. It was observed that as the complexity of lesion increases from benign to carcinoma in situ, to further invasive carcinoma, loss of CD34 occurs. It was reduced from diffuse positivity in stroma to focal positivity and then total negative immunostaining in invasive lesions.

The difference of loss of CD34 expression in premalignant and malignant lesions overall, is highly significant statistically ($p < 0.05$). Both Barth et al. (2002) and Li et al. (2009) in their studies observed similar results and the relationship was found to be statistically significant. Comparable results have been reported in other organs like in invasive ductal carcinoma of the breast, colorectal adenocarcinoma, pancreatic ductal carcinoma and SCC of the oral cavity, pharynx and larynx: normal stroma contains CD34-positive stromal cells, whereas tumour-associated desmoplastic stroma is characterized by the presence of α- SMA-positive myofibroblasts. [14,15]. The mechanisms of myofibroblast formation are not completely understood. Numerous cytokines like PDGF, IGF II, IL-4, and TGF-β1 may be involved in the transdifferentiation of fibroblasts to myofibroblasts, and these cytokines can be derived from several cell types. Among these cytokines, TGF-β1 has a central role in inducing myofibroblast differentiation; high levels of the cytokine are usually associated with myofibroblast containing lesions. [16].

6. Conclusion

CD34 can be used to differentiate between premalignant and malignant lesions of the cervix, where retained or diffuse CD34 expression in the stroma would suggest a benign lesion while loss of stromal CD34 cells would indicate the diagnosis of a malignancy or early stromal invasion.

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