Section: Pathology

Original Research Article

EVALUATION OF CD34 EXPRESSION AS A MARKER OF ANGIOGENESIS IN SQUAMOUS CELL CARCINOMA CERVIX AND ITS CORRELATION WITH HISTOPATHOLOGICAL GRADE OF TUMOUR

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ABSTRACT

Background: Cervical cancer is the second leading cause of cancer related death among women worldwide. In India carcinoma cervix is the third most common cancer, majority being diagnosed at locally advanced stage and in metastasis. Vascular dissemination of tumour cells is an important event in tumour progression and metastasis. Angiogenesis can be quantified by immunohistochemical study of intratumoural microvessel density using marker CD34. CD34 is a highly sensitive marker for vascular endothelial cell. In this study the microvessel density is compared in different histopathological grades of tumour for prognosis assessment, predict recurrence and for targeted therapy. **Materials and Methods:** This is a prospective study conducted in the department of pathology from September 2022- August 2024.Sample size was calculated according to slovins formula. 210 cases were included in the study. Out of 210 cases, 15 cases were hysterectomy specimens and 195 cases are cervix biopsy specimens. **Result:** CD 34 expression was increased in poorly differentiated cervical squamous cell carcinoma compared to well and moderately differentiated cervical squamous cell carcinoma with a significant p value of 0.02. CD 34 expression also has a positive correlation with lymph node positive cases with significant p value of 0.04. **Conclusion:** Mean vascular density of poorly differentiated cervical squamous cell carcinoma cases and lymph node positive cases were higher compared to moderately differentiated and well differentiated cervical squamous cell carcinoma cases and lymph node negative cases.

INTRODUCTION

Cervical cancer has an incidence of 6 -29 % of all cancers. It is the second leading cause of death among women in India. The maximum age of incidence is 50 years. There are two types– squamous cell carcinoma and adenocarcinoma, among which squamous cell carcinoma is the most common type. Major etiology is human papilloma virus infection (HPV 16 and HPV 18). These lesions can progress from low grade squamous intraepithelial neoplasia to high grade squamous intraepithelial neoplasia and then to invasive carcinoma.[1,2]

Risk factors for cervical carcinoma include- Early age of marriage, early onset of sexual activity, multiple sexual partners and low socioeconomic status

Prognostic factors include – Age, size of tumour, lymph node metastasis, stage of presentation and metastasis. Key factors involved in tumour aggressiveness include uncontrolled proliferation activity, tumour cell invasiveness and tumour neoangiogenesis.[3,4]

Though there are many screening tests like VIA, VILI, Pap test, HPV DNA testing and preventable HPV VACCINES, the mortality seems to be increased.[5,6]

Hence a biomarker study which could predict prognosis, metastasis and treatment aspect could improve the survival of the patients. Blood supply to a tumour tissue is essential for nourishment of tumour cells which could increase its growth and

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through these vessels the tumour cells could metastasize also. Intratumoural microvessel density was studied in the year 1991 by Weidner et al with immunohistochemical marker CD34 and CD31 which stains the endothelial lining cells of blood vessels. The microvessel density correlates with histopathological grade of the tumour, which could further predict the chance of recurrence, metastasis and for targeted therapy.[7,8]

Aim and Objective

Aim of this work is to investigate angiogenesis in cervical squamous cell carcinoma by assessing tissue expression of CD34 and to evaluate the association between microvessel density with histopathological grading of tumour for prognostication and initiation of targeted therapy.

MATERIALS AND METHODS

This is a type of prospective study conducted in the department of pathology, Madurai medical College, Madurai from September 2022 to August 2024.

Total number of specimens received was 31678. Number of Gynaecological specimens was 3507. Out of which 527 were cases of cervical malignancy.

According to slovins formula, sample size was calculated. 210 cases were included in the study. Out of 210 cases, 15 cases are hysterectomy specimens. 195 cases are cervix biopsy specimens.

Inclusion criteria: Cervix biopsy specimens histopathologically proven as squamous cell carcinoma Hysterectomy specimens histopathologically proven as squamous cell carcinoma

Exclusion criteria: Cervix biopsy specimens histopathologically proved as benign lesions .Cervix biopsy and hysterectomy specimens histopathologically proven as adenocarcinoma

For immunohistochemical studies, blocks having adequate tissue are required. Hence blocks with inadequate material were excluded

Data collection: 210 cases were included in the study. Detailed clinical history was obtained from each patient who includes age, presenting complaints, family history, past history, treatment history, gyneac and obstetric history. Per vaginal examination findings, imaging details and reports were also collected

Specimens immediately after surgery were fixed in 10 percent formalin. Formalin volume was ten times the volume of the specimen.

The container along with the specimen was received in the department of pathology. After overnight fixation, next day the grossing was done. Measurements of uterus with cervix, bilateral tubes and ovaries were made exactly. The tumour size was measured and distance from the margins was correctly recorded. Bits taken and then subjected to automated tissue processing. Embedding was done with paraffin wax. Using microtome sections of 3 micronmeter thickness were cut. Routine staining was done with hematoxylin and eosin.

Histopathological examination was done. Cases of cervical squamous cell carcinoma were identified along with their grade according to WHO classification of female genital tract. Squamous cell carcinoma was graded as well differentiated, moderately differentiated and poorly differentiated carcinoma.

Well differentiated tumour had squamous cell with pleomorphic hyperchromatic nuclei, abundant eosinophilic cytoplasm and many keratin pearls. Moderately differentiated tumour had squamous cells with occasional keratin pearls and more pleomorphic nuclei. Poorly differentiated squamous cell carcinoma has less evidence of squamous differentiation with hyperchromatic pleomorphic nuclei and scant cytoplasm. Out of the 210 cases 15 were hysterectomy specimen and 195 were small biopsy specimens .Of which, 60 cases with adequate tissue was selected for immunohistochemical studies and the inference was compared with the histopathological grade of tumour.

Immunohistochemical studies: Cervix biopsy cases with adequate tissue were selected and all hysterectomy cases were selected.

Interpretation of CD34

The immunohistochemically stained slides were examined under microscope. Initial assessment was in scanner (4x) view .After scanning, hotspot areas were identified in each slide . Hotspot is defined as highest number of discrete microvessel stained areas. The hotspot areas were then focused in high power.10 fields were examined and average number of microvessels in 10 fields were calculated. The number of microvessels obtained were compared with different grades of cervical squamous cell carcinoma.

Statistical analysis: The strength of association was studied using one way anova test. P value of less than 0.05 percent was considered to be significant.

RESULTS

In the study period from September 2022 to August 2024, conducted in the department of pathology, Madurai medical college, total specimens received were 31678.

Table 1: Total number of specimens received from September 2022 to August 2024

Duration	Total Number of Specimens Received
September 2022-August 2023	14008
September 2023-August 2024	17670

Number of large resection specimens-21760, Number of small biopsy specimens-9918. Total number of malignant cases among the specimens received was – 3294. Number of cervix specimen

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received among the total cases including small and large biopsy specimens were – 3507.Out of 3507 cases – 527 cases were cervical malignancy cases.

Table 2: Number of cervical specimens with malignancy vs other specimens with malignancy

Site of Malignancy	Number	Percentage
Cervical malignancy	527	16%
Non cervical malignancy	2767	84%
Total	3294	100%

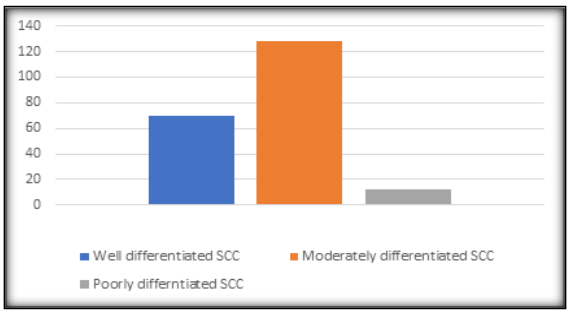
Table 3: Distribution of various types of malignancy among cervical malignancy

Type of malignancy	Number	Percentage
Cervical squamous cell carcinoma	488	92%
Cervical adenocarcinoma	39	08%
Total	527	100%

Our study is being confined to cervical squamous cell carcinoma, which is the most common type of cervical malignancy. Out of 488 cases of cervical squamous cell carcinoma, based on slovin's formula for sample size calculation and considering margin of error to be 5%, sample size calculated is 210 cases. Out of 210 sample cases. Number of well differentiated cervical squamous cell carcinoma cases are- 70. Number of moderately differentiated cervical squamous cell carcinoma cases are- 128. Number of poorly differentiated cervical squamous cell carcinoma cases are- 12.

Table 4: Distribution of various grades of squamous cell carcinoma in the study population (N= 210)

Histological grade	Number of cases	Percentage
Well differentiated SCC	70	33%
Moderately differentiated SCC	128	61%
Poorly differentiated SCC	12	06%



Out of 210 sample cases, number of resected specimens was 15 included total hysterectomies with lymphadenectomy.

Distribution of various grades of squamous cell carcinoma among the resected specimens.Number of well differentiated squamous cell carcinoma among resected specimens- 4. Number of moderately differentiated squamous cell carcinoma among resected specimens-9. Number of poorly differentiated squamous cell carcinoma among resected specimens-2.

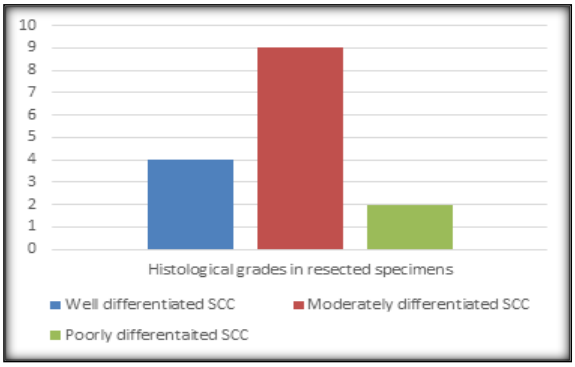


Table 5: Distribution of various grades of squamous cell carcinoma among hysterectomy specimens

Histological grade	Number of cases	Percentage
Well differentiated SCC	4	27%
Moderately differentiated SCC	9	60%
Poorly differentiated SCC	2	13%

Table 6: Microvessel density among different grades of cervical squamous cell carcinoma

Histological grade of squamous cell carcinoma cervix	Micro-vessel density
Grade 1	Minimum – 55 Maximum – 180
Grade 2	Minimum – 70 , Maximum-190
Grade 3	Minimum- 90, Maximum – 218

Table 7: Micro-vessel density with lymph node status in resected specimens

Histological grade of squamous cell carcinoma cervix	Total Number of cases	Number of cases with Lymphnode positivity	Micro-vessel density
Grade 1	4	Nil	Minimum-55 Maximim -180
Grade 2	9	9	Minimum- 70 Maximum- 190

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Grade 3	2	2	Minimum-90 Maximum- 218

Table 8: Micro-vessel density in comparison with tumour size in resected specimens

Histological grade of squamous cell carcinoma cervix	Tumour size	Micro-vessel density
Well differentiated	0.5 cm	Minimum- 55 Maximum- 206
Moderately differentiated	0.5 – 4 cm	Minimum-76 Maximum-210
Poorly differentiated	>4 cm	Minimum-90 Maximum-218

Photographs

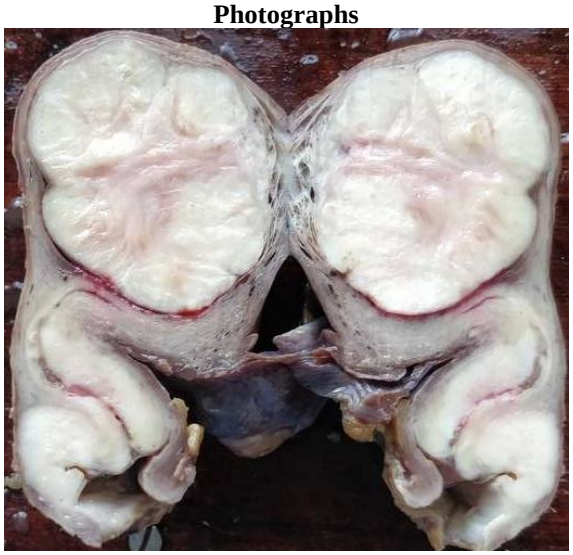

Figure 1: Gross picture of cervical carcinoma -total hysterectomy specimen

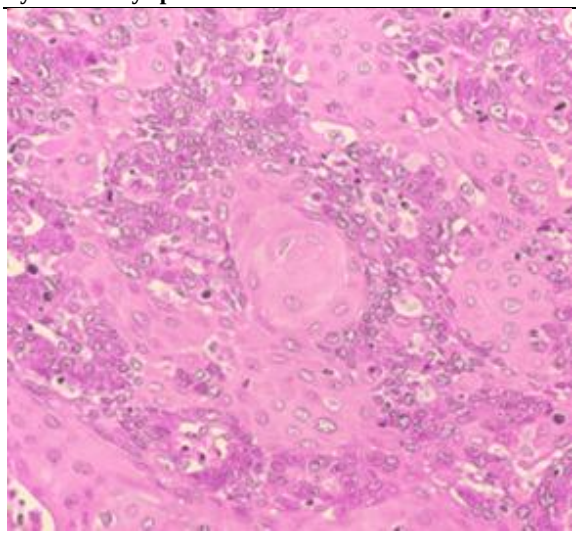

Figure 2: Histopathological section of well differentiated squamous cell carcinoma

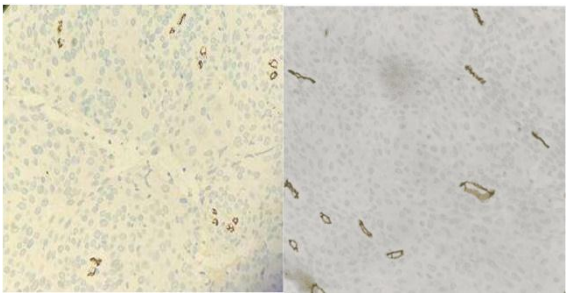

Figure 3: CD 34 expression in well differentiated squamous cell carcinoma cervix

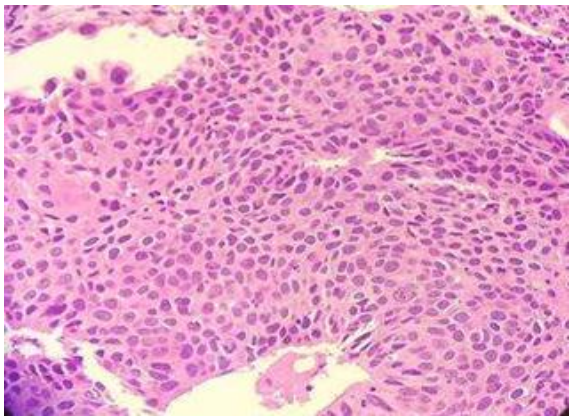

Figure 4: Histopathological sections of moderately differentiated squamous cell carcinoma cervix

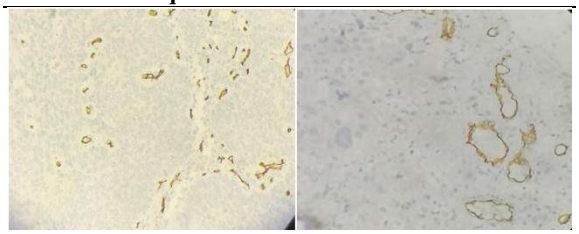

Figure 5: CD34 expression in moderately differentiated squamous cell carcinoma

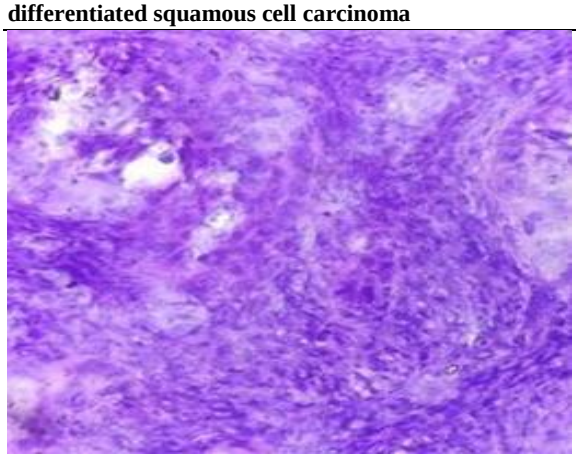

Figure 6: Histopathological section of poorly differentiated squamous cell carcinoma

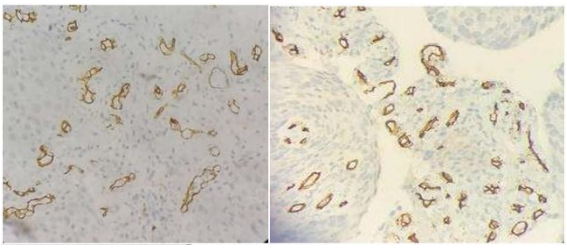

Figure 7: CD34 expression in poorly differentiated squamous cell carcinoma

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DISCUSSION

Table 9: Microvessel Density Assessment in Various Grades of Tumour in Our Study

One Way Anova Test	SCC I	SCC II	SCC III
N	19	35	6
SUM	1403	4869	886
MEAN	7.38	13.9	14.7
STANDARD DEVIATION	16.34	32.73	44.64

P Value- 0.02 (significant)

The above table illustrates the comparison of histopathological grade of tumour with microvessel density. There is found to be statistical difference between the histopathological grade of tumour incomparison to the microvessel density since the p value is found to be 0.03 which is less than 0.05.These results were similar to the findings of Vieira et al , Hirakawa et al, Mondal et al , Ancuta et al , Anan fathy et al ,Cantu et al and Lee at al with significant association and p value less than 0.05.[9-14]

Table 10: Microvessel Density In Comparision With Lymph Node Positivity In Resected Specimens In Our Study

Lymph node status	Number of cases	Percentage	Minimum number of vessels	Maximum number of vessels	Mean	P value
Positive	9	60%	76	218	12.86	0.04
Negative	6	40%	55	206	11.36	

The above table illustrates the comparison of lymph node positive cases among the resected specimens with their microvessel density. It was found that there was statistical significant difference in the microvessel density. The lymph node positive resected specimen cases expressed more number of blood vessels compared to the lymph node negative resected specimen cases.

These results were similar to the findings of Anan fathy et al with p value less than 0.05.

Angiogenesis is an important factor for tumour aggressive and tumour progression and metastasis. In our study there was a significant association between histopathological grade of tumour and microvessel density, lymph node status and microvessel density. Mean microvessel density in various grades of tumour were 7.3 in well differentiated cervical squamous cell carcinoma, 13.9 in moderately differentiated cervical squamous cell carcinoma, 14.7 in poorly differentiated cervical squamous cell carcinoma. These results were similar to the findings of Vieira et al , Hirakawa et al, Mondal et al , Ancuta et al , Anan fathy et al ,Cantu et al and Lee at al with significant association and p value less than 0.05**.** Mean microvessel density in lymph node positive case was 12.86**.** Mean microvessel density in lymph node negative case was 11.34 **.**These results were similar to the findings of Anan fatty et al and p value less than 0.05. Various retrospective studies conducted employed different immunohistochemical stains which include CD31, CD34, Factor VIII. Different methodology was used for quatifying the microvessel density**.** Hence microvessel density could be used as a prognostic marker for angiogenesis assessment which could predict metastasis and recurrence.[9-14]

CONCLUSION

Assessment of microvascular density concluded in the following results-Mean vascular density showed increased values in high grade tumours compared to mean vascular density in low grade tumours. Mean vascular density of moderately and poorly differentiated cervical squamous cell carcinoma were increased as compared to well differentiated cervical squamous cell carcinoma. The mean vascular density for lymph node positive cases were higher compared to lymph node negative cases. Hence our study shows that CD34, one of the angiogenesis assessment immunohistochemical markers can be used as prognostic factor for predicting metastasis, recurrence and period of disease free survival.

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