Original Article

Pathology

PCNA Labelling as a Proliferative Marker in Gynaecological Tumours

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ABSTRACT

Aim: The aim was to study the expression of PCNA in gynaecological tumours and to correlate PCNA expression with types and grades of different gynaecologica tumours.

Materials & Methods: Biopsies from 60 cases of gynaecologica tumours were subjected to Haematoxylin and eosin (H&E) stain and PCNA (proliferating cell nuclear antigen) immunostaining. PCNA scoring was done on each case.

Results: Out of 60 cases, 30 cases (50%) were of cervical lesions, 15 cases (25%) were of endometrial tumours and 15 cases (25%) were of ovarian tumours. In cervical lesions 20 cases (66.6%) were squamous cell carcinoma and positivity was observed with different PCNA proliferative scores. Most of the CIN (cervical intraepithelial neoplasia) cases had low PCNA score and most of the cervical squamous cell carcinomas had a high PCNA score. Among 15 cases endometrial carcinomas, 9 cases (15%) were well differentiated type, 3 cases (5%) were moderately differentiated type and 3 cases (5%) were poorly differentiated type. Of 15 ovarian tumours, 10 cases(16%) were of serous cystadenocarcinoma, 3 cases (5%) were of mucinous cystadenocarcinoma and 2 cases (3%) were of undifferentiated type.

Conclusion: PCNA expression along with other markers in different tumours can be used to predict the proliferative activity of the tumour and subsequent prognosis. It can also be helpful in differentiating cervical intraepithelial neoplasia and squamous cell carcinoma of cervix. The application of PCNA proliferative activity may provide information regarding the clinical stage and histological grade of malignant epithelial ovarian tumours and endometrial adenocarcinomas.

Key Words: Female, Cancer, Diagnosis

INTRODUCTION

Abnormalities and diseases of the female genitalia have been the object of fascination for centuries and the basis for one of the oldest medical specialties. Recent years have witnessed significant developments in the use of immunohistochemistry in diagnostic gynaecologica pathology.

The principle underlying assessment of cell proliferation by immunohistochemical methods is that there are cell cycle associated alterations in the amount or distribution of cellular proteins or other molecules that are recognized as antigens. Immuno-histochemistry is the application of immunologic principles and techniques to the study of cells and tissues. Several procedures are available, the two most commonly used are peroxidase-antiperoxidase immune complex method and biotin-avidin immunoenzymatic technique.

The advantages of immuno-histochemistry are:

1. Remarkable sensitivity and specificity.
2. Applicability to routinely processed material (even if stored for long periods)
3. Feasibility of an accurate correlation with most of the fixatives currently in use.
4. Feasible even in decalcified material or in previously stained microscopic sections.
5. It is sometimes positive even in totally necrotic material.
6. It can also be adopted to cytological preparations and electron microscopy.

PCNA is the marker that is mostly used, together with ki67 for the immuno-histochemical evaluation of proliferative activity in paraffin embedded material. PCNA is a 36 kilodalton (kDa) nonhistone nuclear protein. It is an auxiliary protein of DNA polymerase C and is important in the initiation of cell proliferation. Elevated levels of PCNA appear in the cell in late G1 phase, become maximal during S phase and decline again in G2 and M phases.

Various antibodies to this protein like PC10 and 19A2 have been used to study its association with proliferation kinetics. Studies done in gynaecologica tumours and related lesions showed that PC10 may be useful as a marker for proliferative activity of the cells both in normal and tumour tissues rather than for malignancy. Some studies show that PCNA staining might be prognostically more valuable than its CIN grade in benign and premalignant cervical lesions.

MATERIALS AND METHODS

The present study comprised of 60 cases of excised gynaecologica tumours submitted in a tertiary care hospital. Gross examination of the specimen was done regarding size, shape, consistency, appearance, depth of invasion. The tissues were fixed by using 10% formalin and processed through alcohol and chloroform to form paraffin blocks. The tissues were sectioned at 4 micrometer thickness and subsequently stained with H&E stain.

The tumours were studied and graded initially on H&E stained sections. A single representative tissue block was then selected for immuno-histochemical staining for PCNA expression. Appropriate tissue controls were also used.

For immunohistochemical staining, 3-5micrometer thick sections were cut and fixed on to the freshly prepared Poly-L-lysine coated slides.Sections were then incubated at 37 degree Celsius for 24

hours.Control and test sections were dewaxed in three changes of xylene and hydrated through descending concentrations of alcohol. Deparaffinization was done thoroughly to avoid high background staining of the sections.This was followed by blocking of endogenous peroxidase by incubating specimens with 3% hydrogen peroxide for 20 minutes. Antigen retrieval was done in pressure cooker . The slides were put in a container filled with citrate buffer (pH 6.0). The sections were heated in a pressure cooker for four to five minutes and then cooled down to room temperature. The slides were rinsed in phosphate buffer saline (PBS) for five minutes. Tissues were then incubated with primary monoclonal antibody (mouse anti-proliferating cell nuclear antigen) for two hours. For negative control primary antibody was omitted and two drops of PBS were added. Two washings were given in PBS triton , five minutes each and one washing was given with simple PBS for five minutes. Tissues were then incubated with secondary biotinylated antibody for thirty minutes. Washings were again given with PBS triton and simple PBS for five minutes each. Sections were incubated with avidine-biotin complex for thirty minutes. Washings were again given with PBS triton and simple PBS for five minutes each. Freshly prepared diamino-benzidine was used for five to ten minutes. Slides were washed with distilled water. Counterstaining was done with Haematoxylin for 30 sec. Sections were washed in running water for adequate bluing. Dehydration, clearing and mounting was done . The positive test sections showed positivity in the form of brown coloration of the nuclei.

PCNA immunoreactivity was calculated by counting total of 1000 cells under 40 × magnifications. All the areas of sections were examined under high power field (40×) and blindly graded by two observers using semi-quantitative scale of 1 to 4, corresponding to estimated quartiles of tumour cell nuclear immunostaining

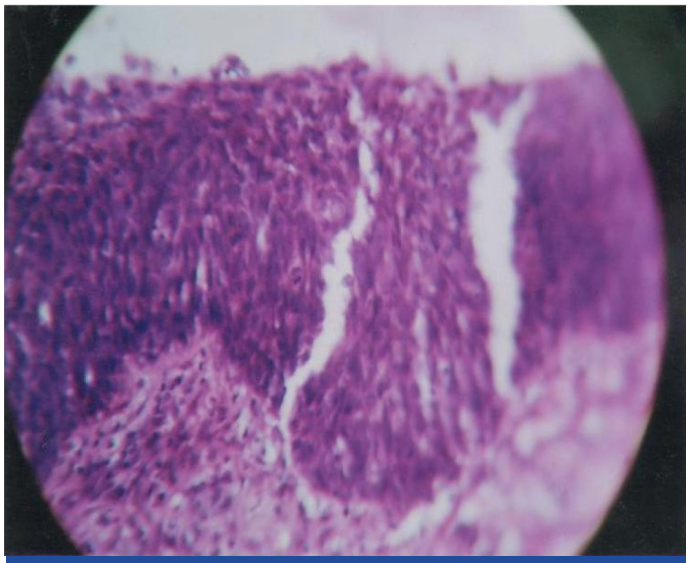
1. 0 to 25% positivity
2. 26% to 50% positivity
3. 51% to 75% positivity
4. 76% to 100% positivity

All immunostained nuclei, independent of intensity were scored as positive. Cells showing positive staining of nucleus as well as cytoplasm were considered negative.

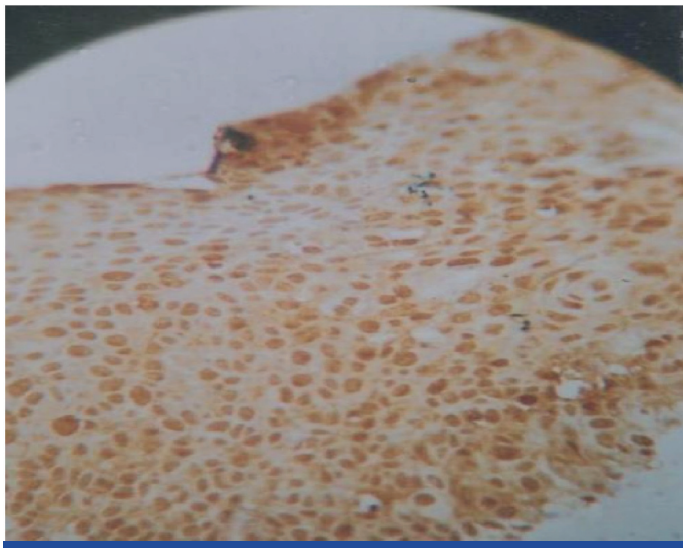
RESULT

A total of 60 cases were studied which included 30 cases of cervical lesions, 15 cases of endometrial tumours and 15 cases of ovarian tumours. Histopathological diagnosis was recorded in each case and then immunostaining for PCNA was done. Immunoreactivity appeared as diffuse or granular nuclear staining and in some rare cases a cytoplasm staining was observed too.

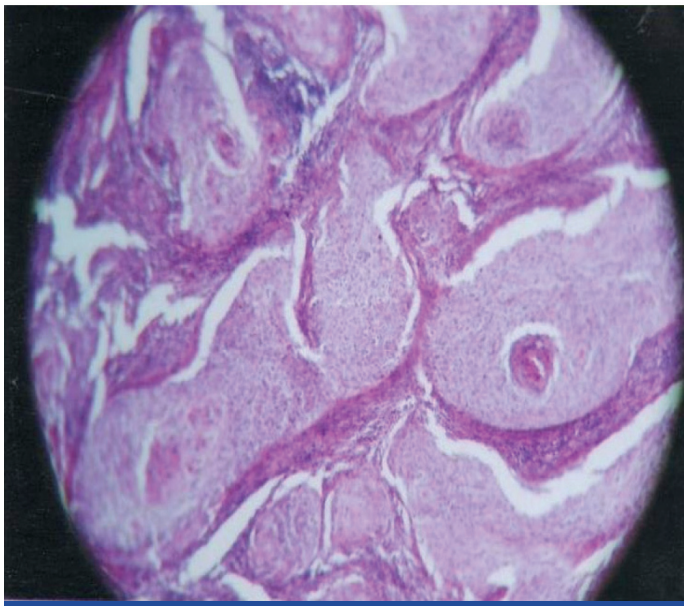
Positive cells in squamous epithelia of control ectocervix were found mainly in the basal layer. In metaplastic squamous epithelia, positive cells were confined to basal and parabasal cell layers. In each cervical intraepithelial neoplasia category, positivity was confined to layers in which dysplastic changes had occurred [Table/Fig-1&2]. In all squamous cell carcinoma cases, positivity was observed with different proliferative scores [Table/Fig-3&4]. In malignant tissues, the localization of the distribution of PCNA positive cells came to be lost and the proportion of positive cells varied from case to case as well as from field to field within the same tissue section. The cervical stromal tissue cells, inflammatory cells and blood vessels were non-reactive to PCNA. The adjacent endocervical glands showed positivity and the number of cell nuclei that stained varied from case to case.



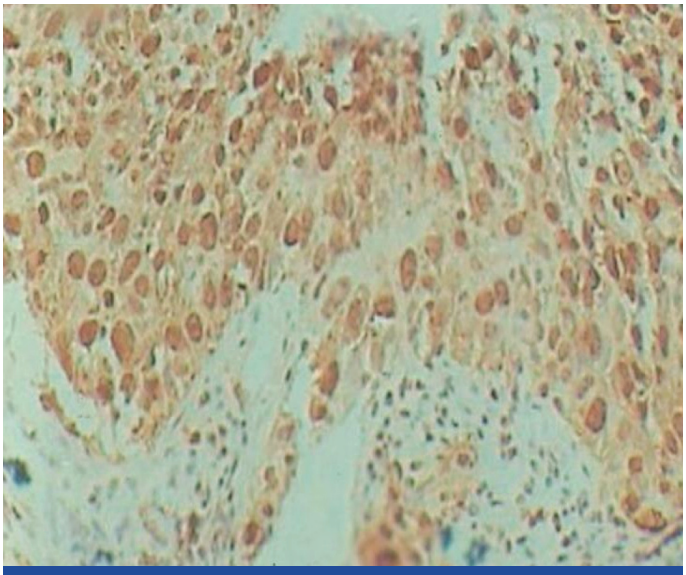
[Table/Fig-1]: Sections showing CIN-III (H&E, 400×)



[Table/Fig-2]: Photograph showing PCNA immunopositivity(>75%) in CIN-III (IHC, 400×)



[Table/Fig-3]: Sections of squamous cell carcinoma large cell keratinizing type(H&E, 100×)



[Table/Fig-4]: Photograph showing PCNA immunopositivity(>75%) in squamous cell carcinoma large cell keratinizing type(IHC, 400×)

PCNA score	Number of cases	Percentage
1	5	16.67
2	5	16.67
3	9	30.00
4	11	36.66
Total	30	100

[Table/Fig-5]: Statistical analysis of cervical lesions according to PCNA score

Thirty cases of cervical lesions were studied, which included 4 cases of CIN-I(cervical intraepithelial neoplasia-I), 3 cases of CIN-II, 3 cases of CIN-III, 10 cases of SCC LCNKT(squamous cell carcinoma large cell non-keratinizing type), 7 cases of SCC LCKT (squamous cell carcinoma large cell keratinizing), 2 cases of SCC (squamous cell carcinoma) small cell type, 1 case of SCC poorly differentiated type.

All the 30 cases of cervical lesions were analyzed according to the PCNA score, independent of histological diagnosis and each case was assigned a score on a semi-quantitative scale of 1 through 4 (Table/Fig-5].

Out of 30 cases, 11(36.66%) cases had a score of 4, 9(30%) cases had a score of 3, 5(16.67%) cases had a score of 2 and another 5(16.67%) cases had a score of one.

[Table/Fig-6] demonstrates the correlation between histological diagnosis of cervical lesions with PCNA percentage positivity. On comparison between all cervical intraepithelial neoplasia cases and all squamous cell carcinoma cases, using t-test, a statistically highly significant difference was observed (p<0.001). Comparison between CIN I and CIN II cases and also between CIN II and CIN III cases revealed a statistical significant difference (p<0.05). Comparison between large cell type of squamous cell carcinoma and other types of squamous cell carcinoma was found to be statistically insignificant (p>0.05).

Fifteen cases of endometrial adenocarcinomas were studied, out of which 9 cases were of well differentiated type, 3 cases were of moderately differentiated type and 3 cases were of poorly differentiated type. All these were analyzed for PCNA score (Table/Fig-7]. Out of 15 cases, 6(40%) cases had a score of 3, 5(33.33%) cases had a score of 4, 3(20%) cases had a score of 2 and 1 (6.67%) case had a score of one.

[Table/Fig-8] demonstrates the correlation between histological grade of endometrial adenocarcinoma and PCNA percentage positivity [Fig-9&10]. A statistically highly significant difference (p<0.001) was found between well differentiated and poorly differentiated endometrial adenocarcinoma. On the other hand, comparison between well differentiated and moderately differen-

			PCNA % positivity				
Histological Diagnosis		No. of cases	Mean	SD	Correlation	t-value	p-value
I	CIN-I	4	19.25	0.96	I/II	3.17	<0.05
II	CIN-II	3	40.00	14.80	II/III	1.27	<0.05
III	CIN-III	3	51.00	7.81	IV/V	7.78	<0.001
IV	All CIN cases	10	35.00	16.32	VI/VII	0.08	>0.05
V	SCC LCKT+ SCC LCNKT	17	75.24	12.31	–	–	–
VI	SCC small cell+ Poorly differentiated	3	74.60	12.78	–	–	–
VII	All SCC cases	20	75.14	12.03	–	–	–

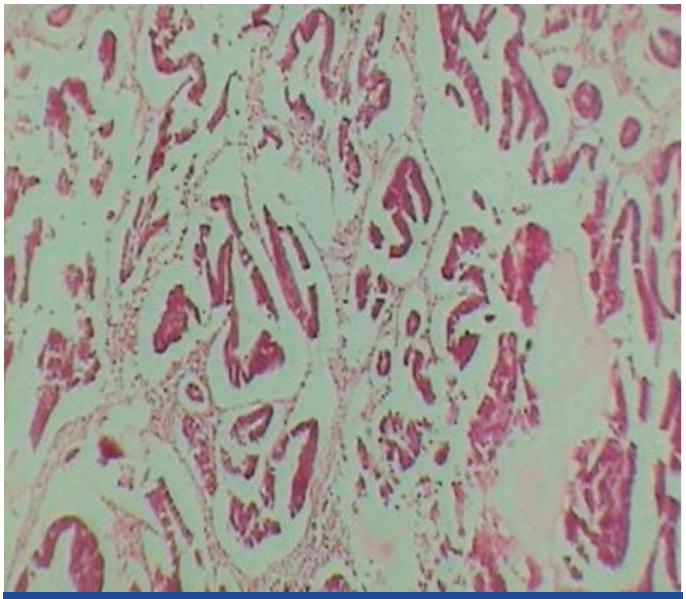
[Table/Fig-6]: Correlation between histological diagnosis of cervical lesions and PCNA percentage positivity

PCNA score	No. of cases of endometrial adenocarcinoma	Percentage
1	1	6.67
2	3	20.00
3	6	40.00
4	5	33.33
Total	15	100.00

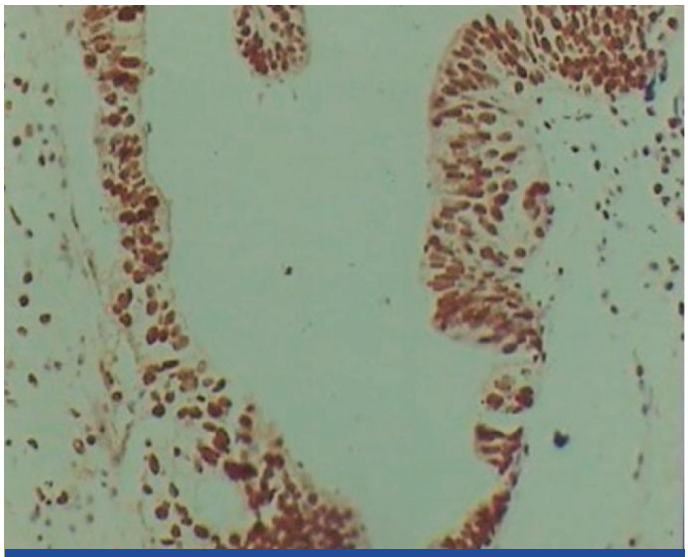
[Table/Fig-7]: Analysis of endometrial adenocarcinoma according to PCNA score

			PCNA%age positivity				
Histological Grade		No. of cases	Mean	SD	Correlation	t-value	p-value
I	Well differentiated	9	57.11	18.29	I/II	1.50	>0.05
II	Moderately differentiated	3	74.00	15.10	I/III	3.35	<0.001
III	Poorly differentiated	3	92.00	2.65	II/III	2.27	>0.05

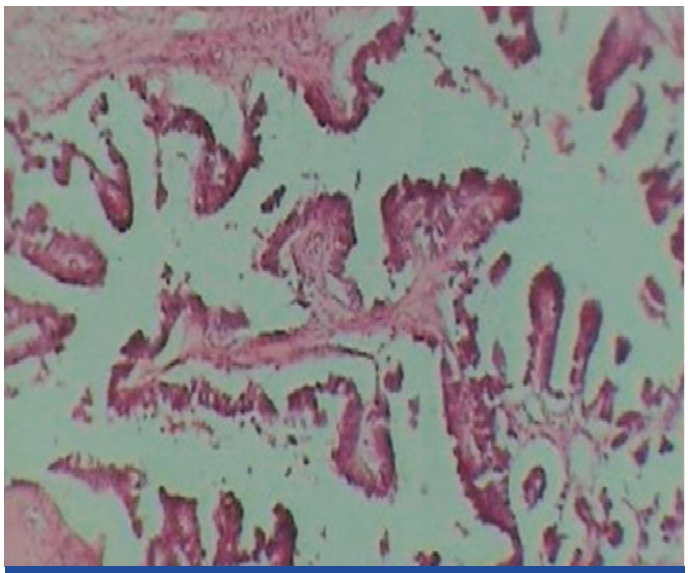
[Table/Fig-8]: Correlation between histological grade of endometrial adenocarcinoma and PCNA percentage positivity



[Table/Fig-9]: Sections of well differentiated adenocarcinoma of endometrium(H&E, 100×)



[Table/Fig-10]: Photograph showing PCNA immunopositivity (>75%) in well differentiated adenocarcinoma endometrium(IHC, 400×)



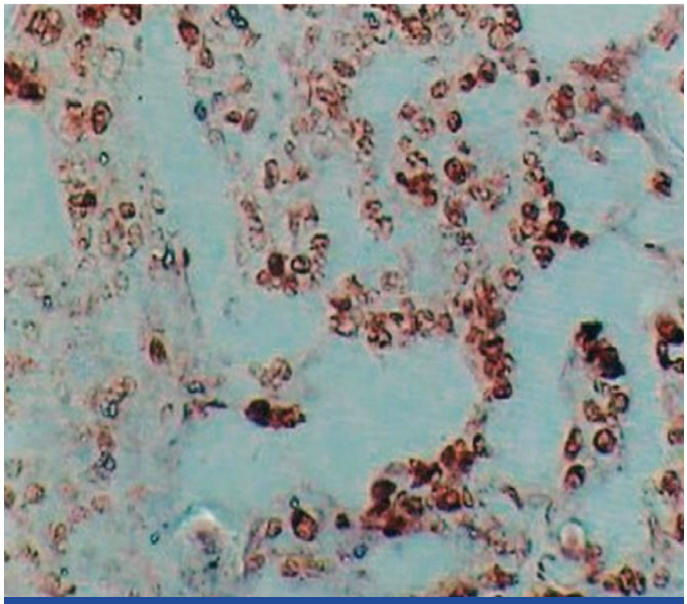
[Table/Fig-11]: Sections showing serous cystadenocarcinoma ovary (H&E, 100×)

tiated adenocarcinoma and also between moderately differentiated and poorly differentiated adenocarcinoma was found to be statistically insignificant (p>0.05).

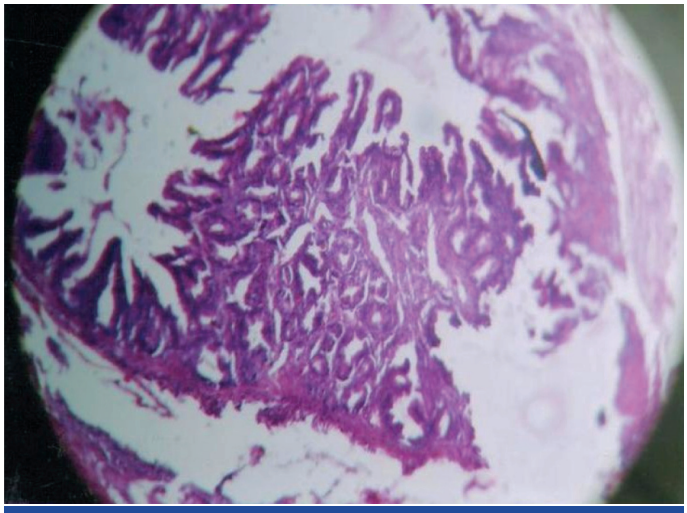
Out of fifteen cases of malignant ovarian tumours studied, majority 10(66.67%) cases were of serous cystadenocarcinoma [Fig-11&12], 3(20%) cases were of mucinous cystadenocarcinoma [Fig-13&14) and 2(13.33%) cases were of undifferentiated type. [Table/Fig-15] shows the distribution of ovarian tumours according to PCNA score.

[Table/Fig-16] demonstrates the correlation between the histological type of ovarian tumours and PCNA percentage positivity. On comparing the above, no statistical significant difference (p>0.05) was observed.

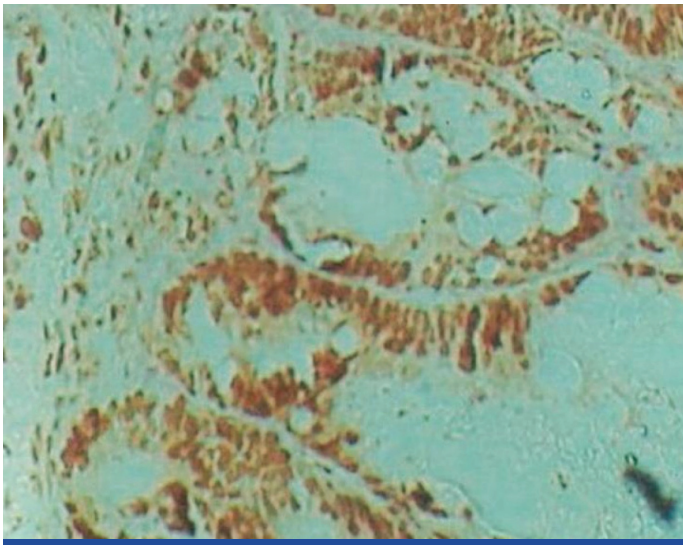
[Table/Fig-17] shows the correlation between histological grade of ovarian tumours and PCNA percentage positivity. On comparing well differentiated and poorly differentiated types of ovarian tumours statistically high significant differences was observed (p<0.001). Also a statistical significant difference (p<0.05) was observed between well differentiated and moderately differentiated ovarian tumours.



[Table/Fig-12]: Photograph showing PCNA immunopositivity (>50%) in serous cystadenocarcinoma ovary(IHC, 400×)



[Table/Fig-13]: Sections of mucinous cystadenocarcinoma ovary(H&E, 100×)



[Table/Fig-14]: Photograph showing PCNA immunopositivity(>50%) in mucinous cystadenocarcinoma ovary(IHC, 400×)

PCNA score	No. of cases of ovarian tumours	Percentage
1	0	–
2	10	66.67
3	2	13.33
4	3	20.00
Total	15	100.00

[Table/Fig-15]: Analysis of ovarian tumours according to PCNA score.

DISCUSSION

The present study used the PCNA scoring using a semi-quantitative scale of 1 to 4, corresponding to estimated quartiles of tumour cell nuclear immunostaining. A similar system was adopted and used in another study [1].

Most of the cases of cervical intraepithelial neoplasia had a PCNA score of either 1 or 2. Among the cervical carcinomas, most of the tumours had a score of either 3 or 4. A statistically high significant difference (p value<0.001) between all the cervical intraepithelial neoplasia and the entire invasive neoplasia group based on PCNA expression (p value<0.05) was observed. This finding was similar to what was observed in other studies [2, 3]. This significant difference between CIN and invasive carcinoma groups suggest that a considerable alteration of biologic behaviour occurs in the progression of carcinogenesis from intraepithelial neoplasia to carcinoma. It was observed that in the CIN lesions there was an increase in the number of PCNA immunoreactive cells with the appearance of positive cells above the basal layer. This finding was similar to what was observed in another study [4]. A statistically significant difference (p value<0.05) between various grades of CIN based on PCNA expression was observed [4,5,6]. These findings suggest that the cell proliferation index as detected immunohistochemically using PCNA may be a useful parameter to indicate the grade of CIN. No statistically significant difference (p value>0.05) was observed in the expression of PCNA between various histological types of squamous cell carcinoma cervix. This finding was in agreement with another study (2).

It was observed that 60% cases of endometrial adenocarcinomas were of well differentiated type, 20% cases were of moderately differentiated type and 20% cases were of poorly differentiated type. Most of the endometrial adenocarcinoma cases had the PCNA score of 3 or 4. A statistically highly significant difference between well differentiated and poorly differentiated endometrial adenocarcinomas based on PCNA expression was seen. These findings were similar to another study which observed a significant positive correlation between the histological grade of endometrial carcinoma and the degree of PCNA expression (7).

Regarding ovarian tumours in the present study, it was observed that 66.67% cases were of serous cystadenocarcinoma, 20% cases were of mucinous cystadenocarcinoma and 13.33% cases were of undifferentiated type.

Majority of the ovarian tumours had PCNA score of 2. No statistical significant correlation (p value>0.05) was observed between different histological types of ovarian tumours and PCNA expression. These findings were in agreement with a study which also observed no significant difference in PCNA expression in different histological types [8]. A statistically high significant difference (p value<0.001) was observed on comparing well differentiated and poorly differentiated types of ovarian tumours based on PCNA expression. Also a statistical significant difference (p value<0.05) was observed between well differentiated and moderately differentiated ovarian tumours. These findings correlate well with another study [9].

CONCLUSION

Actively dividing cells produce a number of unique proteins that may serve as useful antigenic markers in immunological studies of cellular proliferation. The application of PCNA proliferative activity may give information about the proliferative activity of a given cervical intraepithelial lesion with respect to the histologic pattern. Also the PCNA staining and the location of the staining

Histological Diagnosis		No. of cases	PCNA % positivity		Correlation	t-value	P-value
			Mean	S.D.			
I	Serous cystadenocarcinoma	10	50.10	18.94	I/II	1.30	>0.05
II	Mucinous cystadenocarcinoma	3	46.00	19.61	I/III	1.00	>0.05
III	Undifferentiated carcinoma	2	64.00	16.97	II/III	3.46	>0.05

[Table/Fig-16]: Correlation between histologic type of ovarian tumours and PCNA percentage positivity

Histological Diagnosis	No. of cases	PCNA % positivity		Correlation	t-value	P-value
		Mean	S.D.			
Well differentiated	9	39.56	5.50	I/II	5.41	<0.05
Moderately differentiated	1	60.00	0.00	I/III	2.44	<0.001
Poorly differentiated	5	63.00	40.70	II/III	0.13	<0.05

[Table/Fig-17]: Correlation between histological grade of ovarian tumours and PCNA percentage positivity

may be helpful in differentiating cervical intraepithelial neoplasia and squamous cell carcinoma of cervix.

The application of PCNA proliferative activity may provide information regarding the clinical stage and histological grade of malignant epithelial ovarian tumours and endometrial adenocarcinomas.

Many prognostic factors are also important for predicting prognosis such as grade, stage, type of the tumour, other serum biochemical (CA125)/tumour markers (Ki67,BCL2).

Thus PCNA expression along with other markers in different tumours can be used to predict the proliferative activity of the tumour and subsequent prognosis.

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