ORIGINAL RESEARCH

Study to Evaluate the Expression of PAX2 in the Diagnosis of Premalignant and Malignant Lesions of Endometrium

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ABSTRACT

Background: PAX-2 is expressed positive in normal endometrium, gets decreased in endometrial hyperplasia and becomes absent in endometrial carcinoma. PAX2 gene expression reduces in intensity from proliferative endometrium to nonatypical hyperplasia to atypical hyperplasia. Present study was aimed to study the expression of PAX2 in the diagnosis of premalignant and malignant lesions of endometrium.

Material and Methods: Present study was single-center, cross sectional study, conducted in endometrial curettings and hysterectomy resection specimens diagnosed as Endometrial hyperplasia and Endometrial carcinoma.Representative sections were selected for PAX-2 immunohistochemistry (IHC). IHC was performed using polymer kit as per standard protocol.

Results: The mean age of women in our study was 51.13 ± 10.51 years.Among 62 cases, 30 Endometrial Carcinoma and 32 Endometrial Hyperplasia (9 were Atypical Hyperplasia, 23 were Non-Atypical Hyperplasia).Out of the 30 Endometrial carcinoma cases, Endometrioid carcinoma is the most common (80%). The most common FIGO Stage was Stage IB (63%). Among the 30 cases of Endometrial Carcinoma, 25 (83%) showed complete loss of PAX2, while 5 (17%) showed partial loss.There is positive association between premalignant and malignant lesions with PAX2 loss. There is positive association between PAX2 loss and AUBand PMB (p – 0.001).

Conclusion: Decrease in PAX2 expression was significantly associated with progression to endometrial carcinoma. Our study showed significant statistical difference in the PAX2 expression between the premalignant and malignant lesions of endometrium.

Keywords: PAX2 expression, endometrial carcinoma, premalignant lesions, hyperplastic endometrium

INTRODUCTION

Action of unopposed estrogen results in endometrial hyperplasia (EH) which is proliferation of endometrial glands. Endometrial Hyperplasia, if kept unchecked for long can lead to endometrial adenocarcinoma.[1] Till date, histopathological examination is the reference standard to differentiate between benign and premalignant Endometrial Hyperplasia . But this method is characterised by poor inter and intra observer reproducibility. Tissue paucity and unclear features may cause additional problems in diagnosis.[2] So, several IHC markers have been used to increase the reliability of the differential diagnosis between benign, premalignant and malignant lesions of endometrium.[2,3,4]PAX-2 is a new, recent, tumour suppressor gene required during embryonic development, found to be mutated early during endometrial carcinogenesis. PAX-2 gene is second to PTEN (Phosphatase and TENsin homolog), which is also a tumour suppressor gene, established in endometrial carcinoma, controlling cell proliferation.[1] PAX-2 greatly delineates precancerous lesions of the endometrium[1] and is easier to evaluate because of its distinct nuclear staining while PTEN expression is both cytoplasmic and nuclear; and, PTEN shows variable staining-normally expressed in proliferative endometrial glands and stroma; but decreased expression in

normal secretory cycle; whereas PAX2 is less frequently lost in both normal proliferative and secretory endometrium.[2]PAX-2 is expressed positive in normal endometrium, gets decreased in endometrial hyperplasia and becomes absent in endometrial carcinoma. PAX2 gene expression reduces in intensity from proliferative endometrium to nonatypical hyperplasia to atypical hyperplasia. Then it gets lost in endometrial carcinoma.Present study was aimed to study the expression of PAX2 in the diagnosis of premalignant and malignant lesions of endometrium.

MATERIAL AND METHODS

Present study was single-center, cross sectional study, conducted in department of Pathology and the Department of Obstetrics and Gynecology at Sri Manakula Vinayagar Medical college and Hospital, Madagadipet, Puducherry, India. Study duration was of 2 years (January 2020 to December 2022). Study approval was obtained from institutional ethics committee.

Inclusion criteria: Endometrial curettings and hysterectomy resection specimens diagnosed as Endometrial hyperplasia and Endometrial carcinoma

Exclusion criteria

- Autolysed specimen Inadequate material Already treated biopsies Post chemotherapy and radiotherapy Associated ovarian malignancies

The sample size was calculated as 62 using the software open EPI version 3.0. After obtaining informed consent from the study participants, relevant patient information like hospital number was collected from the case records. Relevant clinical data including age, sex, medication history, duration of disease and associated symptoms like abnormal uterine bleeding, loss of weight, loss of appetite were obtained from the study participants using a standardised proforma. The representative paraffin blocks of the corresponding cases were selected and were stained with Hematoxylin and Eosin. They were then be screened under light microscopy to study the histomorphological features and the histological type, grade and stage of the tumour. The histopathological and immunohistochemical parameters were noted instandardized proforma. Representative sections were selected for PAX-2 immunohistochemistry (IHC). IHC was performed using polymer kit as per standard protocol. A golden-brown staining of the cytoplasmic membrane will be taken as a positive reaction. PAX-2 immunohistochemical staining was applied and % loss of PAX-2 staining was evaluated and the results obtained were analysed statistically Data was entered using epi info version 7.2.1.0. Data analysis was done by Statistical Package for Social Sciences (SPSS) software version 24. Description of categorical variables was done using frequency and proportion. Description of continuous variables was done by mean and standard deviation. Association of Hyperplasia and PAX-2 was done by Chi-square test. The results were considered statistically significant if the p value was <0.05.

RESULTS

In present study, 96.7% of premalignant lesions were seen in age group 30-40 years, 81.8% of malignant lesions are seen in more than 51 years. The mean age of women in our study was 51.13 ± 10.51 years.

Table 1: Morphology of endometrial lesion in various age groups

Age group (in years)	Morphology outcome		TOTAL
	PREMALIGN ANT	MALIGNANT	
30-40	29 (96.7 %)	1 (3.3 %)	30
41-50	8 (38.1 %)	13 (61.9 %)	21
> 51	2 (18.2 %)	9 (81.8 %)	11
	39 (62.9 %)	23 (37.1 %)	62

Most common parity index was P3L3; about 44 women had the parity index P3L3; followed by P2L2 the next most common parity index.

Table 2: Parity index

Parity	Frequency	Percent
P1L1A1	1	1.6
P2L2	8	12.9
P2L2A1	2	3.2
P3L3	44	71.0
P4L3A1	1	1.6
P4L3D1	2	3.2
P4L4	1	1.6
P5L3A2	1	1.6
P5L5	2	3.2
Total	62	100.0

History of loss of weight was present in 3 women (4.8 %). History of loss of appetite was present only in 3 women (4.8 %). History of Abnormal Uterine Bleeding was present in 29 women(46.8 %). 69% of carcinoma

cases had H/o AUB. Post menopausal bleeding was present in 27 women(43.5 %). 96.3% of premalignant lesions had H/o postmenopausal bleeding. Most of the women (98.4%) had regular menstrual cycle of 3/30 days not associated with clots. 6 women had BMI of > 25 kg/m^2.

Table 3: General characteristics

	No. of patients	Percentage
Loss of Weight	3	4.8
Loss of Appetite	3	4.8
AUB-Abnormal Uterine Bleeding	29	46.8
Premalignant	9	31
Malignant	20	69
PMB- Post Menopausal Bleeding	27	43.5
Premalignant	26	96.3
Malignant	1	3.7
RegularMenstrual cycle	61	98.4
BMI > 25	6	9.7

In present study, 30 Endometrial Carcinoma and 32 Endometrial Hyperplasia (NAH and AH). Out of 32 Hyperplasias, 9 were Atypical Hyperplasia, 23 were Non-Atypical Hyperplasia.The most common pattern was Endometrioid. There were 2 cases showing endometrioid carcinoma with squamous differentiation, 1 with villoglandular variant. There was 2 cases of serous type. There was a mixed case of both Endometrioid and Serous types. There was a case of Atypical Hyperplasia with polyp, and one with probable invasion. Focal NAH was seen in 1 case. Hyperplasia with no Atypia was seen in 23 cases.

Table 4: Histological characteristics

	Frequency	Percent
Carcinoma/Hyperplasia		
Carcinoma	30	.48.4
Non-atypical and atypicalhyperplasia	32	51.6
• Atypical Hyperplasia	9	14.5
• Nonatypical Hyperplasia	23	37.1
Morphology		
Endometrioid	24	38.7
NAH	21	33.9
AH	7	11.3
Serous	2	3.22
Adenosquamous	1	1.6
AH with Probable Invasion	1	1.6
Endometrioid and Serous	1	1.6
Endometrioid with squamous differentiation	1	1.6
Endometrioid- villoglandular variant	1	1.6
Focal AH with polyp	1	1.6
Focal NAH	1	1.6
Simple NAH	1	1.6

Out of the 30 Endometrial carcinoma cases, Endometrioid carcinoma is the most common one – 24 cases (80%). The second most common is the adenosquamous -2 (6.67%) and serous type – 2 (6.67%), followed by villo glandular variant and mixed type 1 (3.33%)Among the 30 Endometrial Carcinoma cases, 16 (53%) belong to FIGO Grade 1, well differentiated type. FIGO Grade 1 and 2 belong to low grade; FIGO Grade 3 – belong to high grade. Most of the cases – 24 cases (80%) belong to Low grade. 4 cases (13%) showed regional lymph node metastasis. 6 (20%) showed vascular invasion; the rest 24 (80%) did not. In 30 cases, Myometrial Invasion is present in 29 cases (97%) out of which, 25 (86%), showed invasion to > 50% of myometrium, while 4 (14%) showed invasion to < 50% of myometrium. Adenomyosis was present in 6 cases (20%) of the Carcinoma,

Table 5: Histopathological characteristics

	Number and Percentage
Histologic Type	
Endometrioid- most common	24 (80%)
Villo glandular variant of Endometrioid type	1 (3.33%)
Adenosquamous.	2 (6.67%)

Serous	2 (6.67%)
Mixed type - Endometrioid and Serous	1 (3.33%)
FIGO Grading	
Grade 1	16 (53 %)
Grade 2	8 (27 %)
Grade 3	6 (20 %)
2 Tier Grading	
Low Grade	24 (80 %)
High Grade	6 (20 %)
Other	
Regional lymph node metastasis	4 (13 %)
Presence of vascular Invasion	6 (20 %)
Adenomyosis	6 (20 %)
Myometrial Invasion	
Present	29 (97 %)
<50%	4 (14 %)
>50%	25 (86 %)
Absent	1 (3 %)

The most common FIGO Stage is Stage IB- seen in 19 cases (63%) which is Invasion equal to or more than half of myometrium, followed by Stage IA and Stage II.

Table 6: Table showing FIGO Staging of endometrial carcinoma

	Frequency	Percent
Stage IA	3	10%
Stage IB	19	63%
Stage II	3	10%
Stage IIIA	2	7%
Stage III C1	1	3%
Stage III C2	2	7%

Among the 30 cases of Endometrial Carcinoma, 25 (83%) showed complete loss of PAX2, while 5 (17%) showed partial loss.

Table 7: Expression of PAX-2 loss in different subtypes of endometrial carcinoma

Histologic type	PAX-2 loss
Endometrioid (24)	(22) Complete loss; and (2) Partial loss
Villoglandular variant (1) of Endometrioid	Complete loss
Adenosquamous (2)	Complete loss
Serous (2)	Partial loss
Mixed type – Endometrioid & Serous (1)	Complete (Endometrioid component) and Partial loss (Serous component)

In 9 cases of Atypical Hyperplasia, 6 (66.67%) showed complete PAX2 loss, 3 (33.33%) showed partial PAX2 loss. In 23 cases of Non-Atypical Hyperplasia, 15 (65%) showed minimal to no loss of PAX2 (76-100% cells staining), 8 (35%) showed partial PAX2 loss thus showing retained PAX2 in premalignant and normal endometrium and absent in carcinoma.

Table 8: Different grades of PAX2 loss in NAH, AH and Endometrial carcinoma

IHC Score	NAH	AH	Endo Ca
Complete loss (0% cells staining)	0	6 (66.67%)	25 (83%)
Partial loss (1-75% cellsstaining)	8 (35%)	3 (33.33%)	5 (17%)
Minimal to no loss (76-100% cells staining)	15 (65%)	0	0

The present study aims to study the expression of PAX2 in premalignant and malignant lesions of endometrium and so the table showing complete (25 out of 30 carcinoma cases and 7 out of 32 Hyperplasia cases) to partial/minimal loss (25 out of 32 Hyperplasia cases and 5 out of 30 carcinoma cases) in premalignant and malignant cases.

Table 9: Table showing the Loss of expression of PAX2 in Premalignant and Malignant lesions of endometrium

PAX2 loss	**Premalignancy**	**Malignancy**
Complete loss	6 (22%)	25 (83%)
Partial loss/ Minimal loss	26 (78%)	5 (17%)

100% of malignant samples were associated with complete PAX2 loss score, 74.2% of premalignant samples were associated with minimal PAX2 loss score with statistically significant p value < 0.05- There is positive association between premalignant and malignant lesions with PAX2 loss as PAX2 is a gene expressed in normal and premalignant lesions and gets lost in endometrial carcinoma.

Table 10: Association of PAX-2 loss with premalignant and malignant lesions

	Morphology outcome		**Total**	**P value**
	PREMALIGNANT	**MALIGNANT**		
YES	0	31 (100.0 %)	31	0.001
MINIMAL	23 (74.2 %)	8 (25.8 %)	31	

21 patients (72.4 %) with H/o AUB had partial PAX2 loss. There is positive association between PAX2 loss and AUB (p – 0.001).

Table 11: Association of PAX-2 loss score with Abnormal Uterine Bleeding

AUB	**PAX2 loss score**		**Total**	**P value**
	PARTIAL	**COMPLETE**		
Present	21 (72.4 %)	8 (27.6 %)	29	0.001
Absent	10 (30.3 %)	23 (69.6 %)	33	

20 patients (74.1%) with H/o PMB had complete PAX2 loss; 23 patients with no H/o PMB had partial PAX2 loss. There is positive association between PAX2 loss and PMB (p – 0.001).

Table 12: Association of PAX-2 loss score with Post Menopausal Bleeding

PMB	**PAX2 loss score**		**Total**	**P value**
	PARTIAL	**COMPLETE**		
Present	7 (25.9 %)	20 (74.1 %)	27	0.001
Absent	23 (67.6 %)	12 (32.4 %)	35	

There is no association in the expression of PAX2 and the lymph node metastatic status because the p value is 1.0

Table 13: Lymph node status in relation to PAX-2 loss score

Lymph node status	**PAX2 loss score**		**Total**	**P value**
	PARTIAL	**COMPLETE**		
Present	2 (50 %)	2 (50 %)	4	0.001
Absent	29 (50 %)	29 (50 %)	58	

There is no association in the expression of PAX2 and the vascular invasion (p value -0.39)

Table 14: Vascular invasion status in relation to PAX-2 loss

Vascular invasion	**PAX2 loss score**		**Total**	**P value**
	PARTIAL	**COMPLETE**		
Present	4 (66.7 %)	2 (33.3 %)	6	0.001
Absent	27 (48.2 %)	29 (51.8 %)	56	

DISCUSSION

The first symptom of endometrial carcinoma for the diagnosis is abnormal uterine bleeding, most commonly in postmenopausal women. Endometrial carcinoma forms a spectrum of tumours with variable histologic differentiation ranging from well-differentiated to poorly differentiated carcinomas which are originating from glandular epithelial cells.[3]Endometrial carcinoma is rapidly emerging as a leading cancer in developing countries. Atypical hyperplasia presents before endometrial carcinoma. Atypical hyperplasia has a major risk of progression to or concurrent endometrial carcinoma[2]. Subtle cytologic changes in atypical glands can be picked up by comparing with normal glands.Fragmented samples, secretory endometrium, metaplastic changes and small foci of artifactually crowded glands leads to diagnostic dilemma between hyperplastic and carcinomatous endometrium. In these difficult scenario, high diagnostic accuracy is essential in identifying early alterations in endometrium. This early detection has a huge impact on prognosis and management.[5]

Reporting Endometrial biopsies is very subjective and it differs from pathologist to pathologist. PAX2 is a novel immunohistochemical marker which can

be used in diagnostically difficult situations. PAX2 helps diagnosing and differentiating premalignant and malignant lesions of endometrium.Allison KH *et al.*,[5] had proved that loss of PAX2 expression by immunohistochemistry happens early and frequently in endometrial hyperplasia. Their aim was to examine the possible utility of PAX2 as a marker of hyperplastic endometrium. They have found that atypical hyperplasia has the highest risk of progression to or concurrent endometrial carcinoma. They have summarized that PAX2 loss appears to occur early in the development of endometrial precancers and may prove useful in some settings as diagnostic marker for determining normal endometrium from complex and atypical hyperplasia and low-grade carcinomas.The percentage and number of hyperplasia cases in Bedi *et al.*,[1]were comparable with our study; 80% of NAH in Bedi *et al.* to 72% of NAH in our case; there were 10 cases of AH in Bedi *et al.*,[1] study; and comparably 9 cases of AH in our study.Allison *et al.*,[2]evaluated PAX2 expression in 28 samples of normal proliferative and secretory endometrium, 54 samples of Endometrial Intraepithelial Neoplasia(EIN) and 15 cases of endometrioid carcinoma. They found that PAX2 was progressively lost from endometrial hyperplasia to endometrial cancer. They proved that there was significant statistical difference in expression of PAX2 loss in EIN and Endometrial Carcinoma compared to normal proliferative cases. Their study showed that there was 0% complete loss and 17.9% partial loss in normal proliferative and secretory endometrium, 74.1% complete loss & 22.2% partial loss in Endometrial Intraepithelial Neoplasia and 73.3% complete loss & 20% partial loss in endometrial carcinoma. This proved that cases with complete loss increased progressively with increasing severity of hyperplasia.[2]Joiner *et al.*,[6] studied PAX2 expression in simultaneously diagnosed WHO and EIN classification systems,among 67 cases. The result yielded 86.3% complete loss, 11.3% decreased staining and 2.3% increased staining. PAX2 changes are in concordance (92%) with EIN scheme when compared with WHO classification system (88%). They concluded that PAX2 can be used as an adjunct and training tool when there is confusion regarding atypical hyperplasia/EIN.Quick CM *et al.*,[7] concluded that markers like PAX2 can recognize EIN lesions and provide a visual impression of clonal growth and precise delineation of lesion extent that lead to insights with educational and in certain cases, diagnostic value. Clonal loss of PAX2 in EIN lesions is 71% which is high compared to 44% for PTEN. In this study, PAX2 staining was good and scorable (96%) for most of samples.

In study by Monte NM et. al.,[8] loss of PAX2 expression during endometrial carcinogenesis was examined. They found total loss of PAX2 in 77% of carcinoma samples and 71% of EIN samples.Rewcastle E et. al.,[9] observed that PAX2 expression progressively decreased from normal proliferative endometrium to EIN to Endometrioid Endometrial Carcinoma.Trabzonlu*et al.*,[10] concluded that when compared to normal glands, 73.3% of EIN samples showed decrease in PAX2 staining.Diagnosis of Atypical hyperplasia and Endometrioid Intraepithelial Neoplasia is a challenging one. Specific immunohistochemical markers have become an adjuncts in diagnosis of AH/EIN. WHO 2020 Classification recommends loss of PAX2, PTEN and mismatch repair proteins as desirable diagnostic criteria. This study concluded that diagnostically useful immunohistochemical markers such as PAX2, PTEN and Beta-Catenin can detect AH/EIN cases. Hence it should be used as a panel for better results.[11]In our study, we noted that PAX2 loss can be used to differentiate serous and Grade 3, endometrioid carcinoma. Aberrant cytoplasmic expression was seen in one endometrioid carcinoma case in ourstudy.High grade endometrial cancers include FIGO grade 3 endometrioid carcinomas, serous carcinomas, undifferentiated, dedifferentiated carcinomas and carcinosarcomas. There are significant prognostic differences between the histologic subtypes of high-grade endometrial cancers; poorer outcomes with serous carcinoma, clear cell carcinoma compared with grade 3 endometrioid carcinoma. Serous carcinoma had poorer survival than grade 3 endometrioid carcinoma which can be easily differentiated by PAX2.[12]Limitations of our study were, we could not associate between PAX2 loss and endometrial thickness.There was no history of Oral contraceptive pills/ hormonal drugs in the study participants; thus, we could not associate PAX2 loss with drugs.Also, we had only 62 sample size which is a major limitation of our study

CONCLUSION

Decrease in PAX2 expression was significantly associated with progression to endometrial carcinoma. The changes in PAX2 expression can be used in detecting premalignant lesions long before morphological changes occur. PAX2 loss alone cannot be used as a diagnostic criteria. However, this novel immunohistochemical marker PAX2 significantly aids in early detection of endometrial carcinoma. Our study showed significant statistical difference in the PAX2 expression between the premalignant and malignant lesions of endometrium. Hence utility of PAX2 can aid in accurately diagnosing and differentiating hyperplastic endometrium from endometrial carcinoma.

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