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ORIGINAL ARTICLE

Significance of Three-Marker Panel of PAX2, PTEN and Beta-catenin in The Diagnosis of Endometrial Precancers

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ABSTRACT

Background: Endometrial hyperplasia (EH) is a lesion that precedes endometrioid endometrial carcinoma (type 1 EC). Pathologists are aware of the practical challenges associated with accurate atypical hyperplasia/endometrioid intraepithelial neoplasia (AH/EIN) diagnosis. Numerous researchers have concentrated on biomarkers that can forecast the likelihood of progression from endometrial hyperplasia to EC. In order to differentiate AH/EIN from its mimics, the objective of this work is to assess the expression of PAX2, PTEN, and beta-catenin both separately and in combination. **Methods**: This is a case control study that included sixty cases divided into three groups (20 in each group): control group (normal ovulatory cycling endometrium (proliferative and secretory)), EH without atypia, and AH/EIN. Formalin-fixed paraffin-embedded tissue sections were analyzed immunohistochemically for PAX2, PTEN, and beta-catenin. **Results**: The performance characteristics of each marker, the entire panel, and subsets thereof were quantitatively and statistically analyzed. In order of number of cases detected, the most frequently aberrant markers in AH/EIN were Pax2 (80% of cases) with specificity (80%), PTEN (45% of cases) with specificity (85), and B-catenin (40% of cases) with specificity (100%) in distinguishing AH/EIN. Regarding the relationship between three markers in detecting AH/EIN, using β-catenin in addition to PAX2 raises the sensitivity to 90% with specificity (80%). But using a three-marker panel raises sensitivity (95%) with specificity (80%). While using PTEN and B-catenin had the lowest sensitivity (75%). **Conclusions**: Three-marker panel of PAX2, PTEN and B-catenin is significant in differentiation AH/EIN from its benign mimics (EH without atypia).

Keywords: Three-marker panel; PTEN; Beta-Catenin; PAX2; Atypical endometrial hyperplasia

INTRODUCTION:

One of the precursor lesions to endometrioid endometrial cancer (type 1) is endometrial hyperplasia (EH) [1]. Atypical hyperplasia/endometrioid intraepithelial neoplasia (AH/EIN) and endometrial hyperplasia without atypia are two different conditions according to the most recent 2020 World Health Organization (WHO) update. Since up to one-third of patients with atypical endometrial hyperplasia will be diagnosed with carcinoma within a year [3], this distinction is crucial in clinical management [2].

According to other research, between 20 and 50 percent of untreated AEH cases develop into EC, more precisely type I endometrioid adenocarcinoma [4, 5]. An exceptional chance for prevention and better patient care of this often-encountered cancer exists with an earlier and more precise identification of EC, and specifically its histologic antecedents [6].

Though none are now commonly utilized in clinical practice, numerous researchers have concentrated on biomarkers that can forecast the likelihood of progression from endometrial hyperplasia to invasive carcinoma. According to some research, AH/EIN instances exhibit p53 immunohistochemistry expression [7], as well as PRb2/p130 [8]. Additionally, the expression of mismatch repair (MMR) was examined in biopsies containing AH/EIN, and it was found that only 5–10% of AH/EIN showed immunohistochemically significant reduction of Mlh1 expression [9]. Although phospho-Akt and Foxo1 are abnormal in certain AH/EIN, their use as practical biomarkers seems to be limited since it is difficult to detect phosphoproteins in paraffin-embedded tissues [10]. Not all of these indicators are useful in everyday practice.

In the diagnosis of AH/EIN, "loss of immunoreactivity for Paired-box2 (PAX2), Phosphatase, and Tensin Homolog is desirable", according to the 2020 World Health Organization Classification of Female Genital Tumors. B-Catenin and other recently identified AH/EIN indicators would be useful for diagnosis [11,12].

PAX2 functions as a gene that suppresses tumors. Endometrial cancer and precancer development are correlated with decreased Pax2 expression.

To differentiate premalignant EH from benign mimics, the 2017 European Society of Gynecological Oncology (ESGO) guidelines, which were based on the 2016 European Society for Medical Oncology-ESGO-European Society for Radiotherapy & Oncology Consensus Conference, suggest immunohistochemical analysis of paired box 2 protein (PAX2) [11].

PTEN is a tumor suppressor gene located on 10q23 that is a phosphatase and tensin homolog. In human malignancies, it is among the most frequently altered tumor suppressors [13].

PTEN is lost as an early event, according to several studies [7]. According to Steinbakk et al., endometrial hyperplasia development to endometrial cancer was indicated by a decrease in PTEN expression [7].

Beta-catenin is a component of the highly conserved Wnt signaling system, which plays a crucial role in the development of embryos, carcinogenesis, and the transition from epithelium to mesenchymal tissue [14]. The abnormal expression of β-catenin has been widely considered to play important roles in malignant transformation, progression, and metastasis due to its functional significance [15].

One of the most crucial indicators investigated to distinguish between benign EH brought on by the unopposed action of estrogens and premalignant EH is B-catenin [1]. Clinical laboratories can use nuclear B-catenin localization, which is linked to overexpression, for immunohistochemistry detection of β-catenin activation in AH/EIN or endometrial cancer [16].

The goal of a comprehensive study has not yet been determined how many indicators should be employed in AH/EIN diagnosis or whether other recently identified AH/EIN markers, such as B-Catenin, would be useful for diagnosis.

AIM OF WORK:

The aim of this work is to evaluate each of PAX2, PTEN, and Beta-catenin expression individually and in combinations to distinguish AH/EIN from its mimics in a diagnostic approach to endometrial precancers.

METHODS:

This is a case control study that was conducted at the Pathology Department, Faculty of Medicine, Zagazig University, in the period from 2021 to 2023. After receiving approval from the faculty's local ethical committee and the institutional review board (IZU-IRB#10552-7/3-2023) of Medicine, Zagazig University Hospital and a written consent from every patient participating in the study was taken. The study was done according to the code of ethics of the world medical association (Declaration of Helsinki 1979).

Forty cases of endometrial hyperplasia and 20 control cases were enrolled. They were divided into three groups (20 in each group): control

group: ovulatory cycling normal endometrium (proliferative and secretory) (n:20), Atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia (n = 20) and endometrial hyperplasia without atypia (n = 20). All specimens were taken by D&C.

The clinical information of Hormonal status (perimenopausal (30-50 years) or postmenopausal (>50 years), Obesity, and metabolic syndrome were obtained. Histopathology reports, together with haematoxylin and eosin (H&E) stained slides were reviewed to confirm the diagnosis.

Cases with sub diagnostic criteria for AH/EIN or EH without atypia, cases that harbored definitive areas of endometrial adenocarcinoma and cases with prior history of treatment by high-dose progestin for AH/EIN or EH without atypia were excluded.

Histopathological evaluation

The H&E-stained slides were reviewed for each case to verify the original diagnoses and categorized according to the 2020 WHO classification into two groups: (AH/EIN and endometrial hyperplasia without atypia). Criteria used for diagnosis of AH/EIN, including: 1- Gland; stroma ratio >1. 2-Overt nuclear atypia/cytologic demarcation from background endometrium. 3-size ≥ 1 mm. Criteria used for diagnosis of endometrial hyperplasia without atypia including the increase of endometrial glands, known as diffuse or multifocal crowding, is the defining feature of endometrial hyperplasia without atypia, where the glands take up more than 50% of the surface area, resulting in a gland-to-stroma ratio larger than 1. The crowded gland may be uniformly round and tubular or display irregular outlines. Generally, cellular polarity and stratification are preserved. The cells that make up the pseudostratified epithelial lining resemble those found in the mild to late proliferative phase. Ciliated cells and tubal metaplasia are common observations. The cells have elongated nuclei with uniformly distributed chromatin and barely noticeable nucleoli. Commonly seen are mitotic figures and apoptotic bodies [17].

Immunohistochemical study:

In accordance with the manufacturer's instructions, the immunostaining procedure was carried out using the Dako Autostainer Link 48 (Dako, Santa Clara, CA, USA). The subsequent processes of deparaffinization and rehydration were applied to the slides. For 20 minutes, epitope retrieval was carried out at 97°C in a low pH (6.0) solution for β-catenin and a high pH (9.0) Tris/EDTA solution (Agilent) for the other markers (PAX2 and PTEN). The automated staining process involved applying Envision Flex Peroxidase-Blocking Reagent (Dako, Santa Clara, CA, USA) for 10 minutes for B-catenin and 5 minutes for other markers. The primary antibodies were then incubated for 20 minutes for β-catenin (ready to use, monoclonal mouse antibody, clone βcatenin-1, #IR70261-2, Agilent, Santa Clara, CA, USA) and 60 minutes for PAX2 (ready to use, monoclonal rabbit antibody, clone QR060, quartett, Cancer Diagnostics, Potsdam, Germany) and PTEN (prediluted, the used dilution 1/200, polyclonal rabbit antibody, #A11193, ABclonal, Woburn, USA). Incubation with Mouse Linker (Agilent) for β-catenin and Rabbit Linker (Agilent) for Pax2 and PTEN was performed for 10 minutes at room temperature. The incubation period for secondary antibodies (Envision/HRP; Dako, Santa Clara, CA, USA) was 30 minutes for PAX2, 20 minutes for PTEN, and 20 minutes for β-catenin. The substrate chromogen (Substrate Working Solution, Dako, Santa Clara, CA, USA) should then be applied and left at room temperature for ten minutes. A wash buffer solution (Envision Flex Wash buffer, Dako, Santa Clara, CA, USA) was used to rinse the sections after each step. Following the last round of washing, the slides were dehydrated, cleaned, mounted, and counterstained with Harris's hematoxylin. An external positive control for PAX2 was renal cell cancer. B-catenin and PTEN were tested against human breast cancer. For PTEN, the stroma served as an internal positive control, but for PAX2, just the nearby normal endometrial glands were utilized. By

substituting non-immune serum for the main antibodies, a negative control was produced.

Evaluation of immunostaining:

Scoring of PAX2 immunostaining: PAX2 localizes exclusively within nuclei. PAX2 is expressed only in glandular epithelium as strong positive nuclear staining, indicating retained expression. Negative nuclear staining is considered as a loss of PAX2 expression. The adjacent entrapped normal glands serve as internal positive control. The loss of staining should exceed more than 5% (cutoff value) that is used as a threshold for aberrancy (loss of expression) [18].

Scoring of PTEN immunostaining: PTEN is normally expressed (positive staining) in benign endometrial epithelial cells, endometrial stroma, and leukocytes. For the glandular epithelium, it normally stains nucleus, cytoplasm, and cell membrane indicating positive staining (retained expression). Retained stromal expression serves as an internal positive control. PTEN loss (negative staining) is characterized by complete absence of nuclear and cytoplasmic expression in some (heterogenous expression) or the entire glands. The cutoff value is > 5% of the entire glands. A weak staining is also considered as a loss of expression [19].

Scoring of B- catenin immunostaining: B-catenin is predominantly expressed on the membrane with some cytoplasmic and nuclear staining. B-catenin should be assessed in the glandular epithelium. Strong and distinctly nuclear expression in glands is usually associated with overall or overexpression indicating aberrant expression and underlying molecular defect [20]. The nuclear expression staining intensity should be at least as intense as the cell membrane of the same cell. Focal or scattered strong nuclear staining is also considered as an aberrant nuclear expression[16].

Statistical analysis:

Microsoft Excel and the Statistical Package for the Social Sciences (SPSS version 21.0) were used to code and enter the obtained data. The predictive probability was used as a covariate in the construction of receiver operating characteristic curves (ROC) curves. For each marker combination, the diagnostic value was assessed using areas under the curves (AUCs). When a difference's p value was less than 0.05, it was deemed statistically significant; when it was equal to or less than 0.0, it was deemed very significant.

RESULTS:

The mean age is 43.80±10.46 included in the study; 37 (61.7%) were perimenopausal and 23 (38.3%) were postmenopausal. The mean age of patients with atypical endometrial hyperplasia (51.15±7.379) was higher than that of cases of endometrial hyperplasia without atypia (40.0±7.79) and ovulatory cycling normal endometrium (40.250±11.81). The main clinical presentation was abnormal uterine bleeding (AUB).

Patterns of markers expression:

PAX2 expression

Most of the cases of atypical endometrial hyperplasia 16 (80%) showed loss of nuclear expression (negative staining) with retained expression in entrapped normal glands. Most of the cases of ovulatory cycling normal endometrium 17 (proliferative to secretory) (85%) and endometrial hyperplasia without atypia 17 (85%) showed retained expression (positive staining) with loss versus retained <5% of glands in cases of ovulatory cycling normal endometrium with a statistically significant difference (p=0.0001) (Table1) (Figure1).

PTEN expression

Forty five percent of cases with atypical endometrial hyperplasia (9) cases showed loss of nuclear, cytoplasmic, and membranous expression (negative staining) with retained expression in entrapped normal glands while most of cases with endometrial hyperplasia without atypia (80%) (16) and ovulatory cycling normal endometrium (proliferative and secretory) (90.0%) (18) showed retained expression with loss in focal scattered glands (<5% of the glands) in cases of ovulatory cycling normal endometrium (p =**0.031**) **(Table 2) (Figure1).**

B-catenin expression:

Forty percent of cases with atypical endometrial hyperplasia showed positive nuclear expression (aberrant expression), while all cases of ovulatory cycling endometrium (proliferative and secretory) and endometrial hyperplasia without atypia showed non-nuclear (membranous ± cytoplasmic expression), with a statistically significant difference (p = 0.001) **(Table 3) (Figure1).**

Individual marker Evaluation (PAX2, PTEN and B-catenin) in detecting AH/EIN

Using PAX2 in detecting AH/EIN was more sensitive than B-Catenin and PTEN with area under curve (AUC) was 0.80. The sensitivity was 80% compare to 45% and 40% of PTEN and B -catenin. While the Specificity of B-catenin was 100% (**Table 4) (Graph1).**

AUC when using PAX2 in detecting AH/EIN was (0.80) higher than that of PTEN (0.65) and B-catenin (0.7) **(Graph 1).**

Evaluation of markers combination in detecting AH/EIN:

Using more than one marker in detecting AH/EIN, Using B- catenin in addition to PAX2 raises the sensitivity to 90%. But using three-marker panel (PAX2 & PTEN and B-catenin) raises sensitivity (95%). While using PTEN and B-catenin had the lowest sensitivity (75%) **(Table 5).**

AUC when using B -catenin in addition to pax2 in detecting AH/EIN was (0.85). AUC when using a combination of PAX2, PTEN and B-catenin was 0.875 higher than that when using combination of PTEN and B-catenin (0.80) **(Graph 2).**

Table (1): PAX2 immunohistochemical expression in the studied groups.

PAX2	Histological diagnosis			X2	P- value
	Normal endometrium (proliferative to secretory)	Endometrial hyperplasia without atypia	Atypical endometrial hyperplasia		
Loss of expression (negative staining)	3 (15.0%)	3 (15.0%)	16 (80.0%)	20.417	< 0.0001
Retained expression (positive staining)	17 (85.0%)	17 (85.0%)	4 (20.0%)		
Total	20	20	20		

*P: statistically significant < 0.05 ** p < 0.001 highly significant

Table (2): PTEN immunohistochemical expression in the studied groups**.**

PTEN	Histological diagnosis			X2	P- value
	Normal endometrium (proliferative to secretory)	Endometrial hyperplasia without atypia	Atypical endometrial hyperplasia		
Loss of expression (negative staining)	2 (10%)	4 (20.0%)	9 (45.0%)	6.933	0.031*
Retained expression (positive staining)	18 (90%)	16 (80.0%)	11 (55.0%)		
Total	20	20	20		

*P: statistically significant < 0.05 ** p < 0.001 highly significant

Table (3): B-Catenin immunohistochemical expression in the studied groups.

B-catenin	Histological diagnosis			X2	P-value
	Normal endometrium (proliferative to secretory)	Endometrial hyperplasia without atypia	Atypical endometrial hyperplasia		
Non-nuclear	20 (100.0%)	20 (100.0%)	12 (60.0%)	18.462	<0.001
Nuclear (aberrant)	0 (0.0%)	0 (0.0%)	8 (40.0%)		
Total	20	20	20		

*P: statistically significant < 0.05 ** p < 0.001 highly significant

Table (4): Diagnostic markers; PAX2, β-Catenin, and PTEN Immunohistochemistry in detecting AH/EIN

Marker	Area	Sensitivity	Specificity	+PV	-PV
PAX2	0.80	80	80	66.67	88.89
PTEN	0.65	45	85	60	75.56
B- catenin	0.7	40	100	100	76.92

Table (5): Evaluation of markers combination in detecting AH/EIN

Marker	Area	Sensitivity	Specificity	+PV	-PV
PTEN& B-catanin	0.80	75	85	71.42	87.17
PAX2 & PTEN	0.825	85	80	68	91.42
PAX2 & B catanin	0.85	90	80	69.23	94.12
PAX2 & PTEN& B-catanin	0.875	95	80	70.37	96.97

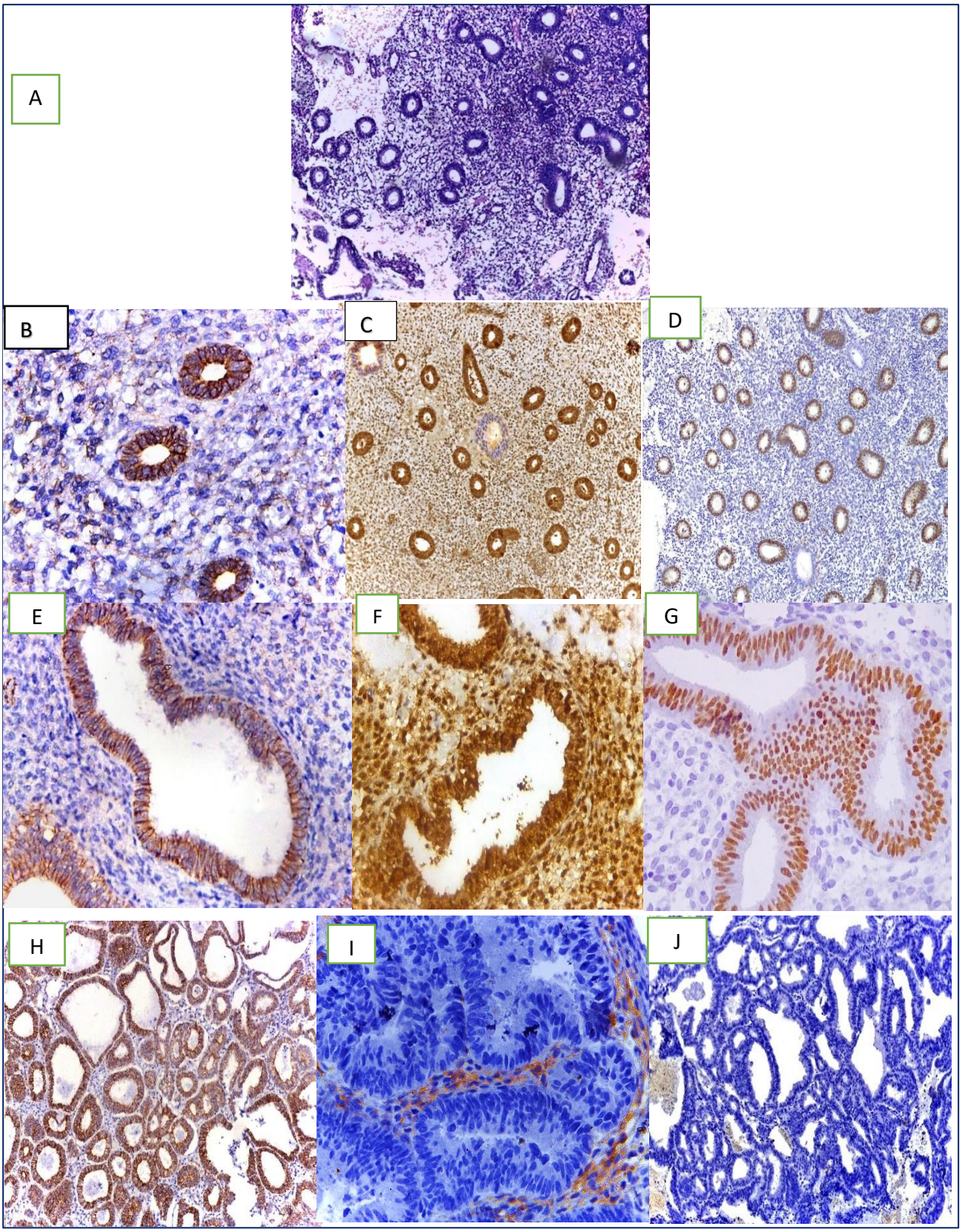
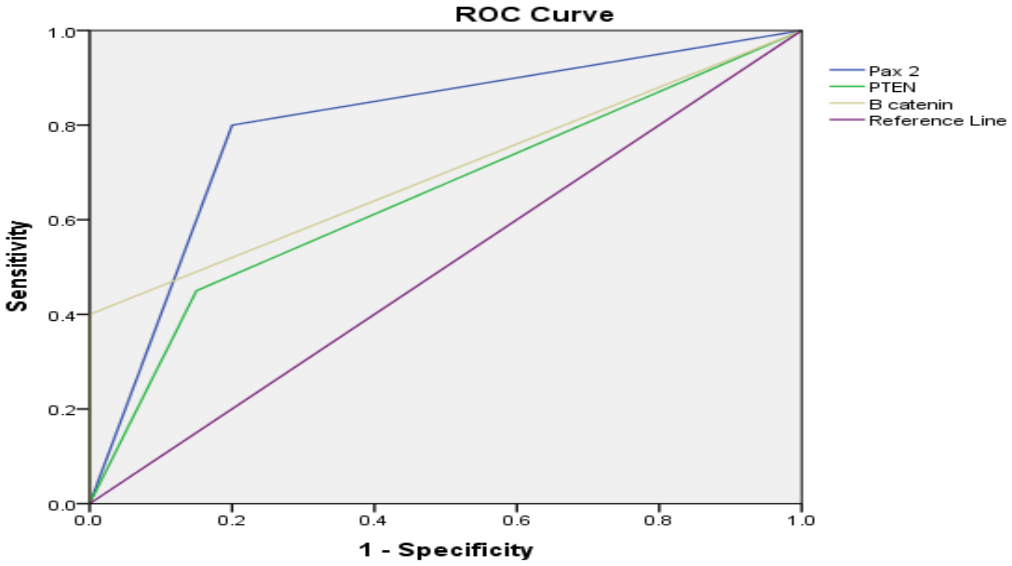
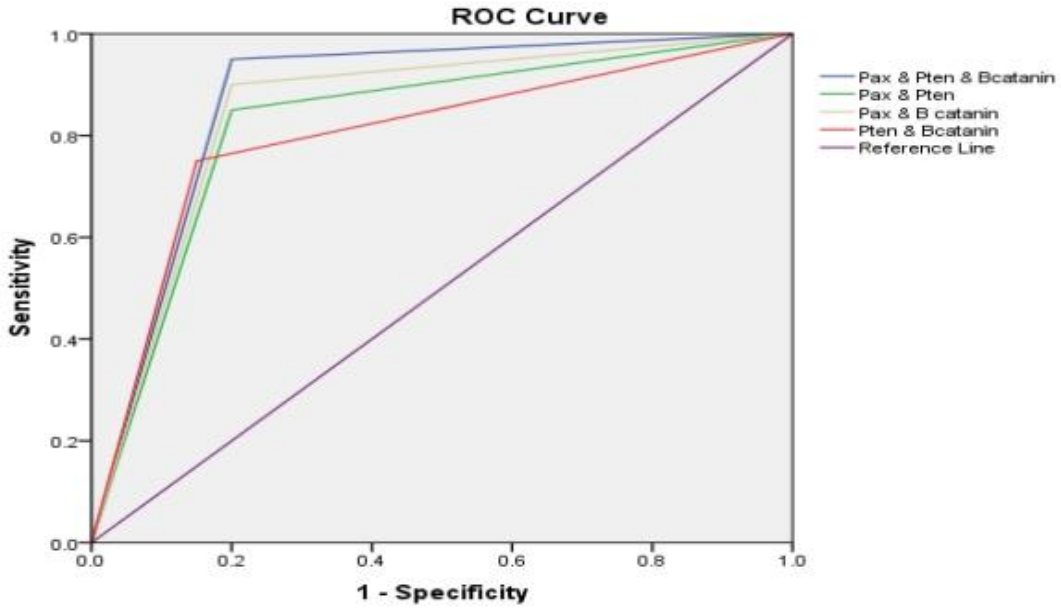


Figure 1; Proliferative endometrium (A) H&E (X200) (B) positive membranous B-catenin(x400) (C) nuclear, cytoplasmic and membranous PTEN with positive stromal expression with single gland loss of expression(x200) (D) Retained nuclear pax2

expression with single gland loss of expression (x200). (E) Endometrial hyperplasia without atypia with positive membranous B-catenin(x400). (F) Endometrial hyperplasia without atypia with nuclear, cytoplasmic and membranous PTEN with positive stromal expression(x400) (G) Endometrial hyperplasia without atypia with retained nuclear PAX2 expression(x400). (H) Atypical endometrial hyperplasia with heterogenous B-catenin staining(x200) (I) Atypical endometrial hyperplasia with loss of PTEN expression with positive stromal staining(x400) (J) Atypical endometrial hyperplasia with loss of PAX2 expression (x200).



Graph (1): Roc curve for individual diagnostic markers; PAX2, β-Catenin, and PTEN Immunohistochemistry in detecting AH/EIN



Graph (2): Roc curve for markers combination evaluation in detecting AH/EIN

DISCUSSION:

Endometrial precancers (endometrial hyperplasia and atypical hyperplasia) represent lesions that have the potential to progress to endometrioid endometrial cancer if left untreated. Early detection and differentiation of these precancerous lesions are critical for guiding appropriate management and preventing progression to invasive cancer [6].

It is commonly known that women with atypical endometrial hyperplasia have a higher risk of developing endometrial cancer than those with endometrial hyperplasia without atypia [5]. Histological analysis remains the cornerstone for distinguishing between benign and atypical hyperplastic glands. However, pathologists are aware of the practical challenges in accurately diagnosing AH/EIN, which include fragmentation of the specimen or limited tissue, changes in glandular architecture during normal cycling, the use of hormonal agents that mask cytological and architectural features, and the presence of endometrial polyp fragments, which typically show significant gland crowding but often harbor precancers. The final challenge is that AH/EIN might vary gradually in gland architecture and be localized or diffuse [21]. Therefore, it is still difficult to make a solid diagnosis of AH/EIN, which is why diagnostically valuable biomarkers for AH/EIN are being sought after and validated [22].

In our study, all specimens are taken by D&C because it is the most common diagnostic method for abnormal uterine bleeding which is the main presenting symptom.

This study aim is to evaluate the performance of the immunohistochemical expression of PAX2, PTEN and B-catenin individually and in combination to distinguish cases of atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia from endometrial hyperplasia without atypia. An additive set of ovulatory cycling endometrium (proliferative to secretory) was included to define optimal diagnostic criteria for markers aberrance.

We evaluated the immunohistochemical expression of PAX2, when endometrioid intraepithelial neoplasia or atypical endometrial hyperplasia occurs, PTEN and B-catenin, both separately and together, endometrial hyperplasia without atypia, and ovulatory cycling normal endometrium (proliferative to secretory), to distinguish atypical endometrial hyperplasia from its mimics.

Atypical endometrial hyperplasia was detected in 13 (56.5%) of postmenopausal cases, and only in 7 (18.9%) of perimenopausal cases with statistically significant difference p=(p=0.004).

Zhao et al. underlined that postmenopause was significantly associated with Atypical endometrial hyperplasia [23].

The mean age of patients with atypical endometrial hyperplasia (51.15±7.379) was higher than that of cases of endometrial hyperplasia without atypia (40.0±7.79) and ovulatory cycling normal endometrium (40.250±11.81).

Allawy et al. discovered that the average age of the groups with endometrial cancer was 50.11 ± 1.27, endometrial hyperplasia without atypia was 46.77 ± 2.75, atypical endometrial hyperplasia was 48.36 ± 2.42, and the control group was 44.76 ± 2.57 [24].

Estrogens increase the expression of PAX2 in neoplastic endometrial epithelium but not in normal endometrium, suggesting that neoplastic tissues have a fundamentally different mechanism for responding to estrogen[25].

PAX2 expression, most of the cases of atypical endometrial hyperplasia (80%) showed loss of nuclear expression (negative staining) with retained expression in entrapped normal glands while most of the cases of ovulatory cycling normal endometrium (proliferative to secretory)(85%) and endometrial hyperplasia without atypia (85%) showed retained expression (positive staining) with loss versus retained <5% of glands in cases of ovulatory cycling normal endometrium with a statistically significant difference (p=0.0001).

In support of our results, Chen et al. revealed that 22 (40.7%) of the 54 original biopsies had

loss of PTEN and 48 (88.9%) had loss of PAX2 expression in regions of atypical hyperplasia. [26]. But Lucas et al. discovered that endometrial polyps (EMPS) had a substantially lower prevalence of PAX2 aberrancy in AH/EIN than nonpolyp AH/EIN (64.8% vs. 81.1%) [27].

Aguilar et al. showed similar results in proliferative and secretory endometrial tissue and atypical endometrial hyperplasia. They discovered that decreased PAX2 expression may aid in the early detection of endometrial cancer and in the diagnosis of atypical endometrial hyperplasia. The possibility of employing PAX2 as a diagnostic marker for endometrial lesions was investigated. According to their research, a low percentage of normal endometrial cases (16.5%) had lost PAX2 nuclear expression, whereas a high percentage of AH/EIN patients (81.1%) did. There were 111 cases of AH/EIN and 79 cases of normal endometrium in this large sample size investigation. [22].

Yildiz et al. reported similar findings where they reported that PAX2 nuclear expression was lost in a high percentage of AH/EIN cases (84%) [28].

A study by Monte et al. evaluated PAX2 expression in a series of endometrial samples, including normal, hyperplastic, and neoplastic lesions. The study found that PAX2 expression was significantly reduced in endometrial hyperplasia with atypia and in endometrial carcinoma compared to normal endometrial tissue. The loss of PAX2 expression was associated with epithelial dedifferentiation, which is a characteristic of more aggressive lesions that may progress to carcinoma [29].

Conversely, Kahraman et al. found that atypical endometrial hyperplasia was associated with a markedly higher level of PAX2 expression, and that the PAX2 positivity in atypical endometrial hyperplasia was much higher than that of proliferative endometrium and endometrial hyperplasia without atypia [30].

It's unclear exactly how PAX2 loss occurs in the endometrium. According to certain research, hypermethylation of the PAX2 promoter may be the cause of epigenetic dysregulation [31]. Notwithstanding the pathobiology of this phenomena, PAX2 deletion as an AH/EIN sign should only be evaluated cautiously and in conjunction with morphology and other markers due to its very high incidence in EMP. However, we cannot rule out the potential that PAX2 depletion is linked to an increased risk of endometrial polyps (EMPs) undergoing neoplastic transformation or that it contributes to this process [27].

An even higher proportion of tumors seem to exhibit decrease of PTEN activity in the absence of mutations. The phosphatase's post-translational changes could well be the cause of this. PTEN-deficient glands may experience clonal expansion and develop AH/EIN or endometrial cancer because unopposed estrogens encourage endometrial development [13].

Forty five percent of cases with atypical endometrial hyperplasia showed loss of nuclear, cytoplasmic, and membranous expression (negative staining) with retained expression in entrapped normal glands while most of cases with endometrial hyperplasia without atypia(80.0%) (16) and ovulatory cycling normal endometrium (proliferative and secretory) (90.0%) (18) showed retained expression with loss in focal scattered glands (<5% of the glands) in cases of ovulatory cycling normal endometrium with a statistically significant difference (p =0.031).

Study by Aguilar et al. investigated how immunohistochemistry can be used to identify PTEN deficiency in endometrial biopsy samples. According to the study, PTEN loss in endometrial hyperplasia with atypia was highly linked to an elevated risk of carcinoma, and PTEN immunohistochemistry may be used as a supplementary method to diagnose and categorize individuals with endometrial lesions [22].

Allithy et al. discovered that when the number of cytological abnormal features increased, the intensity of PTEN immunostaining decreased. Only 6.89% of the atypical EH that was investigated had significant PTEN expression,

in contrast to all of the normal endometrium and EH that were included. PTEN expression was weak in all of the endometrioid ECs that were analyzed [32].

Erkanli et al. and Sharda et al. discovered that normal endometrium (proliferative and secretory endometrium) had a considerably greater PTEN expression than both atypical and endometrial hyperplasia without atypia. They assumed that PTEN played a role in the initial stages of endometrial cancer development [33, 34].

Another study, Tantbirojn et al. discovered that PTEN protein was found in 24% of atypical endometrial hyperplasia and 60% of endometrial hyperplasia without atypia [35].

Aguilar et al. discovered that endometrial hyperplasia without atypia and normal endometrium had lower levels of PTEN expression than atypical endometrial instances [12].

Also, Sarmadi, et al. observed that 75% and 48% of atypical EH and endometrioid endometrial cancer, respectively, tested positive for PTEN, whereas all of the normal proliferative and simple hyperplastic endometrial tissues under examination tested positive for PTEN. Additionally, they observed that PTEN immunostaining in endometrioid ECs and atypical EH varied either within or between endometrial glands [36].

Pten loss may be heterogeneous, occurring in part or all of the AH/EIN glands, and may signify a second "hit" during the tumor's development [19].

Shanmugapriya et al. hypothesized that endometrial pathogenic circumstances (atypical EH and endometrioid ECs) cause a down-regulation of PTEN immunoreactivity. In order to identify precancerous lesions and the early stages of endometrial carcinogenesis, they emphasized the value of PTEN immunohistochemical examination as a screening technique in instances of EH. [37]. But, Cirpan et al. discovered that only 1 out of 24 individuals with AH/EIN had total loss of PTEN immunoreactivity. The tiny sample size could be the cause of this [38].

While all cases of ovulatory cycling normal endometrium (proliferative and secretory) and endometrial hyperplasia without atypia showed non-nuclear (membranous ± cytoplasmic expression), 40% of cases with atypical endometrial hyperplasia showed positive nuclear expression (aberrant expression) for B-catenin. This difference was statistically significant ($p = 0.001$).

A pivotal study by Athanassiadou et al. examined the role of B-catenin expression in endometrial cancer and hyperplasia. According to the study, a subset of atypical hyperplasias had nuclear accumulation of beta-catenin, and these lesions had a higher propensity to develop into endometrial cancer. According to the study, beta-catenin may be a biomarker for atypical hyperplasia that has a higher chance of developing into cancer [39].

In this work, Aguilar et al. discovered that B-catenin had 100% sensitivity in detecting atypical hyperplasia (AH) or EIN. This indicates that there were no false positives for benign hyperplastic lesions when nuclear B-catenin was present, and that it consistently indicated atypical or premalignant lesions. They found that nuclear b-catenin expression is present in 47.7% of AH/EIN. stated that modest levels of nuclear B-catenin are typical and that nuclear staining that is noticeably higher than that of the lateral cell membranes is a sign of significant nuclear expression [22].

Endometrial polyp (EMP) AH/EIN had a considerably higher prevalence of B-catenin aberrancy than nonpolyp AH/EIN (61.9% vs. 47.7%), according to Lucas et al [40].

Some evidence suggests that AH/EIN is defined by nuclear b-catenin localization, which occurs early in endometrial carcinogenesis [41, 42].

The function of nuclear B-catenin localization in the context of AH/EIN is less obvious because of other theories that propose nuclear b-catenin in EIN/AH is not totally specific for b-catenin mutation [43].

Norimatsu et al. revealed that only 26.3% of EIN instances displayed nuclear staining of B-catenin, while all proliferative endometrial cases had negative nuclear staining [44].

Another study revealed that while strong membrane immunoreactivity was diffusely seen in glandular cells of normal endometrial cases, nuclear expression of B-catenin was 0%, 10.8%, and 31.3% in cases of normal endometrium (proliferative and secretory endometrium), endometrial hyperplasia without atypia, and atypical hyperplasia, respectively [41]. But Wright et al. , Aguilar et al. and Yadav et al. shown that nuclear b-catenin expression is present in a significant portion of AH/EIN (42.7%, 47.7%, and 60%, respectively). In order to diagnose atypical EH histopathologically, they proposed using b-catenin expression as a diagnostic adjunct.[20, 22, 45].

Aguilar et al. stated that modest levels of nuclear β-catenin are typical and that nuclear staining that is noticeably higher than that of the lateral cell membranes is a sign of significant nuclear expression [12].

Using more than one marker in detecting AH/EIN, Using B- catenin in addition to PAX2 rise the sensitivity to 90%. But using three-marker panel (PAX2 & PTEN and B-catenin) rises sensitivity (95%). While using PTEN and B-catenin had the lowest sensitivity (75%).

The possibility of combining these two markers to increase diagnostic accuracy in detecting lesions at risk of progression to carcinoma was highlighted by Chen et al.'s examination of PAX2 expression, which revealed a substantial correlation between PAX2 loss and PTEN loss in atypical lesions [26].

The loss of PTEN often correlates with the loss of PAX2, suggesting that as endometrial hyperplasia becomes more atypical, both markers may be downregulated, leading to a greater likelihood of progression to carcinoma. This strong correlation highlights that PTEN and PAX2 may work together in a pathway that governs endometrial epithelial integrity and differentiation[46].

In our study, using PAX2 in detecting AH/EIN was more sensitive than β-Catenin and PTEN with area under curve (AUC) was 0.80. The sensitivity was 80% compare to 45% and 40% of PTEN and B -catenin. While the Specificity of B-catenin was 100%.

In Lucas et al. investigation, a panel of three IHC markers—PAX2, PTEN, and β-catenin—showed high sensitivity in identifying endometrioid intraepithelial neoplasia and atypical hyperplasia [40].

Aguilar et al. discovered that the most helpful marker for diagnosing AH/EIN was PAX2, which was followed by PTEN and B-catenin. However, PTEN and B-catenin immunohistochemistry greatly improved the diagnostic yield over PAX2 alone due to the nonoverlapping patterns of aberrancy of these three markers across AH/EIN. The majority of PAX2-deficient cases were either PTEN or β-catenin aberrant, with 78.4% of cases having both PTEN and B-catenin alone (without PAX2). The majority of AH/EIN using the 3-marker panel were aberrant for at least two markers, and aberrancy for two or more markers can further increase diagnostic confidence when assessing a specific case. When PAX2, PTEN, and β-catenin are combined, they may identify a high number of instances (92.8%), which may make the panel helpful in practice [22].

One of strength points in this study is working on fragments of D&C with its challenges in diagnosis. We also use strict criteria for immunostaining evaluation. One of Limitations of this study is data regarding metabolic syndrome and obesity are lacking. Also, this is a retrospective study, so we couldn’t correlate patient’s outcome and immunohistochemical expression of PAX2, PTEN, B-catenin. Some tissue blocks were with prolonged formalin fixation that causes alteration in the elemental composition of tissues.

Conclusions:

Using three-marker panel (PAX2, PTEN, B-catenin) was more sensitive in detecting AH/EIN than using each marker individually. Combined cocktail immunohistochemical expression of PAX2, PTEN and B-catenin are significant in differentiating atypical endometrial hyperplasia from its mimics. A follow up studies are recommended.

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