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Immunohistochemical and histopathological analysis of hysterectomy cases

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Abstract

To study the immunohistochemical role of PTEN in the diagnosis of endometrial hyperplasia, one of the challenging diagnoses in uterine diseases, we highlight the most important histopathological diagnoses underlying different types of uterine surgeries. Both benign and malignant diseases are now treated by hysterectomy, with an increasing number of such surgeries. The type of surgery depends on the medical staff's experience, facilities, provisional diagnosis, and the patient's desire. Differentiation between typical and atypical endometrial hyperplasia is one of the challenging diagnoses in gynecological lesions, which can decrease the number of hysterectomies when precise. A total of 129 patients were enrolled in this study by collecting data on age, clinical presentation, type of surgery, and pathological diagnosis from hospital data. H&E-stained slides for each case were reviewed by two pathologists with re-diagnosis when needed. Statistical analysis was done with correlations. Cases of endometrial hyperplasia (EH), whether benign or atypical (endometrial intraepithelial neoplasia, EIN), were stained with the immunohistochemical marker for PTEN. The most common histopathological diagnosis was leiomyoma (31.8%), followed by adenomyosis (23.3%) and benign EH. Total abdominal hysterectomy was the most common type of surgery, and irregular vaginal bleeding was the most common complaint, both with significant correlation with the older age group (p-value <0.001, 0.013 respectively). About 58.3 % of cases of AEH showed loss or weak PTEN staining. While benign pathologies (leiomyoma, adenomyosis, and typical EH) were the most common pathological findings, hysterectomy can be avoided when possible. PTEN loss may be of diagnostic value in addition to microscopical features in AEH cases, however, it cannot be depended on alone.

Keywords: Abnormal uterine bleeding, Endometrial hyperplasia, Hysterectomy types, IHC, PTEN.

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1. Introduction

Abnormal uterine bleeding (AUB) is one of the most common public gynecological complaints that medical gynecological staff encounter [1]. It can be caused by different pathological conditions like medical treatment, hormonal imbalance, benign or malignant tumors, infection, and pregnancy [2]. Enodometrial hyperplasia is one of important causes of hysterectomy after diagnosis by curettage . to avoid subjective differentiation between benign typical endometrial hyperplasia EH and atypical (precancerous) endometrial hyperplasia AEH, number of markers had been suggested to aid in such diagnosis and to avoid hysterectomy in young patients and those who seek for fertility [3]. PTEN, a gene located on chromosome 10, is found to be altered in endometrioid carcinoma, resulting in loss of immunohistochemical staining with this marker in different percentages and intensities among researchers. Research suggested that loss of PTEN is an early alteration in the pathogenesis of endometrioid endometrial carcinoma and its precursor lesions [4].

Abnormal uterine bleeding is the most prevalent indication for total or subtotal hysterectomy in developing countries [5]. Hysterectomy in such cases is preferred for patients who don't seek pregnancy in the future and after the failure of medical treatment [6].

Surgical uterine samples include abdominal hysterectomy, laproscopic hysterectomy, vaginal hysterectomy and myomectomy (preferred for young fertile age women with uterine fibroid). Abdominal hysterectomy has become safe and is the most commonly performed by surgeons [7]. Laparoscopic hysterectomy is followed by fewer complications and faster recovery than abdominal hysterectomy [8, 9]. Choice of type of surgery depends on many factors, including: age of the patient, patient's preference, provisional clinical diagnosis depending on radiological findings and pre-operative biopsy (if present), medical staff experiences and facilities [10]. Over the world, hysterectomy is the most common gynecological surgery [11]. in recent years, there has been an increase in nonsurgical treatment of benign gynecological diseases [12, 13]. Indications for hysterectomy are mostly benign and include: gynecological cancers, fibroids, uterine prolapse, and abnormal uterine bleeding [14]. In addition to AUB, clinical gynecological presentation for women who are subjected to surgical treatment also include: chronic pelvic pain, feeling of abdominal mass or fullness, and continuous distressing vaginal discharge. histopathological examination can reach a final diagnosis for most surgical uterine samples, with a low percentage that may need ancillary studies [15]. In a previous study performed in 2022,in Al-Kut city, leiomyoma was the most common cause for hysterectomy [16].

As gynecological surgeries are increasing, our aim in this study is to review the corresponding underlying histopathology that stands behind various types of uterine surgical procedures with immunohistochemical aid using PTEN loss to make the diagnosis of both benign and atypical endometrial hyperplasia more accurate and to avoid surgical treatment in benign cases when possible.

2. Methods and Patients

This study was performed in Babylon City, Iraq, at Al Hilla Hospital for Surgery. A total of 129 cases performed in the period from 2021 to 2024 were included in this study. Data for research, including age, clinical complaint, type of surgery, and histopathological diagnosis, were collected from the archive of the histopathology lab and clinical ward for the same patients. Hematoxylin and eosin-stained sections were reviewed by two pathologists. When H&E slides were found to be insufficient, additional sections from some biopsies were taken and processed for histopathology in some cases. Modification of the diagnosis was done when needed. For cases of endometrial hyperplasia, immunohistochemistry with PTEN is used in an attempt to differentiate between typical and atypical endometrial hyperplasia, looking for PTEN loss in AEH. Paraffin-embedded tissue blocks are sectioned at 3-4 µm thickness, deparaffinized, and the labeled streptavidin-biotin technique is used. Background of stromal cells is used as control, as they should retain positive staining with the PTEN marker. PTEN staining can be detected in the nucleus or cytoplasm of cells, and both the intensity and quantity of staining were scored. Intensity score is 0 for absent stain, +1 for faint staining, and +2 for dark staining. The percentage of staining cells is regarded as negative when less than 10%, +1 (weak) when 10-50%, and +2 (positive) when more than 50%. Both loss of PTEN and weak staining for it are regarded as significant.

Ethical issue: our research was performed after gaining permission and approval from the hospital board. At the same time, preoperative consent was consistently obtained from patients in the surgery department. Furthermore, a document requesting consensus for the practical use of pathology reports and clinical data was prepared.

2.1. Data Analysis

Statistical analysis was performed using SPSS version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). The ANOVA test was used to relate means among three or more sets. Pearson's chi-squared test and Fisher-Freeman-Halton's Exact test were used to discover the relationship between categorical variables. A P value ≤ 0.05 was considered significant.

3. Results

Table 1: Distribution of patients with hysterectomy, including the age of patients and clinical presentation. The mean age of patients was (47.50 ± 9.45) years, with older participants being 74.0 years and younger participants being 25.0 years. Less than half of the patients (N=59, 45.7%) presented in the age group (40-50 years). Regarding clinical presentation, in excess of half of patients (N=67, 51.9%) suffered from irregular vaginal bleeding.

Table 1.
Distribution of cases with hysterectomy in relation to age of patients and clinical presentation (N=129).

Study variables	Number	%
Age (Years)		
< 30 Y	3	2.3%
30-40 Y	17	13.2%
40-50 Y	59	45.7%
50-60 Y	33	25.6%
≥ 60 Y	17	13.2%
Total	129	100%
Clinical presentation		
Irregular vaginal bleeding	70	54.2%
Chronic pelvic pain	52	40.3%
Abdominal mass	7	5.4%
Total	129	100%

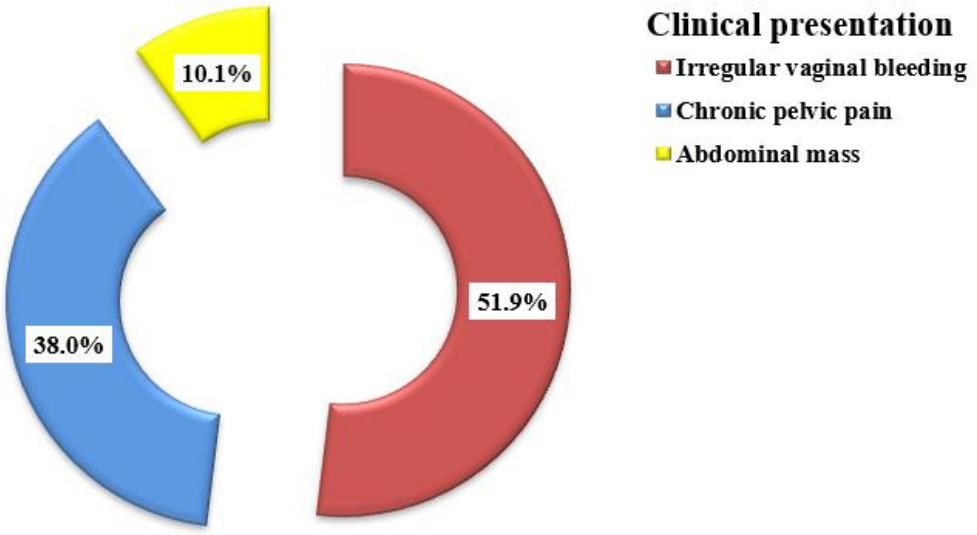

Figure 1.
Distribution of cases with hysterectomy in relation to clinical presentation (N=129).

Table 2: Distribution of patients with hysterectomy according to pathological diagnosis including (Leiomyoma, Endometrial carcinoma, Adenomyosis, Endometrial polyp, Endometrial hyperplasia, Inflammatory changes, and No remarkable pathology). The most common pathological diagnosis was Leiomyoma, which represents less than one third of patients (N=41, 31.8%), followed by endometrial hyperplasia and adenomyosis.

Table-2.
Arrangement of cases with hysterectomy in relation to pathological diagnosis (N=129).

Pathological diagnosis	Number	%
Leiomyoma	41	31.8%
Endometrial carcinoma	3	2.3%
Adenomyosis	30	23.3%
Endometrial polyp	16	12.4%
Endometrial hyperplasia (EH) Typical EH Atypical EH	34 22 12	17.0% 9.3%
Inflammatory changes	3	2.3%
No remarkable pathology	2	1.5%
Total	129	100.0%

On immunohistochemical examination for PTEN loss, it was found that 7 cases (58.3%) of endometrial hyperplasia, that was diagnosed microscopically as atypical, showed either loss of PTEN in their endometrial crowded glands or faint stain, while only 22.7% of benign EH showed weak stain (Figure 2).

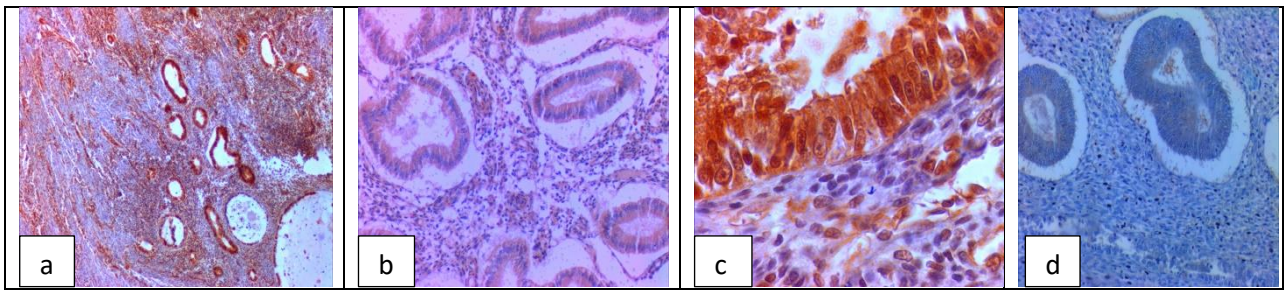

Figure 2.
A- normal proliferative endometrium tissue is used as control, it show positive strong stain in both gland and stroma.b-endometrial hyperplasia without atypia show for PTEN.c-strong nuclear and cytoplasmic stain in another case of benign endometrial hyperplasia.d-loss of PTEN stain in atypical endometrial hyperplasia.

Table 3: Distribution of cases with hysterectomy in relation to the type of surgery, including Total Abdominal Hysterectomy, Subtotal Hysterectomy, and Myomectomy. More than three-quarters of patients were treated by Total Abdominal Hysterectomy (N=102, 79.1%).

Table 3.
Distribution of cases with hysterectomy in relation to type of surgery (N=129).

Type of surgery	Number	%
Total abdominal hysterectomy	102	79.1%
Subtotal hysterectomy	23	17.8%
Myomectomy	4	3.1%
Total	129	100.0%

Table 4: The relationship among study variables comprising (age of patient, clinical presentation, and pathological diagnosis) and type of surgery, including (Total Abdominal Hysterectomy, Subtotal Hysterectomy, and Myomectomy) among study patients. There were significant mean differences in the age of patients according to the type of surgery. Patients with Total Abdominal Hysterectomy were older than other patients, with a mean age of (49.76 ± 8.75) years, while those with Subtotal Hysterectomy had a mean age of (39.35 ± 6.23) years, and patients with Myomectomy had a mean age of (36.50 ± 10.02) years.

Table 4.
The relationship among study variables comprising (age of patient, clinical presentation and pathological diagnosis) and type of surgery among study patients (N=129).

Study variables	Type of surgery			Total (N=129)	P-value
	TAH (N=102)	STH (N=23)	Myomectomy (N=4)		
Age (years)	49.76 ± 8.75	39.35 ± 6.23	36.50 ± 10.02	47.50 ± 9.45	**<0.001***
Pathological diagnosis					
Leiomyoma	31 (30.0)	7 (31.8)	3 (75.0)	41 (31.8)	0.708
Endometrial carcinoma	3 (2.9)	0 (0.0)	0 (0.0)	3 (2.3)	
Adenomyosis	23 (22.3)	6 (27.2)	1 (25.0)	30 (23.3)	
Endometrial polyp	15 (14.5)	1 (4.5)	0 (0.0)	16 (12.4)	
Endometrial hyperplasia	29 (28.1)	5 (22.7)	0 (0.0)	34 (26.3)	
No remarkable pathology or inflammatory	2 (1.9)	3 (13.6)	0 (0.0)	5 (3.8)	
Total	103 (100.0)	22 (100.0)	4 (100.0)	129 (100.0)	

Table 5: The link among study variables counting (age of patient and pathological diagnosis) and clinical presentation including (Irregular vaginal bleeding, Chronic pelvic pain and Abdominal mass) among study patients. There was significant mean differences in the age of patients according to clinical presentation. Patients with Irregular vaginal bleeding were older than other patients with mean equal (49.60 ± 10.61) years, while those with Chronic pelvic pain had mean age equal (44.45 ± 6.08) years, patients with Abdominal mass had mean age equal (48.15 ± 11.06) years. There was a significant association between clinical presentation and pathological diagnosis. More than one third of patients with irregular vaginal bleeding (38.5%) diagnosed with endometrial hyperplasia and (N=16, 22.9%) diagnosed as Endometrial polyp, less than half of patients with chronic pelvic pain (N=24, 46.0%) diagnosed as Adenomyosis. all patients with Abdominal mass (100%) diagnosed as Leiomyoma.

Table 5.
The link among study variables, counting (age of patient and pathological diagnosis) and clinical presentation among study patients (N=129).

Study variables	Clinical presentation			Total (N=129)	P-value
	Irregular vaginal bleeding (N=70)	Chronic pelvic pain (N=52)	Abdominal mass (N=7)		
Age (years)	49.60 ± 10.61	44.45 ± 6.08	48.15 ± 11.06	47.50 ± 9.45	0.013*
Pathological diagnosis					
Leiomyoma	17 (24.2)	17 (32.7)	7 (100.0)	41 (31.8)	
Endometrial carcinoma	3 (4.3)	0 (0.0)	0 (0.0)	3 (2.3)	
Adenomyosis	6 (8.5)	24 (46.0)	0 (0.0)	30 (23.3)	
Endometrial polyp	16 (22.8)	0 (0.0)	0 (0.0)	16 (12.4)	<0.001*
Endometrial hyperplasia	27 (38.5)	7 (13.4)	0 (0.0)	34 (26.3)	
No remarkable pathology or inflammatory	1 (1.4)	4 (7.6)	0 (0.0)	5 (3.8)	
Total	70 (100.0)	52 (100.0)	7 (100.0)	129 (100.0)	

Table 6 the mean differences in the age of patients (years) according to pathological diagnosis, including Leiomyoma, Endometrial carcinoma, Adenomyosis, Endometrial polyp, Endometrial hyperplasia, inflammatory changes, and no remarkable pathology, among study patients were analyzed. There were significant mean differences in the age of patients according to pathological diagnosis. Patients with Endometrial carcinoma were older than other patients, with a mean age of 57.57 ± 14.47 years.

Table 6.
The mean variances of age of patient (years) in relation to pathological diagnosis among cases of study (N=129)

Study variable	Pathological diagnosis	Number	Mean ± SD	P-value
Age (years)	Leiomyoma	41	44.90 ± 7.21	**0.004***
	Endometrial carcinoma	3	57.57 ± 14.47	
	Adenomyosis	30	47.10 ± 8.67	
	Endometrial polyp	16	52.69 ± 8.93	
	Endometrial hyperplasia	34	46.05 ± 10.45	
	No remarkable pathology or inflammatory	5	47.07 ± 9.15	

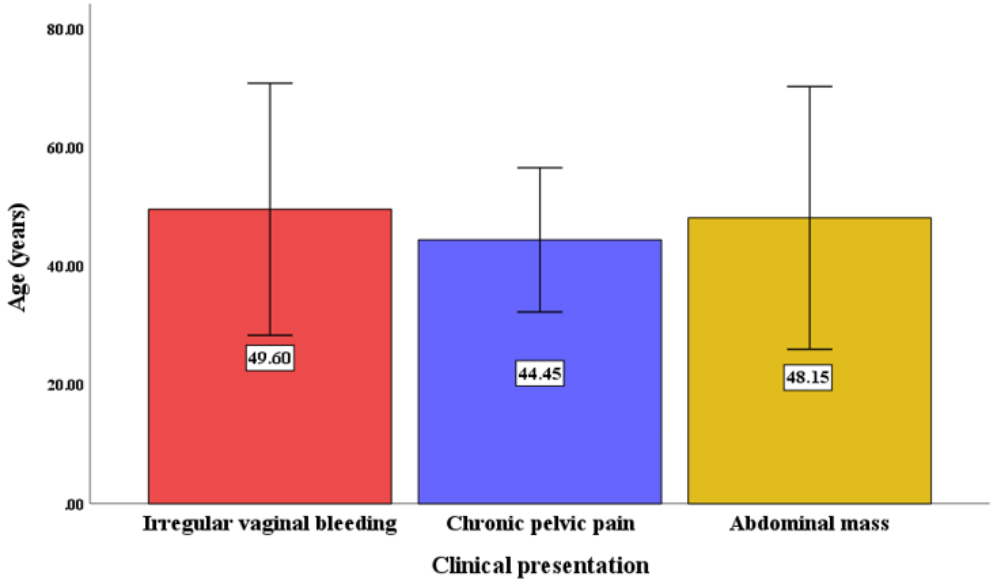

Figure 3.
The mean variances of patients ages in relation to clinical presentation among study cases (N=129).

4. Discussion

This study aimed to analyze types of gynecological pathologies present in uterine surgical biopsies with a focus on discrimination between typical and atypical EH, a difficult task since it depends just on microscopic features which might be subjective to certain limits or queries for a biggener pathologist. Immunohistochemical expression of PTEN was used for this purpose. The research also highlighted the most likely age groups and clinical complaints that undergo uterine surgery to treat such conditions. The mean age of patients who had uterine surgeries in this study was (47.50 ± 9.45) years. We found a significant correlation between age and type of surgery, as TAH was done mostly in the older age group (p-value < 0.001),

and this may be related to the desire of the patient and surgeon to overcome their fear of future gynecological disease. this is similar to the results in the study of Gifty et al. [17], which revealed the highest hysterectomy rates in women aged from 45 to 49 years for total and subtotal hysterectomy in Germany, and highest at 65 to 69 years for radical hysterectomy Gifty et al. [17]. In a study by Marwa et al. [16], most of the patients in the TAH group were between 41-50 years old. Kantarci et al. [10] found that TAH was 60% among hysterectomy methods in Turkey, and leiomyoma was the most common pathological diagnosis in those patients. Laparoscopic hysterectomy was absent in our study center, this may be related to the lack of experience of staff with such surgery, and most likely due to that more complications can be encountered with such surgery than with TAH. Furthermore, preceding studies have stated that main complications are more prevalent with laparoscopic hysterectomy than other types [18] and in study of Kantarci, et al. [10] laparoscopic hysterectomies reported (2.9 %) bladder and (2%) ureteral damages while, the incidence of lower urinary tract injury was only 1.1% (n = 24) in the TAH patients[10]

The most prevalent histopathological finding in patients seeking surgical treatment for gynecological problems was leiomyoma (31.8%), followed by adenomyosis (23.3%) and endometrial benign hyperplasia (17.0%). The most common clinical complaint for our patients was irregular vaginal bleeding, with a significant correlation with age (p-value 0.013) and the highest prevalence in the older age group. This finding was in agreement with

.Marwa et al. [16], Rahiem and Salman [6], and Anuradha et al. [2], and it was in agreement with other studies [19, 20]

In the article review of Allison et al. [21], they found that the use of PTEN immunohistochemistry was powerful in differentiating between benign and precancerous endometrial hyperplasia, and they recommended its use together with other markers like BAX. In this study, we found that PTEN use is also of good value for this purpose, since a good number of cases of hysterectomy are done after diagnosis of EH on curettage specimens, with fear of progression to cancer. However, the use of PTEN should be aided by microscopical features and other markers. In the study of Sarmadi et al. [22], they depended on the intensity of staining of PTEN, which was weak in AEH and endometrioid carcinoma in comparison with benign proliferative or secretory endometrium, with a significant percentage of 75% of staining for AEH and 48% for endometrioid carcinoma. Kapucuoglu et al. [4] found complete loss of PTEN in 20% of atypical complex samples. In Mutter et al. [23] study, most precancer cases show loss of PTEN in groups of glands among other positive glands.

There was a significant association between clinical presentation and pathological diagnosis. All patients with an Abdominal mass were diagnosed as Leiomyoma (p-value <0.001). significant association between age and pathological diagnosis (p-value 0.004), with endometrial polyp being the most common benign finding in the older age group (52.69 ± 8.93) after exclusion of two malignant cases. This association was identical to other studies[1]. A study performed by Saera and Yasmeen [24] revealed that endometrial hyperplasia was the most common pathology; this is different from our study (endometrial hyperplasia represents 26.3%) and other studies mentioned above. No remarkable pathology group in our study represented cases with physiological changes like proliferative phase, secretory phase, or atrophic changes in the elderly, which constituted 1.5%, while proliferative endometrial changes constituted 36.36% of specimens in a study conducted by Sawsan TS in Diyala city [25]. This may reflect a more rapid decision for surgery in their region.

5. Conclusion and Recommendation

While benign pathologies (leiomyoma, adenomyosis, and benign endometrial hyperplasia) were the most common pathological findings, more conservative therapeutic modalities can be tried to decrease the performance of TAH, which is the most common type of gynecological surgery. The PTEN marker (whether weak or loss of stain in AEH) can aid significantly in the diagnosis of AEH, along with microscopical features.

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