PAX2 Expression in Endometrial Intraepithelial Neoplasia

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Abstract

Endometrial intraepithelial neoplasia (EIN) is a clonal growth of glands of the endometrium, regarded as a precancerous condition because of its highcorrelation with endometrioid adenocarcinoma of the endometrium of uterus.

Objective: To evaluate the expression of PAX2 in EIN and to correlate the expression with clinical parameters.

Materials and Methods: - This is a retrospective study for **(57)**totalcasesof D&C and endometrial pipple biopsies, **(21)** cases where diagnosed as hormonal imbalance, **(26)** cases where diagnosed as EIN and **(10)** cases where diagnosed as endometrioid endometrial adenocarcinoma.

Results: -Total number of cases that showed completely loss of PAX2 expression in all this study groups 25/57 and cases that showed partial loss 10/57. In hormonal imbalance group: all the cases showed normal expression of PAX2. In EIN: (65.4%) of cases were with complete loss of PAX2, (30.4%) with partial loss. In endometrial endometrioid cancer group:80% of cases with complete PAX2 expression, while partial loss or decrease PAX2 expression did occur in only (20%) ofcases.The association between study groups and PAX2 expression:decrease and complete loss of PAX2 staining were significantly (P= 0.001) occurred in cases of EIN and endometrial endometroid adenocarcinoma, while there was insignificant association (P=0.635) between study groups (EIN and endometrial endometroid adenocarcinoma), and results of PAX2 expression.

Conclusion: -Loss of PAX2 immunomarkerexpressionhappens early and during of EIN using the WHO diagnostic categories.

Keyword*: Endometroid carcinoma, PAX2,Endometrial intraepithelial neoplasia*

Introduction

Endometrial carcinoma is the 6th most commonly happeningcarcinoma in females and the 15th most commonly happening carcinoma worldwide.(1)

Iraq cancer registry results in 2015 reveal this cancer was not included within the ten commonest cancer in Iraq. (2)

Endometroid carcinoma account about 75-80% from endometrial cancer. (3)Endometrial intraepithelial neoplasia (EIN) is a clonal growth of the glands of the endometrium, considered as a precancerousstatebecause of itshigh correlation with coincident and/or succeeding adenocarcinoma of endometrioid type of endometrium. EIN have a 45-fold of increasinghazardof a future (after 1 year) adenocarcinoma diagnosis.(4)

EIN diagnosisneedsaccurate application of histological criteria and it is an important element of a

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definite diagnosis. [5]

PAX2 is a part of a paired box gene familyshowedin the embryonic development and organogenesis. [6]

Embryonic paired-box 2 (PAX2) containing gene expression is needed for amelioration of the kidneys and ureters, the uterus and oviducts in females and the vas deferens and epididymis in males. [7]

In proliferating and regeneration of epithelial cells of endometrium, PAX2 behaves as a tumor suppressor. [8]

Materials and Method

This is a retrospective study of Formalin fixed, paraffin embedded tissue blocks were gathered from archived materials in medical city complex /teaching laboratories institute in Baghdad (covering the period from January 2018 to May 2019). The paraffin blocks represent **(57)** cases of D&C and endometrial pipple biopsies tissue of the uterus, **(21)** cases where diagnosed as hormonal imbalance, **(26)** cases where diagnosed as EIN and **(10)** cases where diagnosed as (well to moderate differentiated) endometrioid endometrial adenocarcinoma (ECC).

All the cases were presented by abnormal uterine bleeding.

Two sections of 5µm thickness were taken from each block, the first was stained with H&E for histological revision, the other section was stained by PAX2 marker.

Exclusion criteria for EIN: -

· Insufficient material.

· EIN association with endometritis.

· EIN concurrent with adenocarcinoma.

Exclusion criteria of hormonal imbalance: -

· Co-existing pathological endometrial condition (polyps, endometritis...).

· Current history of sex hormone use.

· Prior history of tamoxifen use.

Exclusion criteria of adenocarcinoma: -

· Poorly differentiated endometrioid endometrial adenocarcinoma.

· Other types of endometrial carcinoma.

Immunohistochemical expression of PAX2 in normal endometrial glands should be intense brown nuclear stainingand, in this study, PAX2 expression analysis are as below: [9]

1-normal (preserved of nuclear expression of PAX2as the same intensity of the normaladjacent endometrial glands).

2-changed [complete loss, obviously decreased, or increased staining as compare with background glands].

This analysis is alternative tothe numerical quantitative scoring system because the numerical scoring systems for PAX2 can be difficult as they cannotable to consider the geographic nature of a clonal premalignant glands when made a comparison with the background glands and the assessment of PAX2 marker is strongly dependent on the distribution and intensity of the nuclear staining in the atypical glands compared with the normal adjacent endometrialglands. [9]

Results

A total of **(57)** cases were the subjects of this study.

They were divided into three groups:

Hormonal imbalance cases were 21/57, endometrial intraepithelial neoplasia (EIN)cases were 26/57 and endometrioid endometrial adenocarcinoma (EEC) cases were 10/57.

Patient´s age ranges from 25-80 years with a mean of 52.05. The most common presentation was metrorrhagia in hormonal imbalance group of patients. While post-menopausal bleeding was the most common presentation in Endometrial intraepithelial neoplasia and Endometrial endometrioid adenocarcinoma groups of patients. The total number of dilation and curettage (D&C) were 45/57 and total number of endometrial biopsies were 12/57.

Both groupsEIN and endometrioid carcinoma cases had a significant higherendometrial thickness compared with hormonal imbalance (18.65 mm and 21.9 mm vs 8.01 mm).There was significant p value (0.001) between (endometrial intraepithelial neoplasia, endometrial endometroid adenocarcinoma) and hormonal imbalance groups.Total number of cases that showed completely loss of PAX2 in all study groups 25/57 and cases that showed partial loss 10/57.

In hormonal imbalance group; all the cases (100%) showed normal nuclear expression of PAX2 marker, as shown in figure 2(a).

In EIN group; 17/26 cases (65.4%) were with complete loss of PAX2, 8/26 (30.4%) with partial loss, and one case (3.8%) was with normal expression, as shown in figure 2(b).

In endometroid carcinoma group; the number of cases with complete PAX2 loss was 8/10 (80%), while partial loss or decrease PAX2 expression did occur in only 2/10 cases (20%), as shown in figure 2(c).

The association between study groups and PAX2 staining: decrease and complete loss of PAX2 expression were significantly (P= 0.001) occurred in cases of endometrial intraepithelial neoplasia and endometrial endometroid adenocarcinoma, while there was insignificant association (P= 0.635) between study groups (endometrial intraepithelial neoplasia and endometrial endometroid adenocarcinoma), and results of PAX2 expression.

Table 1: Comparison in PAX2 expression by endometrial thickness

PAX2 Expression	Endometrium Thickness/mm Mean ± SD	P - Value
Normal	8.18 ± 1.91	0.001
Decrease	18.20 ± 6.44	
Loss	20.24 ± 5.1	

Table 2: Comparison of PAX2 expression in EEC & hormonal imbalance

PAX2 Expression	Study Groups		Total (%) n= 31	P- Value
	EEC (%) n= 10	Hormonal imbalance (%) n= 21		
Normal	0 (0)	21 (100.0)	21 (67.7)	0.001
Decrease	2 (100.0)	0 (0)	2 (6.5)	
Loss	8 (100.0)	0 (0)	8 (13.3)	

Table 3: Comparison between PAX2 expression in EIN & hormonal imbalance

PAX2 Expression	Study Groups		Total (%) n= 47	P- Value
	EIN (%) n= 26	Hormonal imbalance (%) n= 21		
Normal	1 (4.5)	21 (95.5)	22 (46.8)	0.001
Decrease	8 (100.0)	0 (0)	8 (17.0)	
Loss	17 (100.0)	0 (0)	17 (36.2)	

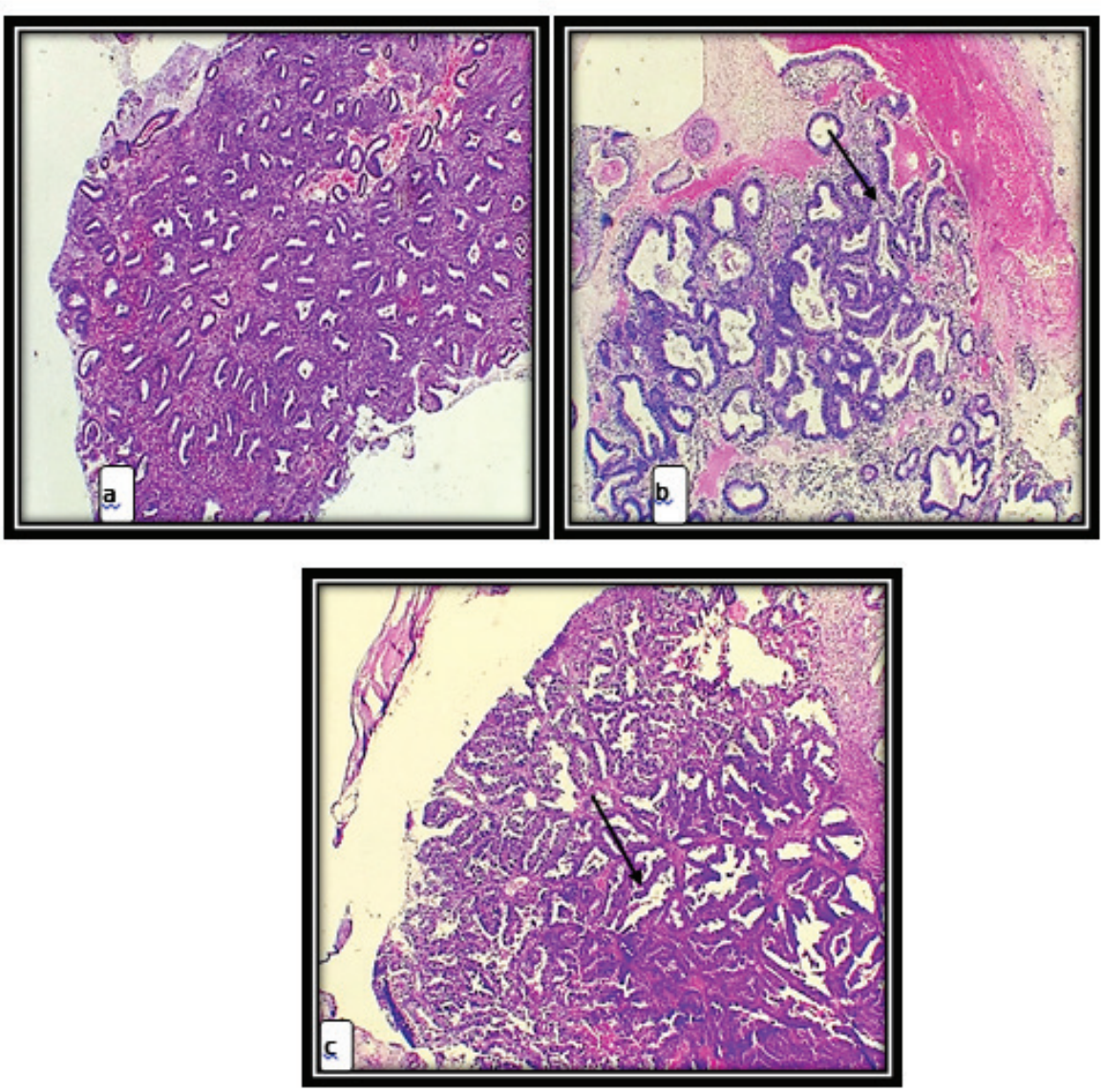

Fig.1(a) D&C from a patient diagnosed with hormonal imbalance showing normal endometrial glands separated by stoma (H&E x10).(b) D&C from a patient with EIN showing irregular crowded glands with no or little intervening stroma(arrow) (H&E X10). (c) D&C from a patient diagnosed as EEC showing complex glandular architecture(arrow) (H&E x10)

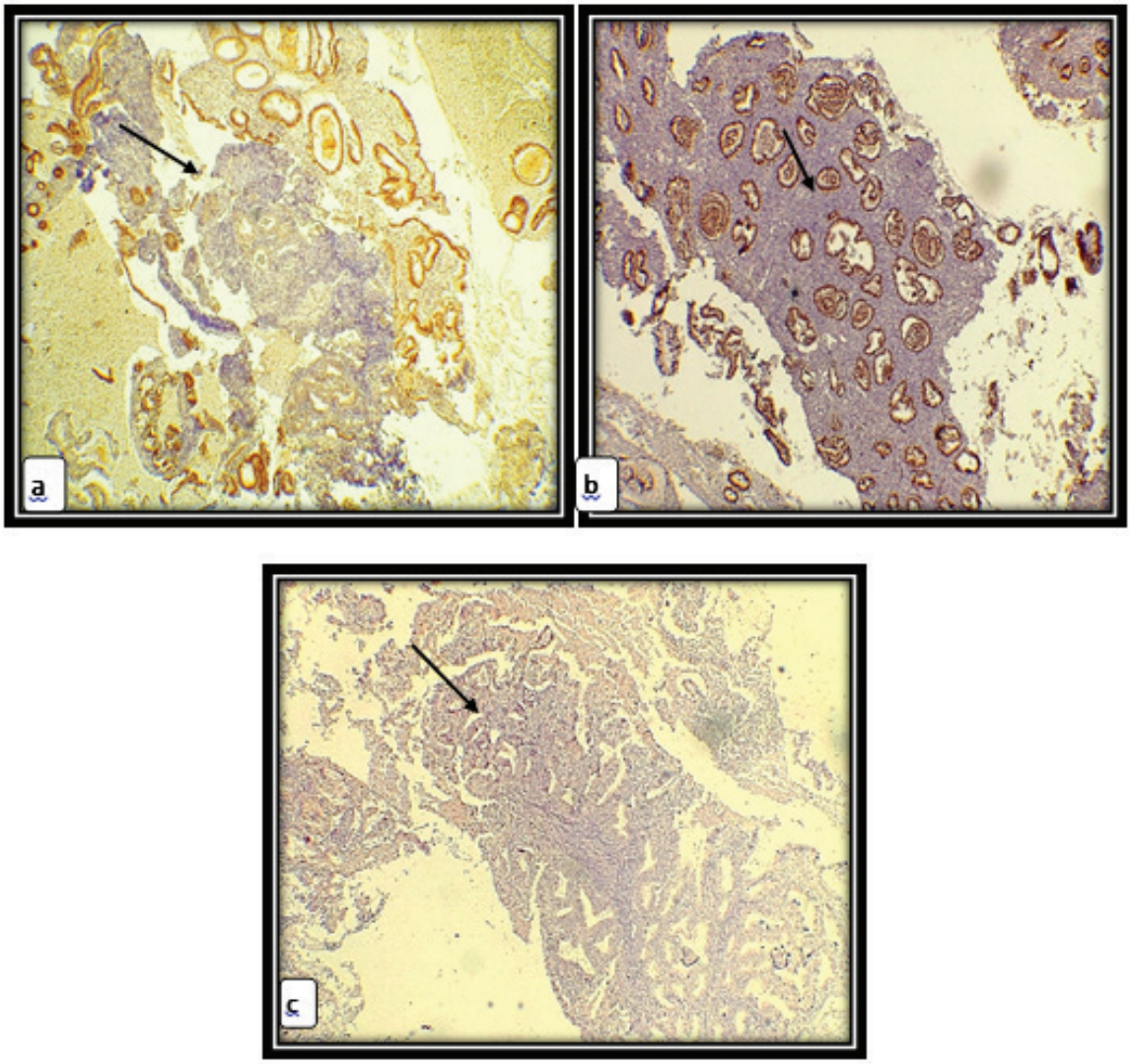

Figure 2 (a):D&C from a patient with hormonal imbalance showing strong and uniform nuclear PAX2 expression in endometrial glands (arrow) (IHC x10),(b) D&C from a patient diagnosed with EIN showing loss of PAX2 expression in the glands at the center(arrow) as compared with background glands at the periphery that showing normal nuclear expression of PAX2 marker (IHC x10),(c) D&C from a patient diagnosed with EEC showing completely loss of PAX2 nuclear expression in malignant glands (arrow) (IHC x10)

Discussion

The mean age of women in this study was ranging from 25 to 80 years with a mean of 52.05 and standard deviation of ± 11.75 years.

In hormonal imbalance group (A) :13 (61.9%) of women and in EIN group(B) 12 (46.2%) aged between 25-45 years, while 50% of those in EEC group (C) aged > 45 years.

In this study endometrial thickness measurements of the cases were obtained from request papers (results of ultrasonographic examination).

The mean thickness of hormonal imbalance, EIN and EEC were 8.01 ± 1.76, 18.65 ± 4.73, 21.50 ± 7.01respectively. Both EIN and adenocarcinoma cases had a significant higher endometrialthickness compared with control group(P=**0.001**).

No significant difference was found in endometrial thickness between EIN and adenocarcinoma cases (18.65mm versus 21.50mm, P= 0.091)

A study done by Singhet al. (2019)found that no abnormal endometrial abnormality was founded when ET was below <5 mm and hyperplasia with atypia was detected when ET was ≥11–16 mm.[10]

Getpook et al. (2006) founded that (Endometrial thickness ≤8 mm is less likely to have malignancy in premenopausal uterine bleeding...)[11]

So, in comparing these studies with the current study, US is a useful, non-invasive and not expensive screening tool for precancerous and cancer cases in gynecological patients with abnormal uterine bleeding.

Till now histological examination is the stone corner in differentiation between benign and atypical hyperplastic glands. However; there are some pitfalls in this method likepoor inter- and intra-observer reproducibility, unobviouscriteria for diagnosis or small amounts of tissue., etc.,which may cause diagnostic confusion.[12]

A number ofimmunomarkers have been suggested to make more reliability of the differential diagnosis.[13]

The immunohistochemical evaluation of (PAX2) has been recommended by the European Society of Gynecological Oncology (ESGO) 2018 roles (according to 2016 European Society for Medical Oncology (ESMO) Consensus Meeting),to differentiate betweenEINand benign mimicker.[14]

The EIN criteria for diagnosis have been adjusted by WHO in its newest 2014 announcement as the new classification strategy for premalignantendometrial lesions.[15]However, pathologists requiremore time to acquire skillto apply the new system and general use will be Kind ofretard,because of the wide acceptance of the 1994 WHO system over the last years. The most cases of EINs diagnoses arebasedon just H&E staining.[16]

Application of the EIN diagnostic criteria can also be some kind be difficult in the setting of doubtful or scanty non-lesional background endometrial glands, very fragmented tissues, or in case of a secretory backgroundglands. Soimmunomarker may be important for diagnosis in somecases to assist in detection of premalignant lesions whichhavea high risk of progression to cancer. [9]

Nerveless,the classification system is used in assessment of premalignant lesions of the endometrial glands, PAX2 is a favorableimmunomarker marker in this situation. [9]

The changes of PAX2staining in endometrial carcinogenesis are not clear yet, with usual suggestion that PAX2 expression decreases or loses in endometrial endometrioid carcinoma andEINexcept few studies have an oppositeopinion. [13]

In this study; PAX2 expression decreased progressively from hormonal imbalance to EIN to EEC were observed.There were no significant differences in this study (p=0.056) in women´s age regarding PAX2 expression.

The results showed **Normal** expression of PAX2 inhormonal imbalance group but there were **lost** or **decrease** of expression in EIN group and **completely lost** in endometrioid endometrial adenocarcinoma.

In EIN group (complete loss of PAX2 was in a percent of 65.40% and partial loss in a percent of 30.80%) and in endometrioid carcinoma (complete loss 80%, decrease in 20%). There were statistically remarkable differences in PAX2 loss in EIN and EEC when compared separately with hormonal imbalance (P<0.001) and insignificant differences in PAX2 loss in EIN as compared with EEC (P>0.635).

These findingsare compatible with many separated studies: -A study done by **Allison** et al (2012). This study evaluated PAX2 expression in 15 cases of endometrioid endometrialcancer, 54 cases of EIN, and 28 of normal proliferative and secretary endometrium. In their study, the PAX2 staining had a progressive loss through the spectrum from endometrial hyperplasia to endometrialcarcinoma. The percentage of cases with total loss increased with increasing severity of hyperplasia;(0% complete loss, 17.9% partial loss in normal proliferative and secretory endometrium); (74.1% complete loss, 22.2% partial loss of EIN), and (73.3%

complete loss, 20% partial loss in endometrioid cancers). There were statistically significant differencesin PAX2 loss in EINand endometrial cancer when comparedin the other hand with normal proliferative samples (P<0.001). (16)

Other study done by **Joiner et al.** (2015)that stated **(**Most common sample of change was total loss of PAX2 nuclear staining (86.3%) then decreased staining (11.3%) in the EIN as compared with the normal background staining). (9)

A study done by **Monte et al (**2010) noticed that the most of EIN cases (71%) and carcinoma cases (77%) demonstrate absolute loss of PAX2staining). (8)

Other study done by**Rewcastle et al** (2018)showed thata progressive decrease in PAX2 expression from proliferative endometrium to EIN to EEC were observed. (17)

A study done by **Trabzonlu et al**. (2019)showed that 73.3%of EIN cases had decreased PAX2 stainingas compare tonormalendometrial glands. (18)

Only one study found todisagreed with this study done by **Kahraman et al** (2012) showed that PAX2 expression was detected in all of the endometrial samples: -80.8% in proliferative endometrium, 92.7% and 99.2% in atypical hyperplasia and endometrial adenocarcinoma respectively). (19)

These findings might show thatPAX2gene has a dualjob (tumor suppressor and oncogene) in carcinogenesis of endometrium, also suggested in carcinogenesis of ovary.(20,21)

About increasing of PAX2 expression, moreresearches are required to know its significance and its probableadvantages in differentiation between benign and premalignantendometrial hyperplasia.

In this study when comparing PAX2 expression between EIN ,ECC and hormonal imbalance cases ,there was a significant statistical correlation with a p value of (0.001), the result was comparable to many other studies showed significant loss of PAX2 in EIN and ECC as comparison with benign glandular epithelium so PAX2 is a consonant and powerfulimmunostain for a nuclear transcription factor expressed in the normal endometrial glandular epithelium and lostin the EIN lesionand it is especially helpfulin EINlesion presenting againsta secretory backgroundglands so PAX2 marker loss demarcate the premalignant glands .(22)

In this study there was a significant statistical correlation between PAX2 expression and mean endometrial thickness with a p value of 0.001.

These may be due to the fact that increase endometrial thickness is associated with progression of endometrial pathology from benign endometrium to EIN to ECC and increasing of PAX2 gene mutation.

No such relation was found in other studies to compare with.

Conclusion

Loss of staining of PAX2 immunomarkerhappenes early and in the spectrum of EIN using the WHO diagnostic groups.

PAX2 loss appears to be a goodimmune-marker for EIN and Endometrioid carcinoma and is seldomly completely lost in normal glandular epithelium of endometrium.Therefore, it may be helpfulin some caseswhen the neoplastic nature of a given case is query.

Recommendations: -

1.Further prospective studies with a sample of larger size and clinical follow-up of patients to confirm the diagnostic significance of PAX2 expression in EIN and endometrial adenocarcinoma in hysterectomy samples.

2.Molecular studies (as Polymerase chain reaction "PCR", northen blot analysis) are recommended to study the PAX2 gene abnormalities in patients with EIN and EEC.

3.Immunohistochemical studies of other markers useful in detecting EIN (PTEN ,BCL-2, P53,KRAS) and to correlate them with PAX2 expression in EIN and EEC.

No Conflicts of Interest

Source of Funding: Self

Ethical Clearance: was taken from the scientific committee of the Iraqi Ministry of health.

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