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P16 expression as a diagnostic parameter of endometrial carcinoma and endometrial hyperplasia

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Abstract
Background: Endometrial cancer is malignant epithelial neoplasm originating from endometrium; endometrial cancer is the sixth most common cancer in women and the fifteenth most common cancer worldwide. The aim of study is to evaluate the expression of P16 IHC in endometrial carcinoma; and its diagnostic role in different histopathological types of endometrial carcinoma.
Method: Retrospective study of 70 cases with collection of paraffin embedded tissue blocks and clinical data of patients with endometrial carcinoma and endometrial hyperplasia. H&E staining of slides done to confirm the histopathological diagnosis by specialist pathologist and then stained immunohistochemically for P16 marker.
Results: In the current study, the average age of women was 32 to 78 years with a mean age of 53.42. More than half of the women with endometrial carcinoma (EC) (66.6%) presented in the age group between 50 and 70 years, half of the patients with atypical hyperplasia (50%) fall in the same age group as endometrial carcinoma, and the rest of the atypical hyperplasia patients and 65% of those without atypia presented in younger ages (30-50 years' age group). (63.3%) of patients with endometrial cancer had postmenopausal bleeding; 70% of patients with atypical endometrial hyperplasia and 75% of those without atypia had excessive monthly bleeding (HMB). Histopathology correlated with patient age and clinical presentation. P16 immune marker expression did not correlate with stage at diagnosis of endometrial cancer in TAH & BSO instances. All endometrial serous carcinoma and more than half of EEC had positive p16 IHC. Endometrial hyperplasia had no P16 IHC expression, although more than half of endometrial cancer cases did.
Conclusion: All endometrial serous carcinomas expressed high P16 IHC stain. More than half of endometrioid carcinomas had p16 IHC stain, with significant expression in high-grade morphological sections (FIGO 3). Most low-grade endometrioid carcinomas are P16-negative. P16 loss in all endometrial hyperplasia. P16 immunohistochemistry marker aids endometrial carcinoma diagnosis.

Keywords: P16 expression, endometrial carcinoma, endometrial hyperplasia

Introduction
The sixth most frequent cancer in women and the fifteenth most common cancer overall is endometrial cancer (EC), a malignant epithelial tumor that arises from the endometrium [1]. According to 2015 data from the Iraqi cancer registry, this cancer was not one of the top ten cancers diagnosed there [2]. While it may affect people of any age, it mainly affects older women, with 80% of the patients being postmenopausal at the time of diagnosis. Most endometrial adenocarcinomas in women under the age of 40 are of the endometrioid type, early-stage tumours that are well to moderately differentiate. On the other hand, older individuals' tumours are more likely to have higher grades and more advanced illness when they are diagnosed [3]. Endometrioid endometrial carcinoma, clear cell carcinomas, uterine serous carcinomas, undifferentiated carcinomas, and tumours with mixed epithelial-mesenchymal differentiation are only a few of the pathophysiological variants of primary endometrial carcinoma (carcinosarcomas) [4]. P16 protein, also known as multiple tumour suppressor type 1 (MTS1), is a cell cycle regulator encoded by CDKN2A gene, act as inhibitor of cyclin-dependent kinases, and the tumour suppressor p16 has continued to gain widespread importance in malignancy. It is now thought that loss of p16 is an early and frequently crucial event in tumour progression. Hence, p16 is a significant tumour suppressor gene whose frequent loss occurs early in a lot of human malignancies [5]. Immuno-histochemistry for p16ink4a is most often utilized as a surrogate maker for high-risk human papilloma virus infection in formalin fixed paraffin embedded tissues.

The lower anogenital tract is where p16 immunostaining is most often used, accepted, and explored. Moreover, p16 is often utilised to treat oropharyngeal squamous cell carcinoma in the oropharynx. Its uses have been expanded to include gynecologic malignancies unrelated to HPV [6]. The aim of study is to evaluate the expression of P16 IHC in endometrial carcinoma; and its diagnostic role in different histopathological types of endometrial carcinoma.

Method

This is a cross sectional retrospective study carried out in the Department of Pathology and Forensic Medicine, Faculty of Medicine, Babylon University. the study sample consist of formalin fixed, paraffin embedded tissue blocks were collected from archived material in Al-Hilla Teaching hospital, Al-Sadiq teaching hospital, Baghdad medical city and private Laboratories; during the period from April 2020 to November 2021. The paraffin blocks represent (70) cases including: (29) D&C samples and (41) endometrial tissue sample from hysterectomy specimen; with patients age range from 32 to78 years. Of these endometrial tissue samples; (20) cases were diagnosed as endometrial hyperplasia without atypia; (20) cases were diagnosed as atypical endometrial hyperplasia; (30) cases were diagnosed as endometrial carcinoma. All the cases were presented by abnormal uterine bleeding (HMB or PMB) which have been adopted from clinical data. Two sections of 5µm thickness were taken from each block, the first was stained with hematoxylin and eosin stain (H & E) for histopathological revision, the other section was stained immune-histo-chemically for P16 marker. SPSS 22 was used for statistical analysis, and the mean, median, and standard deviation were calculated for numerical data. Chi-square utilised for evaluated relationship between variables. A statistically significant p-value is less than or equal to 0.05.

Results

Seventy cases of endometrial hyperplasia without atypia, atypical endometrial hyperplasia and endometrial carcinoma were included in this study. Thirty (42.8%) cases had been diagnosed as endometrial carcinoma, twenty (28.6%) cases had been diagnosed as hyperplasia without atypia and twenty (28.6%) cases had been diagnosed as atypical hyperplasia, the age of patients ranged from 32 to 78 years. Forty-one (58.6%) patients undergone TAH; while the rest (41.4%) of them had D&C. Table 1 shows the association between P 16 including (positive and negative) and age group among patients with endometrial carcinoma. There was no significant association between P16 and age group of EC.

Table 1: Association between P 16 and age group of endometrial carcinomas

Age groups	P16		Total	P-Value
	Positive	Negative		
30-50 years	2 (11.8)	3 (23.1)	5 (16.7)	0.105
50-70 years	10 (58.8)	10 (76.9)	20 (66.6)	
≥ 70 years	5 (29.4)	0 (0.0)	5 (16.7)	
Total	17 (100.0)	13 (100.0)	30(100.0)	

*P value ≤ 0.05 was significant

Table 2 shows the association between P16 IHC expression; (including positive and negative) and staging of endometrial carcinoma (after exclusion of six patients with D&C) among patients with EC. There was no significant association between P16 and staging of tumor.

Table 2: Association between P 16 and staging of endometrial carcinoma (N=24)

Staging	P16		Total	P-Value
	Positive	Negative		
Stage I	3 (42.9%)	4 (57.1%)	7(29.2%)	0.31
Stage II	9 (81.8%)	2 (18.2%)	11(45.8%)	
Stage III	3 (60.0%)	2 (40.0%)	5(20.8%)	
Stage IV	1(100.0%)	0 (0.0)	1(4.2%)	
Total	16 (100.0)	8 (100.0)	24(100.0%)	

*P value ≤ 0.05 was significant.

Table 3 shows the association between type of EC including (Adenosquamous, Endometrioid, Secretory and Serous carcinoma) with Grading of EC "after exclusion of six patients with D&C". There was significant association between type of EC and grading of EC.

Table 3: Association between type of Endometrial carcinoma and grading (N=24)

Grading	Type of Endometrial carcinoma				Total	P-Value
	Adenosquamous carcinoma	Endometrioid	Secretory carcinoma	Serous carcinoma		
Grade I	0 (0.0)	8 (57.1)	0 (0.0)	0 (0.0)	8 (33.3)	< 0.001*
Grade II	2 (100.0)	4 (28.6)	1 (100.0)	0 (0.0)	7 (29.2)	
Grade III	0 (0.0)	2 (14.3)	0 (0.0)	7 (100.0)	9 (37.5)	
Total	2 (100.0)	14 (100.0)	1 (100.0)	7 (100.0)	24 (100.0)	

*P value ≤ 0.05 was significant.

Table 4 shows the association between type of EC including (Adenosquamous, Endometrioid, Secretory and Serous carcinomas) and P16 IHC expression (after exclusion of 6 cases of D&C). There was significant association between type of EC and P16 IHC expression.

Table 4: Association between type of Endometrial carcinoma and P16 (N=24)

P16	Type of Endometrial carcinoma				Total	P-Value
	Adenosquamous carcinoma	Endometrioid	Secretory carcinoma	Serous carcinoma		
Positive	0 (0.0)	5 (35.7)	1 (100.0)	7 (100.0)	13 (54.2)	0.01*
Negative	2 (100.0)	9 (64.3)	0 (0.0)	0 (0.0)	11 (45.8)	
Total	2 (100.0)	14 (100.0)	1 (100.0)	7 (100.0)	24(100.0)	

Discussion

In the current study, the average age of women was 32 to 78 years, with a mean age of 53.42. Most women with endometrial carcinoma were between 50 and 70 years old, and half of those with atypical endometrial hyperplasia were in the same age group (30-50 years age group). Most patients with endometrial cancer had postmenopausal bleeding (PMB), whereas most with atypical and non-atypical hyperplasia had significant menstrual bleeding (HMB). Histopathological diagnosis (endometrial hyperplasia without atypia, atypical, and cancer) correlated with patient age and clinical presentation (PMB and HMB). In this investigation, the clinical presentation and age groups largely matched those of Clarke *et al.* (2018) from the US and Wu *et al.* (2018) from China, who both found an association between endometrial cancer risk and postmenopausal bleeding in older women Also agreed with Japanese research by Ogane *et al.* (2018): Type II endometrial cancer is more common in older women than younger ones [7-9]. This study's clinical presentation matched Pennant, *et al.* (2016)'s UK study: Premenopausal women with abnormal uterine bleeding had a low risk of endometrial cancer or atypical hyperplasia [10]. Agreed with clinical presentation of Begum *et al.* research from India. (2019): Postmenopausal women with new or recurring bleeding symptoms should be re-evaluated as PMB increases the risk of endometrial hyperplasia or cancer [11]. Histological testing was used to distinguish premalignant endometrial hyperplasia from endometrial cancer until recently. Nevertheless, this approach has certain drawbacks, such as low inter- and intra-observer repeatability, ambiguous characteristics, tissue scarcity, etc. To improve differential diagnosis, numerous markers have been suggested. Many indicators have been suggested to improve differential diagnosis [12]. Like the P53 immuno-histochemical stain, the P16 stain may add value to existing diagnostic biomarkers and function as an immunological marker to help distinguish endometrial cancer subtypes (especially endometrial endometrioid carcinoma and endometrial serous carcinoma). p16, a CDK pathway protein, interacts with cyclins, CDKs, and their inhibitors. This protein is linked to many gynaecological malignancies, although its role in endometrial cancer is unknown [13]. P16 overexpression in tumour cells may imply functional inactivation of the pRb pathway and absence of negative feedback, causing P16 accumulation. Gene expression studies showed that endometrial serous carcinoma cells elevated P16 gene, unlike normal endometrial cells and endometrioid carcinoma cells [14]. 45.8% of endometrial cancer patients who underwent TAH &BSO had stage II disease, and there was no significant association between stage at diagnosis and P16 immune marker expression. This study's staging of EC agrees with a 2003 German study by Semczuk, *et al.* on p16 IHC expression and P16 gene alterations, which found abnormal P16 immunostaining in endometrial carcinomas and no statistically significant relationship between altered p16 nuclear expression and patient clinical stage or other factors. This research contradicted the German study by Ignatov *et al.* (2008), which demonstrated a link between p16 gene alterations, notably deletions, and metastases in endometrial cancer patients [15]. The differences between Ignatov, *et al.* (2008) and our study may be explained by the fact that the former study had incorporated the P16 gene study in conjunction with P16 immuno-histochemical testing, showing that gene mutation and especially deletions have higher risk of metastasis [15]. P16 gene mutation led to accumulation of P16 protein in the nucleus and cytoplasm (similar to pathogenesis of P53 gene mutation). In this investigation, all patients with grade III endometrial cancer had positive P16 immunostaining, whereas almost one third of grade I patients and more than half of grade II patients had positive P16 IHC. This research found a substantial association between high-grade endometrial cancer and immunohistochemistry expression of numerous immune markers, including P16 [16-17]. This research further verified the typically poor and localised expression pattern in FIGO grades 1 and 2 endometrioid carcinomas, but found greater expression in FIGO grade 3, serous endometrial carcinoma, clear cell carcinomas, and the epithelial component of carcinosarcomas [18]. In this research, all endometrial serous carcinomas and more than half of EEC "particularly high-grade EEC" expressed p16 immunohistochemistry stain. P16 immunological marker correlated with endometrial cancer histopathology. This result agreed with the US study of Espinosa *et al.* (2016), which found that immunostaining for P53 and P16 reclassified up to 90% of endometrial carcinomas as serous [19]. And concurred with the US research by Nicholson, *et al.* (2006): Serous carcinomas displayed robust and diffuse P16 expression, indicating this may be utilised to differentiate endometrioid adenocarcinoma [18]. This research also agreed with Vettero *et al.* (2007) from Ohio, USA: Immunohistochemical Overexpression of P16 and P53 in Uterine Serous Carcinoma and Ovarian High-grade Serous Cancer. All endometrial serous carcinomas and half of ovarian serous carcinomas substantially expressed P16 retrospectively [20]. In this study, all cases of endometrial hyperplasia, including those without atypia, expressed negative P16 immunohistochemical marker, while more than half (56.7%) of patients with endometrial carcinoma expressed positive P16 IHC, including all cases of serous carcinoma and more than half of endometroid carcinoma, especially grade 2 and grade 3.

Conclusion

All instances of endometrial serous carcinoma had high P16 immunohistochemical staining. More than half of uterine endometrioid carcinomas had positive expression of P16 immunohistochemical stain, with robust expression of P16 IHC stain in the sections with high-grade morphology (FIGO 3). The majority of low grade endometrial endometrioid carcinomas exhibit weak positive or deletion of P16 immunohistochemical marker expression. P16 immunohistochemical marker is lost in all forms of endometrial hyperplasia. The examination of the endometrial cancer diagnosis may benefit from the use of the P16 immunohistochemistry marker.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, *et al.* Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International Journal of Cancer.

2019 Apr 15;144(8):1941-1953.
2. Iraqi cancer board. Iraq cancer registry center ministry of health result of Iraqi cancer registry; c2015. p. 25.
3. Goldblum JR, Lamps LW, McKenney JK, Myers JL, Ackerman LV. Rosai and Ackerman's surgical pathology. Eleventh edition. Philadelphia, PA: Elsevier. Chapter 33; Uterus: corpus/endometrial carcinoma; general and clinical feature; c2018. p. 1306-1312.
4. Crum C, Nucci M, Howitt B, Granter S, Parast M. Diagnostic Gynecologic and Obstetric Pathology. Third edition. Elsevier Masson. Chapter19; Adenocarcinoma, carcinosarcoma and other epithelial tumors of endometrium: Introduction; c2019. p. 1525-1526.
5. Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor gene p16. International journal of cancer. 2012 Apr 15;130(8):1715-1725.
6. Mahajan A. Practical issues in the application of p16 immunohistochemistry in diagnostic pathology. Human pathology. 2016 May 1;51:64-74.
7. Clarke MA, Long BJ, Morillo AD, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of endometrial cancer risk with postmenopausal bleeding in women: a systematic review and meta-analysis. JAMA internal medicine. 2018 Sep 1;178(9):1210-1222.
8. Wu Y, Sun W, Liu H, Zhang D. Age at Menopause and Risk of Developing Endometrial Cancer: A Meta-Analysis. BioMed Research International; c2019. 8584130.
9. Ogane N, Hori S, Yano M, Katoh T, Kamoshida S, Kato H, *et al.* Preponderance of endometrial carcinoma in elderly patients. Molecular and Clinical Oncology; c2018.
10. Pennant M, Mehta R, Moody P, Hackett G, Prentice A, Sharp S, *et al.* Premenopausal abnormal uterine bleeding and risk of endometrial cancer. BJOG: An International Journal of Obstetrics & Gynaecology. 2016;124(3):404-411.
11. Begum J, Samal R. A clinicopathological evaluation of postmenopausal bleeding and its correlation with risk factors for developing endometrial hyperplasia and cancer: A hospital-based prospective study. Journal of Mid-life Health. 2019;10(4):179.
12. McCluggage WG. My approach to the interpretation of endometrial biopsies and curettings. Journal of Clinical Pathology. 2006 Aug 1;59(8):801-12.
13. Netzer I, Kerner H, Litwin L, Lowenstein L, Amit A. Diagnostic Implications of p16 Expression in Serous Papillary Endometrial Cancer. International Journal of Gynecological Cancer. 2011;21(8):1441-1445.
14. Yemelyanova A, Ji H, Shih I, Wang T, Wu L, Ronnett B. Utility of p16 Expression for Distinction of Uterine Serous Carcinomas from Endometrial Endometrioid and Endocervical Adenocarcinomas. American Journal of Surgical Pathology. 2009;33(10):1504-1514.
15. Ignatov A, Bischoff J, Schwarzenau C, Krebs T, Kuester D, Herrmann K *et al.* P16 alterations increase the metastatic potential of endometrial carcinoma. Gynecologic Oncology. 2008;111(2):365-371.
16. Murali R, Davidson B, Fadare O, Carlson J, Crum C, Gilks C *et al.* High-grade Endometrial Carcinomas. International Journal of Gynecological Pathology. 2019;38:S40-S63.
17. Hu S, Hinson J, Matnani R, Cibull M, Karabakhtsian R. Are the uterine serous carcinomas underdiagnosed? Histomorphologic and immunohistochemical correlates and clinical follow up in high-grade endometrial carcinomas initially diagnosed as high-grade endometrioid carcinoma. Modern Pathology. 2017;31(2):358-364.
18. Reid-Nicholson M, Iyengar P, Hummer A, Linkov I, Asher M, Soslow R. Immunophenotypic diversity of endometrial adenocarcinomas: implications for differential diagnosis. Modern Pathology. 2006;19(8):1091-1100.
19. Espinosa I, D'Angelo E, Palacios J, Prat J. Mixed and Ambiguous Endometrial Carcinomas. American Journal of Surgical Pathology. 2016;40(7):972-981.
20. Chiesa-Vottero A, Malpica A, Deavers M, Broaddus R, Nuovo G, Silva E. Immunohistochemical Over-expression of p16 and p53 in Uterine Serous Carcinoma and Ovarian High-grade Serous Carcinoma. International Journal of Gynecological Pathology. 2007;26(3):328-333.

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