Differential protein expression of PTEN and PI3K mutation markers and expression of CD68 and IL-6 inflammatory markers in determining malignancy of endometrioid and clear cell ovarian carcinoma with underlying endometriosis

Nor Haslinda Abd Aziz
Universiti Kebangsaan Malaysia https://orcid.org/0000-0002-4632-7135
Noorazizah Arsad
Universiti Kebangsaan Malaysia
Nur Maya Sabrina Tizen Laim
Universiti Kebangsaan Malaysia
Reena Rahayu Md Zin
Universiti Kebangsaan Malaysia
Abdul Muzhill Hannaan Abdul Hafizz
Universiti Kebangsaan Malaysia
Mogesh Sababathy
Universiti Kebangsaan Malaysia
Mohamad Nasir Shafiee (nasirshafiee@hotmail.com)
Universiti Kebangsaan Malaysia

Research

Keywords: endometrioid adenocarcinoma, clear cell carcinoma, ovarian carcinoma, endometriosis, PTEN, PI3K, interleukin-6 (IL-6) and macrophage (CD68)

Posted Date: March 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-159192/v1

Differential protein expression of PTEN and PI3K mutation markers and expression of CD68 and IL-6 inflammatory markers in determining malignancy of endometrioid and clear cell ovarian carcinoma with underlying endometriosis

Nor Haslinda Abd Aziz[1], Noorazizah Arsad[1], Nur Maya Sabrina Tizen Laim[2], Reena Rahayu Md Zin[2], Abdul Muzhill Hannaan Abdul Hafizz[1,2], Mogesh Sababathy[1], Mohamad Nasir Shafiee[1*]

[1]Department of Obstetrics and Gynaecology, [2]Department of Pathology, Faculty of Medicine Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

*Corresponding author

E-mail: nasirshafiee@hotmail.com

Declaration of interest: None

ABSTRACT

Objective: The aim of this study is to determine the differential protein expression of Phosphatase and tensin homolog (PTEN), Phosphatidylinositol-4,5-biphosphate3-kinase, catalytic subunit alpha (PI3K), macrophage (CD68) and interleukin-6 (IL-6) in women diagnosed with endometrioid adenocarcinoma or clear cell carcinoma of the ovary underlying with and without endometriosis. **Methods:** We constructed a tissue microarray (TMA) slides from paraffin blocks of ovarian endometrioid and clear cell carcinomas, collected over a period of 9 years. There are 19 paraffin blocks which representing 19 ovarian cancer cases, which were divided into two groups; i.e ovarian cancer with endometriosis (n=10) vs ovarian cancer without endometriosis (n=9). Four markers were used; PTEN, PI3K, IL-6 and CD68. Comparisons were subsequently made in terms of clinicopathological characteristics of the ovarian malignancy, as well as any differences in expressions between the 2 groups. **Results:** Protein expression of PTEN, PI3K, IL-6 and CD68 were analyzed by immunohistochemistry. Results showed lower PTEN expression (100% vs 88.9%, p=0.47) and higher PI3K expression (80% vs 77.8%, p=1.00) in ovarian cancer with endometriosis. Higher expression of IL-6 was observed in ovarian carcinoma with endometriosis (70% vs 11.11%, p=0.35). Macrophage (CD68) in contrary was expressed much less in ovarian cancer with underlying endometriosis (66.67% vs 40%, p=0.16). **Conclusion:** Ovarian endometrioid adenocarcinoma and clear cell carcinoma with endometriosis expressed greater PTEN protein expression and PI3K overexpression. They also demonstrated higher IL-6 expressions.

KEYWORDS: endometrioid adenocarcinoma, clear cell carcinoma, ovarian carcinoma, endometriosis, PTEN, PI3K, interleukin-6 (IL-6) and macrophage (CD68)

INTRODUCTION

Endometriosis is a benign gynecological condition whereby endometrial glands or stroma are abnormally implanted outside the endometrium, commonly at the pelvic organs including uterine surface, ovary, bowels and bladder and peritoneum, as well as distant sites like the lungs and skin [1]. The incidence is reported at 5 - 15% in the reproductive age females although this could have been an underestimation as the diagnosis requires direct visualisation of the pelvis; either with or without tissue biopsy [1, 2].

Despite being a benign lesion, endometriosis has several characteristics mimicking cancerous cells, which includes epithelial ovarian cancer (EOC) with an overall risk of nearly 50% in developing ovarian cancer [3]. Endometriosis associated ovarian carcinoma (EAOC) endometriosis is associated with increased risk for specific histotypes of ovarian carcinoma, most notably endometrioid and clear cell as well as low-grade [4]. Somigliana et al. (2006) reported that, endometriotic-associated ovarian carcinoma was commonly seen in clear cell ovarian carcinoma (35% in 390 cases), followed by endometrioid ovarian carcinoma (27% in 648 cases) and much less seen in serous epithelial carcinoma (5% in 1372 cases) and mucinous carcinoma (4% in 614 cases) [5]. Clear cell ovarian carcinoma accounts for 3.7 - 12.1% of all histologic subtypes among epithelial ovarian cancers and is found in association with endometriosis, ranging in frequency from 9 – 70 % [6]. In Malaysia, Cancer Registry Report 2007-2011 stated that the total incidence of ovarian cancer was 6.1% in women, 6.6% from the overall cases were of clear cell adenocarcinoma and 7.1% were endometrioid adenocarcinoma [7].

PTEN (phosphatase and tensin homolog) and PI3K (phosphatidylinositol-4,5-biphosphate3-kinase, catalytic subunit alpha) are among frequent oncogenic mutations which occurred in EAOC [8]. PTEN loss of function or mutations are well documented in 40% of endometroid ovarian cancer, whereas Romero and Bast (2012) quoted that 20% of endometroid

and 35% of clear cell ovarian carcinoma have documented PI3K mutations [9]. Alteration of this PI3K/AKT pathway leads to tumor proliferation, growth, migration, invasion and evasion of apoptosis therefore promoting malignant transformation [10]. In addition, PTEN also plays an important role in regulation of the cell cycle and induction of cell apoptosis as well as autophagy which involves angiogenesis and transduction of various signaling pathways of cells [11]. Therefore, this pathway has already been the pathway of interest in the targeted therapy for ovarian carcinoma [12, 13]. Although PTEN and PI3K alteration were found histologically specific, its correlation with possible underlying endometriosis has never been addressed.

Endometriosis is also known to cause chronic local inflammatory reactions that activates macrophages as well as several cytokines and chemokines such as IL-6. This reaction further induces DNA damage and mutations [14]. Specifically, for epithelial ovarian cancer, Isobe et al (2015) demonstrated that out of 94 patients, increased expression of IL-6 were observed in 55% of patients with clear cell, 50% with mucinous and 45.5% with endometrioid carcinoma in comparison to patients with serous (38.2%) and other ovarian carcinoma (15.4%) [15]. Similarly, IL-6 level was found to be high in endometriosis and endometrioid endometrial adenocarcinoma compared to normal endometrium [16]. This is not surprising, as both endometrial and ovarian endometrioid carcinoma was hypothesized to evolve from similar precursor endometrial epithelial cells. Nonetheless, the association between high IL-6 level with CD68 is not clear and to date, no study has been done to assess the level of these inflammatory markers in ovarian carcinoma with endometriosis.

In view of the above, this study aims to detect alteration of molecular signatures in ovarian cancer with endometriosis patients. Specifically, we identified and analyzed the expressions of differential protein including PTEN, PI3K, as well as IL-6 and CD68 level in endometrioid and clear cell ovarian carcinoma; which are closely linked to endometriosis. Comparison is be made between patients with underlying endometriosis versus without

endometriosis. The findings will be linked with recruited patient's clinical and pathological data.

MATERIALS AND METHODS

We retrospectively examined surgical specimens of ovarian tumor who underwent exploratory laparotomy at UKM Medical Centre with histopathological confirmation of both primary clear cell carcinoma and endometrioid adenocarcinoma of the ovary. The paraffin embedded tissue blocks were collected between January 2007 until December 2015 from the archives of the pathology department. PTEN, PI3K, IL-6 and CD68 expression were examined by immunohistochemistry (IHC) method in tissue samples from 19 cases of ovarian tumor comprising of n=10 and n=9 for endometrioid and clear cell ovarian carcinoma with endometriosis and endometrioid and clear cell ovarian cancer without endometriosisrespectively.

Immunohistochemistry

Desired formalin-fixed, paraffin-embedded tissue blocks were retrieved, along with their corresponding hematoxylin and eosin (H&E) stained slides. The tissue blocks (donor block) were chosen to construct tissue microarray (TMA) blocks using a tissue microarrayer, Alphelys Minicore® 3 Tissue Arrayer (Alphelys, Plaisir France). Immunohistochemical staining for PTEN, PI3K, IL-6 and CD68 were performed in all cases using the protocol from EnVision™ FLEX Mini Kit, High pH (Dako, Glostrup,Denmark). All sections were stained with primary antibodies of PTEN (6H2.1; 1:100; Dako, Glostrup, Denmark), PI3K (EPR3951; 1:100; Abcam, Massachusetts, USA), CD68 (PG-M1; Dako, Glostrup, Denmark) and IL-6 (ab9324; 1:200; Abcam, Massachusetts, USA).

Immunohistochemical scoring was evaluated based on semi-qualitative analysis meanwhile the interpretation of immunohistochemical staining was done based on respective primary antibody. The analysis was assessed in a blinded fashion by pathologists who examined each slide independently. Cells presenting with an organized cytoplasm and nucleus are considered positive for PTEN. On the other hand, scores were assigned following the intensity of the stain ranging from 0 for negative staining, 1 for weak staining, 2 for moderate staining and 3 for strong staining. Cases staining at level 0 or 1 were considered to have undergone an inactivation of PTEN and level 2 or 3 were considered as normal expressions. The cells which are stained brown in the cytoplasm or in the membrane are considered positive for PI3K. Similar to PTEN scoring method, staining intensity for PI3K was given a score on a scale of 0 to 3 which corresponds to negative, weak, moderate and strong intensity respectively. Scores of 2 and 3 reflect PI3K overexpression. CD68 expressions were also graded on a scale of 0 to3 based on the proportion of positive mononuclear cells infiltrated in each TMA core. Cutoff of 1% (grade 0), 5% (grade 1), 25% (grade 2) and 50% (grade 3) for each positive cell per infiltrated immune cells were used. Meanwhile for IL-6, each tumor core was scored based on the intensity of staining; i.e. 0 for none, 1 for weak and 2 for strong IL-6 expressions. Each core was viewed in 3 microscopic fields for each case at ×100 magnification and individually scored. Average score for each core was used in the analysis.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences for Windows (SPSS 24.0). Mean, standard deviation (SD), median, interquartile range (IQR) and frequencies (percentage) were used to show the patient's characteristics. The mean values of continuous variables within normal distribution between the two groups were compared by Student t-test. Mann-Whitney U tests were used to compare the median values of continuous

variables with non- normal distribution. To examine the interrelationship of categorical data, a chi-squared test or Fisher's exact test was utilized with a level of significant of 0.05.

RESULTS

Demographic and clinical details

The study involved 19 subjects of different ages divided into 2 major groups that includes ovarian carcinoma with endometriosis and without endometriosis of 10 and 9 subjects respectively. The mean age of the subjects involved in this study was 49.10±10.97 years old in endometriosis group and 49.78±8.82 years old in the non-endometriosis group. The race distributions showed that the majority of the patients were Malays (60.0% vs 77.8%), followed by Chinese (30.0% vs 11.1%) and Indians (10.0% vs 11.1%) in both groups. The subjects were slightly overweight in the non–endometriosis group, although the difference is not statistically significant (BMI of 25.37±5.78 kg/m^2 vs 24.05±3.22 kg/m^2, p=0.562). Most the subjects were also yet to reach menopause at the time of diagnosis. The demographic distributions are shown in (Table 1).

Subjects in the group of ovarian carcinoma with endometriosis consisted of mainly endometrioid adenocarcinoma rather than clear cell carcinoma (90.0% vs 10.0%). All of the cases in this group were in early stages of diseases (100.0% were of stage 1 and 2) and well-differentiated tumor (50.0%). On the other hand, the distribution of cancer types showed similar pattern with predominant endometrioid adenocarcinoma subtype (77.8%) in the other group consisting of subjects of ovarian carcinoma without endometriosis. However, majority were in early stages of the disease (77.7% stage 1 and 2), while advanced stage of carcinoma was seen in 22.2% with greater proportions of moderate and poorly differentiated tumor (55.5% vs 50.0%). The mean tumor diameter from ovarian carcinoma with endometriosis was also smaller compared to ovarian carcinoma without endometriosis (14.55±6.20 cm vs 16.22±6.53

cm). Nevertheless, all of these differences were not statistically significant to suggest any association between presence of endometriosis with the cancer subtype, disease stage, tumor grade or size (Table 2).

Protein expression of PTEN, PI3K, IL-6 and CD68

TMA serial sections from 19 ovarian cancer subjects were used to assess the protein expressions of PTEN, PI3K, IL-6 and CD68, and was evaluated by immunohistochemistry. Each sample was scored based on the percentage of positive cells and the intensity of the staining. Cytoplasmic or membranous staining was seen in the cases of ovarian carcinoma with endometriosis (Figure 1). Low expression of PTEN of score 0 – 1 was recorded reflecting its inactivation. PTEN inactivation was seen in all samples of ovarian cancer with endometriosis and much lesser in ovarian cancer without endometriosis (100.0% vs 88.9%, p=0.47) (Table 3). 11.1% of ovarian cancer without endometriosis had normal PTEN expression with intensity score of 2 (Table 3). Over expression of PI3K with scores of 2 and 3 (Figure 2) was significantly higher in ovarian cancer with endometriosis rather than without endometriosis (80% vs 77.8%, p=1.00) (Table 3). IL-6 expression was higher in ovarian cancer with endometriosis (Figure 3), with percentage of 70% vs 11.1% (p=0.35) (Table 3). CD68 infiltration in the tumor cells was found to be higher in the ovarian cancer without endometriosis patients (66.67% vs 40%, p=0.16) (Table 3) contradicting to our hypothesis as seen in Figure 4.

DISCUSSION

Modern biotechnology advancement promotes further genetic and molecular characterization of EAOC. This is important in understanding the potential role of mutations and the ovarian microenvironment that may contribute to malignant transformation. In addition, recent studies have shown a growing interest and emerging towards applying genetic and molecular

identification methods in finding new targeted therapy for ovarian malignancy. Precision medicine in treating EAOC would be a useful tool to classify risk for transformation to ovarian cancer, which could serve as the basis of prevention. Although molecular studies assist in the prognostication of EAOC, immunohistochemistry remains a powerful, cheap and reliable method in detecting early malignancies [17].

Previous publications showed that mutation of PTEN, which leads to its inactivation and loss of heterozygosity at locus 10q23 is associated with endometrioid and clear cell carcinoma in both endometrial and ovarian cancers [18]. Similar findings were seen in benign endometriotic cyst, as well as in concurrent ovarian cancer with adjacent endometriosis, supporting its role in the pathogenesis of EAOC [19]. In this study, we demonstrated that PTEN inactivation was seen in all cases (100%) with background of endometriosis (10 out of 10), and in 88.9% (8 out of 9) cases of ovarian cancer without endometriosis. This number is much higher compared to another study which quoted that negative PTEN expression was demonstrated in 71.8% of EAOC [19]. The percentage score of PTEN expression was also lower in the ovarian cancer with endometriosis group, although the different is not statistically significant (0.40%, vs 0.67%, p=0.37).

PI3K mutation is highly specific as it seen in 20 - 40% of endometrioid and 20 - 40% of clear cell carcinoma of the ovaries [20]. The same signalling pathway consists on PI3K-Akt-mTOR has also been found to be activated in ovarian endometriosis and the activity is higher in ovarian endometriosis than in the normal endometrium [21]. We postulated that PI3K expressions is exaggerated in ovarian carcinoma patients with endometriosis, and the results were in favour of endometriosis group as 80% patients (8 out of 10) showed over expression of PI3K vs 77.8% (7 out of 9) in patients without endometriosis. This is relatively higher that quoted in above mentioned literatures.

Among the cytokines, IL-6 is one of the important markers found to be present in the ovarian cancer microenvironment, whereby higher serum and ascites IL-6 levels correlate to the disease extent and poor clinical outcome [22]. We report here that the IL-6 expression is higher in ovarian cancer with endometriosis patients. As inflammation plays an important role in pathogenesis of endometriosis, presence of macrophage and pro-inflammatory mediators are expected to be markedly significant as reported before [23]. These macrophages activate angiogenesis, sustain resistance to apoptosis, and stimulates proliferation as well as invasion of precursor cells in order to establish the endometriotic tissues' progression and propagation.

Our study demonstrates that the IL-6 level is higher in the ovarian cancer with endometriosis 70% (7 out of 10) hence we would expect that the macrophage (CD68) level will also be similar in trend. However, we found that the macrophage (CD68) expression is lower in ovarian cancer with endometriosis 60% (6 out of 10). This finding could have been explained by the macrophage phenotype involved in ovarian cancer with endometriosis. Macrophages can be classified into 2 main phenotypes; M1 (classically activated macrophage) which involves in inflammatory response, pathogen clearance and anti-tumour immunity and M2 (alternatively activated macrophage) which influences an anti-inflammatory response, wound healing and pro-tumorigenic properties. In advanced epithelial ovarian cancer, macrophages in the ascites are polarized to M2 macrophages by processes stimulated by IL-6, leukaemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF) [14]. Therefore, protein level expression of CD68 which is hypothesized to be higher in ovarian cancer with endometriosis may not be a true reflection of the CD68 of type M2. Future study is required to establish the differences in CD68 type M1 and CD68 type M2 levels in ovarian carcinoma, endometriosis and EAOC.

This is a preliminary study comparing the immunohistochemically expression of PTEN and PI3K markers and CD68 and IL-6 inflammatory markers in endometriosis associated

ovarian cancers tissues. However, immunohistochemical analysis could only be performed on a low number of cases available, possibly due to the fact that endometrioid and clear cell subtypes are not common and accounted for less than 10% of ovarian carcinoma [24]. The potential of PTEN, PI3K and CD68 and IL6 inflammatory markers as a prognostic marker and indicator for early ovarian carcinogenesis in endometriotic patients should be explored on a larger sample size to further understand the role of endometriosis in disease progression.

CONCLUSION

Endometrioid adenocarcinoma and clear cell ovarian cancer with underlying endometriosis demonstrate more PTEN inactivation, PI3K overexpression and higher IL-6 level compared to ovarian carcinoma without endometriosis. Our findings showed protein expression trends similar to those observed by others eventhough the number of samples were relatively smaller. Despite this, our findings are notably important considering the significant contribution of PTEN and PI3K protein which could be a potential diagnostic marker in identifying women with endometriosis at risk of developing endometrioid and clear cell ovarian carcinoma. Consequently, proper disease surveillance and stratification can be tailored for them in predicting malignant transformation of endometriosis.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose. Funding for this study was provided by the Ministry of Education, Malaysia coded FRGS/2017/SSK02/UKM/02/1. Furthermore, the study protocol was approved by the Universiti Kebangsaan Malaysia medical ethics committee and the project code given was FF-2017-294.

Address for correspondence: Professor Dr. Mohamad Nasir Shafiee, Department of Obstetrics and Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia.
E-mail: nasirshafiee@hotmail.com

Author's contribution

Nor Haslinda Abd Aziz first author responsible for the manuscript construct and result analysis of the manuscript, authors Noorazizah Arsad and Nur Maya Sabrina Tizen Laim responsible for the performing of experiment according to the requirement standard, author Reena Rahayu Md Zin is the senior pathologist Reena Rahayu Md Zin for validation of analysed data, authors Abdul Muzhill Hannaan Abdul Hafizz and Mogesh Sababathy involved in manuscript writing and table/images and corresponding author Mohamad Nasir Shafiee responsible for the development and idea of the project and also leader of the project/ fund owner.

REFERENCES

1. Dewhurst J. Dewhurst's textbook of obstetrics and gynaecology: John Wiley & Sons; 2012.
2. Krawczyk N, Banys-Paluchowski M, Schmidt D, Ulrich U, Fehm T. Endometriosis-associated malignancy. Geburtshilfe und Frauenheilkunde. 2016;76(02):176-81.
3. Pearce CL, Templeman C, Rossing MA, Lee A, Near AM, Webb PM, et al. Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case–control studies. The lancet oncology. 2012;13(4):385-94.
4. Nezhat FR, Apostol R, Nezhat C, Pejovic T. New insights in the pathophysiology of ovarian cancer and implications for screening and prevention. American journal of obstetrics and gynecology. 2015;213(3):262-7.
5. Somigliana E, Parazzini F, Stoppelli S, Giambattista E, Vercellini P. Association between endometriosis and cancer: a comprehensive review and a critical analysis of clinical and epidemiological evidence. Gynecologic oncology. 2006;101(2):331-41.
6. Orezzoli J, Russell A, Oliva E, Del Carmen M, Eichhorn J, Fuller A. Prognostic implication of endometriosis in clear cell carcinoma of the ovary. Gynecologic oncology. 2008;110(3):336-44.
7. Azizah AM, Nor Saleha I, Noor Hashimah A, Asmah Z, Mastulu W. Malaysian national cancer registry report 2007–2011. Malaysia cancer statistics, data and figure Putrajaya: National Cancer Institute, Ministry of Health. 2016.
8. Cheaib B, Auguste A, Leary A. The PI3K/Akt/mTOR pathway in ovarian cancer: therapeutic opportunities and challenges. Chinese journal of cancer. 2015;34(1):4-16.
9. Romero I, Bast Jr RC. Minireview: human ovarian cancer: biology, current management, and paths to personalizing therapy. Endocrinology. 2012;153(4):1593-602.
10. Musa F, Schneider R. Targeting the PI3K/AKT/mTOR pathway in ovarian cancer. Translational Cancer Research. 2015;4(1):97-106.

11. Wang L, Wang C, Jin S, Qu D, Ying H. Expression of NF-κB and PTEN in primary epithelial ovarian carcinoma and the correlation with chemoresistance. International journal of clinical and experimental pathology. 2015;8(9):10953.
12. Kalamanathan S, Bates V, Lord R, Green J. The mutational profile of sporadic epithelial ovarian carcinoma. Anticancer research. 2011;31(8):2661-8.
13. Vestergaard AL, Thorup K, Knudsen UB, Munk T, Rosbach H, Poulsen JB, et al. Oncogenic events associated with endometrial and ovarian cancers are rare in endometriosis. Molecular human reproduction. 2011;17(12):758-61.
14. Li S, Fu X, Wu T, Yang L, Hu C, Wu R. Role of interleukin-6 and its receptor in endometriosis. Medical science monitor: international medical journal of experimental and clinical research. 2017;23:3801.
15. Isobe A, Sawada K, Kinose Y, Ohyagi-Hara C, Nakatsuka E, Makino H, et al. Interleukin 6 receptor is an independent prognostic factor and a potential therapeutic target of ovarian cancer. PloS one. 2015;10(2):e0118080.
16. Yousefi H, Momeny M, Ghaffari SH, Parsanejad N, Poursheikhani A, Javadikooshesh S, et al. IL-6/IL-6R pathway is a therapeutic target in chemoresistant ovarian cancer. Tumori journal. 2019;105(1):84-91.
17. Kriplani D, Patel MM. Immunohistochemistry: A diagnostic aid in differentiating primary epithelial ovarian tumors and tumors metastatic to the ovary. South Asian journal of cancer. 2013;2(4):254.
18. Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, et al. Loss of heterozygosity on 10q23. 3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. Cancer research. 2000;60(24):7052-6.

19. Makker A, Goel MM, Das V, Agarwal A. PI3K-Akt-mTOR and MAPK signaling pathways in polycystic ovarian syndrome, uterine leiomyomas and endometriosis: an update. Gynecological Endocrinology. 2012;28(3):175-81.
20. Hao T, Ge A, Dong W, Han F, Song H. Clinical significances of PTEN and HER-2 protein expression in endometriosis associated ovarian carcinomas and their correlations. 2017.
21. Yamamoto S, Tsuda H, Takano M, Iwaya K, Tamai S, Matsubara O. PIK3CA mutation is an early event in the development of endometriosis-associated ovarian clear cell adenocarcinoma. The Journal of pathology. 2011;225(2):189-94.
22. Kuo K-T, Mao T-L, Jones S, Veras E, Ayhan A, Wang T-L, et al. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. The American journal of pathology. 2009;174(5):1597-601.
23. Lane D, Matte I, Rancourt C, Piché A. Prognostic significance of IL-6 and IL-8 ascites levels in ovarian cancer patients. BMC cancer. 2011;11(1):1-6.
24. Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Archiv. 2012;460(3):237-49.

Table 1. Demographics for ovarian endometrioid adenocarcinoma and clear cell ovarian carcinoma with and without endometriosis

Demographics	CA with endometriosis		CA without endometriosis		p-value
	N(%)	Mean (SD)	N (%)	Mean (SD)	
Age (years)		49.10±10.97		49.78±8.82	0.885[a]
Race					
Malay	6 (60.0)		7 (77.8)		0.777[b]
Chinese	3 (30.0)		1 (11.1)		
Indian	1 (10.0)		1 (11.1)		
BMI (kg/m2)		24.05±3.22		25.37±5.78	0.562[a]
Menopause					
Yes	4 (40.0)		3 (33.3)		1.000[b]
No	6 (60.0)		6 (66.7)		
Types of surgery					
Unilateral SO	3 (30.0)		1 (11.1)		0.433[b]
TAH+unilateral SO	6 (60.0)		8 (88.9)		
TAH+BSO	1 (10.0)		0 (0.0)		
Omentectomy					
Yes	2 (20.0)		0 (0.0)		0.474[b]
No	8 (80.0)		9 (100.0)		
LN clearance					
No	5 (50.0)		1 (11.1)		0.125[b]
Unilateral	0 (0.0)		2 (22.2)		
Bilateral	5 (50.0)		6 (66.7)		

[a]Independent t-test, [b]Fisher's Exact Test

CA – carcinoma, SO – salpingo-oophorectomy, TAH – total abdominal hysterectomy, BSO – bilateral salpingo-oophorectomy

Table 2. Clinical and pathological characteristics for endometrioid and clear cell ovarian carcinoma with and without endometriosis

Characteristics	CA with endometriosis		CA without endometriosis		p-value
	N (%)	Mean (SD)	N (%)	Mean (SD)	
Type of Cancer					
Endometrioid	9 (90.0)		7 (77.8)		0.582[a]
Clear cell	1 (10.0)		2 (22.2)		
FIGO staging					
1	9 (90.0)		4 (44.4)		
2	1 (10.0)		3 (33.3)		0.324[a]
3	0 (0.0)		0 (0.0)		
4	0 (0.0)		2 (22.2)		
Tumour grade					
Well differentiated	5 (50.0)		4 (44.4)		1.000[a]
Moderately differentiated	3 (30.0)		2 (22.2)		
Poorly differentiated	2 (20.0)		3 (33.3)		
Largest tumour diameter (cm)		14.55±6.20		16.22±6.53	0.575[b]

[a]Fisher's Exact Test, [b]Independent t-test

CA – carcinoma, FIGO – International Federation of Gynaecology and Obstetrics

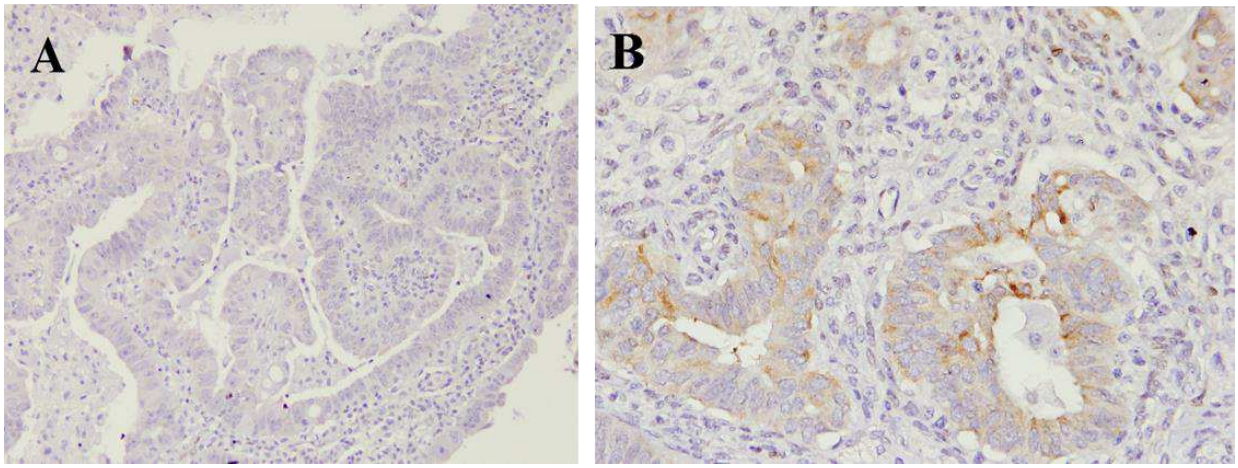

Figure 1. Representative photographs of PTEN protein in (A) ovarian carcinoma (endometrioid) with endometriosis; low expression (inactivation of PTEN) (B) ovarian carcinoma (endometrioid) without endometriosis; normal expression of PTEN. All images are magnified 40x.

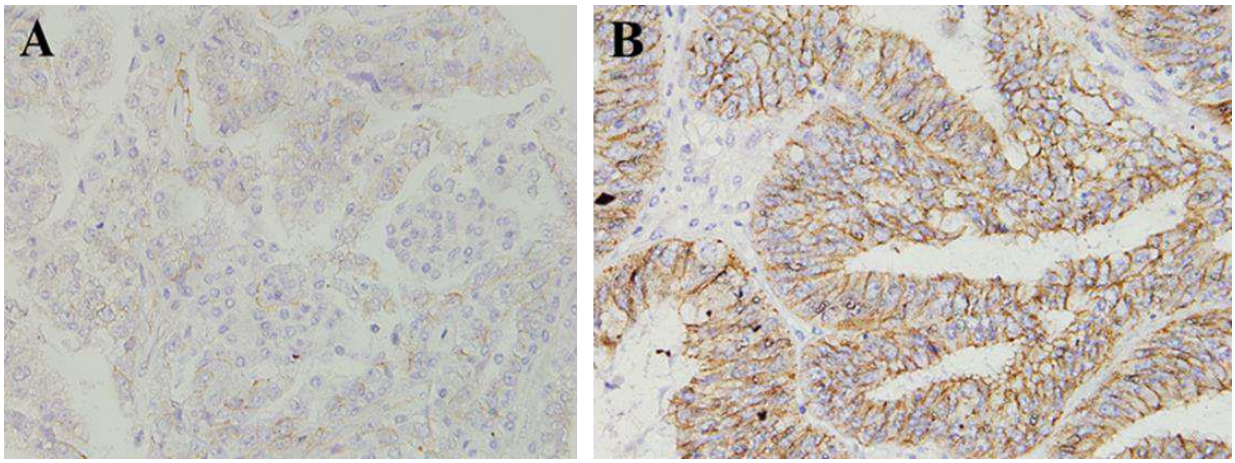

Figure 2. Representative photographs of PI3K protein in (A) ovarian carcinoma (endometrioid) without endometriosis; weak expression (B) ovarian carcinoma (endometrioid) with endometriosis; over expression of PI3K. All images are magnified 40x.

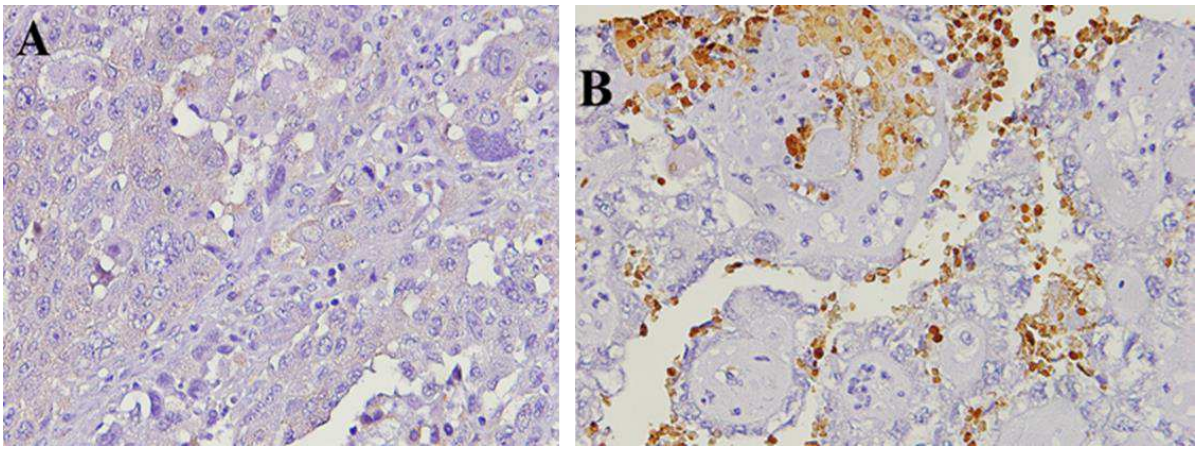

Figure 3. Representative photographs of IL-6 protein in (A) ovarian carcinoma (endometrioid) without endometriosis; low expression (B) ovarian carcinoma (endometrioid) with endometriosis; high expression of IL-6. All images are magnified 40x.

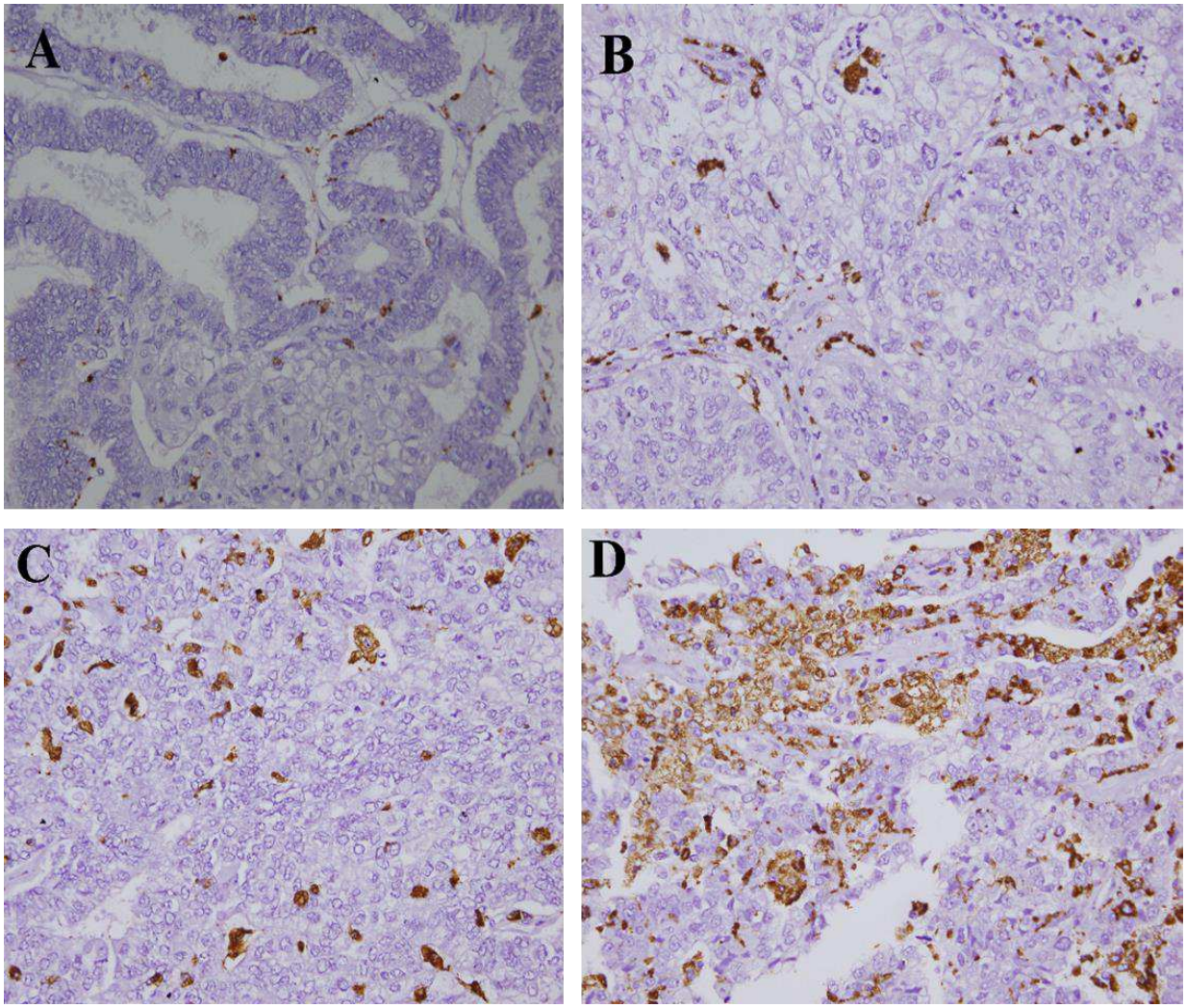

Figure 4. Representative photographs of CD68 macrophage in (A) ovarian carcinoma (endometrioid) with endometriosis; 1 % infiltration (B) ovarian carcinoma (endometrioid) with endometriosis; 5% infiltration. (C) ovarian carcinoma (endometrioid) without endometriosis; 25% infiltration. (D) ovarian carcinoma (endometrioid) without endometriosis; 50% infiltration. All images are magnified 40x.

Table 3. PTEN, PI3K IL-6 and CD68 (macrophage) protein expression in ovarian cancer with and without endometriosis

Group	N (%)	Score, N (%)				P value
		0	1	2	3	
PTEN						
Ovarian cancer with endometriosis	10	6 (60.0)	4 (40.00)	0 (0.00)	0 (0.00)	p= 0.47
Ovarian cancer without endometriosis	9	4 (44.44)	4 (44.44)	1 (11.11)	0 (0.00)	
PI3K						
Ovarian cancer with endometriosis	10	1 (10.00)	1 (10.00)	5 (50.00)	3 (30.00)	p=1.00
Ovarian cancer without endometriosis	9	0 (0.00)	2 (22.22)	2 (22.22)	5 (55.56)	
IL-6						
Ovarian cancer with endometriosis	10	0 (10.00)	3 (30.00)	3 (30.00)	4 (40.00)	p=0.35
Ovarian cancer without endometriosis	9	3 (33.33)	5 (55.56)	1 (11.11)	0 (0.00)	
CD68						
Ovarian cancer with endometriosis	10	4 (40.00)	2 (20.00)	4 (40.00)	0 (00.00)	p=0.16
Ovarian cancer without endometriosis	9	1 (11.11)	2 (22.22)	5 (55.56)	1 (11.11)	

Figures

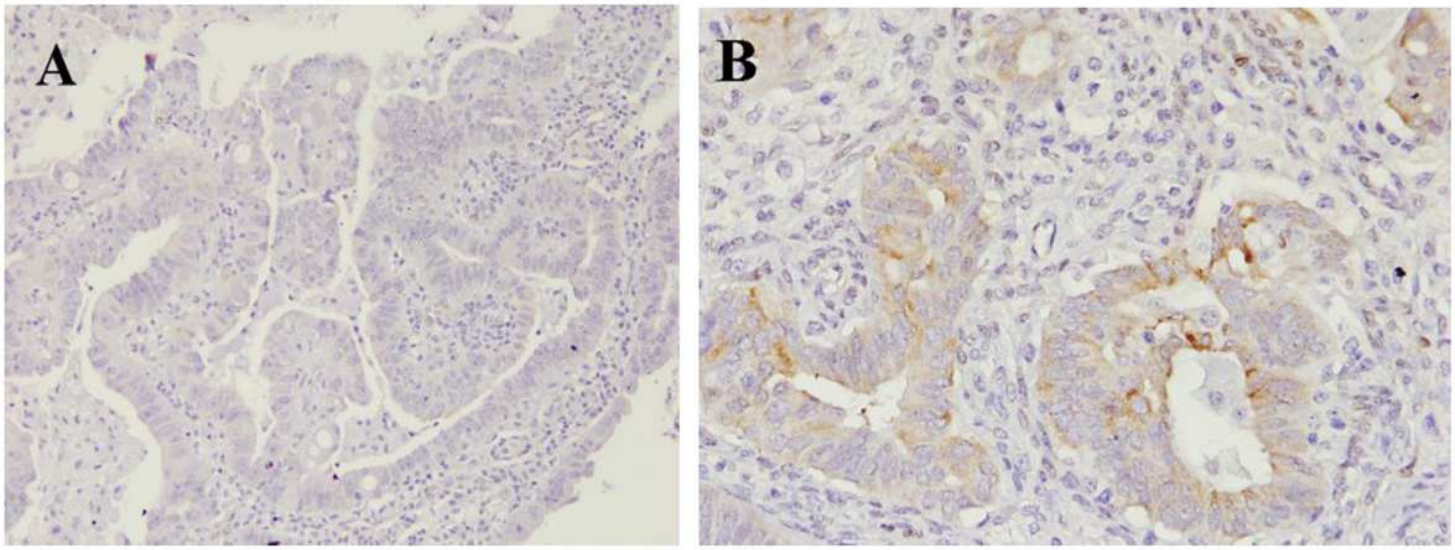

Figure 1

Representative photographs of PTEN protein in (A) ovarian carcinoma (endometrioid) with endometriosis; low expression (inactivation of PTEN) (B) ovarian carcinoma (endometrioid) without endometriosis; normal expression of PTEN. All images are magnified 40x.

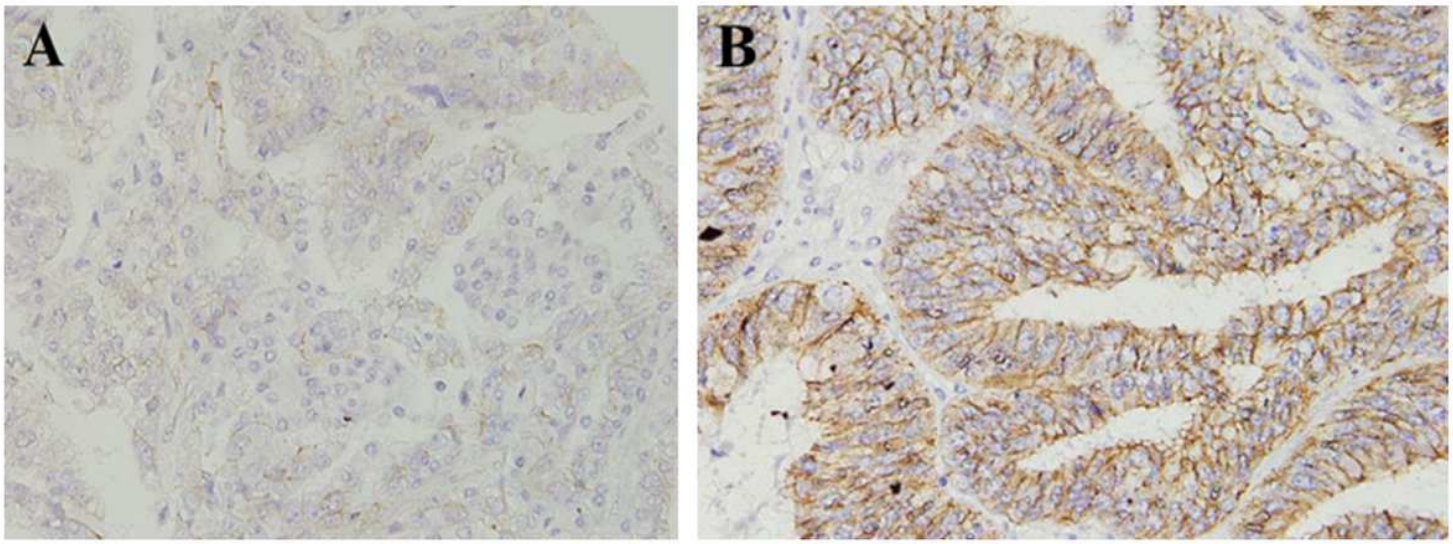

Figure 2

Representative photographs of PI3K protein in (A) ovarian carcinoma (endometrioid) without endometriosis; weak expression (B) ovarian carcinoma (endometrioid) with endometriosis; over expression of PI3K. All images are magnified 40x.

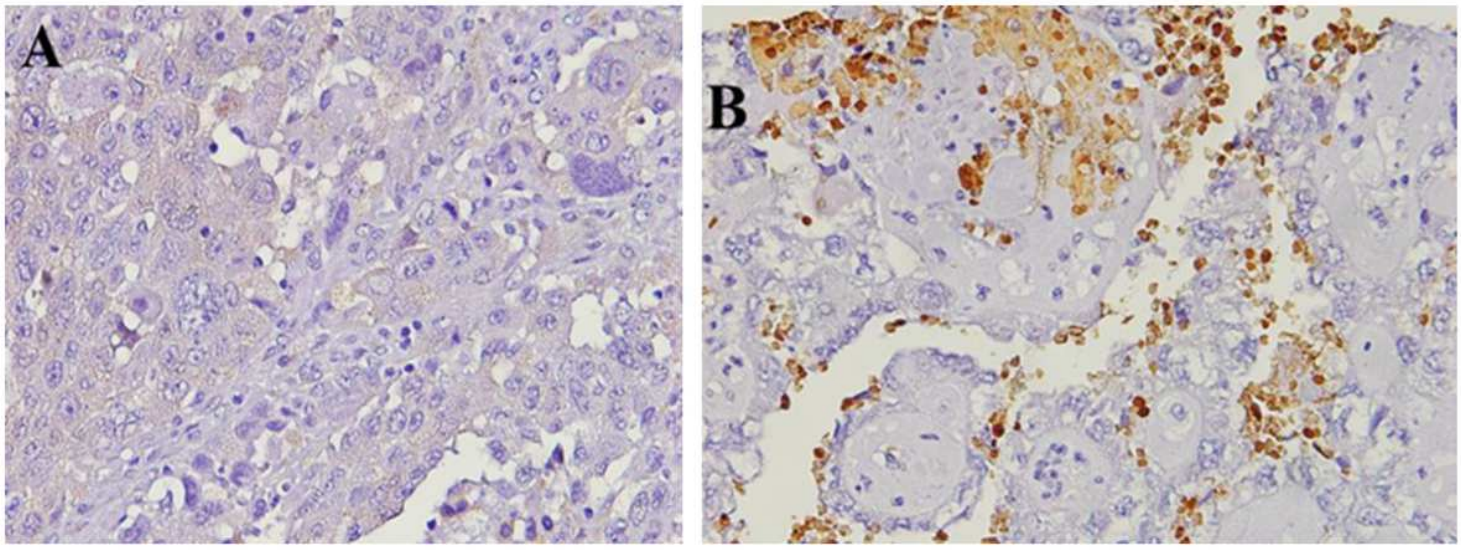

Figure 3

Representative photographs of IL-6 protein in (A) ovarian carcinoma (endometrioid) without endometriosis; low expression (B) ovarian carcinoma (endometrioid) with endometriosis; high expression of IL-6. All images are magnified 40x.

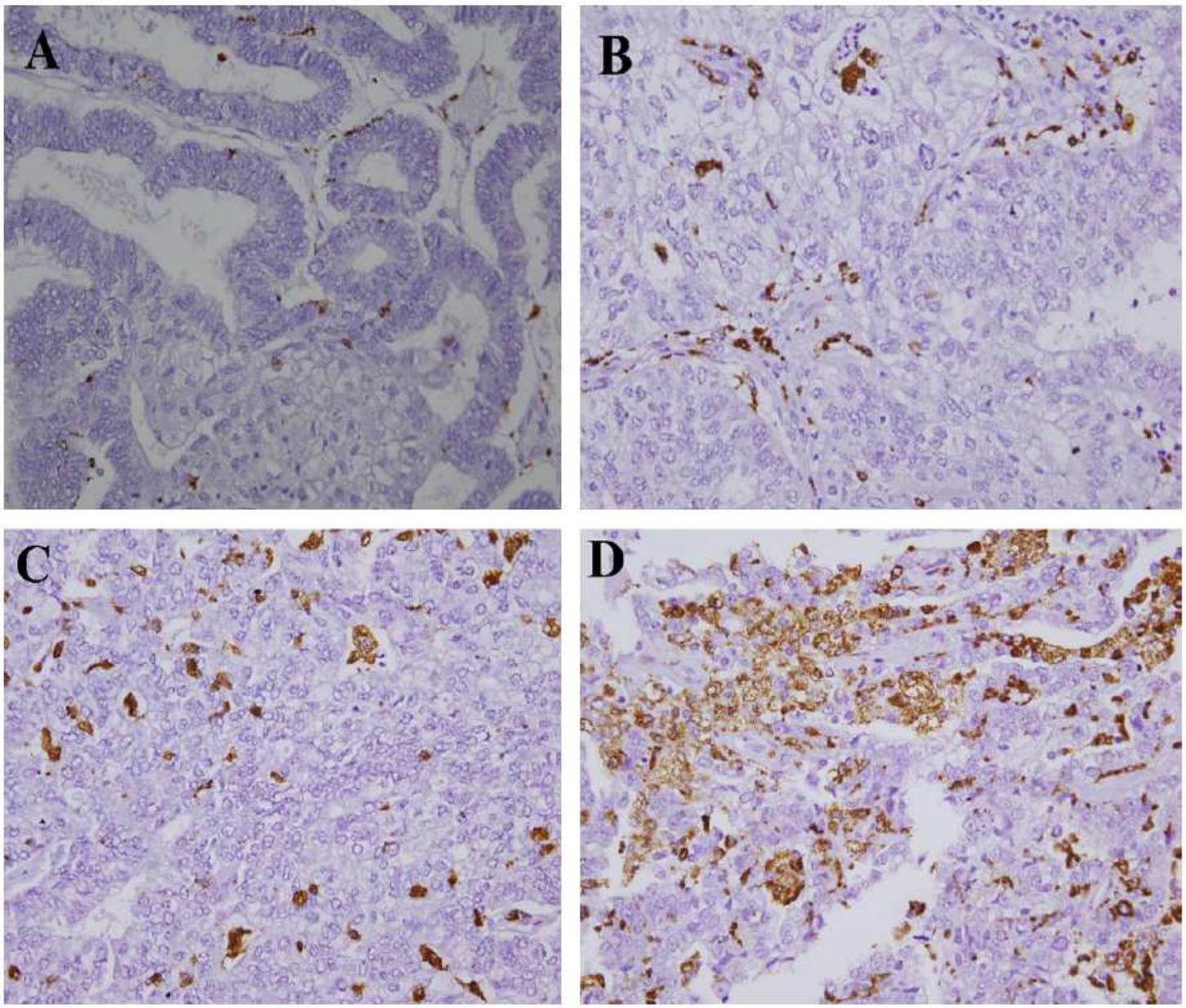

Figure 4

Representative photographs of CD68 macrophage in (A) ovarian carcinoma (endometrioid) with endometriosis; 1 % infiltration (B) ovarian carcinoma (endometrioid) with endometriosis; 5% infiltration. (C) ovarian carcinoma (endometrioid) without endometriosis; 25% infiltration. (D) ovarian carcinoma (endometrioid) without endometriosis; 50% infiltration. All images are magnified 40x.