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ORIGINAL ARTICLE

PTEN and PI3K Mutation Markers and Expression of CD68 and IL-6 Inflammatory Markers in Endometrioid and Clear Cell Ovarian Carcinoma with Underlying Endometriosis

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ABSTRAK

Objektif penyelidikan ini adalah untuk memastikan sama ada terdapat perbezaan dalam tahap ekspresi protein fosfatase dan tensin homolog (PTEN), fosfatidilinositol-4,5-bifosfat3-kinase (PI3K), makrofaj (CD68) dan interleukin-6 (IL-6) di kalangan wanita yang telah didiagnosis menghidap karsinoma ovari (subjenis endometrioid dan sel jernih) yang mendasari endometriosis. Slaid tisu mikrorai (TMA) telah disediakan dengan menggunakan blok parafin terfiksasi formalin (FFPE) penderma yang mengandungi spesimen tisu karsinoma ovari dengan subtaip endometrioid dan karsinoma sel jernih pada ovari yang telah diambil untuk tempoh sembilan tahun. Sebanyak 19 blok FFPE terdiri daripada 19 kes kansasinoma ovari, yang dikategorikan kepada dua kumpulan iaitu kanser ovari dengan endometriosis (EAOC, n=10) dan kanser ovari tanpa endometriosis (n=9). Kesemua ekspresi protein ini telah dianalisis menggunakan teknik imunohistokimia (IHC). Setelah itu, perbandingan berkaitan dengan ciri-ciri klinikal dan patologi karsinoma ovari, serta sebarang variasi yang boleh dilihat dalam tahap ekspresi antara kedua-dua kumpulan telah dilakukan. Dalam EAOC, ekspresi PTEN adalah lebih rendah (88.9% berbanding 100%, P=0.47) berbanding mereka yang tidak mempunyai endometriosis. Penemuan kami mendedahkan peningkatan PI3K dan IL-6 dalam EAOC daripada mereka yang tidak mempunyai endometriosis; masing-masing terdapat 80% berbanding 77.8%, P=1.00 dan 70% berbanding 11.11%, P=0.35. Sebaliknya, CD68 adalah jauh lebih rendah dalam kanser ovari yang berkaitan dengan endometriosis berbanding mereka yang tidak mempunyai endometriosis

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(40% berbanding 66.67%, P=0.16). Kesimpulannya, terdapat perbezaan ketara dalam pengubahsuaian penanda mutasi dan keradangan antara karsinoma sel jernih dan adenokarsinoma endometrioid ovari dengan endometriosis.

Kata kunci: Adenokarsinoma endometrioid; adenokarsinoma sel jelas; CD68; endometriosis; interleukin-6; karsinoma ovari; PI3K; PTEN

ABSTRACT

The objective of this research endeavour was to ascertain whether there existed a distinction in the protein expression levels of phosphatase and tensin homolog (PTEN), phosphatidylinositol-4,5-biphosphate3-kinase (PI3K), macrophage (CD68), and interleukin-6 (IL-6) among women who had been diagnosed as having ovarian carcinoma (endometrioid and clear cell subtypes) underlying endometriosis. Tissue microarray (TMA) slides were fabricated using donor's formalin-fixed paraffin-embedded (FFPE) blocks that comprised specimens of ovarian carcinomas tissues with the subtypes of endometrioid and clear cell that were retrieved for a duration of nine years. A total of 19 FFPE blocks represented 19 cases of carcinoma of the ovary, which were categorised into two groups: ovarian cancer with endometriosis (EAOC, n=10) and ovarian cancer without endometriosis (n=9). All of these protein expressions were analysed using immunohistochemistry (IHC) technique. Following that, comparisons were made regarding the clinical and pathological characteristics of the ovarian carcinomas, as well as any discernible variations in expression levels between the two groups. In EAOC, PTEN expression was lower (88.9% vs. 100%, P=0.47) compared to those without endometriosis. Our findings revealed an increase of PI3K and IL-6 in EAOC than those devoid of endometriosis; 80% vs. 77.8%, P=1.00 and 70% vs 11.11%, P=0.35, respectively. In contrast, CD68 was significantly lower in endometriosis-associated ovarian cancer compared to those without endometriosis (40% vs. 66.67%, P=0.16). In conclusion, there were no significant difference in the mutational and inflammatory marker modifications between clear cell carcinoma and endometrioid adenocarcinoma of ovary with endometriosis.

Keywords: CD68; clear cell adenocarcinoma; endometrioid adenocarcinoma; endometriosis; interleukin-6; ovarian carcinoma; PI3K; PTEN

INTRODUCTION

Endometriosis is a benign gynaecological condition distinguished by the atypical protrusion of endometrial glands or stroma beyond the confines of the uterus. This phenomenon is frequently observed in several anatomical locations, including the peritoneum, uterine

surface, ovary, intestines, bladder, and even remote regions such as the skin and lungs (Dewhurst 2012). The documented prevalence among reproductive-aged women varies between 5 to 15%. Nevertheless, this range may be underestimated due to the diagnostic requirement of direct pelvic visualisation, with or without tissue biopsy, which could potentially interfere with quality of their marital life (Dewhurst 2012; Krawczyk et al. 2016; Rao et al. 2023).

Notwithstanding its benign nature, endometriosis exhibits numerous attributes that resemble those of malignant cells; this includes the potential for epithelial ovarian cancer (EOC), which carries an approximate 50% overall progressional risk (Pearce et al. 2012). An increased vulnerability to particular histotypes pertaining to ovarian carcinoma, encompassing clear cell and endometrioid subtypes, has been observed in individuals with endometriosis-associated ovarian carcinoma (EAOC) (Nezhat et al. 2015). As indicated by the findings Somigliana et al. (2006), clear cell ovarian carcinoma exhibited the highest prevalence of EAOC (35% in 390 cases), followed by endometrioid ovarian carcinoma at 27% in 648 cases, whereas serous epithelial carcinoma and mucinous carcinoma were significantly less frequent, occurring at respective frequencies of 5% in 1372 cases and 4% in 614 cases (Somigliana et al. 2006). There is a correlation between clear-cell ovarian carcinoma and endometriosis ranging from 9 to 70%; this subtype accounts for 3.7 to 12.1% of all EOC (Orezzoli et al. 2008). Based on the Cancer Registry Report 2007-2011, the overall prevalence of ovarian cancer among women in Malaysia amounted to 6.1%; with endometrioid and clear cell adenocarcinoma constituted 7.1 and 6.6%, respectively (Azizah et al. 2016).

Among the several oncogenic mutations that have been observed in EAOC, phosphatase and tensin homolog (PTEN) and phosphatidylinositol-4,5-biphosphate3-kinase, catalytic subunit alpha (PI3K) are among the most common (Cheaib et al. 2015). In 40% of EOCs, PTEN dysfunction or mutations are well known, whereas Romero and Bast (2012) identified PI3K mutations present in between 20-35% of endometrioid and clear cell ovarian carcinomas. Alteration of this PI3K/AKT pathway promotes malignant transformation by promoting tumour proliferation, growth, migration, invasion, and apoptosis evasion, as well as microRNA (miRNA) alteration in endometriosis (Ankasha et al. 2018; Azam et al. 2022; Musa & Schneider 2015). Furthermore, PTEN assumes a critical function in the modulation of many cellular signalling pathways, including autophagy, which encompasses angiogenesis and apoptosis activation, and cell cycle regulation (Wang et al. 2015). PTEN was also discovered to affect the myometrial invasion of cancer cells and prognosis outcomes (Shafiee et al. 2021). Consequently, this pathway is already of interest for the targeted therapy of ovarian carcinoma (Kalamanathan et al. 2011; Vestergaard et al., 2011). While histological

specificity has been established for PTEN and PI3K alterations, the relationship between these markers and the presence of endometriosis as an underlying condition remains unexplored.

Chronic local inflammatory responses induced by endometriosis have been observed to stimulate macrophage activation and release of several cytokines and chemokines, IL-6 being among them. This response generates DNA damage and mutations further (Li et al. 2017). Isobe et al. (2015) substantiated the existence of IL-6 overexpression in 45.5% of individuals diagnosed with endometrioid cancer, 55% of those who have been identified as having clear cell carcinoma, and 50% of patients with mucinous carcinoma among 94 patients with EOC. While patients with serous carcinoma (38.2%) and other subtypes of ovarian cancer did not exhibit this significant variation (15.4%) (Isobe et al. 2015). Similarly, IL-6 levels in endometriosis and endometrioid endometrial adenocarcinoma were found to be higher than in normal endometrium (Yousefi et al. 2019). This finding is not unexpected, considering the hypothesis that both endometrial and ovarian endometrioid cancer originate from comparable precursor endometrial epithelial cells. However, the relationship between elevated levels of IL-6 and CD68 remains ambiguous, and to far, there has been a lack of research examining the expression of these inflammatory markers in cases of EAOC.

Considering the aforementioned literatures, even though molecular biomarkers as a diagnostic tool in endometriosis remains inconclusive (Abdul Karim et al. 2020); thus, our study initiated to determine the altered molecular signatures in EAOC patients. With regard to endometrioid and clear cell ovarian cancer, which are intricately associated with endometriosis, we conducted a targeted identification and analysis of the variations in protein expression for PTEN, PI3K, IL-6, and CD68 in patients with endometriosis underlying the disease with those who did not have endometriosis. The findings were linked to clinical and pathological patient information.

MATERIALS AND METHODS

Sample Collection

Surgical samples representing ovarian tumours from patients whom an exploratory laparotomy was performed at the UKM Medical Centre (UKMMC) and were diagnosed with the primary clear cell carcinoma and endometrioid adenocarcinoma of the ovary were retrospectively analysed. The donor's formalin-fixed paraffin-embedded (FFPE) blocks were retrieved from the pathology department's archives throughout the period from January 2007 to December 2015. Concurrently, the corresponding haematoxylin and eosin (H&E) slides were also acquired. The cases included endometrioid and clear cell ovarian carcinoma with endometriosis (n=10) and endometrioid and clear cell ovarian cancer without endometriosis (n=9).

Immunohistochemistry (IHC)

The FFPE blocks, specifically the donor FFPE blocks were selected for the construction of tissue microarray (TMA) blocks utilising the Alphelys Minicore® 3 Tissue Arrayer, a tissue microarrayer (Alphelys, Plaisir France). Initial screening was performed to choose the appropriate FFPE blocks and cancer location for TMA creation in order to guarantee the production of high-quality TMA blocks for this study. All cases were stained immunohistochemical for PTEN, PI3K, IL-6 and CD68 using the instructions provided in EnVision™ FLEX Mini Kit, High pH (Dako, Glostrup,Denmark). Each section of TMA slides was stained with primary antibodies that targeted PI3K (EPR3951; 1:100; Abcam, Massachusetts, USA), PTEN (6H2.1; 1:100; Dako, Glostrup, Denmark), CD68 (PG-M1; Abcam, Massachusetts, USA), and IL-6 (ab9324; 1:200; Abcam, Massachusetts, USA). Semi-qualitative analysis was utilised to assess IHC scoring, whereas the interpretation of immunohistochemical staining was performed utilising the appropriate primary antibody. The analysis was evaluated by two pathologists who separately inspected each slide.

IHC analysis identified the presence of PTEN in cells containing a well-organised cytoplasm and nucleus. Conversely, scores were allocated in accordance with the stain's intensity: Negative staining was denoted by 0, weak staining by 1, moderate staining by 2, and strong staining by 3. Cases exhibiting staining levels of 0 or 1 were classified as PTEN inactivate, whereas staining levels of 2 or 3 were considered to be normal. Positive for PI3K staining were cells with brown cytoplasmic or membrane. Analogous to the PTEN scoring system, on a scale from 0 to 3, the strength of PI3K staining was categorised as follows: strong, weak, moderate, and negative, respectively. Scores of 2 and 3 indicated overexpression of the PI3K protein.

The determination of the percentage of positive mononuclear cells that infiltrated each TMA core, CD68 protein expressions were graded from 0 to 3. The grades were delineated by the following cutoff values: 1% (grade 0), 5% (grade 1), 25% (grade 2), and 50% (grade 3) were applied to each positive cell in relation to infiltered immune cells. In contrast, the quantification of IL-6 was determined for each tumour core by assessing the intensity of staining, with a score of 0 for no expression, 1 for weak expression, and 2 for strong expression. An independent assessment of each core was conducted by examining it in three microscopic fields at a magnification of 100x. In the analysis, the mean score for each core was utilised.

Statistical Analysis

Software Package for the Social Sciences (SPSS 24.0) for Windows was utilised to conduct every statistical analysis in this study. Patients' characteristics were denoted by means, standard deviations (SD), medians, interquartile ranges (IQRs), and frequencies (percentage). The Student t-test was employed to compare the means of continuous variables

that followed a normal distribution between the two groups. On the other hand, the Mann-Whitney U test was utilised to compare the median values of continuous variables that followed a non-normal distribution data. In order to analyse the correlation between categorical data, a chi-squared test or Fisher's exact test was applied at a significance level of 0.05.

RESULTS

Demographic and Clinical Details

A total of 19 individuals, aged varied, participated in the research. They were separated into two groups: those with ovarian cancer and endometriosis (n=10), and those without endometriosis (n=9). The participants in this study had a mean age of 49.10 ± 10.97 years for those with endometriosis and 49.78 ± 8.82 years for those without endometriosis. The patients in both groups were predominantly Malays (60.0% vs. 77.8%), followed by Chinese (30.0% vs. 11.1%) and Indians (10.0% vs 11.1%). The non-endometriosis group exhibited a marginally lower BMI (25.37 ± 5.78 kg/m^2 vs. 24.05 ± 3.22 kg/m^2, P=0.562), although the disparity did not reach statistical significance. The majority of individuals were not yet in the menopausal stage at the time of diagnosis. The distributions of the demographics were illustrated in Table 1.

Within the subset of EAOC, endometrioid adenocarcinoma was more prevalent than clear cell carcinoma (90.0% vs. 10.0%). Each individual within this cohort exhibited the disease in its nascent stages (stage 1 and 2) and exhibited a well-differentiated tumour. Conversely, the abundance of distinct forms of cancer exhibited a comparable trend, wherein the subtype of endometrioid adenocarcinoma predominated (77.8%) among ovarian carcinoma patients without endometriosis. A higher percentage of moderate and poorly differentiated tumours were identified, which was identified in 22.2% of the cases (55.5% vs 50.0%). Likewise, ovarian carcinoma with endometriosis exhibited a comparatively smaller average tumour diameter (14.556 ± 6.20 cm vs. 16.22 ± 6.53 cm) than ovarian carcinoma without endometriosis. However, it was important to note that none of these disparities exhibited sufficient statistical significance to indicate a correlation between endometriosis and the specified cancer histological features (Table 2).

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

The scoring of each sample was determined by assessing the strength of staining and the percentage of cells that were positive and stained with PTEN, PI3K, IL-6, and CD68 antibodies. Our observations revealed that PTEN protein expression in EAOC patients was either cytoplasmic or membrane-bound (Figure 1). PTEN inactivation, as measured by a score from 0 to 1, was detected in every sample of EAOC, and to a lesser degree in ovarian cancer devoid of endometriosis (100%

TABLE 1: Demographics for ovarian carcinoma with and without endometriosis

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

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

Demographics	Ovarian carcinoma with endometriosis		Ovarian carcinoma without endometriosis		P-value
	N (%)	Mean (SD)	N (%)	Mean (SD)	
Type of Cancer					0.582[a]
Endometrioid	9 (90.0)		7 (77.8)		
Clear cell	1 (10.0)		2 (22.2)		
FIGO staging					0.324[a]
1	9 (90.0)		4 (44.4)		
2	1 (10.0)		3 (33.3)		
3	0 (0.0)		0 (0.0)		
4	0 (0.0)		2 (22.2)		
Tumour grade					1.000[a]
Well differentiated	5 (50.0)		4 (44.4)		
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
FIGO: International Federation of Gynaecology and Obstetrics.

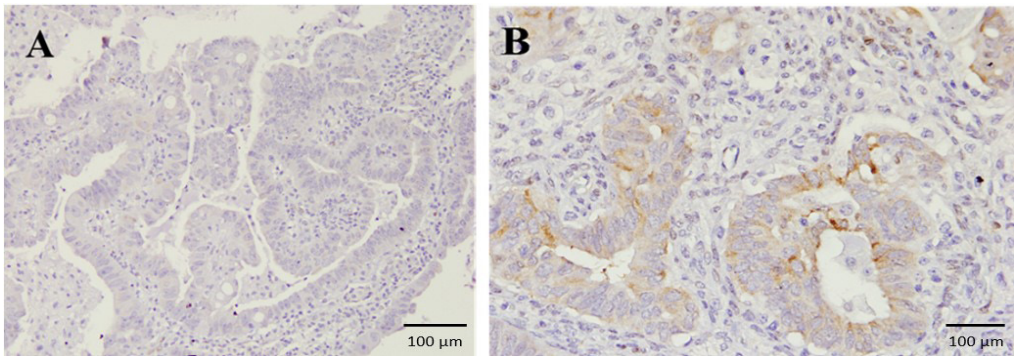

FIGURE 1: Representative photographs of PTEN protein in (A) ovarian carcinoma (endometrioid) with endometriosis; low expression (inactivation of PTEN) (B) ovarian carcinoma (endometrioid) without endometriosis; normal expression of PTEN. All images were magnified at 40X

versus 88.9%, P=0.47) (Table 3). With an intensity score of 2, normal PTEN protein expression was observed in 11.1% of ovarian cancer cases devoid of endometriosis.

In ovarian cancer linked with endometriosis, overexpression of PI3K protein with scores of 2 and 3 (Figure 2) was notably higher than in cancer unrelated to endometriosis (80% vs. 77.8%, P=1.00). The percentage of IL-6 protein expression was greater in ovarian cancer with endometriosis (70% vs. 11.1%; P=0.35) (Figure 3). In contrast to our prediction, as illustrated in Figure 4, more CD68 infiltration of tumour cells was observed in ovarian cancer patients without endometriosis (66.67% vs. 40%, P=0.16).

TABLE 3: PTEN, PI3K IL-6 and CD68 (macrophage) protein expression in ovarian carcinoma 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)	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)	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)	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)	0.16
Ovarian cancer without endometriosis	9	1 (11.11)	2 (22.22)	5 (55.56)	1 (11.11)	

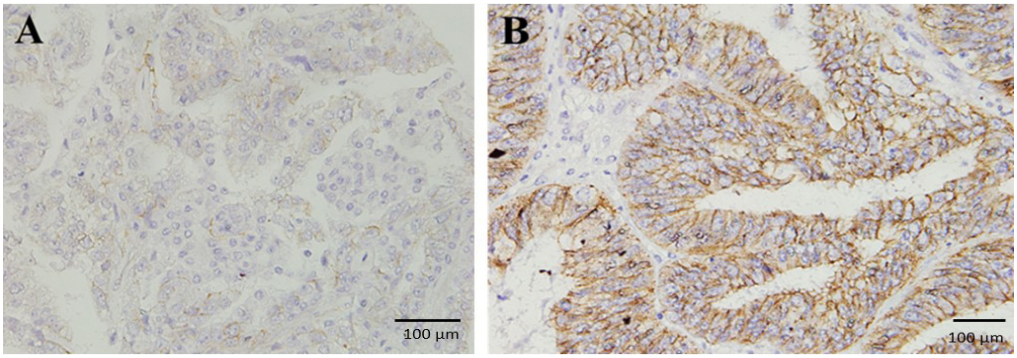

FIGURE 2: Representative photographs of PI3K protein in (A) ovarian carcinoma (endometrioid) without endometriosis; weak expression (B) ovarian carcinoma (endometrioid) with endometriosis; over expression of PI3K. All images were magnified at 40x

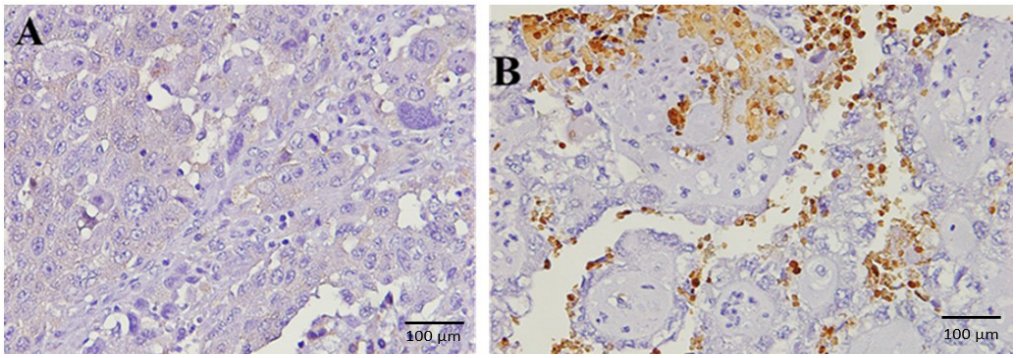

FIGURE 3: Representative photographs of IL-6 protein in (A) ovarian carcinoma (endometrioid) without endometriosis; low expression (B) ovarian carcinoma (endometrioid) with endometriosis; high expression of IL-6. All images were magnified at 40x

DISCUSSION

The progression of contemporary biotechnology facilitates the molecular and genetic characterisation of EAOC. Understanding the potential influence of mutations and the ovarian microenvironment on malignant transformation requires this information. Furthermore, an increasing number of studies have revealed a renewed fascination with the utilisation of molecular and genetic identification methods in order to create novel targeted treatments for ovarian cancer. In the treatment of EAOC, precision medicine could be a valuable instrument for categorising the likelihood of progression to ovarian cancer, thereby providing a foundation for preventative measures. While molecular studies contribute to the prediction of EAOC, immunohistochemistry continues to be a dependable, cost-effective, and highly effective method for identifying early cancers (Kriplani & Patel 2013).

Endometrioid and clear cell

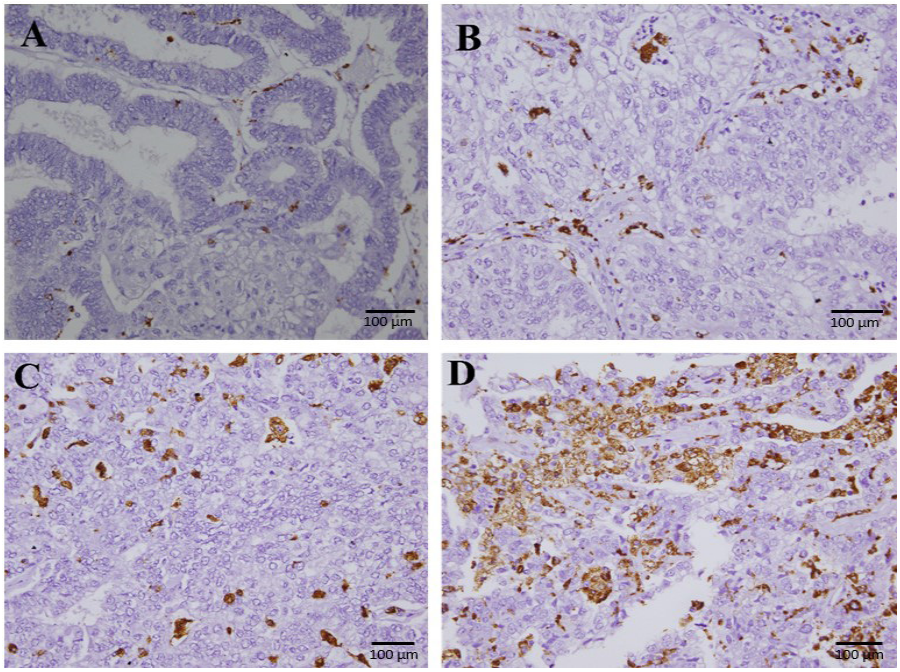

FIGURE 4: Representative photographs of CD68 macrophage in (A) ovarian carcinoma (endometrioid) with endometriosis; 1% infiltration (B) ovarian carcinoma (endometrioid) with endometriosis; 5% infiltration; (C) ovarian carcinoma (endometrioid) without endometriosis; 25% infiltration. (D) ovarian carcinoma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

carcinoma, which are subtypes of endometrial and ovarian malignancies, have been linked to PTEN mutations at locus 10q23 that result in the loss of heterozygosity and inactivation of the protein (Sato et al. 2000). Consistent outcomes were observed in ovarian cancer cases that occurred simultaneously with endometriosis and benign endometriotic cysts, which provided more support for its role in the development of EAOC (Makker et al. 2012). PTEN inactivation was observed in every case (100%) of ovarian cancer with a history of endometriosis (10 out of 10) and in 88.9% (8 out of 9) cases of ovarian cancer without endometriosis, as reported in this study. This proportion was considerably more than that of a prior investigation that identified negative PTEN expression in 71.8% of EAOC (Makker et al. 2012). PTEN expression was less prevalent in the group with endometriosis and ovarian cancer (0.40% versus 0.67%, P=0.37), although the statistical analysis did not indicate a substantial difference.

The fact that 20–40% of endometrioid and clear cell carcinoma of the ovaries contain the PI3K mutation, indicating its high prevalence (Hao et al. 2017). Activation of the PI3K-Akt-mTOR signalling pathway has also been observed in ovarian endometriosis, with activity levels being significantly elevated compared to those of the healthy endometrium (Yamamoto

et al. 2011). Our hypothesis was that ovarian carcinoma patients with endometriosis would have elevated PI3K expression; the results supported this conclusion, as 80% of patients (8 out of 10) with endometriosis exhibited PI3K overexpression, compared to 77.8% (7 out of 9) in patients without endometriosis; however, the difference was not statistically significant. This finding significantly surpassed the quantities documented in prior research as above mentioned, but not specifically in comparison with or without endometriosis.

Increased levels of IL-6 in both blood and ascites fluid have been linked with disease progression and unfavourable clinical outcomes in ovarian cancer microenvironments, making it one of the most significant cytokine indicators (Kuo et al. 2009). We presented herein evidence that IL-6 expression was higher in patients with EAOC. Given the substantial contribution of inflammation to the development of endometriosis, it is anticipated that the existence of macrophages and pro-inflammatory mediators will be of the utmost importance (Lane et al. 2011). Promoting the development and proliferation of endometriotic tissues, these macrophages induce angiogenesis, maintain resistance to apoptosis, and increase precursor cell invasion and proliferation.

As 70% (7 out of 10) EAOC had elevated IL-6 protein expression levels, we hypothesised that the level of macrophages (CD68) will follow a comparable pattern. However, it was observed that the expression of the macrophage (CD68) protein reduced to 60% (6 out of 10) in EAOC cases compared to cases without endometriosis. The observed outcome might have been accounted for by the macrophage phenotype that is correlated with EAOC patients. There are two primary phenotypes of macrophages: M1 (macrophages that are naturally activated) and M2 (alternatively activated macrophage). M1 facilitates the inflammatory response, pathogen clearance, and anti-tumour immunity. On the other hand, M2 is responsible for wound healing and pro-tumorigenic properties. The activities triggered by IL-6, leukaemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF) lead to the polarisation of ascites macrophages into M2 macrophages in advanced epithelial ovarian cancer (Li et al. 2017). Hence, the expression level of CD68 protein, which is postulated to be increased in cases of EAOC, might not precisely represent CD68 of type M2. Further investigation is warranted to ascertain the distinctions in CD68 type M1 and CD68 type M2 expression levels across endometriosis, ovarian cancer, and EAOC.

Tissues of ovarian tumours associated with endometriosis in this preliminary study were compared the IHC results of PTEN and PI3K markers, as well as CD68 and IL-6 protein inflammatory markers. Nevertheless, the utilisation of IHC was restricted to a fraction of the accessible cases, potentially attributable to the rarity of endometrioid and clear cell subtypes of ovarian cancer, which comprise less than 10% of the disease (Prat

2012). With the aim of gaining a more comprehensive understanding of the progression of endometriosis, it is imperative to conduct further research on the potential prognostic and indicator functions of inflammatory markers PTEN, PI3K, CD68 and IL-6 in endometriotic patients. Additionally, a greater sample size ought to be employed in order to perform this.

CONCLUSION

Ovarian carcinoma without endometriosis exhibits lower levels of IL-6, PI3K overexpression, and PTEN inactivation compared to clear cell ovarian cancer and endometrioid adenocarcinoma with underlying endometriosis; however, the difference is non-significant. Despite the fact that our sample size was relatively smaller than that of previous studies, our findings revealed similar patterns of protein expression. Potential diagnostic markers for identifying women with endometriosis who are at risk of developing clear cell ovarian cancer and endometrioid carcinoma may be derived from the substantial contribution of PTEN and PI3K proteins. Therefore, in order to forecast the malignant evolution of endometriosis, appropriate disease surveillance and classification can be implemented specifically for them.

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AUTHOR'S CONTRIBUTION

The authors confirm contribution to the paper as follows: the development and idea of the project and also leader of the project/fund owner: Mohamad Nasir Shafiee; the study conception and design: Noorazizah Arsad, Nor Haslinda Abd Aziz and Mohamad Nasir Shafiee; data collection: Noorazizah Arsad; performing experiment, analysis, interpretation and validation of results: Noorazizah Arsad, Reena Rahayu Md Zin, Nur Maya Sabrina Tizen Laim and Abdul Muzhill Hannaan Abdul Hafizz; draft manuscript preparation: Noorazizah Arsad, Nor Haslinda Abd Aziz, Abdul Muzhill Hannaan Abdul Hafizz; finalising the and reviewed final report and manuscript: Abdul Muzhill Hannaan Abdul Hafizz and Mohamad Nasir Shafiee. All authors reviewed the results and approved the final version of the manuscript.

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