

The infiltrating ability of abdominal wall endometriosis is associated with ectopic endometrial glandular activity

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Introduction: The infiltration pattern of endometriosis is one of the most important pathophysiological features of this lesion. Recent studies indicate the infiltrating nature of endometriosis is associated with a lesion's genetic characteristics. However, related research regarding abdominal wall endometriosis (AWE) is limited. The aim of this study is to investigate whether AWE lesions with different infiltrating depth have different clinical and pathological features. **Material and methods:** A retrospective review of all cases of surgically excised AWE from 2001 to 2018 was performed from the records at Dalian Medical University and its affiliated hospitals. Descriptive data were collected and analyzed. Hematoxylin-eosin stained (H&E) slides were re-evaluated by pathologists for the density of ectopic endometrial glands (DOG). **Results:** Ninety-one cases were included in this study. Cases were divided into three types according to the depth of infiltration of the lesion: the fascia type (38 cases), the muscle type (40 cases) and the peritoneum type (13 cases). The primary analysis showed that mass size ($P = 0.009$), serum CA125 levels ($P = 0.04$) and operation time ($P = 0.02$) were significantly different among the three groups. Analysis showed that the diameter of the lesion was positively correlated with the infiltrating depth of the lesion, as well as the level of serum CA125 and the operation time. Even for lesions larger than 3 cm, serum CA125 levels and operation time still showed positive correlation with the lesion diameter ($P = 0.02$ and $P < 0.01$, respectively). Further histological research indicated that ectopic endometrial glands in deep lesions were more active and had higher density compared to masses in the superficial layers. **Conclusion:** This study suggests the three types of AWEs have different clinical and pathological features. When a lesion infiltrates deep into the abdominal wall, it has a larger size, is associated with increased serum CA125

levels, and needs longer time for surgical excision. The different infiltrating ability of AWEs is associated with different activities of ectopic endometrial glands.

Keywords

Abdominal wall endometriosis; Infiltrating ability; Glandular activity

1. Introduction

Abdominal wall endometriosis (AWE) is a special type of endometriosis that often occurs secondary to caesarean section or other gynecological surgery. It usually presents as an abdominal mass caused by the growth of an ectopic endometrium. As AWE bears the symptoms of cyclic local pain, constant mass expansion and the risk of malignant transformation, it has increasingly attracted the attention of clinicians worldwide [1–4].

As previously reported [5], AWE can be divided into three subtypes according to the infiltrating depth into different layers of the abdominal wall. These are the superficial, or fascia (F) type; the intermediate, or muscle (M) type; and the deep, or peritoneum (P) type (Fig. 1). The infiltration pattern is one of the most important pathophysiological features of endometriosis. Recent studies indicate the infiltrating nature of endometriosis is associated with the lesions' specific genetic characteristics. Endometriosis arising in different sites, such as the ovary, peritoneum or rectovaginal pouch, are thought to have considerably different pathogenesis [6–12].

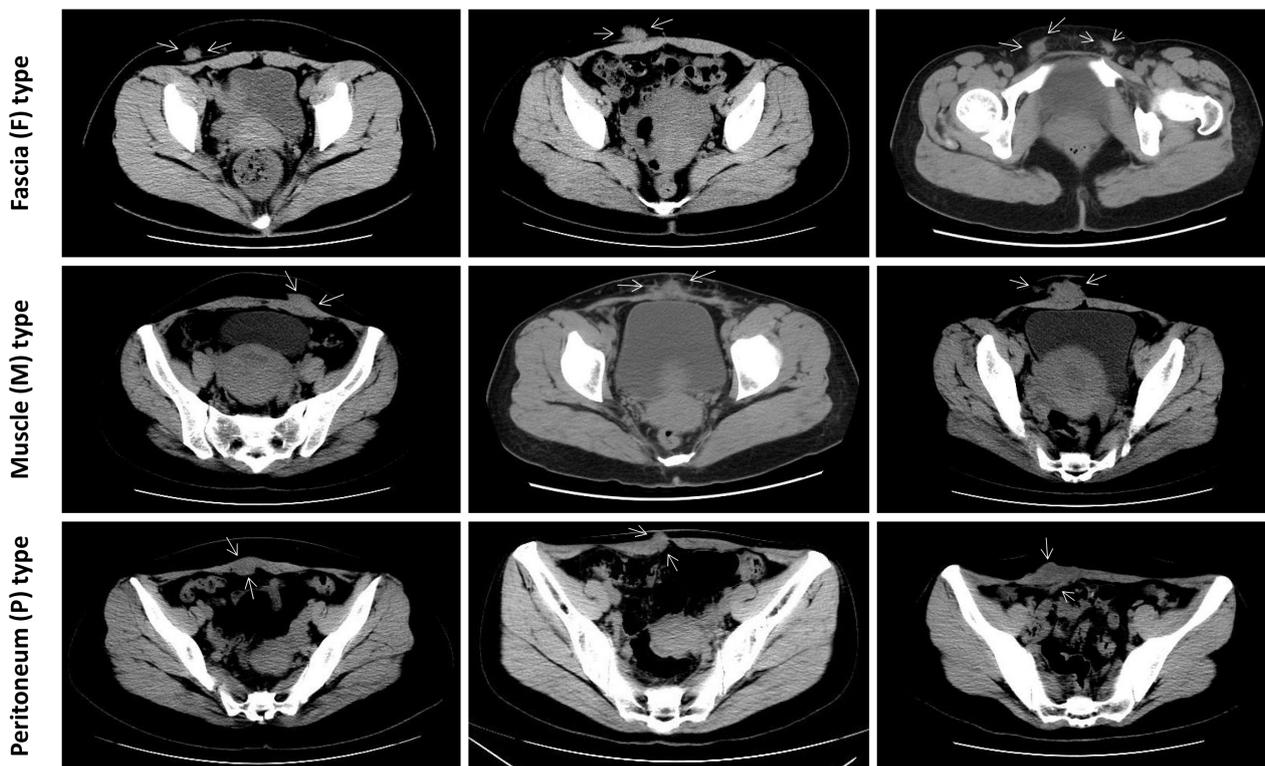


Fig. 1. Three types of AWEs classified according to their infiltrating depth into different layers of the abdominal wall as seen in computer tomographic images. The arrows indicate AWE lesions. In Fascia (F) type, the lesion is restricted to the fat layer of the abdominal wall; in Muscle (M) type, the lesion is located within the layer of rectus abdominus and its sheath; while in Peritoneum (P) type, the endometriotic lesion invades the peritoneum.

Although numerous studies have been performed on intra-peritoneal endometriosis [9, 10, 13], research on abdominal wall endometriosis is limited. Moreover, there is little published data regarding the clinical and pathologic characteristics of different infiltrating AWEs. The aims of this study are therefore to analyze the clinical and pathological features of the three subtypes of AWE, and to identify factors correlated with the infiltrating ability of AWEs.

2. Materials and methods

A retrospective medical record search was manually conducted for patients treated at First Affiliated Hospital of Dalian Medical University between January 2001 to December 2018 with diagnosis of AWE. A total of 91 cases with the pathological diagnosis of AWE were recruited for analysis. All the research was approved by the institutional review of First Affiliated Hospital of Dalian Medical University. The following data were retrieved from patient charts: age at AWE surgery, age at past caesarean section (CS), previous surgical history, gestational week of CS delivery, category of health care institution (tertiary or not) where the CS was performed, number of CS, latent time (months from last CS to the appearance of symptoms), mass size (largest diameter of the lesion, measured from computer tomography images), lesion location (the layer that the deepest margin of AWE lesion infiltrated, as described in surgery records), and

the serum CA125 level recorded in medical chart before the operation. All haematoxylin and eosin (H&E) stained slides were re-examined for density of ectopic endometrial glands (DOG), defined as the portion of gland area in a 40X low power field (calculated as area of glands/ entire area in a 40X low power field).

Data were expressed as mean \pm standard deviation or as a percentage. In analyzing quantitative results, student's *t*-test and Chi-square (X^2) test were used for two group comparisons, whereas the analysis of variance and a post hoc test (Student-Newman-Keuls) was used for multiple comparisons. $P < 0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed using SPSS 21.0 (Chicago, USA) and graphs were produced using Graphpad Prism 8.0. DOG was assessed using the digital pathology software QuPath [14].

3. Results

3.1 Social-demographic characteristics were similar in the three AWE patient groups

A total of 91 cases of pathologic AWE were included in the analysis. Several social demographic characteristics including age, gestational week, incision type, previous CS institution and previous operation times were compared among the three AWE groups (Table 1). No significant differences between the three groups were found for any of these character-

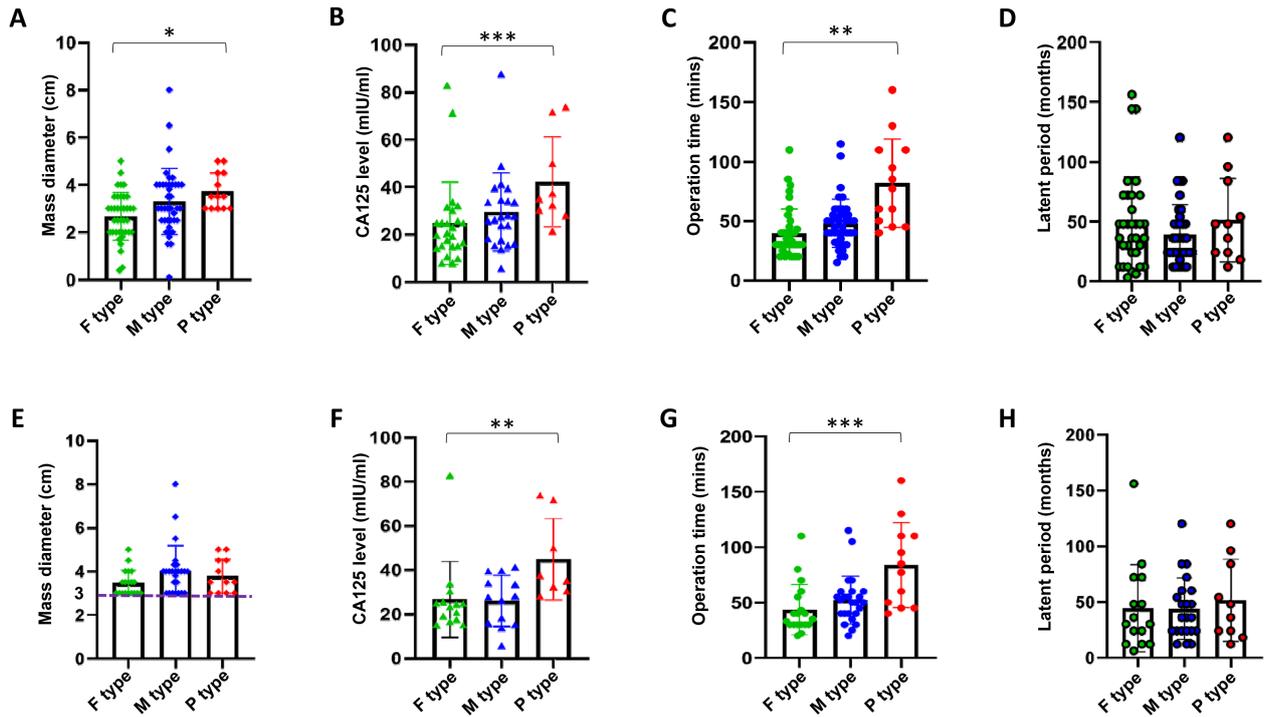


Fig. 2. Clinical manifestations of the three types of AWEs. Fig. 2A-D shows the distribution and differences in mass diameters, serum CA125 levels, operation time and latent time among 91 cases of the three types of AWEs ($P = 0.009, 0.041, < 0.001, 0.588$, respectively). Fig. 2E-H shows the distribution and differences in the same four parameters among 56 patients with a mass diameter larger than 3cm for the three types of AWEs ($P = 0.146, 0.021, < 0.001, 0.881$, respectively).

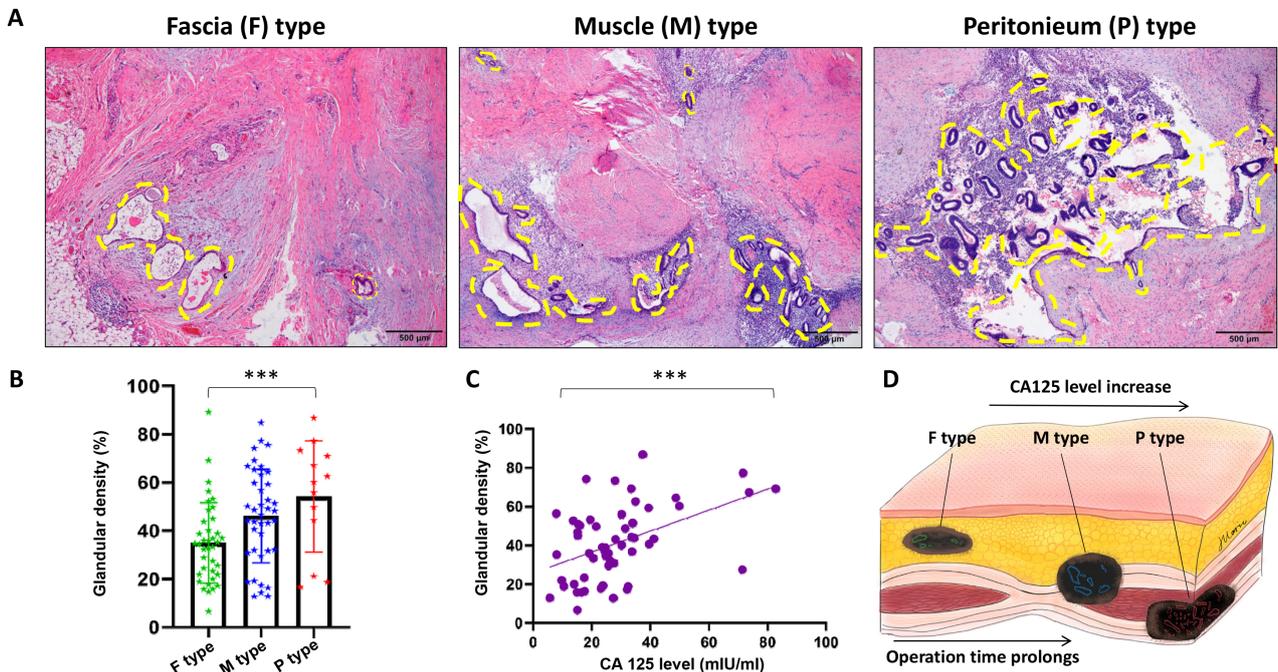


Fig. 3. Deeply infiltrating lesions have a greater microscopic ectopic endometrial glandular density. A. The region within the yellow dotted lines is the glandular area selected for calculation of DOG. B. The average DOG value in H&E slides of AWE patients is lowest in those of F type and highest in those of P type ($P < 0.001$). C. The DOG value in H&E slides correlates with the patient's serum CA125 levels (Pearson correlation coefficient = 0.465, $P < 0.001$). D Schematic presentation of the conclusion of this study.

Table 1. Demographic features of patients among three groups.

	F type (n = 38)	M type (n = 40)	P type (n = 13)	P value
Age	32.8 ± 3.77	32.8 ± 3.19	32.2 ± 5.31	0.858
Gestational week	39.4 ± 1.13	40.1 ± 1.34	39.5 ± 0.84	0.161
Incision type (%)				
verticle	5.3	10	23.1	0.178
transverse	94.7	90	76.9	
Previous CS institu- tion (%)				
non-tertiary hospital	60.7	59.3	42.9	0.683
tertiary hospital	39.3	40.7	57.1	
Previous CS times				
1	94.7	100	92.3	0.281
> 1	5.3	0	7.7	

istics. The average age of patients with the F, M and P types was 32.8, 32.8 and 32.2 years, respectively ($P = 0.858$). The average gestational period for the three groups was 39.4, 40.1 and 39.5 weeks, respectively ($P = 0.161$). Most patients had a transverse incision when CS was performed, with 94.7% in the F type, 90% in the M type and 76.9% in the P type ($P = 0.178$). In the F and M types, more than half the patients had CS performed in a non-tertiary hospital (60.7% and 59.3%, respectively). In contrast more than half the CS (57.1%) in P type patients were performed in a tertiary hospital, although there was no statistical difference ($P = 0.683$). Most patients in all three groups had just one previous CS, with very few having two CS ($P = 0.281$).

3.2 With different infiltration, the three AWE types presented with distinct clinical manifestations

The average mass size was largest in the P type and smallest in F type (Fig. 2A). In the F type, the mass diameter ranged from 0.5 cm to 5 cm, with an average diameter of 2.67 cm. In the M type, the diameter ranged from 1 cm to 8 cm, with an average value of 3.29 cm. Lesions invading the peritoneum were the largest, with an average diameter of 3.73 cm and range from 3 to 5 cm. The difference among these groups was statistically significant (Table. 2) ($P = 0.009$).

As not all cases had information on the serum CA125 level, only 58 cases could be used for CA125-related analyse. The serum level of CA125 increased with the depth of AWE infiltration (Fig. 2B). Among the F type patients, the serum CA125 level ranged from 7.95 mIU/mL to 82.84 mIU/mL, with an average value of 24.8 mIU/mL. In the M type, the CA125 level ranged from 5.73 mIU/mL to 87.66 mIU/mL, with an average of 29.45 mIU/mL. The CA125 value was highest in P type patients, with an average value of 42.26 mIU/mL and range of 21.59 mIU/mL to 73.79 mIU/mL. The difference between the groups was statistically significant (Table. 2) ($P = 0.04$).

Our analysis also showed that a prolonged operation time was associated with increasing infiltration of AWE (Fig. 2C). Patients with F type AWE generally had a 40 minute opera-

tion time, M type patients a 50 minute operation time, and P type patients an average of 82 minute operation time (Table. 2) ($P = 0.02$).

To determine whether there is a link between potential growth time and the infiltrating depth of AWE, we compared the latent time from the gynaecological operation until the development of symptoms. No significant difference was found between the three groups for this factor (Fig. 2D) ($P = 0.588$).

Since mass size could determine the clinical features of the three groups, cases with a mass diameter of at least 3 cm were selected for comparison (Table 2). The analysis of serum CA125 level and operation time showed the same correlations, with P values of 0.02 and < 0.01 , respectively. However, mass size and latent time showed no significant difference among the three groups (Fig. 2E-H, Table 3).

3.3 Microscopic ectopic endometrial glandular density is associated with infiltrating ability

As CA125 is secreted by Mullerian epithelium, we hypothesized that elevated levels of this glycoprotein might be correlated with higher activity and density of ectopic endometrial glands (DOG) in AWE lesions. To evaluate DOG (Fig. 3A), all H&E slides were re-examined for all cases. The average DOG was found to increase with the depth of endometrial infiltration into the abdominal wall (Fig. 3B,C).

4. Discussion

This retrospective study of AWE analyzed 91 cases of AWE diagnosed at one medical center over an 18 year period. Our novel idea was to focus on the three types of infiltration (fascial, muscular and peritoneal) of AWE lesions and to evaluate the correlations between these three types as well as the clinical and pathological characteristics of these cases.

Our analysis found that AWE lesions that infiltrate into deeper abdominal layers are usually larger in size, characterized by higher serum CA125 levels, and require longer time for surgery compared with superficial lesions. There was also a difference in the microscopic DOGs within the masses between the three types of AWE.

Since there is a positive correlation between AWE infiltration and mass diameter, we sought to determine if lesion size was a confounding factor that contributes to different clinical manifestations. It is also rational to think that the larger the mass, the deeper it infiltrates. However, when cases with a size larger than 3 cm were selected for comparison, the results obtained were similar to those of the first analysis. This indicates that deep infiltrating AWEs may present in a specific clinical manner that is distinct from superficial AWEs, which have no association with mass size. This finding is in consistent with previous studies [5, 15–17].

The present study revealed that CA125 serum levels correlated with the lesion's infiltration depth, with this relationship being maintained even after the mass size had been stratified. This observation is also consistent with several other reports [3, 16–20]. It is well known that CA125 is highly ex-

Table 2. Distinct clinical manifestations between three types of AWEs.

	Mass diameters		Serum CA125 levels		Operation time		Latent period	
	Mean ± SD (cm)	P value	Mean ± SD (IU/mL)	P value	Mean ± SD (min)	P value	Mean ± SD (mons)	P value
F type (n = 38)	2.67 ± 1.01		24.78 ± 17.29		39.82 ± 20.51		43.55 ± 39.65	
M type (n = 40)	3.29 ± 1.39	0.009	29.45 ± 16.43	0.041	48.05 ± 20.29	0.000	36.00 ± 26.44	0.588
P type (n = 13)	3.73 ± 0.78		42.26 ± 18.92		82.08 ± 37.25		43.38 ± 37.24	

Table 3. Clinical manifestations between three types of AWEs when selecting cases with mass diameter larger than 3 cm.

	Mass diameters		Serum CA125 levels		Operation time		Latent period	
	Mean ± SD (cm)	P value	Mean ± SD (IU/mL)	P value	Mean ± SD (min)	P value	Mean ± SD (mons)	P value
F type (n = 19)	3.47 ± 0.59		26.72 ± 17.07		43.48 ± 22.59		44.40 ± 39.20	
M type (n = 25)	4.03 ± 1.16	0.146	26.07 ± 11.43	0.021	52.28 ± 21.69	0.000	46.13 ± 31.42	0.881
P type (n = 12)	3.79 ± 0.78		44.84 ± 18.45		83.91 ± 38.29		51.60 ± 31.79	

pressed in tissue of Mullerian origin including endometrium, fallopian tubes and cervix [18–20]. The increased level of CA125 in deep AWE may be caused by the more active ectopic endometrial tissue present in the lesion. Based on this hypothesis, we re-evaluated the H&E slides of all patients and performed a digital pathological analysis for the density of ectopic endometrial glands (DOG). The data shown in Figure 3 indicates that the deeper the mass infiltration, the higher the DOG. However, although a causal relationship between CA125 values and DOGs cannot be drawn here, this correlation warrants further investigation regarding the pathogenesis of various AWEs.

Longer operation time was also correlated with increasing infiltration depth, regardless of the lesion size. This has also been reported in other studies [3, 5, 7, 15–17, 21]. The probable cause for the prolonged operation time with deeply infiltrated lesions is that more time is needed for the surgeon to carefully removed these lesions. More time is also needed to reconstruct the abdominal wall.

Although numerous reports have summarized the clinical features of AWE, the present study is to our knowledge the first to focus on the infiltrating nature of this disease and its implications. We revealed major differences in clinical characteristics depending on the infiltration depth of the lesions, suggesting a possible underlying mechanism at the histopathologic level. More importantly, this study of AWE also provides clinical evidence for the theory that deep infiltrated endometriosis (DIE) has a different pathology compared to common endometriosis [9, 13]. The histopathologic result indicates that microscopic DOG is associated with increasing infiltrating ability, and may be further correlated with the activity level of the ectopic endometrial glands (Fig. 3D).

Because this study is a retrospective review of a relatively rare disease, it has several limitations. First, the sample size was low for the peritoneum group. Second, not all cases had information on the serum CA125 level and hence only 58 cases could be used for CA125-related analysis. Thus, although correlations were seen between infiltration depth, serum CA125 level and DOG, the causal relationships still re-

quire further research.

In summary, this study demonstrated the importance of AWE classification into the three types of infiltration of the abdominal wall (fascia, muscle and peritoneum type), as shown by the correlation of these types with clinical features, serum CA125 levels and length of operation. These characteristics are likely to be determined by the level of activity of ectopic endometrial glands (Fig. 3B). We report here for the first time a relationship between DOG and various characteristics of AWE revealed through our novel digital assessment of H&E stained slides using the digital pathology software Qupath. The strong correlation between DOG and the infiltration depth of AWE not only has clinical significance, but also sheds light on the pathogenesis of deeply infiltrating AWE.

Author contributions

ZX, XGX, XZ and YYH designed the research study. XZ, YYH, NJ, ZGS performed the research. FY provided help and advice on the image analysis. XZ, YYH analyzed the data. ZX, XGX, XZ and YYH wrote the manuscript. YC, FL and FXS helped revise the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethic reference number is YJ-KY-FB-2020-16.

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Conflict of interest

The authors declare no competing interests.

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