

The Correlation between Serum and Peritoneal Fluid CA125 Level in Women with Pelvic Endometriosis

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Abstract

Background: Despite a high prevalence of endometriosis, there still exist many challenges in diagnosing the disease. This study aims to evaluate non-invasive and practical diagnostic methods by measuring serum and peritoneal fluid CA 125 levels in patients with endometriosis. A secondary aim is to determine the correlation between these markers with the stage of disease as well as the relationship of the two markers with each other.

Materials and Methods: This is a cross-sectional study of 60 women who underwent laparoscopy for benign conditions. Based on laparoscopic findings and biopsy results, patients were divided to two groups; one group included patients with pelvic endometriosis (35 patients) and the second enrolled patients free from endometriosis (25 patients). Serum and peritoneal fluid specimens were provided at the time of laparoscopy and CA125 levels were then assessed by electrochemiluminescence immunoassay.

Results: Mean serum and peritoneal fluid CA125 levels were significantly higher in women with endometriosis as compared to the control group (26.42 ± 24.34 IU/ml versus 12.64 ± 6.87 IU/ml in serum and 2203.54 ± 993.19 IU/ml versus 1583.42 ± 912.51 IU/ml in peritoneal fluid, $p < 0.05$). CA 125 levels also varied proportionally with the stage of endometriosis; but showed a significant difference only in higher stages of the disease, both in serum and peritoneal fluid. We calculated the cut-off value suggesting a diagnosis of pelvic endometriosis as 14.70 IU/ml for serum and 1286.5 IU/ml for peritoneal fluid CA125. A linear correlation between CA 125 levels in serum and peritoneal fluid in patients with pelvic endometriosis has also been observed.

Conclusion: Serum and peritoneal fluid CA 125 levels are simple and non-surgical tools for diagnosing and staging pelvic endometriosis. These markers are of greater diagnostic value in higher stages of the disease.

Keywords: Endometriosis, Carcinoma Antigen, Peritoneal Fluid, Laparoscopy

Introduction

Endometriosis can be regarded as one of the most prevalent gynecologic diseases of women in reproductive age. This disease can be found in all ethnic and social groups. Its prevalence rate has been reported to range between 20% and 90% in women suffering from either chronic pelvic pains or infertility. Clinically, the disease can be asymptomatic or present itself with infertility, dysmenorrhea, dyspareunia, or chronic pelvic pain (1).

Contrary to the importance and high prevalence of the disease, there still exist many shortcomings and difficulties in its diagnosis. Clinical examination might only be helpful in diagnosis of deep endometriosis with infiltration to the cul-de-sac and rec-

to vaginal septum. Ultrasonography is only helpful in diagnosis of ovarian endometrioma. On the other hand, endoscopic examination using a laparoscope, which is considered as the gold standard method for diagnosis of the presence, intensity and relapse of endometriosis, is both invasive and expensive (2). In recent years, there have been extensive efforts to create non-invasive, faster and less expensive diagnostic methods including different serum and peritoneal fluid markers. But measurement of these markers, especially CA125 which has been assessed more extensively in comparison with the other markers, has always been accompanied with limitations in the diagnosis of endometriosis especially in the earlier stages of the disease (3).

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This study has been conducted with the aim of evaluating serum and peritoneal fluid CA125 levels and determining the correlation between these markers to facilitate the diagnosis of endometriosis through non-invasive accessible tools.

Materials and Methods

This study has been conducted as a cross-sectional controlled study on 60 women who underwent laparoscopy in their early follicular phase because of infertility, chronic pelvic pain, or recurrent abortion in the *in vitro* fertilization (IVF) center of Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences during 2008- 2009.

Women who received hormonal therapies within six months prior to laparoscopy, women with ovarian neoplasia and other cancers, those with pelvic inflammatory disease (PID) or large uterine myomas were excluded from the study. This study has been approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences and informed consent was taken from all participants.

Based on the laparoscopic findings, patients were divided to two groups; one included 35 cases with pelvic endometriosis and the second included 25 women without pelvic endometriosis as the control group. All endometriosis cases were confirmed by pathologic evaluation of the biopsy specimen provided at the time of laparoscopy. Staging of endometriosis was performed during the laparoscopy procedure by using the classification method introduced by the American Society for Reproductive Medicine (1997) (4).

Peripheral blood samples were taken from the brachial vein before general anesthesia induction in the operation room in order to measure serum CA125. The blood samples were transferred to dry tubes so that blood clotting occurred. Peritoneal fluid was also aspirated from the posterior cul-de-sac and anterior uterovesical fossa before patients were placed in the Trendelenburg position. The peritoneal fluid was held in sterile tubes and centrifuged at 1500 g for ten minutes. The supernatant was collected and stored at -18° C until assayed.

CA125 levels were measured in duplicate in all serum and peritoneal fluid specimens by using a 2010 Elecsys kit (Roche Diagnostic GmbH, D-68298 Mannheim, USA) by the electrochemiluminescence immunoassay (ECLIA) method. The sensitivity of Elecsys 2010 for CA125 is 0.60 IU/ml (lowest measurable level) with a measurement range of 0.600- 5000 IU/ml.

Independent T test was used in order to compare the mean CA125 levels in serum and peritoneal fluid

of the patients with and without endometriosis. The mean serum and peritoneal fluid CA125 levels of different stages of endometriosis were compared using one-way analysis of variance. Pearson Correlation coefficient was used to specify the relationship between CA125 levels in serum and peritoneal fluid of patients with endometriosis. Cut-off points for serum and peritoneal fluid CA125 levels were delineated through ROC curves. Statistical analysis of the data was performed using SPSS- version 16. $p < 0.05$ was considered significant.

Results

There were 46 (66.7%) patients had undergone laparoscopy because of primary infertility and 10 (16.7%) as a result of secondary infertility. Chronic pelvic pain and recurrent abortion were the reasons for laparoscopy in 7(11.7%) and 3 patients (5%), respectively.

Overall, 38.3% (23 patients) had complaints of dysmenorrhea and 16.7% (10 patients) suffered from dyspareunia. The mean age was 28.36 ± 4.02 in the control group and 28.94 ± 4.34 in the group of patients with endometriosis ($p=0.599$).

The mean of serum CA125 levels was 12.64 ± 6.87 IU/ml (range: 3.0 - 31.4 IU/ml) in women without endometriosis and 26.42 ± 24.34 IU/ml (range 6.4 - 100.5 IU/ml) in women with pelvic endometriosis which depicted a significant difference between the two groups ($p=0.003$). The mean of peritoneal fluid CA125 levels was also 138 times higher than its serum levels.

The mean of peritoneal fluid CA125 levels was 1583.42 ± 912.51 in women without pelvic endometriosis, ranging from 33.5 to 3204 IU/ml. Among women with pelvic endometriosis, the mean peritoneal fluid CA125 level was 2203.54 ± 19.993 IU/ml, ranging from 524 to 4336 IU/ml which was significantly higher than the control group ($p=0.017$).

Analysis of variance was used in order to compare serum CA125 levels in different stages of endometriosis. The results revealed that the mean serum CA125 levels showed significant differences in different stages of the disease ($p < 0.001$). As depicted in Fig 1, the mean serum CA125 levels increased as the stages of endometriosis increased (Fig 1).

The results of the multiple comparisons of the means in different stages of endometriosis and the control group by using the Tukey method revealed that this difference was not significant up to Stage II ($p > 0.05$) but the mean serum CA125 levels in Stage III was significantly different from both earlier stages and Stage IV ($p < 0.05$). The mean serum CA125 levels in Stage IV also showed a significant

difference from other stages of the disease as well as from the mean in the control group ($p < 0.05$).

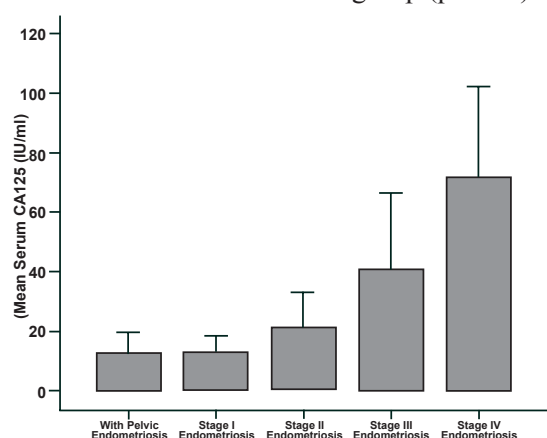


Fig 1: Mean serum CA 125 levels in different stages of endometriosis and the control group.

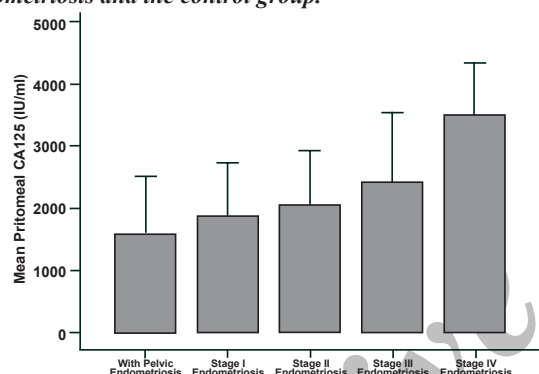


Fig 2: Mean peritoneal fluid CA125 levels in different stages of endometriosis and the control group.

The mean peritoneal fluid CA125 levels also showed an increasing trend in different stages of endometriosis (Fig 2) although the results of the multiple comparison of the means in different stages of endometriosis and the control group revealed that this difference was not significant up to Stage III ($p > 0.05$); but the mean serum CA125 levels in Stage IV showed a significant difference from other

stages of the disease as well as from the mean in the control group ($p < 0.05$). Table 1 illustrates the mean serum and peritoneal fluid CA125 levels in different stages of endometriosis.

Pearson correlation coefficient was used to assess the relationship between serum and peritoneal fluid CA125 levels. The results revealed that in patients without pelvic endometriosis, there was no linear relationship between serum and peritoneal fluid CA125 levels ($p = 0.668$, $r = 0.090$) while in patients with pelvic endometriosis a positive linear relationship was observed between the serum and peritoneal fluid CA125 levels ($p = 0.001$, $r = 0.557$). Using linear regression, an equation of "peritoneal fluid CA125 = $1602.7 + 22.74 \times \text{serum CA125}$ " with a co-efficient of determination (r^2) of 0.311 was obtained for the correlation of CA125 in serum and peritoneal fluid (Fig 3).

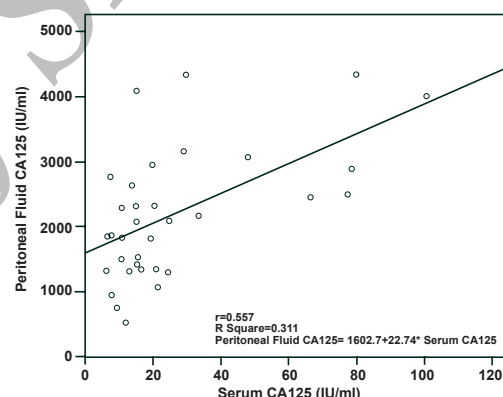


Fig 3: Correlation of serum and peritoneal fluid CA125 levels in women with pelvic endometriosis.

ROC curves were used in order to determine the cut-off values. The cut-off value of serum CA125 was 14.70 IU/ml, with a sensitivity of 65% and specificity of 68%. This was 1286.5 IU/ml for peritoneal fluid, with a sensitivity of 87% and specificity of 52% (Table 2 and Fig 4).

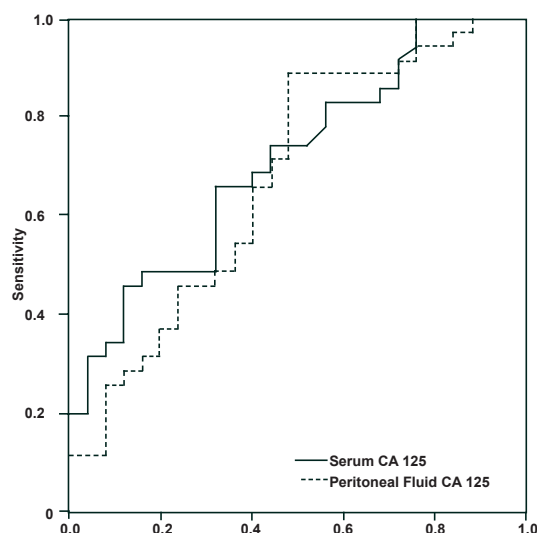
Table 1: Mean serum and peritoneal fluid CA125 levels in different stages of endometriosis and the control group.

Stage	Number of patients (percent %)	Serum CA125 (IU/ml) (mean±SD)	Peritoneal fluid CA125 (IU/ml) (mean±SD)
Without pelvic endometriosis	25 (41.7%)	12.64 ± 6.87 a	1583.42 ± 912.51 a
With pelvic endometriosis	Stage I	17 (28.3%)	13.06 ± 5.35 a
	Stage II	8 (13.3%)	21.24 ± 11.78 a
	Stage III	6 (10.0%)	40.97 ± 25.46 b
	Stage IV	4 (6.7%)	71.72 ± 30.24 c

Identical letters depict non-significant differences while non-identical letters illustrate a significant difference at the 0.05 level.

Table2: Cut-off values for serum and peritoneal fluid CA125 levels in pelvic endometriosis.

Cut off value (IU/ml)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P-Value
Serum CA125 (14.70)	65	68	0.711 (0.581-0.841)	0.006
Peritoneal fluid CA125 (1285.5)	87	52	0.674 (0.533-0.816)	0.022

**Fig 4: ROC curves delineating the cut-off value.**

Discussion

CA125 is a high molecular weight glycoprotein of epithelial origin and is secreted from the celomic epithelium in the embryonic period. In adults, this marker is also secreted from both normal and neoplastic epithelium with celomic origin. The relationship between endometriosis and increased CA125 levels has been revealed in the mid- 80s (5). The increased levels of this marker among women with endometriosis has been attributed to its higher concentration in ectopic endometrium as compared to the entopic endometrium (6).

In a study conducted by Xavier et al. in 2004, through comparison of 25 patients with high-grade endometriosis with 13 controls, it was observed that the mean serum CA125 levels were higher in patients with advanced endometriosis when compared to women without pelvic endometriosis (33 vs. 12.1 IU/ml) (7). This finding is also confirmed in E. Somingliana's study (mean serum CA125 levels of 23.4 IU/ml and 11.4 IU/ml in women with endometriosis and those without endometriosis, respectively). The research team reported higher levels of the marker in cases with more advanced disease (2). This finding was confirmed by later studies as well (8-10). In the present study, the mean CA125 levels were also significantly higher in women with pelvic endometriosis as compared to the control group (26.42 ± 24.34 IU/ml versus 12.64 ± 6.87 IU/ ml) and an increasing trend

was observed in serum CA125 levels with higher stages of the disease, confirming the results of the previous studies.

In this research, the mean peritoneal fluid CA125 levels were almost 138 times higher than levels in the peripheral blood. This finding is concordant with the results of previous studies [Kitawaki et al. (8), H Falconer et al. (9), V Ferreira do Amarel et al. (10)].

Williams and Moen could not show a significant difference between peritoneal fluid CA125 levels among women with and without pelvic endometriosis (12, 13); but in the study conducted on baboons in 2005 by H. Falconer it was revealed that induction of pelvic endometriosis would increase peritoneal fluid CA125 levels. In their study, the mean peritoneal fluid CA125 levels were reported to be 1313 ± 1171 IU/ml and 2523 ± 836 IU/ ml before and after induction of endometriosis, respectively (9). In our study, the mean peritoneal fluid CA125 levels were significantly higher in women with pelvic endometriosis as compared to women spared from the disease (2203.54 ± 993.19 IU/ ml vs. 1583.42 ± 912.51 IU/ ml) and an increasing trend was observed as the stage of endometriosis increased. The increase in production of CA125 in the peritoneal fluid of women with pelvic endometriosis might either originate from the ectopic endometrium or an inflamed and traumatized peritoneum following retrograde menstruation (9).

In a meta-analysis conducted on 23 papers by Mol et al. researchers came to the conclusion that the diagnostic value of serum CA125 levels was higher in severe endometriosis compared to milder cases of the disease. Overall, in this study, the sensitivity of the test for all stages of endometriosis was 28% with a specificity of 90%; specificity declined to 72% as sensitivity rose to 50%. Meanwhile, in case of severe endometriosis, sensitivity of the CA125 test was 47% with a specificity of 98%. With sensitivity of 60%, specificity of the test dropped to 81% (5).

Despite the rising trend in serum and peritoneal fluid CA125 levels with increasing stages of endometriosis in this study; pairwise comparisons of the means of these markers revealed the fact that the aforementioned rise has a more significant difference in higher stages of the disease. Thus, it seems that serum and peritoneal fluid CA125 levels are of higher diagnostic value in higher stages

of the disease.

The cut-off value most commonly considered in literature for CA125 (especially for diagnosis and follow up of ovarian carcinoma) is 35 IU/ml (5, 14). Xavier et al. calculated the cut-off value of 22.6 IU/ml for diagnosis of endometriosis based on serum CA125 levels; a fig which is definitely lower than the values considered in literature (7). Kitawaki et al. recommended two cut-off values of 20 and 30 IU/ml instead of 35 IU/ml for diagnosis of endometriosis in the absence of endometrioma (8). In our study, the cut-off value for diagnosing pelvic endometriosis equals 14.70 IU/ml, which is also a lower fig than mentioned in the literature (35 IU/ml). Based on this finding, CA125 levels higher than 14.7 IU/ml in peripheral blood in the follicular phase of the menstrual cycle can be suggestive of pelvic endometriosis without any other pelvic pathology. The cut-off value of 1286.5 IU/ml is also achieved for peritoneal fluid CA125 levels for diagnosis of pelvic endometriosis, a factor which has hardly received attention in previous studies. Considering the lower number of patients with higher stages of endometriosis, design and implementation of a study with a larger sample population is deemed necessary to find out the accurate cut-off value.

Ferreira do Amaral et al. found a linear correlation between CA125 levels in the serum and peritoneal fluid of 35 patients suffering from pelvic endometriosis and simultaneously they indicated a rise in the mean serum and peritoneal fluid CA125 levels as the stage of endometriosis increased (10). In our study, a linear relationship between CA125 levels in the serum and peritoneal fluid of patients with pelvic endometriosis was also confirmed, while such a relationship was not observed in patients without pelvic endometriosis. Taking this linear correlation into consideration, it can be assumed that blood CA125 levels can represent the disease feature in the pelvic cavity. This highlights the value of serum CA125 in the diagnosis of patients with pelvic endometriosis.

In 1999, Martin and Koninckx concluded that superficial endometriosis caused a more significant rise in peritoneal fluid CA125 levels, whereas cases of deep infiltration of the disease were accompanied with higher CA125 levels in the peripheral blood and lower levels of the marker in peritoneal fluid (15). Similar to Ferreira do Amaral et al.'s study (10), our study does not confirm this finding.

Conclusion

In this study we evaluated serum and peritoneal fluid CA125 levels to see their relationship with

endometriosis and the stages of the disease. They were also assessed as adjacent non-surgical tools for suggesting the diagnosis of endometriosis. We certainly are not considering these tests as a substitute of laparoscopy and biopsy for diagnosing endometriosis. But despite the limitations available in use of CA125 marker in the diagnosis of pelvic endometriosis, and taking the direct relationship between serum and peritoneal fluid CA125 levels into consideration; this study illustrates the fact that this marker could be deemed as a non-surgical, simple, and non-expensive tool for the diagnosis and staging of patients with pelvic endometriosis. At the same time, it has been revealed that the marker was of higher diagnostic value in higher stages of the disease as compared to the lower stages.

Further studies are required to assess diagnosis and staging of patients in lower stages of the disease by the means of serum and peritoneal fluid markers. Simultaneous use of multiple serum and peritoneal fluid markers in order to increase the capability to diagnose the disease can also be considered in these studies.

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