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Original Article

Differential diagnosis of benign ovarian cysts using tumor markers in serum and cyst fluid

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ABSTRACT

Differentiating the type of benign ovarian cyst can result in better care. Aims: To measure CA-125, CA 19-9, Carcinoembryonic Antigen (CEA), and Alpha-fetoprotein (AFP) in serum and cyst fluid of patients with benign ovarian cysts and whether these biomarkers can be used to identify the type of the cyst. Methods and materials: Patients with benign ovarian cysts undergoing laparoscopic cystectomy were included. Cyst types were determined histologically. Levels of CA 125, CA 19-9, CEA and AFP were measured in serum and cyst fluid. Results: 98 cysts (25 functional, 12 endometrioma, 15 dermoid, 28 mucinsous cystadenoma, 18 serous cystadenoma) were evaluated. There was a significant difference in levels of CA 125 and CA 19-9 in serum and CA-125, CA 19-9, and CEA in cyst fluid. For diagnostic purposes, a ≥35 IU/mL value for serum CA 125 predicted endometriomas with a sensitivity of 91.7% and a specificity of 91.9%. A value of ≤22.5 IU/mL for cyst fluid CA 19-9 predicted functional cysts with a sensitivity of 92% and specificity of 95.2%. A ≥100 ng/mL value for cyst fluid CEA predicted mucinous cysts with a sensitivity of 96.4% and a specificity of 96.7%. Conclusion: Levels of CA-125, CA 19-9, and CEA in serum and cyst fluid of patients with benign ovarian cysts can be used as a diagnostic tool for inpatient evaluation with acceptable sensitivity and specificity. This finding can be used in conjunction with other methods such as ultrasound, especially in cases that are harder to diagnose.

Keywords: Ovarian cyst, tumor marker, CA 125, CA 19-9, CEA, AFP

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INTRODUCTION

It is estimated that about 7% of the women worldwide experience ovarian cysts at least once in their lifetime [1]. These cysts are usually asymptomatic and are detected during the pelvic examination and imaging [2]. Most of these cysts are benign and have a low chance of malignant transformation [2-4]. Symptoms (if any) are usually nonspecific, such as pain or sensation of pelvic pressure [2-4] although cyst rupture and ovarian torsion might lead to more severe manifestations [4].

Functional cysts (such as follicular, corpus luteum, and theca lutein cysts) are the most common type of ovarian cysts and result from a variation of the normal ovulation [2-4]. Other types of ovarian cysts include (but are not limited to): endometriomas, dermoid cysts, mucinous and serous cystadenomas [2].

Most studies in the literature focus on differentiating between malignant and benign cysts. Several methods have been proposed with different levels of accuracy [3,5], including ultrasound, tumor markers, and scoring systems combining various factors [6-9].

Differential diagnoses of benign cysts have remained more obscure. Some proposed methods include ultrasound and tumor markers [10-12]. Definitive diagnosis of ovarian cysts requires surgical removal of the cyst and subsequent histopathological analysis [13]. Determining the type of the cysts would help to decide on appropriate treatment [5,11,14]. For example, functional cysts will usually resolve spontaneously and require no intervention [3,5,15]. Other types of ovarian cysts such as endometriomas or dermoid cysts might require surgical intervention[11,16-18]. Precise identification of the cyst could prevent unnecessary interventions and surgeries.

In the present study, we measured levels of a panel of tumor markers including cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) in sera and cyst fluids of patients with different types of benign ovarian cysts. Our aim was to determine if the levels of these tumor markers are significantly different among various types of ovarian cysts and whether these can be used to identify the type of the cyst.

MATERIAL AND METHODOLOGY

Patient selection

This prospective study was conducted for one year from April 2018 until April 2019, at Imam Hossein Hospital, Tehran, Iran. Patients admitted to gynecological ward with benign ovarian cysts were included in this study. We excluded pregnant patients, smokers, and patients with inflammatory bowel disease (IBD). Hemorrhagic and/or malignant cysts were also excluded.

Measurements

Blood samples were obtained from patients before the surgery and sent to the lab for analysis. Cysts were removed by laparoscopic ovarian cystectomy and sent to the laboratory within 30 minutes of removal. Levels of CA-125, CA19-9, CEA, and AFP were measured in serum and cyst fluid using Electrochemiluminescence (ECL) with Cobas e 411 analyzers (Roche Diagnostics, Tokyo, Japan). Each of the cysts were sectioned using a microtome and stained using hematoxylin and eosin. A certified pathologist examined these histological slides and determined cyst types using World Health Organization (WHO) classification 19. The pathologist and the laboratory staff were blind to the result of each other's work.

Statistical Analysis

The Normality of data for tumor marker levels in each category was evaluated using Shapiro–Wilk test. The data did not follow a normal distribution in most of the categories. Therefore, a non-parametric test (Kruskal-Wallis H) test was used to compare differences between groups. Post hoc Mann–Whitney U test evaluated pairwise differences among individual groups, adjusted for multiple comparisons using Bonferroni correction. Ultimately, we used median and interquartile ranges to describe the level of tumor markers. One-way analysis of the variance test (ANOVA) was used to evaluate differences in age among patients

since it roughly followed a normal distribution. We used mean and standard deviation to describe the age of the patients. Parity was described using frequency and percentage. Differences in cyst histology between nulliparous and multiparous patients was investigated using the chi-square test of independence and Cramer's V, followed by a post-hoc analysis of adjusted standardized residuals corrected by the Bonferroni approach. A p-value≤ 0.05 was considered statistically significant in all cases (unless corrected by the Bonferroni approach). All statistical analysis was performed using SPSS software (Version 25.0, IBM Corp).

Study ethics

All patients in this study provided informed written consent beforehand, and their names remain confidential. This study is in accord with the Declaration of Helsinki of 1946 and was approved by the Institutional ethics committee.

RESULTS

Patient characteristics

103 patients met the study criteria. The type of the cysts determined by histological examination were: 25 (24.3%) functional, 12 (11.7%) endometrioma, 15 (14.6%) dermoid, 28 (27.2%) mucinous cystadenoma, 18 (17.5%) serous cystadenoma. Five (4.9%) cysts did not belong to any of the groups and were excluded from the analysis, leaving a total of 98 patients. A total of 98 sera and 88 cyst fluids were analyzed. Ten (seven endometrioma and three dermoid) cyst fluids were excluded from analysis due to high viscosity. The mean age of the patients was 30.55 (SD=10.44, Range=14-70). There was no statistically significant difference in the age of the patients (P=0.218). Characteristics of the patients are shown in table I, categorized by cyst type.

Tumor markers in serum

Median (IQR) and range for tumor marker levels in sera of different cyst types are shown in table II. A Kruskal-Wallis H test revealed a statistically significant difference between levels of CA 125 and CA 19-9 in sera of different cyst types (P<0.001 for both cases). Post hoc analysis using the Mann-Whitney U test showed that CA 125 levels in endometriomas were significantly higher than all other cyst types (P<0.001 for all cases).

Table 1. Characteristics of the patients, categorized by cyst type

	Age		Nulliparity
	Mean (SD)	Range	Frequency (%)
Overall (n=98)	30.6 (10.4)	14-70	35 (35.7)
Functional (n=25)	31.7 (11.1)	15-56	10 (40)
Endometrioma(n=12)	31.3 (7.7)	21-45	8 (66.7)
Dermoid (n=15)	25.73 (8)	18-44	9 (60)
Mucinous cystadenoma (n=28)	29.57 (7.8)	18-45	3 (10.7)
Serous cystadenoma (n= 18)	34.00 (15)	14-70	5 (27.8)

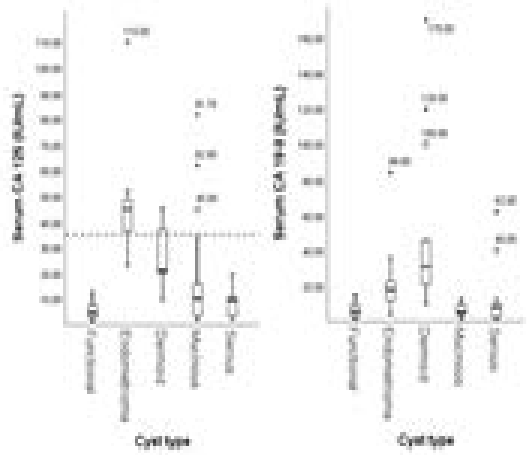

Figure 1. Tumor markers in serum (outliers not shown for clarity)

TCA 125 levels in dermoid cysts were significantly higher than functional and serous cysts (P<0.001 in both cases). CA 125 levels in mucinous cystadenomas were significantly higher than functional cysts (P=0.005). No other statistically significant difference in serum CA 125 levels were

observed. CA 19-9 levels were significantly higher in endometriomas than functional, mucinous, and serous cysts (P≤0.001 for all cases). CA 19-9 levels in dermoid cysts were significantly higher than functional, mucinous, and serous cysts (P<0.001 for all cases). No other statistically significant difference in serum CA 19-9 levels were observed. There was no statistically significant difference between CEA and AFP levels among sera of different cyst types (P=0.095 and P=0.840, respectively). Comparisons with statistical significance are shown in figure 1.

Tumor markers in cyst fluid

Median (IQR) and range for tumor marker levels in cyst fluid of different cyst types are shown in table III. A Kruskal-Wallis H test revealed a statistically significant difference between levels of CA 125, CA 19-9, and CEA in cyst fluids of different cysts types (P<0.001 for all cases). Post hoc analysis using the Mann-Whitney U test showed that CA 125 levels in functional cysts were significantly lower than all other cyst types (P≤0.001 for all cases). No other statistically significant difference in cyst fluid CA 125 levels were observed. CA 19-9 levels in functional cysts were significantly lower than all other cyst types (P<0.001 for all cases). CA 19-9 levels in serous cystadenomas were significantly lower than dermoid and mucinous cysts (P=0.002 and P=0.001, respectively). No other statistically significant difference in cyst fluid CA 19-9 levels were observed. CEA levels in mucinous cystadenomas were significantly higher than all other cyst types (P<0.001 in all cases). CEA levels in dermoid cysts were significantly higher than functional and serous cysts (P<0.001 for both cases). No other statistically significant difference in cyst fluid CEA levels were observed.

Table 2: Tumor markers in serum

		CA 125	CA 19-9	CEA	AFP
Functional	Median (IQR)	4.60 (5.2)	5.7 (6.6)	2.6 (5.9)	1.4 (3.2)
	Range	1.20-12.98	1.40-15.2	0.8-9.61	0.3-9.49
Endometrioma	Median (IQR)	45 (39.9)	18 (42.1)	2.7 (2.7)	1.6 (1.4)
	Range	23-110	3.9-84	1.16-5.29	0.8-4.52
Dermoid	Median (IQR)	21(20.8)	31 (63)	2.7 (5.7)	1.4 (1.3)
	Range	9.6-45.3	10-170	1.5-12	0.8-5.5
Mucinous cystadenoma	Median (IQR)	10.1 (12.6)	5.2 (6.2)	2.2 (3.2)	1.5 (1)
	Range	1.76-81.7	1.00-12.9	0.8-12	0.6-4.6
Serous cystadenoma	Median (IQR)	9.5 (7.7)	7.9 (7.1)	4.7 (4.7)	1.3 (1.5)
	Range	1.8-19.7	1.42-62	0.9-8.56	0.2-3.8

Table 3: Tumor markers in cyst fluid

		CA 125	CA 19-9	CEA	AFP
Functional	Median (IQR)	23 (48.7)	8.7 (7.4)	0.7 (0.8)	0.9 (1.1)
	Range	0.40-7501	2.7-52	0.1-3.9	0.1-3.2
Endometrioma	Median (IQR)	5001(3363.5)	1001 (585)	0.9 (37.3)	0.6 (1.6)
	Range	274-5001	131-1201	0.1-52	0.2-2.5
Dermoid	Median (IQR)	2044(4000)	1051(860.8)	45(54.6)	0.4 (0.8)
	Range	51.1-5001.1	0.5-1401	10-773	0.1-1.6
Mucinous cystadenoma	Median (IQR)	3001(3900)	1001(375.8)	1101(175)	0.5(0.5)
	Range	48-5001	21.5-120	40-1401	0.3-1.67
Serous cystadenoma	Median (IQR)	5001 (3250)	57 (67.75)	0.6 (0.8)	0.4 (0.6)
	Range	90-7501	20-1201	0.1-2.5	0.1-3.3

The values for CA 125 and CA 19-9 are in IU/mL. The values for CEA and AFP are in ng/mL.

There was no statistically significant difference between AFP levels among cyst fluids of different cyst types (P=0.097). Comparisons with statistical significance are shown in figure 2.

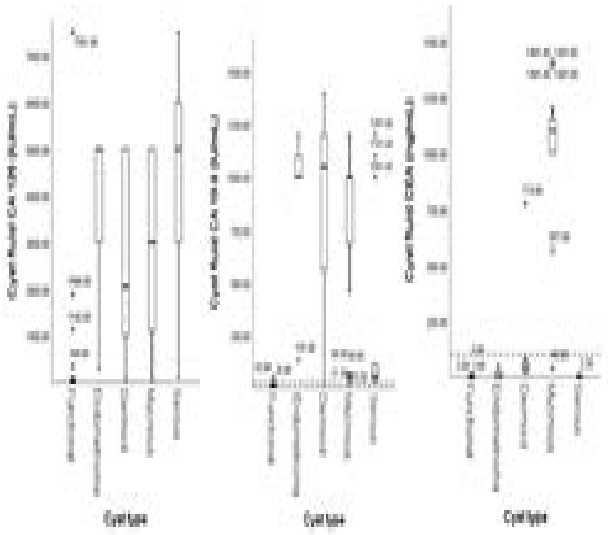

Figure 2. Tumor markers in cysts fluid

Using tumor markers for differential diagnosis

In this study, a high level of serum CA 125 was observed in endometriomas. A cut-off value of ≥35 IU/mL for serum CA 125 predicted endometriomas with a sensitivity of 91.7% and a specificity of 91.9%.

Functional cysts had lower tumor marker levels than most other histotypes. Using a cut-off value of ≤22.5 IU/mL for cyst fluid CA, 19-9 resulted in a sensitivity of 92% and specificity of 95.2% for detecting functional cysts.

Exceptionally high levels of cyst fluid CEA were observed in mucinous cysts. Using a cut-off value of ≥100 ng/mL cyst fluid, CEA provided a sensitivity of 96.4% and specificity of 96.7% for mucinous cysts.

Using tumor markers for other cyst types and/or using a combination of tumor markers was of little clinical value in this sample.

Differences in cyst types between parity groups

There were 35 (35.7%) nulliparous patients in this study. The Pearson chi-square test of independence showed at least one count of difference between groups (p-value=0.002). The Cramer's V test showed a strong association (V=0.419). In the post-hoc analysis, the only significant comparison after Bonferroni correction was the difference between mucinous cysts between nulliparous and multiparous patients (p-value= 0.001). The frequency of cyst types in different parity groups is depicted in figure 3.

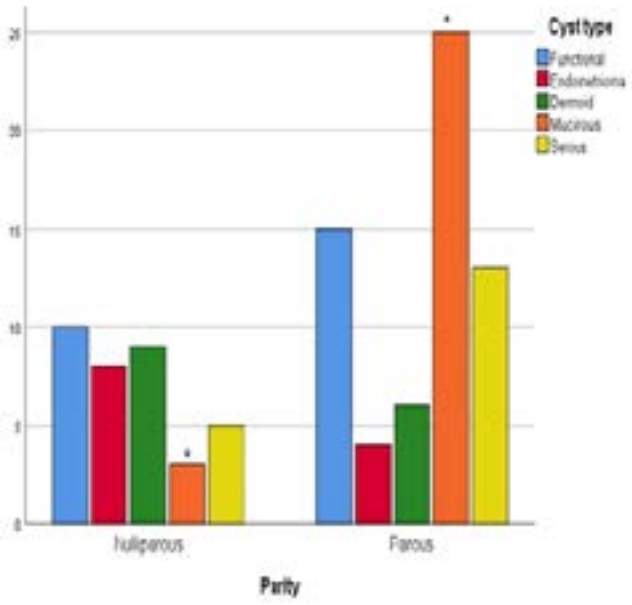

Figure 3. Cyst types categorized by parity

DISCUSSION

Our results indicate that serum concentrations of CA 125 and CA 19-9 and cyst fluid concentrations of CA 125, CA 19-9, and CEA significantly differ between various cyst types. Therefore, these tumor markers might be of use for differential diagnosis of benign ovarian cysts. There was no significant difference in serum concentrations of CEA and AFP and cyst fluid concentrations of AFP between various cyst types. Therefore, measuring these tumor markers in ovarian cysts is of little value.

Only a handful of studies in the literature focus on differences between biomarker levels among different types of benign ovarian cysts. Furthermore, results from these studies are not unanimous, and some of these studies even report no statistically significant difference in biomarker levels [19].

Halila et al. [20] found high levels of cyst fluid CEA in all mucinous cystadenomas of their sample. We confirm this finding. In fact, the levels of cyst fluid CEA in our sample was so high that a cut-

off value of 100 ng/mL would predict mucinous cystadenomas with a sensitivity of 96.4% and a specificity of 96.7%, as mentioned earlier. They reported significantly higher CA 125 levels in the cyst fluid of serous cystadenomas compared to mucinous cystadenomas. We found no such association.

Similar to our findings and those of Halila et al., higher CEA levels in cyst fluid were associated with mucinous histotype in the study by Pinto et al. [12]. The investigators found low concentrations of CA 125, CA 19-9, and CEA in functional cysts' fluid, which is mostly consistent with our findings. They reported high CA 125 and low levels of CEA and AFP in cyst fluid of serous cystadenomas. We reached the same result about CEA, but not the other two tumor markers. We also agree that AFP is of low diagnostic value for ovarian cysts.

Koninckx et al. [11] observed higher CA 125 in cyst fluid of endometriomas compared to corpus luteum (functional) cysts. In contrast to our study, Koninckx et al. used 10,000 IU/mL as the cut-off point for distinguishing endometriomas and functional cysts (with 100% sensitivity and specificity), whereas, in our study, the highest cyst fluid CA 125 level for endometriomas was 5001.00 IU/mL. Although, our results should be treated with caution as only five endometrioma cystic fluids were included in our study.

In the study by Vercellini et al. 14, serum CA 125 in endometriomas was significantly higher than other cyst types, which is consistent with our results. Similar to our study, they chose a cut-off value of 35 IU/mL for serum CA 125 in endometriomas with a sensitivity and specificity of 61.8% and 94.3%, respectively. Furthermore, they designated serum CA 19-9 level of 16 IU/mL as the cut-off value for endometriomas, which contradicts our study as 13 (86.66%) of our patients with dermoid cysts exceeded this cut-off point. They also reported a significantly higher serum CA 19-9 in endometriomas than mucinous cystadenomas, which we confirm.

Serum CA 125 levels in a study by Van Calster 21 were generally higher than our results (median=37), and using 30 IU/mL in their result identified 63% of endometriomas. They discarded CA 125 as a clinically valuable diagnostic tool for ovarian cysts. We believe that CA 125 levels alone should not be used to determine cyst type. Levels of this tumor marker can elevate in other conditions such as malignancies (e.g., ovarian cancer), diseases (e.g., pelvic inflammatory disease), and physiological conditions (e.g., pregnancy and menstruation) [3,22].

As mentioned before, a definitive diagnosis of an ovarian cyst requires surgical excision and histopathological analysis. Currently, ultrasound is considered the most accurate approach for preoperative diagnosis of benign ovarian cysts, yielding a sensitivity between 88% to 100% and a specificity between 62% and 96% in some studies 15. However, the precision of ultrasound mainly depends on the skill of operator [2]. Even with an experienced sonographer, mistakes are sometimes unavoidable since some cysts are harder to differentiate. This case is especially true in cysts that appear in many forms or share morphological features with other cyst types [4,11,15]. For example, mucinous and serous cystadenomas have standard features that make them difficult to differentiate in ultrasound. It has been suggested not to rely on ultrasound in such instances [15]. In such cases, using our cut-off value of 100 ng/mL of CEA in cyst fluid would facilitate the diagnosis. Endometriomas are also difficult to diagnose on ultrasound [15], and serum CA 125 levels can act as a complement to ultrasound.

The relationship between parity and cyst types is not well studied, but there seems to be a higher risk of overall incidence of ovarian cysts in women with lower parity [23].

CONCLUSION

In the present study, there was no meaningful correlation between parity and cyst type, except for the fact that multiparous patients showed a significantly higher incidence of mucinous cysts compared to nulliparous ones. Therefore, any definitive opinion for this matter requires further investigation.

This study only relied on tumor markers for differential diagnosis of benign ovarian cysts. We suggest combining the results of this study with other approaches (especially ultrasound) to obtain accurate results. There might be other biomarkers

valuable in the diagnosis, which were not measured in this study. Further studies with higher sample populations are required to validate the results of this study.

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