RESEARCH ARTICLE

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D-dimer, Fibrinogen and Tumor Marker Levels in Patients with benign and Malignant Ovarian Tumors

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Abstract

Limited studies have investigated the differences between the levels of plasma coagulants and tumor markers in ovarian cancer. Therefore, we conducted this study to determine and compare the level of coagulation, fibrinolysis and tumor markers in patients with benign and malignant ovarian tumors. This cross-sectional study was conducted between January 2022 and February 2023 in Imam Hossein Hospital on patients with ovarian mass. Laboratory tests included platelet count, PT, INR, PTT, fibrinogen and D-dimer were sent to the pathology laboratory to be examined by a pathologist. Based on histopathology, patients were divided into benign, borderline and malignant groups. Logistic regression was used for determine predictors of malignancy. Receiver operating characteristics (ROC) curves and their corresponding 95% CI were determined for the predictor value of the full model. From 141 investigated patients, tumor type in 124 (87.94%) patients were benign, in 12 (8.51%) was malignant and in 5 (3.55%) was borderline. D-dimer, Ca-125 and HE4 were significantly higher in the patients with malignant tumor type (P<0.001), whereas AFP was significantly higher in patients with borderline tumor type (P<0.001). With one-unit increase in D-dimer odds of borderline/malignant tumor 0.3% increases (OR=1.003, 95% CI: 1.001, 1.006) and with one-unit increase in Ca-125 odds of borderline/malignant tumor 1% increases (OR=1.01, 95% CI: 1.003, 1.02). We found that plasma fibrinogen, D-dimer and Ca-125 levels are independently associated with malignant ovarian tumors and combined use of these markers has the high discriminant power for distinction of benign and malignant ovarian masses.

Keywords: D-dimer- Fibrinogen- tumor marker- ovarian tumor- malignant tumor- Benign tumor

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Introduction

Ovarian cancer is the seventh most common cancer in women, with 313,959 women diagnosed with ovarian cancer worldwide and 207,252 women dying from this cancer in 2020 (Sung et al., 2021). This cancer is the most common neoplasia of the reproductive system in the first 2 decades of life (Huang et al., 2022). Ovarian tumors are very diverse due to their histological structure, and there is also a great diversity in the prevalence of ovarian cancers and also the types of ovarian tumors in different societies. The highest rate of ovarian cancer is reported in Scandinavia, followed by Israel and the United States, and the lowest rate is in developing countries and Japan (Momenimovahed et al., 2019).

Coagulation caused by tumors is closely related to the growth of tumors. Malignant diseases may show signs of venous thromboembolism (Prandoni et al., 2005). A vicious cycle is established between procoagulant proteins and malignant tumor cells by promoting neovascularization and metastasis (Falanga et al., 2009). There is evidence that activated fibrinogens prevent the removal of tumor cells by natural killer cells, increase the survival of circulating tumor cells, increase the risk of tumor metastasis, and lead to a poor prognosis (Palumbo et al., 2005). Therefore, D-dimer levels have clinical value for predicting prognosis and differential screening of benign and malignant tumors (Ryu et al., 2019).

D-dimer levels may be an effective tool to use as a diagnostic marker and design more effective and individualized treatment strategies. In addition, disruption of the coagulation system may be the first sign of tumor cell proliferation. For this reason, D-dimer may be useful in cancer screening or monitoring for cancer recurrence. Finally, measurement of plasma D-dimer level is a non-invasive and cost-effective test that can be useful in early cancer screening, monitoring cancer recurrence, and as a cancer prognostic tool (Hanna et al., 2013). Activation of blood coagulation can be an obvious indicator of latent cancer, which has been confirmed by clinical trials in

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cancer-free subjects, which show that hypercoagulation is a risk factor for possible death from cancer (Hong et al., 2010). One study reported that tumor cells directly stimulate the coagulation system, disrupt endothelial vessel wall integrity, and increase fibrinolytic protein and platelet activity (Neises et al., 1993). However, further research is needed to clarify the biological mechanism of how high D-dimer affects malignancy in cancer patients. In another way plasma fibrinogen is a key acute phase protein and known to be raised in patients with ovarian cancer (Hefler-Frischmuth et al., 2015). The role of some coagulation factors in tumor progression have been studied previously (Sampson and Kakkar, 2002).

However, limited studies have investigated the differences between the levels of plasma coagulants and tumor markers in ovarian cancer. Therefore, this study was designed to determine and compare the level of coagulation, fibrinolysis and tumor markers in patients with benign and malignant ovarian tumors.

Materials and Methods

The current cross-sectional (descriptive-analytical) study was conducted between January 2022 and February 2023 in Imam Hossein Hospital affiliated to Shahid Beheshti University of Medical Sciences, Iran on patients with ovarian mass.

In this study the inclusion criteria were: age over 18 years, willingness to participate in the study; and admission to the women's department for elective surgery. Moreover, exclusion criteria were pregnancy; other gynecological malignancies (cervix or uterus), history of deep vein thrombosis (DVT), pulmonary thromboembolism (PE), or history of chemotherapy or radiotherapy; and receiving anticoagulants (such as clopidogrel and warfarin).

In this research, data was collected based on the census method and patients entered to the study through consecutive available sampling. The Ethics Committee of the Shahid Beheshti University of Medical Sciences approved the study. Before participating in the study, the study protocol was read to the patients and all participants completed the informed consent form. First, patients were interviewed by a member of the research team to assess age, body mass index (BMI) and history of co-morbidities. On the day of the operation at 8:00 AM, a blood sample was taken from the peripheral veins of each patient by a trained nurse. Then the samples (citrated plasma) were quickly sent to the hematology laboratory of Imam Hossein Hospital at a temperature of 20 ° C. Laboratory tests included platelet count, PT, INR, PTT, fibrinogen and D-dimer. The tissue samples obtained from the operation were sent to the pathology laboratory to be examined by a pathologist. Based on histopathology, patients were divided into benign, borderline and malignant groups. In addition, malignant pathologies were evaluated according to the FIGO staging system. Finally, the laboratory results were compared between the stages.

The coagulation factors and tumor marker levels were compared between three groups formed according histopathology results using ANOVA test. Categorical variables were compared between groups through Chi-square and fisher- exact test.

We used logistic regression model for determine predictors of malignancy. In this model outcome was dichotomous (benign or borderline/ malignant). Hosmer and lemeshow approach was used for model building and variables with the P-value less than 0.2 in the crude model were entered to the adjusted model. Receiver operating characteristics (ROC) curves and their corresponding 95% CI were determined for the predictor value of the full model. All data were analyzed using Stata 14 software (Stata Corp, Texas). The significant level was considered less than 0.05.

Results

A total of 141 patients were included to the study during the mentioned time period. Tumor type in 124 (87.94%) patients was benign, in 12 (8.51%) was malignant and in 5 (3.55%) was borderline. The mean age in patient with benign, malignant and borderline tumor was 47.53, 53.33 and 54.4 years, respectively (P=0.17). Moreover, the mean

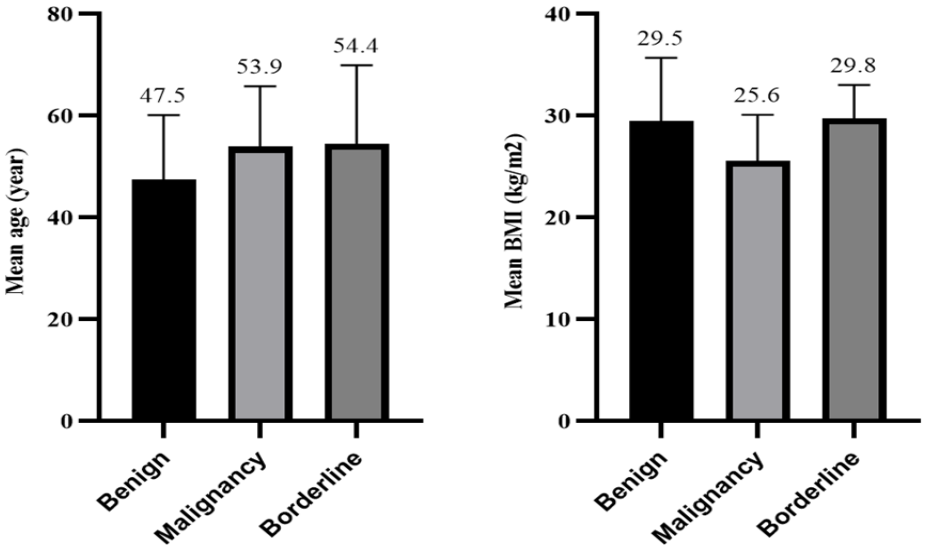

Figure 1. The Mean Age and BMI of the Patients According the Tumor Type

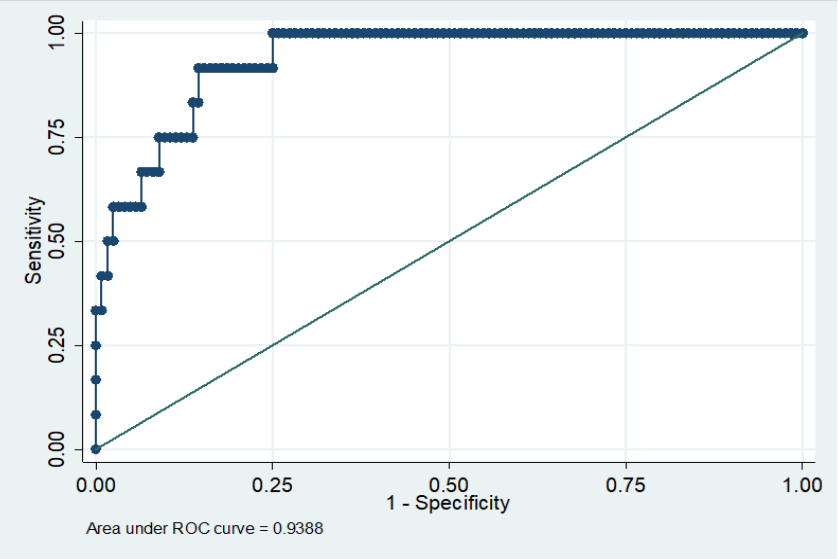

Figure 2. Area under the Curve (AUC) for Assessing the Discriminant Power of the Explanatory Variables for the Occurring of Borderline/Malignant Tumor

Table 1. Biochemical Characteristics of the Patients According the Tumor Type

Variable	Tumor type			P-value
	Benign N=124	Malignant N=12	Borderline N=5	
Hemoglobin	12.68±1.68	12.25±2.36	13.26±0.97	0.53
Platelets	268153.2±66241.13	286333.33±97888	246600±51451.9	0.52
PT	11.9±1.22	12.19±1.63	11.26±0.61	0.38
PTT	24.65±4.89	25.42±2.21	24.12±2.64	0.83
INR	1.62±3.50	1.09±0.17	1.01±0.05	0.81
Fibrinogen	204.39±53.22	235.08±66.82	182.4±29.85	0.11
D-Dimer	275.97±249.33	733.42±703.26	417.6±321.15	<0.001
Ca-19.9	19.63±29.3	43.68±84.03	29.08±18.96	0.12
Ca-125	28.68±61.04	229.89±451.82	16.79±13.89	<0.001
B-hCG	2 ± 0.39	2	2	0.99
AFP	2.06±1.44	3.15±2.81	18.63±30.54	<0.001
CEA	1.44±1.3	1.56±0.69	2.49±1.4	0.19
HE4	47.6±22.1	180.19±29171	52.54±17.44	<0.001

HE4, human epididymis protein 4; Ca-125, Cancer antigen 125; Ca-19.9, carbohydrate antigen 19.9; CEA, carcinoembryonic antigen

BMI in these patients was 29.49, 25.6 and 29.76 kg/m^2, respectively (P=0.05) (Figure 1).

In Table 1 we compared biochemical characteristics of the patients according the tumor type. As shown D-dimer, Ca-125 and HE4 were significantly higher in the patients with malignant tumor type (P<0.001), whereas AFP was significantly higher in patients with borderline tumor type (P<0.001). Results of the Chi-square test showed that there was not a significant relationship between tumor type of the patients and their menopause status (P=0.35)

Table 2. Relation between Ttumor Type and Menopause Status and Co-Morbidity with Other Diseases

Variable		Tumor type			P-value
		Benign	Malignant	Borderline	
Menopause status	Pre- menopause	74 (59.68)	5 (41.67)	2 (40)	0.35
	Post-menopause	50 (40.32)	7 (58.33)	3 (60)	
Co-morbidity	Hypertension	29 (23.39)	4 (33.33)	2 (40)	0.46
	Diabetes	19 (15.32)	4 (33.33)	0	0.19
	Thyroid disease	19 (15.32)	1 (8.33)	1 (20)	0.73
	HLP	14 (11.29)	2 (16.67)	1 (20)	0.53

Table 3. Biochemical Characteristics of the Patients According the Tumor Type and Menopause Status

Variable	Pre-menopause			Post-menopause		
	Benign	Malignant	P-value	Benign	Malignant	P-value
Hemoglobin	12.21±1.66	13.59±1.02	0.03	13.39±1.46	11.82±2.34	0.007
Platelets	280932.4±70405.3	254142±87008.5	0.34	249240±54936	289000±88905.7	0.07
PT	12.07±1.29	11.16±0.61	0.07	11.66±1.09	12.45±1.65	0.06
PTT	25.24±3.79	25.53±2.96	0.84	23.77±6.11	24.7±1.88	0.64
INR	1.36±2.39	0.98±0.02	0.68	2.01±4.69	1.22±0.17	0.55
Fibrinogen	198.26±53.71	223.71±58.82	0.24	213.48±51.69	216.7±67.91	0.87
D.Dimer	260.97±228.19	396.43±181.95	0.13	298.18±278.64	811.4±767.19	<0.001
Ca-19.9	20.18±31.99	27.25±16.53	0.57	19.28±21.32	43.52±83.98	0.07
Ca-125	30.36±54.43	74.74±81.64	0.05	26.2±70.22	231.95±501.64	0.006
AFP	2.09±1.42	20.6±28.99	<0.001	-	-	-
CEA	1.23±1.29	1.26±0.66	0.96	1.74±1.27	2.18±1.08	0.33
HE4	42.57±13.74	45.7±16.59	0.61	54.93±29.17	197.06±302	0.009

HE4, human epididymis protein 4; Ca-125, Cancer antigen 125; Ca-19.9, carbohydrate antigen 19.9; CEA, carcinoembryonic antigen

Table 4. Predictors of Borderline/Malignant Status Using Multivariable Logistic Regression

Variable	Odds Ratio	95% Confidence interval	P.value
BMI	0.70	0.55, 0.91	0.006
Diabetes	5.820	0.98, 34.38	0.050
PT	0.510	0.2, 1.26	0.140
Fibrinogen	1.012	0.998, 1.025	0.070
D-dimer	1.003	1.001, 1.006	0.003
Ca-125	1.010	1.003, 1.02	0.009

and co-morbidity with other diseases (P>0.05) (Table 2).

In Table 3 we compared biochemical markers according the tumor type in premenopausal and postmenopausal patients separately. In premenopausal patients, hemoglobin, Ca-125 and AFP was significantly higher in patients with malignant tumor (P<0.05). In postmenopausal patient's hemoglobin was significantly higher in patients with benign tumor (P=0.007), whereas Ca-125, AFP and HE4 was significantly higher in the patients with malignant tumor (P<0.05).

The results regarding the predictors of occurring malignant or borderline tumor using multivariable logistic regression is presented in Table 4. As shown, with one-unit increase in fibrinogen odds of borderline/malignant tumor 1% increases (OR=1.012, 95% CI: 0.998, 1.025). With one-unit increase in d-dimer odds of borderline/malignant tumor 0.3% increases (OR=1.003, 95% CI: 1.001, 1.006) and with one-unit increase in Ca-125 odds of borderline/malignant tumor 1% increases (OR=1.01, 95% CI: 1.003, 1.02). As shown in Figure 2, The above final model that involved all the significant determinants has a high discrimination power (AUC= 0.94), with the 92% sensitivity and 79% specificity.

In patients with benign tumor, Serous cyst adenoma, simple cyst and follicular cyst in the 25.81%, 18.55%, and 11.29% of cases were the common subtype. High grade papillary Serous was the common subtype in malignant cases (58.33% of cases) and serous borderline tumor was the common subtype in borderline cases (60% of cases) (Table 5).

Table 5. Tumor Sub-Types in the Patients

Tumor subtype	Tumor type		
	Benign	Malignant	Borderline
Hemorrhagic cyst	7 (5.65)	-	-
Serous cyst adenoma	23 (18.55)	-	-
Mucinous adenoma	13 (10.48)	-	-
Fibrothecoma & fibroma	5 (4.03)	-	-
Endometrioma	13 (10.48)	-	-
Mucinous adenocarcinoma	-	1 (8.33)	-
Follicular cyst	14 (11.29)	-	-
Simple cyst	32 (25.81)	-	-
Stroma ovary	1 (0.81)	-	-
Mature teratoma	11 (8.87)	-	-
Advanced endometriosis	5 (4.03)	-	-
High grade papillary Serous	-	7 (58.33)	-
Low grade serous carcinoma	-	2 (16.67)	-
Serous borderline tumor	0	-	3 (60)
Granulose cell tumor	-	1 (8.33)	-
Clear cell carcinoma	-	1 (8.33)	-
Mucinous borderline	-	-	2 (40)
Total	124	12	5

Discussion

The purpose of this study was to levels of plasma coagulants and tumor markers in patients with benign, borderline and malignant ovarian tumors. The results of the study indicated that, in premenopausal patients, hemoglobin, Ca-125 and AFP was significantly higher in patients with malignant tumor. In postmenopausal patient's hemoglobin was significantly higher in patients with benign tumor, whereas Ca-125, and HE4 was significantly higher in the patients with malignant tumor.

We could not find a significant relationship between tumor type of the patients and their menopause status and co-morbidity with other diseases. In this study, BMI, diabetes status, fibrinogen, D-dimer and Ca-125 were the significant predictors of borderline or malignant tumor with the 94% discrimination power.

In our study, after adjusting on the other covariates, plasma fibrinogen was the significant differential diagnostic marker of malignant tumor. The effect of elevated plasma fibrinogen levels on malignant tumor in ovarian cancer has proven in other studies (Polterauer et al., 2009; Hefler-Frischmuth et al., 2015). Results of the conducted study by Qiu et al. revealed that elevated plasma fibrinogen levels is associated with the poorer response to chemotherapy (Qiu et al., 2012). Given that inflammatory response occurred following malignant tumor, it seems that the raised fibrinogen level in these patients is associated with the increased systemic inflammatory response caused by tumor progression (Falanga et al., 2009).

In the present study elevated D-dimer was higher in ovarian cancer patients compared to benign masses. Association between increased D-dimer in plasma with poor prognosis in other cancers has been showed in the conducted studies in this regards (Erdem et al., 2014; Li et al., 2020; Liu et al., 2020). Discrimination power of D-dimer between benign and malignant ovarian tumors had been proven in other studies (Den Ouden et al., 1998; Worasethsin and Narkwichean, 2013).

Results of the study by Gadducci et al. revealed that if both test results od D-dimer and CA-125 be in the below cut-off value, combined use of D-dimer and CA-125 had 100% specificity to exclude malignant ovarian tumor (Gadducci et al., 1996). Therefore, Blood coagulation and fibrinolytic pathway activated by ovarian cancer cells can be proven by measurement of fibrin degradation product such as serum D-dimer (Worasethsin and Narkwichean, 2013). In the study by Stukan et al., (2019) a combination of two simple ultrasound predictors and D-dimers better diagnosed malignant disease from benign ovarian lesions compared with CA 125 and RMI.

Although in our study elevated Ca-125 was associated with was higher in ovarian cancer patients compared to benign masses, however evidence showed that this marker alone has the low sensitivity and specificity for disease severity differentiation (Molina et al., 1992; Bast et al., 2005). This tumor-marker has the higher sensitivity in the high stages of the disease (Zheng et al., 2018). Therefore, researchers have suggested the combined use of serial CA-125 and ultrasonography in postmenopausal asymptomatic women to early diagnosis of ovarian cancer (Jacobs and Menon, 2004; Bast et al., 2005). Although, the American College of Obstetricians and Gynecologists use of CA-125 for disease classification and differentiation, but neither is comprehensively accepted in the United States (American College of Obstetricians Gynecologists, 2002).

Choosing the patients from the single center with the consistent diagnostic criteria, eligibility criteria and treatment modalities, and the obtained interesting results can be considered as the strengths of the study. However, low sample size in the malignant and borderline groups and small numbers within strata of categorical factors causes sparse-data bias is a limitation of the study.

In conclusion, we found that plasma fibrinogen, D-dimer and Ca-125 levels are independently associated with malignant ovarian tumors and combined use of these markers has the high discriminant power for distinction of benign and malignant ovarian masses.

Author Contribution Statement

FF Conceived and designed the analysis. All authors had the same contribution in the other parts of the study including data collection, analysis of the data and wrote the paper.

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Ethic approval

The Ethics Committee of the Shahid Beheshti University of Medical Sciences approved the study.

Conflict of interest

The authors claimed no conflict of interest.

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