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Original Article

THE ROLE OF THREE PLASMA PROTEINS IN THE DIAGNOSIS OF OVARIAN TUMORS

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Summary

Ovarian cancer is not common, but it is still the fifth leading cause of death from malignant diseases among women worldwide. More than 200,000 women are diagnosed with ovarian cancer each year globally. Due to its asymptomatic course, most patients are diagnosed at a late stage. Therefore, ovarian cancer (OC) has the highest mortality among gynecological malignancies. Unfortunately, there is no adequate screening program for the early detection of ovarian cancer, and as a result, this diagnosis escapes clinicians. The protocol for early diagnosis of OC is currently a combination of elevated cancer antigen 125 (CA 125) and transvaginal ultrasonography (TVUS). However, it does not meet the necessary cost-effectiveness criteria and is therefore not recommended by any working group to screen ovarian cancer in the general population. The biomarkers with the highest informative value should be selected individually or combined in multi-biomarker panels from the many biomarkers strongly associated with OC. Numerous such panels of biomarkers and algorithms have been developed for the early diagnosis and differentiation of OC from other benign ovarian diseases. These panels or biomarkers need to be sufficiently reliable and show measurable changes in non-invasive samples obtained from patients with early-stage OC. Their reliability would significantly reduce mortality from this aggressive disease and improve the patient's prognosis.

Keywords: ovarian cancer, transthyretin, apoA1 lipoprotein, CA 125, diagnostic reliability

Introduction

Cancer of the ovary (OC) is the eighth most common cancer in women worldwide and the second most commonly diagnosed gynecological malignancy. Mortality from this disease exceeds that of any other gynecological cancer [1]. According to the Bulgarian National Cancer Registry, OC is the fifth most common in women and represents 5.5% of all malignancies [2]. The disease is asymptomatic in the early stages, which is why 85% are diagnosed at an advanced stage when it is advanced or metastatic, and the

survival rate is low. The survival rate is increased if effective detection of the early stages of OC is possible [3]. Standard methods for proving OC are gynecological examination, TVUS, and serum CA 125 levels [4]. The ultrasound finding does not provide information about malignancy, so an invasive procedure is needed, i.e., a biopsy. CA 125 is a routinely used tumor marker for diagnosing and monitoring OC. However, it is not sensitive enough as an individual marker to detect all cases of early ovarian cancer, as it increases by only 50% in the early stages of the disease. Many benign gynecological diseases also cause an increase in the concentration of CA 125 [5]. Such an increase shows its poor specificity, making it difficult to distinguish between benign and malignant ovarian diseases. Efforts to develop a biomarker for early detection of OC have shown that no individual biomarker, including CA 125, can provide sufficient diagnostic sensitivity (DSn) with high diagnostic specificity (DSp). Therefore, there is an urgent need for developing additional informative biomarkers to complement CA 125 to achieve the required diagnostic efficiency (DE) [6]. Given the low prevalence of OC, any proposed strategy according to Jacobs IJ and Menon U must demonstrate a minimum DSp of 99.6% and Dsn > 75% to achieve a positive predictive value (PPV) of 10% and avoid the unacceptable level of false-positive results [7, 8].

By determining the serum concentrations of three plasma proteins - transthyretin (TTR), ApoA1lipoprotein (ApoA1LP), and CA125, this study aims to assess their role in diagnosing women with ovarian tumors. TTR is the major transport protein of serum thyroxine and facilitates retinol transport via retinol-binding protein. The lower plasma concentration of this protein is associated with an increased rate of malignant transformation in the ovarian epithelium. According to some studies, TTR shows 47% sensitivity with 95% specificity in ovarian cancer [26]. Some authors have found that TTR levels are inversely related to tumor volume in OC [27].

ApoA1LP is the main protein component of high-density lipoproteins. There is evidence that the levels of ApoA1LP in the serum of patients with ovarian cancer are reduced [15-17]. This reduction is thought to be related to free radical damage to cell biomembranes, leading to lipid peroxidation. Malondiadlehydrate (MDA) is a by-product of lipid degradation. MDA-DNA complexes are likely to be promutagenic, causing mutations in oncogenes and tumor suppressor genes observed in human tumors [28]. CA125 is the gold-standard biomarker for ovarian cancer. The serum levels of CA125 are widely used to distinguish benign from malignant ovarian tumors and monitor the clinical course of patients with ovarian cancer. [29]. According to some authors, it is a poor diagnostic tumor biomarker in premenopausal women, non-serous histologies, and early-stage diseases because only 50% -60% of women with early-stage OC have elevated serum CA125 levels [30]. Falsely elevated levels are common in some benign conditions such as pregnancy, uterine fibroids or intra-abdominal infections, and other intraperitoneal pathology [31]. According to some researchers, when CA 125 combines with ApoA1LP and TTR, there is a significant improvement in overall sensitivity and specificity: 93.9% sensitivity at 95% specificity [9], or 74% sensitivity and 97% specificity, according to others [10].

The present study describes experiments designed to determine whether changes in the concentrations of these proteins occur in an established ovarian tumor and whether these proteins, alone or in combination, can help preoperatively distinguish benign from malignant conditions. As far as we know, such a study among women in the Bulgarian population has not been conducted. According to many authors, combining these three parameters helps to significantly increase the diagnostic reliability (DR) in proving the OC [9-14]. These assumptions provoked us to establish the DR of these indicators among women in the Bulgarian population.

Materials and Methods

We conducted a prospective study on patients divided into a target and a control group. The target group included 120 women with ovarian tumors, proven by a gynecological examination and TVUS and operated in a gynecological clinic at the St. Marina University Hospital in Pleven. Of these, 60 patients had histologically proven OC, and the remaining 60 had ovarian cysts re-diagnosed by histopathological biopsy.

The other group of patients were 60 healthy controls without proven ovarian tumor during gynecological examination and TVUS, referred to the St. Marina University Hospital for a preventive examination. All patients signed informed consent. Pleven Medical University funded the study, which the University Ethics Commission approved. The group of patients with OS (n = 60) included women aged 57.67 (range 29-83). The group of women with ovarian cysts (n = 60) included patients aged 40.23 (range 21-70). The control group included women aged 39.92 (range 22-68).

We analyzed 5 ml of venous blood from Greiner's Vacuette tubes in all patients. We centrifuged the blood at 3500 rpm (rpm) for 15 minutes. The separated serum was transferred to Eppendorf tubes and stored at -20 ° C until analysis (within 3 months). The serum concentrations of the three plasma proteins were investigated in the Clinical Laboratory of the St. Marina University Hospital. We determined the concentrations of TTR and ApoA1LP using an immunoturbidimetric method of biochemical analysis (AU 480 Beckman Coulter). The level of CA125 was measured by enzyme-linked fluorescence analysis on an immunoassay Tosoh AIA 360. Statistical analysis was performed using the Mann-Whitney U comparative test of patient results and the controls. P values <0.05 were assessed as statistically significant. We determined the diagnostic reliability (DR) by calculating the DSn, DSp, DE, PPV, and negative predictive value (NPV) of each indicator separately and in different combinations between them in three directions. First, we aimed to prove its change in women with ovarian tumors. The second objective was to determine the significance of its change is in the differential diagnosis of OC and benign ovarian diseases and see if we can use it to detect the OC.

Results

We determined the serum concentrations of TTR, ApoA1LP, and CA 125 in 180 women. We adopted reference values for serum TTR 0.2-0.4 g/l, for serum ApoA1> 1.40 g/l and 125 0-35 IU / mL for serum CA 125 0-35 IU/ mL. We made the measurements in three stages. We first compared women with a proven ovarian tumor with healthy controls. There were 120 patients with a proven ovarian tumor. The average serum concentration of TTR is 0.20 g / l (range 0.03-0.33 g / l), of ApoA1LP - 1.76 g / l (range 1.08 -2.62 g / l) and CA125 - 343.07 IU / mL (range 2.1-1892 IU / mL). The mean serum concentration of CA125 in the controls was 14.92 IU / mL (range 3-51.6 IU / mL), the TTR was 0.27 g / l (range 0.18-0.32 g / l), and the ApoA1LP is 1.80 g / l (range 1.30-2.32 g / l). We compared the mean values of the laboratory parameters we studied in the two groups and found a statistically significant difference for TTR and CA 125, presented in Table 1.

Table 1. Statistically significant differences in women with ovarian tumors and controls

Parameter	Ovarian tumors	controls	P
TTR	0.20 (SD 0.07)	0.27 (SD 0.04)	< 0.0001
ApoA1LP	1.76 (SD 0.27)	1.80 (SD 0.24)	0.169
CA 125	343.07 IU/mL (SD 490.7)	14.92 IU/mL (SD 9.30)	< 0.0001

Table 2. DR of TTR, ApoA1LP and CA 125 individually and in combination in the detection of ovarian tumor

	DSn%	Dsp%	DE%	PPV%	NPV%
TTR	44.2 %	90%	59.4%	89.8%	44.6%
ApoA1LP	10.8%	95 %	38.9%	81.3%	34.8%
CA 125	63.3%	96.7%	74.4%	97.4%	56.9%
TTR+ApoA1LP+ CA 125	39.4%	93.9%	57.6%	89.5%	45.4%
ApoA1LP+ CA 125	37.1%	95.9%	56.7%	89.4%	45.9%
TTR+ CA 125	53.8%	93.4%	66.9%	93.6%	50.8%

We determined the DR separately for each indicator and in combinations between them, presented in Table 2.

We found that CA 125 was the best DR regarding ovarian tumors.

In the second stage, to distinguish OC from benign ovarian diseases, we compared women with histologically proven OC (n = 60) and those with a histological diagnosis of ovarian cyst (n = 60). In the group diagnosed with OS, the mean serum concentrations were as follows: TTR 0.17 g/l (range 0.03-0.33 g/l), ApoA1LP 1.68 g/l (range 1.08-2.36 g/l)), and CA125 - 640.70 IU/mL (range 4.2-1892 IU/mL). In women diagnosed with ovarian cyst, the serum level of CA 125 was 45.43 IU/mL (range 2.1-450.5 IU/mL), the TTR is 0.23 g/l (range 0.14-0.31 g/l), and ApoA1LP 1.84 g/l (range 1.38-2.62 g/l).

We found a statistically significant difference for all three parameters Table 3.

We determined the DR of these three plasma proteins alone and in combination. The data are presented in Table 4.

We found the highest DSn, DE, and NPV of CA125 and the highest DSp and PPV of ApoA1LP.

In the third stage of our study, we compared the studied indicators in women with OC (n = 60) and healthy controls (n = 60). We found a statistically significant difference for TTR and CA 125. The average values of the concentrations of the three parameters are presented in Table 5.

We determined the DR of these three plasma proteins alone and in combination, comparing these two groups. The results showed that the DR of CA125 was again the highest. The data are presented in Table 6.

Table 3. Statistically significant differences between patients with OC and ovarian cyst

Parameter	OC	**Ovarian cyst**	P
TTR	0.17 g/l (SD 0.08)	0.23 g/l (SD 0.05)	< 0.0001
ApoA1LP	1.68 g/l (SD 0.29)	1.84 g/l (SD 0.23)	0.00174
CA 125	640.70 IU/mL (SD 548.38)	45.43 IU/mL (SD 69.08)	< 0.0001

Table 4. DR of TTR, ApoA1LP, and CA 125 in differentiating OC and benign ovarian diseases

	DSn%	**DSp%**	**DE%**	**PPV%**	**NPV%**
TTR	65%	76.7%	70.8%	73.6%	68.7%
ApoA1LP	20%	98.3 %	59.2%	92.3 %	55.1%
CA 125	90%	63.3%	76.7 %	71%	86.4%
TTR+ApoA1LP+ CA 125	58.3%	79.4%	68.9%	79%	70.1%
ApoA1LP+ CA 125	55%	80.8%	68%	81.7%	70.8%
TTR+ CA 125	77.5%	70%	73.8%	72.3%	77.6%

Table 5. Statistically significant differences in women with OC and controls

Parameter	OC	**Controls**	P
TTR	0.17 (SD 0.08)	0.27 (SD 0.04)	< 0.0001
ApoA1LP	1.68 (SD 0.29)	1.80 (SD 0.24)	0.017
CA 125	640.0 IU/mL (SD 540.38)	14.92 IU/mL (SD 9.30)	< 0.0001

Table 6. DR of TTR, ApoA1LP, and CA 125 separately and in combination when proving OC

	DSn%	**DSp%**	**DE%**	**PPV%**	**NPV%**
TTR	65%	90%	77.5%	86.7%	72%
ApoA1LP	20%	95%	57.5%	80%	54.3%
CA 125	90%	96.7%	93.3%	96.4%	90.6%
TTR+ApoA1LP+ CA 125	58.3%	93.9%	76.1%	87.7%	72.3%
ApoA1LP+ CA 125	55%	95.9%	75.4%	88.2%	72.5%
TTR+ CA 125	77.5%	93.4%	85.4%	91.6%	81.3%

Discussion

Our study focused on women with ovarian tumors. Comparing the group of women with a proven ovarian tumor and the group of healthy controls, we showed that the TTR and ApoA1LP levels were lower in women with ovarian tumors and the concentration of CA 125 in the same group of patients was higher. Comparing the women with OC and those with ovarian cysts, we showed that plasma concentrations of TTR and ApoA1LP were lower in women with OC, and the concentration of CA 125 was significantly higher. Comparing women with OC to the healthy controls, we found the same dependencies. These results indicate that, in the presence of an ovarian tumor, there are changes in the concentrations of these three plasma proteins, which are statistically significant: the values of TTR and ApoA1LP decrease, and those of CA 125 increase. The relationship between OC and ApoA1LP, TTR, and CA125 has been discussed in publications. [15-17]. TTR and ApoA1LP are characterized as negative acute-phase proteins. Some authors have established a link between OC and inflammation [18-20]. Ovulation itself is a natural inflammatory process that involves cyclic rupture and healing of the ovarian cortex, and according to some authors, it is a major factor in ovarian cancer [21,22]. Also, patients with endometriosis or pelvic inflammatory disease are at an increased risk of ovarian cancer [23,24]. According to some researchers, oral contraceptives that suppress ovulation reduce the risk of ovarian cancer [24]. Hysterectomy has been shown to protect against OC by preventing the retrograde spread of proinflammatory factors to the ovaries [23-25].

Conclusions

In our study, we found that the DR of the combination of the three plasma proteins, as well as the different combinations between them in pairs, was significantly lower than the DR of CA 125 not only for diagnosing ovarian tumors but also for distinguishing OC from benign diseases of ovaries and proving OC. Identifying more sensitive and specific biomarkers for the early detection of OC remains a challenge. Furthermore, implementing such biomarkers will lead to a significant increase in survival rates. It is essential to check the DE of different multi-label panels and validate them in large populations.

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