Preoperative serum vascular endothelial growth factor (VEGF) in ovarian masses

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Summary

Purpose of the investigation: To determine the diagnostic value of serum vascular endothelial growth factor (VEGF) in the preoperative assessment of the nature of ovarian masses.

Materials and Methods: A propective cohort study was conducted from August 2001 to September 2002 on 40 premenopausal and 23 postmenopausal patients with ovarian masses. During preoperative workup, patient age, serum Ca-125 levels, serum VEGF levels, and tumor volume based on ultrasonographic examination were determined. Laparoscopic (n=23) or laparotomic (n=39) approaches were undertaken to obtain the final pathologic result. According to the final ovarian pathology, follicular cysts, corpus luteum cysts and endometriomas were grouped as non-neoplastic ovarian masses (n=40, group I). Serous or mucinous cyctadenomas, dermoid tumors and fibromas were allocated into the neoplastic benign ovarian mass group (n=10, group II). Primary malignant ovarian neoplasms were categorized into the neoplastic-malign group (n=12, group III).

Results: Mean ages of cases among groups I, II and III were 39.0 ± 2.0, 42.2 ± 5.2 and 56.9 ± 4.2, respectively. As age of the cases enrolled in this sudy increased, the more likely was the occurrence of neoplastic malign ovarian pathologies ($p < 0.001$). Among postmenopausal cases diagnosed with an ovarian mass, serum Ca-125 levels were 113.5 ± 20 IU/ml compared to those in premenopausal cases (85.8 ± 16.0, $p = 0.05$). The values for serum VEGF values among pre- and postmenopausal ovarian masses were 46.2 ± 6.7 pg/ml and 68.2 ± 7.9, respectively ($p = 0.04$). In group 1, serum VEGF levels of endometriomas (56.5 ± 1.5 pg/ml) were higher compared to those of follicular or corpus luteum cysts (30.6 ± 2.8, $p = 0.05$). In contrast, tumor size appeared to be larger in non-endometriotic, non-neoplastic cysts (10.1 ± 2.0 cm), compared to endometriomas (6.4 ± 0.6 cm, $p < 0.01$). Serum VEGF levels of group III were higher than other groups ($p < 0.001$). With respect to the discriminating benign or malign nature of the mass, with a specific cut-off value of serum VEGF level of 68.7 pg/ml, the sensitivity, specificity, positive and negative likelihood ratios were 92.3%, 88.0%, 3.3 and 0.1, respectively. For serum Ca-125 levels, the sensitivity, specificity, positive and negative likelihood ratio with a statistically relevant cut-off value of 102 IU/ml were, 76.9%, 76.0%, 3.21 and 0.3, respectively. Area under curve (AUC) for serum VEGF and Ca-125 values were 0.938 (95% CI: 0.81-0.96) and 0.769 (95% CI: 0.64-0.86), respectively ($p = 0.02$). Among the postmenopausal group, AUC for serum VEGF and Ca-125 was detected as 0.902 (95% CI: 0.70-0.98) and 0.873 (95% CI: 0.66-0.91) ($p = 0.14$).

Conclusion: Serum VEGF has the potential to be considered as a tumor marker with a good diagnostic relevance in differentiating the nature of ovarian masses.

Key words: Vascular endothelial growth factor; Ovarian mass; Diagnosis.

Introduction

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a homodimeric 34-42kDa heparin-binding glycoprotein with potent angiogenic, mitogenic and vascular premeability enhancing actions for various physiologic and pathologic events in the ovary [1]. Angiogenesis takes part in several processes in ovarian physiology like folliculogenesis, follicular selection and corpus luteum support [2]. In addition, VEGF is considered to be a potent angiogenic growth factor most strongly implicated in several ovarian pathologies such as, aging ovaries (due to hypoxic environment), polycystic ovary syndrome, ovarian hyperstimulation syndrome and benign or malignant ovarian masses [3]. Current data pertaining to the angiogenesis of the ovary have revealed a correlation between tumor neovascularisation, aggressiveness of the tumor growth and metastatic spread [4, 5].

This study was designed to demonstrate the diagnostic relevance of serum VEGF and Ca-125 levels among benign and malignant ovarian masses

Revised manuscript accepted for publication December 12, 2002

Materials and Methods

Forty premenopausal and 23 postmenopausal women diagnosed with pelvic masses were enrolled in this prospective cohort study from August 1991 to September 2002 in a university hospital setting. All cases were preoperatively assessed with a thorough clininal examination, ultrasonographic evaluation of the tumor, serum Ca-125 and VEGF level determinations. Following the decision of a surgical approach, either laparoscopy (n=23) or laparotomy (n=39) was performed in all cases. According to the final histopathologic result of the tumor, tumor specimens were allocated into non-neoplastic (group I, n=40), neoplastic-benign (n=10, group II) or neoplastic-malign (n=12, group III). Follicular cysts, inclusion cysts, corpus luteum cysts and endometriomas were asssigned as non-neoplastic ovarian masses. Serous or mucinous cystadenomas, dermoid cysts, fibroids or benign Brenner tumors were categorised into neoplastic-benign lesions. Primary ovarian malignant neoplasms were allocated into the neoplastic-malign group. All the patients were examined with transvaginal or transabdominal gray-scale and real-time pulsed Doppler ultrasonography (Toshiba SSA-250A). Due to inconsistency in the routine Doppler assessment, especially in postmenopausal cases, together with the lack of routine utility of color Doppler measurements, the Doppler findings were not taken into account for statistical analyses. Since, among postmenopausal cases, ultrasonographic features of the pelvic mass (tumor volume, solid/cystic appearence, presence

and thickness of septa, uni- or bilaterality, intratumoral papillary excrescences), together with serum tumor markers were considered to be suggestive of a malignant nature of the ovarian mass.

Venous samples were taken by venipuncture from the antecubital arm and stored with heparin-enriched sterile tubes. After 30 minutes for completion of coagulation of samples, the tubes were centrifuged at 1200 g for 10 minutes and then kept at –70°C. VEGF level was determined by using the quantitative sandwich ELISA technique (Quantikine, human VEGF immunoassay, Research and Diagnostic Systems Inc., Minneapolis, MN), as previously described [6]. Intra-assay and inter-assay coefficients of variation (CV) of the test were 4.5% and 7.0%, respectively. The concentration of vascular endothelial growth factor in plasma samples was represented as picogram per milligram of protein (pg/ml). Serum Ca-125 levels were assessed by a standard immunoradiometric assay and represented as IU/ml.

Statistical analyses were performed using the Statistical Package Programme for Social Sciences (SPSS 10.0, Chicago, IL, USA). The unpaired Student's t-test and ANOVA with posthoc Tukey HSD were performed for continuous data analyses. Correlation analysis was performed with the Pearson correlation analysis. Receiver operating curves and areas under curves (AUC) with specific sensitivities, specificies, positive and negative likelihood ratios for different cut-off values were calculated (Medcalc® software version 6.16, Belgium). Confidence intervals were expressed within 95% limits. Data were expressed as mean ± SEM; a p value of < 0.05 was considered statistically significant.

Results

The mean ages of cases in groups I, II and III were 39.0 ± 2.0, 42.2 ± 5.2 and 56.9 ± 4.2, respectively). As the age of the cases enrolled in this study increased, the more likely was the occurence of neoplastic malign ovarian pathology ($p < 0.001$). Pathologic findings of non-neoplastic, neoplastic-benign and neoplastic-malign cases are shown in Table 1. Non-neoplastic pathologies consisted of 15 follicular cysts, nine corpus luteum cysts, ten endometriomas, three tubo-ovarian abscesses and three inclusion cysts. For the neoplastic-malign group, apart from ovarian malignancies, one synchronous ovarian and endometrial carcinoma of endometrioid histiologic type were present. Of the ovarian malignant tumors, eight tumors were of epithelial and three were of germ cell origin. Among postmenopausal cases diagnosed with ovarian masses, serum Ca-125 levels were 113.5 ± 20 IU/ml compared to those in premenopausal cases (85.8 ± 16.0, $p = 0.05$). The values for serum VEGF values among pre- and postmenopausal ovarian masses were 46.2 ± 6.7 pg/ml and 68.2 ± 7.9, respectively ($p = 0.04$) (Table 2). Serum VEGF levels of group III were higher than the other groups ($p < 0.001$), as presented in Figure 1.

There was a fairly relevant correlation between serum Ca-125 and VEGF levels in the study group (r_p: 0.389, $p < 0.01$). However, no correlation was observed between tumor volume and serum Ca-125 level (r_p: 0.01, $p > 0.05$) and serum VEGF levels (r_p: –0.07, $p > 0.05$) among the study group. As shown in Table 2 tumor volume in the neoplastic-benign group appeared to be higher compared to group I and group III ($p < 0.001$). In group I serum VEGF levels in endometriomas (56.5 ± 1.5 pg/ml) were higher compared to those of follicular or corpus luteum

Table 1. — *Final pathologic results among non-neoplastic (n=40), neoplastic-benign (n=10) and neoplastic-malign groups (n=13), respectively.*

Findings	n
Non-neoplastic (n=40)	
Follicular cysts	15
Corpus luteum cysts	9
Inclusion cysts	3
Endometrioma	10
Tubo-ovarian abscess	3
Neoplastic-benign (n=10)	
Serous cystadenoma	3
Mucinous cystadenoma	2
Fibroma	1
Mature cystic teratoma	1
Benign Brenner tumor	1
Thecoma	1
Neoplastic-malign (n=12)	
Serous cystadeocarcinoma	5
Serous borderline tumor	1
Mucinous cystadenocarcinoma	2
Clear cell carcinoma	1
Yolk sac tumor	1
Endodermal sinus tumor	1
Ovarian ca + endometrium ca	1

Table 2. — *Age, tumor volume, serum Ca-125 and serum VEGF level distributions among non-neoplastic, neoplastic-benign and neoplastic-malign groups. Parentheses represent 95% confidence intervals (CI).*

Variables	Non-neoplastic (n=40)	Neoplastic-benign (n=10)	Neoplastic-malign (n=12)
Age	39.0 ± 2.0	2.2 ± 5.2	56.9 ± 4.2
	(34.9 ± 43.2)	(30.3 ± 54.2)	(47.7 ± 66.1)*
Tumor volume	8.4 ± 1.3	10.2 ± 1.2	8.3 ± 1.1
	(5.8 ± 11.0)	(7.3 ± 13.0)**	(5.9 ± 10.8)
Ca-125	87.2 ± 21.4	73.2 ± 21.4	228.7 ± 72.8
	(43.8 ± 130.5)	(24.8 ± 121.6)	(70.0 ± 387.4)π
VEGF	41.2 ± 6.7	53.2 ± 2.1	95.3 ± 7.8
	(27.5 ± 54.8)	(43.1 ± 63.4)	(78.1 ± 112.4)τ

* $p < 0.001$, compared to groups I and II; ** $p < 0.001$, compared to groups I and III; π $p = 0.003$ compared to group I and $p = 0.008$ compared to group II; τ $p < 0.001$, compared to groups I and II.

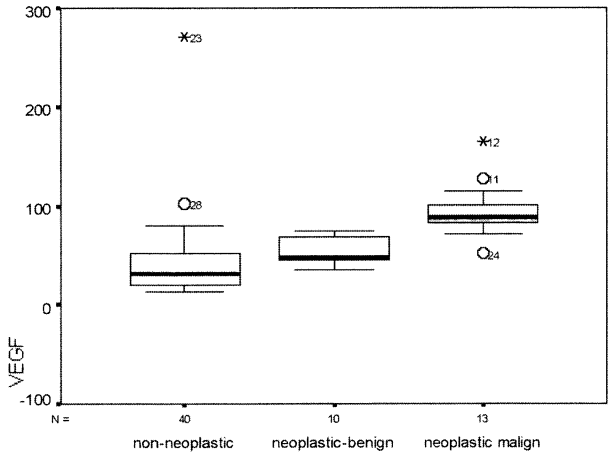

Figure 1. — Serum VEGF values (pg/ml) among three pathologic subgroups (*$p < 0.05$ compared to other groups).

cysts (30.6 ± 2.8 pg/ml, p = 0.05). In contrast, tumor size appeared to be larger in non-endometriotic, non-neoplastic cysts (10.1 ± 2.0 cm), compared to endometriomas (6.4 ± 0.6 cm). Hence, in the non-neoplastic group, serum VEGF levels were correlated with the nature of the ovarian mass rather than the tumor volume.

Among the study groups, for the discrimination of the benign and malign nature of the ovarian mass – with 68.7 pg/ml as a cut-off value for serum VEGF – the sensitivity, specificity, positive and negative likelihood ratios were 92 %, 88.%, 3.3 and 0.1, respectively (Table 3). For serum Ca-125 levels with a statistically relevant cut-off value of 102 IU/ml, the sensitivity, specificity, positive and negative predictive values were 76.9%, 76.0%, 3.2 and 0.3, respectively.

ROC curves for both cut-off values were constructed and are illustrated in Figures 2 and 3. Area under curve (AUC) for serum VEGF and Ca-125 values were, 0.938 (95% CI: 0.81-0.96) and 0.769 (95% CI: 0.64-0.86), respectively (p = 0.02) (Figure 4). In the postmenopausal group, AUC for serum VEGF and Ca-125 was detected as 0.902 (95% CI: 0.70-0.98) and 0.873 (95% CI: 0.66-0.91) (p = 0.14) (Figure 5).

Discussion

This study has demonstrated that although only a small number of cases were enrolled, serum VEGF determination can be considered as a preoperative tumor marker to predict the likelihood of the benign or malign nature of ovarian masses. However, in our study it was not possible to assess the value of serum VEGF and serum Ca-125 in the premenopausal subdivision of the total cases, since only two neoplastic malign cases were detected and statistical analyses with those small number of cases did not reveal any significance. Hence, only in postmenopausal adnexal masses was statistical analysis possible, which constituted one limitation of our study.

Table 3. — *Different cut-off values for serum VEGF and Ca-125 levels with sensitivity, specificity, positive likelihood ratios (LR+) and negative likelihood ratios (LR–) indicated among the study group.*

Cut-off values	Sensitivity (%)	Specificity (%)	LR (+)	LR (–)
Serum VEGF(pg/ml)				
12.8	100	0	100	–
48.5	100	72	3.5	0.10
51.2	92	72	3.3	0.11
68.7*	92	88	3.3	0.11
71.1	84	94	7.0	0.16
74.9	84	94	14.1	0.17
78.6	76	94	12.8	0.25
100	23	96	5.7	0.80
Serum Ca-125 (IU/ml)				
37	92	54	2.0	0.10
41	76	54	1.6	0.40
102*	76	76	3.2	0.30
121	69	80	3.4	0.38
135	61	86	4.4	0.45
181	38	88	3.2	0.70

* cut-off value of best sensitivity and specificity.

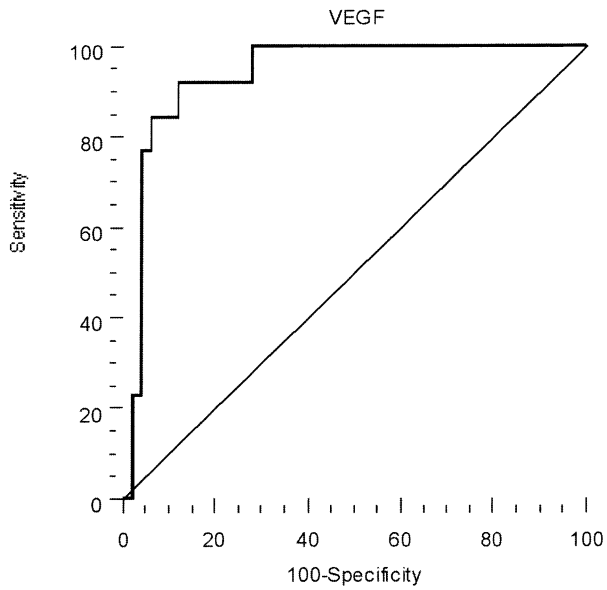

Figure 2. — Receiver operating curve for VEGF levels in the study groups (sensitivity of 92% and specificity of 88% at 68 pg/ml cut-off value).

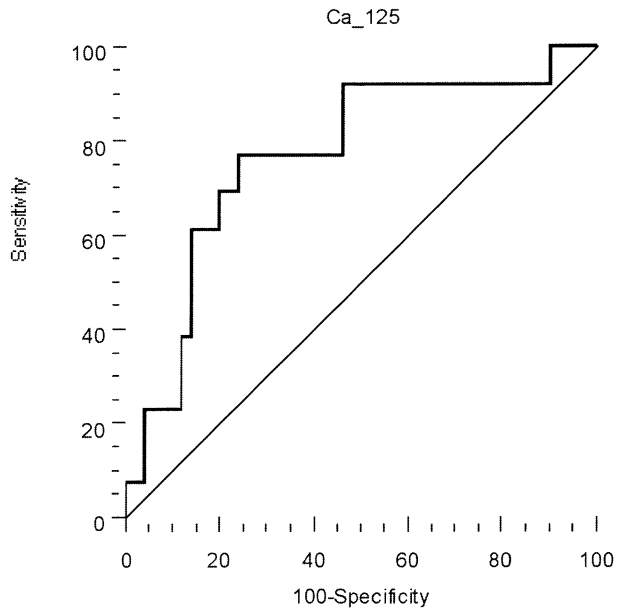

Figure 3. — Receiver operating curve for Ca-125 levels among the study groups (sensitivity of 76% and specificity of 76% at 102 IU/ml cut-off value).

Several researchers have suggested that serum VEGF levels vary with different ovarian tumors of different histologic types, with the highest levels detected in malignant tumors [7-9]. An analysis of 61 patients with ovarian neoplasms (41 carcinomas, 20 cystadenomas) has shown that the sensitivity and specificity of serum VEGF levels to predict the malignant nature of ovarian masses were 71% and 65%, respectively [5]. In our study, the serum VEGF cut-off level of 68.7 pg/ml yielded a sensitivity and specificity of 92% and 88%, respectively,

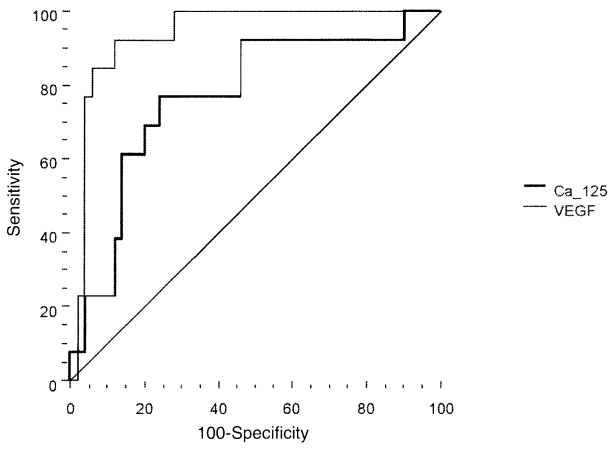

Figure 4. — Receiver operating curves of VEGF and Ca-125 levels in the study groups (AUC_{VEGF}: 0.938, $AUC_{Ca\text{-}125}$: 0.769, p = 0.02).

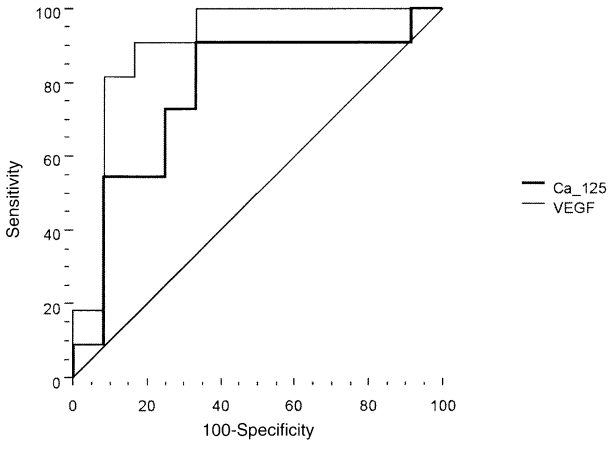

Figure 5. — Receiver operating curves (ROC) for serum VEGF and Ca-125 levels among postmenopausal ovarian masses. (AUC_{VEGF}: 0.902, $AUC_{Ca\text{-}125}$: 0.873, p = 0.14).

which underlines the diagnostic relevance of serum VEGF despite the fact that our study comprised a small number of cases.

Chen *et al.* [10], has suggested that preoperative VEGF serum levels might be regarded as an additional factor in predicting the outcome of malignant ovarian tumors and the recurrence. Tempfer *et al.* [11] has shown that elevated pretreatment serum VEGF levels correlated significantly with a poorer disease-free and overall survival and suggested that in a subset of cases with ovarian tumors, such as those with early-stage or lymph node-negative ovarian cancers, serum VEGF could be used for prognostic information. However, according to another study using a logistic regression model on benign (n=81) and ovarian cancer patients (n=44), at 363.7 pg/ml, serum VEGF achieved a sensitivity of 54% and a specificity of 77% [12]. With respect to differentiating a benign and malignant ovarian mass, based on the result of that study, serum Ca-125 ($p < 0.0001$) but not VEGF ($p = 0.22$) predicted the presence of malignancy. In contrast to this finding, according to our study AUC for VEGF (0.938) was higher than that of Ca-125 levels (0.769), suggesting that serum VEGF better predicted the nature of the tumor. The lower cut-off value for serum VEGF obtained in our series could be related to the small number of cases enrolled in the study, which necessitated that the study be continued until reaching a larger data set. However, another limitation of our study was the fact that not all malignant ovarian masses were epithelial in origin or of great number, which limited the diagnostic accuracy of serum Ca-125 levels with respect to differentiating benign or malign ovarian masses.

As a conclusion, preoperative serum VEGF level has both diagnostic and prognostic value in the clinical management of ovarian masses. Future studies with a larger case series will help the physician to better understand the value of angiogenic markers in both the diagnosis and postoperative follow-ups of the cases.

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p53 overexpression as a prognostic indicator in endometrial carcinoma

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Summary

Purpose: To investigate the prognostic value of p53 overexpression in endometrial adenocarcinoma cases of different stages and histologic subtypes.

Methods: One hundred and eleven surgically staged endometrial carcinoma (EC) cases from 1996 to 2000 constituted this retrospective study group. Prognostic factors determined through the evaluation of surgery specimens by co-author pathologist, were surgical stage, tumor size, histology, histologic and nuclear grade, myometrial invasion, adnexal/serosal metastasis, peritoneal cytology, retroperitoneal lymph node involvement p53 overexpression was assessed via immunohistochemical staining. Tissues that expressed p53 were considered as positive p53 staining. In terms of degree of staining, 1-29%, 30-90% and 80-100% of tumoral tissue stained with p53 were considered to be mild, moderate and high p53 staining, respectively.

Results: Mean age and follow-up period of the study group were 58.2 ± 10.6 years and 33.4 ± 2.7 months, respectively. Percentages of cases surgically staged as early (I-II) and advanced (III-IV) FIGO stages were 65.8% (n: 73) and 34.2% (n: 38), respectively. Cases with positive p53 staining had a significantly high mean survival period compared with those with negative p53 staining (86.6 ± 6.0 vs 49.1 ± 8.1, $p < 0.001$). p53 overexpression was statistically detected to be high in Stage III-IV tumors, non-endometrioid histologic subtypes ($p = 0.019$), histologic and nuclear grade 2-3 tumors ($p < 0.001$), adnexal/serosal metastasis ($p = 0.001$), lymph node involvement ($p = 0.012$), and positive peritoneal cytology ($p = 0.017$). The degree of p53 staining was remarkably correlated with survival. In cases with mild and high p53 staining, mean survival times were 47.1 ± 7.0 months and 57.0 ± 13.1 months, respectively ($p = 0.0003$) compared to those with high p53 staining. On univariate analysis, all of the prognosticators, including p53 staining ($p < 0.001$) and degree of p53 staining ($p < 0.001$) appeared to be independent risk factors for poor prognosis. On multivariate analysis, only pelvic lymph node involvement ($p = 0.03$), serosal/adnexal involvement ($p = 0.004$), and positive peritoneal cytology ($p = 0.01$) were found to be independent prognosticators of survival while p53 expression ($p = 0.743$) and degree of p53 staining ($p = 0.802$) were not detected as independent prognosticators.

Conclusion: p53 overexpression is strongly related to poor prognostic indicators in endometrial adenocarcinoma. Although in this study p53 overexpression was not detected as an independent prognosticator, additional studies with large data set are needed to evaluate the prognostic value of p53 expression.

Key words: p53 overexpression; Endometrial carcinoma; Prognosis.

Introduction

p53 mutation and abnormal p53 accumulation, mostly due to missense mutation, inhibits the function of *'wild type'* p53 protein and leads to uncontrolled cell proliferation [1]. Although several genetic abnormalities are known to occur in endometrial carcinoma (EC), including p53 gene mutation, the pathogenesis of this common malignancy remains poorly defined [2]. Previous reports on oncocogenes and tumor supressor genes and proliferation markers in EC have resulted in conflicting findings regarding their prognostic value [3]. p53 overexpression has been suggested as an independent predictor of poor survival, aggressive histologic subtypes, higher grades and recurrent disease in endometrial cancer [4, 5].

The aim of this study was to determine the presence and the prognostic value of p53 overexpression in endometrial carcinoma.

Revised manuscript accepted for publication November 29, 2002

Materials and Methods

One hundred and eleven surgically staged EC cases from 1996 to 2000 constituted this retrospective study group. Prognostic factors determined through the evaluation of surgery specimens by co-author pathologist were surgical stage, tumor size, histology, histologic and nuclear grade, myometrial invasion, adnexal/serosal metastasis, peritoneal cytology and retroperitoneal lymph node involvement. The pathologist was blinded to the clinical outcome of the cases. p53 overexpression was assessed via an immunohistochemical staining method. Paraffinized tissue blocks 4 μ in size, following treatment with poly-L-lysine solution, were deparaffinized and rehydrated. After being treated with antigen retrieval solution (1/10 diluted citrate buffer, pH: 6.0) they were incubated in a microwave oven for 10 minutes. Afterwards, the tissue specimens were cooled at room temperature for 20 minutes and treated with tris buffer solution for five minutes. Primary p53 antibodies were added to the blocks, which later on were incubated at room temperature for 60 minutes. Following the washing with buffer solution for five minutes, secondary p53 antibodies were added to the blocks. They were then kept at room temperature for 10 minutes. Following treatment with buffer solution, AEC chromogramine solution was dropped onto the blocks which were then kept at room temperature for 10 minutes. Counterstaining was done with hematoxylin.

Tissues that expressed p53 were considered as positive p53 staining. In terms of degree of staining, 1-29%, 30-90% and 80-100% of tumoral tissue stained with p53 were considered to be mild, moderate and high p53 staining, respectively. Statistical analyses were performed using the Statistical Package Program for Social Sciences, Computer Program (SPSS 10.0, Inc, Chicago, IL). Fisher's exact chi-square test, $\chi 2$ test with Yates'correction, and Kruskal-Wallis analysis were applied for discrete data analyses. The Cox proportional-hazards model was used for univariate and multivariate analysis. Univariate analysis of overall survival was performed as outlined by Kaplan and Meier. Values are presented as mean ± standard error of mean (SEM). A p value of < 0.05 was considered significant.

Results

Mean age and follow-up period of the study group were 58.2 ± 10.6 years and 33.4 ± 2.7 months, respectively. Percentages of cases surgically staged as early (I-II) and advanced (III-IV) stages were 65.8% (n: 73) and 34.2% (n: 38), respectively. The number of cases with p53 staining positive and p53 staining negative were 42 (42.3%) and 64 (57.7%), respectively. With regard to degree of p53 staining, percentages mild, moderate and severe p53 stained ones were (17.1%), (11.7%) and (13.5%), respectively.

Cases with positive p53 staining had a significantly higher mean survival period compared with those with negative p53 staining (86.6 ± 6.0 vs 49.1 ± 8.1, $p < 0.001$). p53 overexpression was statistically detected to be high in Stage III-IV tumors, non-endometrioid histologic subtypes ($p = 0.019$), grade 2-3 tumors ($p < 0.001$), adnexal/serosal metastasis ($p = 0.001$), para-aortic lymph node involvement ($p = 0.012$), positive peritoneal cytology ($p = 0.017$), as presented in Table 1. Tumor volume, myometrial invasion and pelvic lymph node metastasis did not differ among the two groups ($p > 0.05$). The degree of p53 staining was remarkably correlated with survival. In cases with mild, intermediate and severe p53 staining, mean survivals were 47.1 ± 7.0 months, 35.2 ± 11 and 57.0 ± 13.1 months, Paradoxically, in cases with severe p53 staining, survival time was found to be comparably longer than in those with mild and intermediate staining. This result may be due to the small number of cases that were enrolled in the statistical analyses.

On univariate analysis, p53 staining ($p < 0.001$) and degree of p53 staining ($p < 0.001$) appeared to be independent risk factors for poor prognosis. On multivariate analysis, only pelvic lymph node involvement ($p = 0.03$), serosal/adnexal involvement ($p = 0.004$), and positive peritoneal cytology ($p = 0.01$) were found to be independent prognosticators of survival. p53 overexpression ($p = 0.743$) and degree of p53 staining ($p = 0.802$) were not detected to be independent prognosticators. Log-rank analyses of survival curves of p53 staining and degree of p53 staining are presented in Figures 1 and 2.

Table 1. — *Distribution of various prognostic variables among cases with p53 + and (-) staining (2x2 contingency tables).*

Prognostic factors	p53 negative n (%)	p53 positive n (%)	p
Histologic type			
Endometrioid	44 (67.7)	21 (32.3)	
Non-endometrioid	20 (43.5)	26 (56.5)	< 0.05*
Histologic grade			
Grade I	26 (78.8)	7 (21.2)	
Grade II	33 (60)	22 (40)	
Grade III	5 (21.7)	18 (78.3)	< 0.001*
Nuclear grade			
Grade I	23 (76.7)	7 (23.3)	
Grade II	34 (60.7)	22 (39.3)	
Grade III	7 (28)	18 (72)	< 0.001*
Tumor size			
< 2 cm	16 (69.6)	7 (30.4)	
2-4 cm	15 (55.6)	12 (44.4)	
> 4 cm	33 (54.1)	28 (45.9)	> 0.05
Myometrial invasion			
< 1/2	36 (64.3)	20 (35.7)	
> 1/2	28 (50.9)	27 (49.1)	> 0.05
Serosal and/or adnexal metastasis			
Negative	55 (67.9)	26 (32.1)	
Positive	9 (30)	21 (70)	< 0.001*
Lymph node metastasis			
Negative	57 (64)	32 (36)	
Positive	7 (31.8)	15 (68.2)	< 0.05*
Pelvic lymph node metastasis			
Negative	58 (61.1)	37 (38.9)	
Positive	6 (37.5)	10 (62.5)	> 0.05
Para-aortic lymph node metastasis			
Negative	61 (61.6)	38 (38.4)	
Positive	3 (25)	9 (75)	< 0.05*
Peritoneal cytology			
Negative	62 (61.4)	39 (38.6)	
Positive	2 (20)	8 (80)	< 0.05*
Surgical stage			
Stage I-II	51 (69.9)	22 (30.1)	
Stage III-IV	13 (34.2)	25 (65.8)	< 0.001*

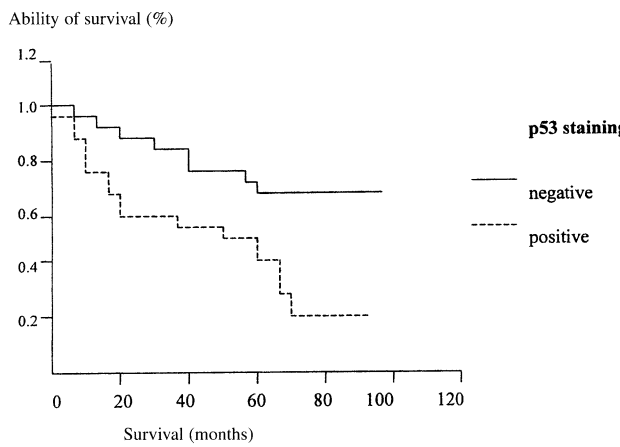

Figure 1. — Probability of overall survival in 111 patients with endometrial cancer with (+) and (–) p53 staining in the primary tumor (log-rank test, $p < 0.001$).

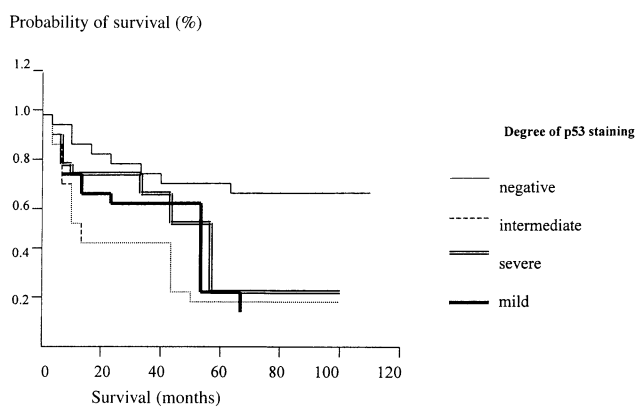

Figure 2. — Probability of overall survival in 111 patients with endometrial cancer with regard to degree of p53 staining (log-rank test, p = 0.0005).

Table 2. — *Evaluation of various prognosticators via univariate and multivariate analysis.*

	Univariate			Multivariate		
	B	B/SE	P	B	B/SE	P
Variables						
Age	0.027	0.017	0.222	0.035	0.023	0.120
Stage	0.397	0.060	< 0.0001*	0.125	0.157	0.425
Nuclear grade	0.559	0.250	0.025*	–2.780	1.451	0.055
Histologic grade	0.523	0.248	0.034*	2.511	1.391	0.071
Myometrial invasion	1.118	0.331	0.0001	0.384	0.475	0.419
Pelvic LN metastasis	2.317	0.356	< 0.0001*	1.682	0.815	0.039*
Para-aortic LN metastasis	2.534	0.414	< 0.0001*	1.030	0.719	0.152
Adnexal metastasis	2.262	0.384	< 0.0001*	1.790	0.614	0.004*
Peritoneal cytology	2.094	0.379	< 0.0001*	1.539	0.653	0.018*
Tumor size	0.599	0.263	0.027*	0.184	0.368	0.617
Tumor histology	0.240	0.071	0.0008*	–0.046	0.121	0.702
p53 staining	1.335	0.352	0.0002*	–0.268	0.818	0.743
Degree of p53 staining	0.457	0.131	0.0005*	0.084	0.334	0.802

*statistically significant.

Discussion

Previous reports have indicated that p53 overexpression in EC cases is regarded to be an independent prognostic factor in the prediction of recurrent disease and is associated with advanced tumor stage, more aggressive histologic subtypes, lymph node involvement, higher tumor grade, and positive peritoneal cytology [6-9]. The present study also highlighted the fact that p53 overexpression was associated with those independent prognostic variables except tumor size, myometrial invasion and pelvic lymph node metastasis. In one study, p35 overexpression in endometrioid and serous papillary type endometrial carcinoma was observed in 52% and 84% of the cases [10].

Salvesen *et al.* [11] has shown that age, FIGO stage, microvessel density, Ki-67 and p53 overexpression had an independent prognostic impact in EC cases. In early stage of endometrial cancer, immunohistochemical evaluation of p53 overexpression has been suggested to predict the outcome. Powell *et al.* [12], have demonstrated that, on multivariate analysis among early-staged EC cases, myometrial invasion and p53 overexpression were found to provide strong prognostic information for the outcome of endometrial carcinoma patients. Moreover, p53 overexpression, loss of apoptotic markers such as bax gene frame shift mutation and loss of bcl-2 expression, can also lead to the local invasive behaviour of endometrial cancer [13-14]. Furthermore, the absence of p53 expression is correlated with less aggressive behaviour and other molecular features of the primary tumor such as the presence of PTEN mutation and microsatellite instability [15]. Recent molecular studies have emphasized that nuclear DNA content and p53 expression were stronger predictors of relapse-free survival than the individual prognostic factors [16, 17].

As a conclusion, the present study pointed out that p53 overexpression is an independent prognostic variable despite, on multivariate analysis, a contrary findinng was observed. However, studies based on a larger set of data are in needed to favor the contention that p53 overexpression is a potent prognostic factor.

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