Immunohistochemical Expression of CD44V6, P53 and BCL-2 in Epithelial Ovarian Tumors

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Abstract: Ovarian epithelial cancer is a leading cause of death among gynaecological malignancies due mainly to advanced stage at presentation. **Aim of the work**: The purpose of this study was to investigate the expression and prognostic significance of CD44v6, Bcl-2 and P53 in ovarian tumors of low malignant potential (LMP) and primary epithelial ovarian carcinomas **Experimental Design:** We analyzed the immunohistochemical expression of CD44v6, Bcl-2 and P53 in 100 patients with primary ovarian carcinoma (64) serous 26 mucinous and 10 undifferentiated carcinoma) and 50 patients with ovarian tumors of (LMP). **Results:** CD44v6 was expressed in 25 cases of ovarian tumors of LMP (50%) and in 9 cases of ovarian carcinomas (9%). CD44v6 expression was correlated with early clinical FIGO stage (p=0.003) and the grade of the tumor. (p <0.05). P53 expression was detected in 21 cases of ovarian tumors of LMP (42%) and in 52 cases of ovarian carcinomas (52%) and was associated with high grade and advanced clinical FIGO stage (p <0.004). Bcl-2 was expressed in 43 cases of ovarian tumors of LMP (86%) and in 66 cases of epithelial ovarian carcinoma (66%) with positive correlation between Bcl-2 expression and early clinical stage and low grading of the tumors (p <0.001). **Conclusion:** CD44v6 and Bcl-2 expressions were correlated with LMP and, early- stage tumor with good prognosis, whereas P53 expression was associated with less differentiated, advanced-stage tumor and unfavorable prognosis

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1. Introduction

The incidence of ovarian cancer is high in the industrial countries of the world, particularly in the Western and Northern Europe and North America. Among the middle-east countries, the age standardized incidence rate of ovarian cancer was 5.4/ 100000 women in Egypt. The highest proportions of ovarian cancer cases were in the age range 50-69 year (17.7%). Carcinoma was the most commonly observed type of ovarian tumors accounting for 77.8%-93.2% and serous carcinoma was the most commonly observed specific type of ovarian carcinoma in all countries including Egypt (Parkin *et al.,* 2002).

CD44 characterizes a polymorphic family of cell surface glycoprotein which is involved in cell to cell and cell to extracellular matrix adhesive interaction, cell trafficing, lymphocyte homing, wound healing and inflammation (Visca *et al.,* 2002). Recent studies have identified CD44 glycoproteins as potentially important component of tumor progression and metastasis (Indinnimeo *et al.,* 2003). In the metastatic process, CD44 is the primary receptor for hyaluronan. The attachment of tumor ovarian cells to the peritoneal mesothelium is mediated through the interaction between CD44 expressed in the malignant cells and hyaluronic acid expressed on the mesothelial surface. (Kokenyesi, 2001). P53 is a tumor suppressor gene that has been studied extensively as a prognostic indicator in ovarian cancer. In several studies, p53 over-expression has been observed in at least 50% of advanced stage ovarian cancers Mutations and over-expression of p53 has also been studied in borderline ovarian tumors (Aunoble *et al.,* 2000& Nielsen *et al.,* 2004,). Bcl-2 gene, a member of Bcl-2 family has been shown to exert antiapoptotic activity in ovarian carcinoma cells (Sagarra *et al.,* 2002). Overexpression of Bcl-2 is a feature in 24-80% of epithelial ovarian carcinomas and is associated with resistance to chemotherapy (Kupryjanczk *et al.,* 2003). All histological types of epithelial ovarian carcinomas can be divided into two designated type I and type II which correspond to two pathways of tumorgenesis. Type I tumors include low grade serous carcinomas, mucinous carcinomas, endometrioid and clear cell carcinomas that develop slowly in a stepwise manner from premalignant condition or borderline tumors. Type II tumors include the high grade serous carcinoma, carcinosarcoma and undifferentiated carcinoma which grow rapidly and typically have spread beyound the ovaries at presentation. These tumors are rarely associated with morphologically recognizable precursor lesions and it has been propodsed that they develop de novo. (Shih and Kurman, 2004 & Okamura and Katabuchi, 2005).

In this study, we tried to calrify the association between the apoptotic regulator (Bcl-2) and a tumor suppressor gene (P53) in the process of carcinogenesis of epithelial ovarian carcinomas especially high grade, advanced stage groups. We studied the expression of CD44v6, Bcl-2 and P53 by immunohistochemical

technique in ovarian tumors of low malignant potential and epithelial ovarian carcinoma and compared their expression with the clinico-pathological factors and overall prognosis.

2. Patients and Methods

The biopsies for this study were collected from 150 women admitted to the Department of OB/Gyn, Sohag University hospital during the period from March 2009 to August 2011. These patients were diagnoses in the Pathology Department and were divided into 100 cases of epithelial carcinoma (64 serous, 26 mucinous and 10 undifferentiated carcinoma) and 50 cases ovarian tumors of low malignant potential (LMP) (35 serous and 15 mucinous) according to International Histological Classification of Tumors (Scully, 1998, Tavassoli *et al.,* 2003).The studied cases were divided into 54 Grade I, 26 Grade II, 10 Grade III and 10 Grade IV. The relevant clinical data were retrieved from the patients' medical records files. Tumors staging was based on the standard FIGO staging system classification (Berek *et al.,* 2007).The patients were divided into 48 Stage I, 16 Stage II, 34 Stage III and 2 Stage IV, while all patients with ovarian tumors of LMP were at stage I. All patients were operated upon according to their tumor clinical stage. The median follow up time was 12 months for all patients. The clinico-pathological characteristics of the included patients were summarized in Tables (1, 2).

CD44v6, Bcl-2 and P53 immunohistochemical staining method:

Of all tumors, tissue sections were stained with H&E and histopathological typing, grading and staging of the tumors were evaluated. Tissue sections of 5um thickness of the selected paraffin blocks were deparaffinized and rehydrated then were put in the microwave oven in citrate buffer (pH 6) for 15 min (3x5min), left to cool at room temperature and then washed in PBS buffer. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide for 10 min followed by wash in PBS. The sections were incubated with the primary antibody as followed: CD44v6 50U/slide (Ab-1(Clone vff-7) cat # MS-1093-R7) (7.0ml) 1/40 concentration for 12 hours in humid chamber at room temperature and goat serum (1micron/slide) overnight at room temperature in humidified chamber. Bcl-2, 50U/slide (Bcl-2, DAKO, Clone 124), 1/100 for 1 hour, at humid chamber at room temperature and P53 50U/slide (DAKO, Clone Do7+) 1/50 concentration and incubated in a humidified chamber for 2 hours at room temperature. The slides were incubated with the Biotinylated goat Antipolyvelant for 10 min and washed with PBS. The slides were then incubated for 15 min in Streptavidin-Biotin peroxidase in humidified chamber then washed with PBS. DAB-Chromogen 1/25 concentration was added to the slides then washed with PBS. The slides were immersed in Mayer's Hematoxyline, washed many times with tap water, dehydrated in upgrading alcohols, cleared and mounted in DPX. Tonsiller samples were used as a positive control for CD44v6, Membranous staining of the cancer cells were considered CD44v6 positive and the intensity of expression was categorized into two groups according to the median percentage of positive tumor cells into low expression in ≤ 10% of the tumor cells and high expression if >10% of the tumor cells. (Anwar and Wood, 2000).

The positive control for P53 was colon cancer tissue and expression of P53 was considered positive (strong or weak) if there is distinct nuclear staining was detected even focally in the tumor cells (Diebold *et al.,* 1996)

Bcl-2 is typically cytoplasmic and was graded into negative, weakly positive (+), and strongly positive (++) and follicular lymphoma tissue was the used positive control (Henriksen *et al.,* 1995)

Statistical analysis

Statistical analysis was used to evaluate correlations between expression of CD44v6, p53, Bcl-2 and clinicopathological parameters. SPSS computerized program, was used with statistical significance defined as $p<0.05$, using Chi-square and Student test.

3. Results

Formalin-fixed paraffin embedded 100 primary epithelial ovarian carcinoma and 50 ovarian tumors of LMP tissue biopsies were used for the detection of expression of CD44v6, Bcl-2 and P53 by immunohistochemical technique. The included cases were 64 cases of serous carcinoma, 26 mucinous and 10 undifferentiated carcinoma and 50 ovarian tumors of low malignant potential (LMP). The age range of the patients with ovarian tumors was (30-70 y) with the mean age (50.6y) Figure (1). CD44v6 was expressed in LMP tumor tissue more than in carcinoma cases (25/50 cases of ovarian tumors of LMP (50%) were CD44v6 (+) while only 9/100 cases of ovarian carcinoma were positive for CD44v6. In Univariate analysis, there was a statistically significant relation between CD44v6 expression and LMP ovarian tumors ($p<0.001$). In carcinoma cases CD44v6 expression was statistically correlated with low tumor grading (7 cases in Grade I and 2 in Grade II) ($p<0.05$) and early clinical FIGO stage as all the 9 positive cases were at early clinical stage (stage I) ($p<0.003$). There was no statistically significant correlation between the CD44v6 expression and the histological type of ovarian carcinoma. P53 was expressed in (21/50) cases of ovarian tumors of LMP (42%) and in (52/100 cases) of ovarian carcinoma (in 39/64 cases of serous carcinoma (56%) and in (10/26 cases) of mucinous carcinoma (27%) and

in (3/10 cases) of undifferentiated carcinoma). There was a statistically significant correlation between the P53 expression and the serous histological type (p<0.005). There was a significant statistical correlation between the P53 expression and the advanced clinical FIGO stage with (32/52 cases of p53 positive cases) (61%) were at advanced clinical stage (at stage III, IV) (p< 0.004) and high grade tumors (14/20 cases of grades III and IV) were P53 positive (p<0.005). Bcl-2 was expressed in (43/50 cases) of tumors of LMP (86%), and in (66/100 cases) of ovarian carcinoma. Bcl-2 expression was statistically correlated with early clinical stage (44/66 positive cases) (66%) were at early clinical stages I and II (p<0.005) and low grade tumors (62/66 of positive cases) (93%) were low Grades I and II) (p<0.005) Tables (3, 4, 5, 6) and Figures (2, 3, 4). The staining intensity in different cases did not show enough variability to warrant grading of intensity of reactivity.

Table (1): The clinicopathological characteristics of the patients

Histological type	Stage I	Stage II	Stage III	Stage IV	Total
Serous	30	10	22	2	64
Mucinous	16	2	8	-	26
Undifferentiated	2	4	4	-	10
Total	**48**	**16**	**34**	**2**	**100**

Table (2) Different tumor grades of the studied cases.

Histological type	Grade I	Grade II	Grade III	Grade IV	Total
Serous	41	14	9	-	64
Mucinous	13	12	1	-	26
Undifferentiated	-	-	-	10	10
Total	**54**	**26**	**10**	**10**	**100**

Table (3)The expression of P53, Bcl-2 and D44v6 and clinical stage of the tumor

	Stage I	Stage II	Stage III	Stage IV	Total
P53	10/48	10/16	30/34	2	52/100
Bcl-2	32/48	12/16	22/34	-	66/100
(P53+Bcl-2+)	-	6	18	-	
CD44v6	9	-	-	-	9/100

Table (4):CD44v6, Bcl-2 and P53 expression in different tumors grades

	Grade I	Grade II	Grade III	Grade IV	Total
P53	22/54	16/26	8/10	6/10	52
Bcl-2	40/54	22/26	4/10	-	66
(P53+Bcl-2+)	8	12	2	-	
CD44v6	7/54	2/26	-	-	9

Table (5)CD44v6, P53 and Bcl-2 expression in different histological types

Histological type	No	CD44v6	Bcl-2	P53
Serous	64	7	48	39
Mucinous	26	2	18	10
Undifferentiated	10	-	-	3
Total	**100**	**9**	**66**	**52**

Table (6) P53, Bcl-2 and D44v6 in tumors of (LMP).

	P53	Bcl-2	CD44v6
LMP	**21(50%)**	**43(86%)**	**25 (50%)**
(P53 +& Bcl-2+)	**14**		

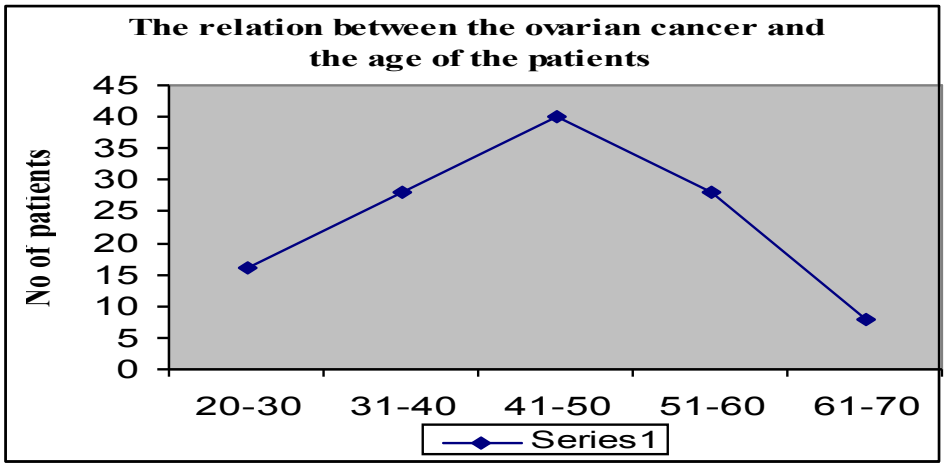
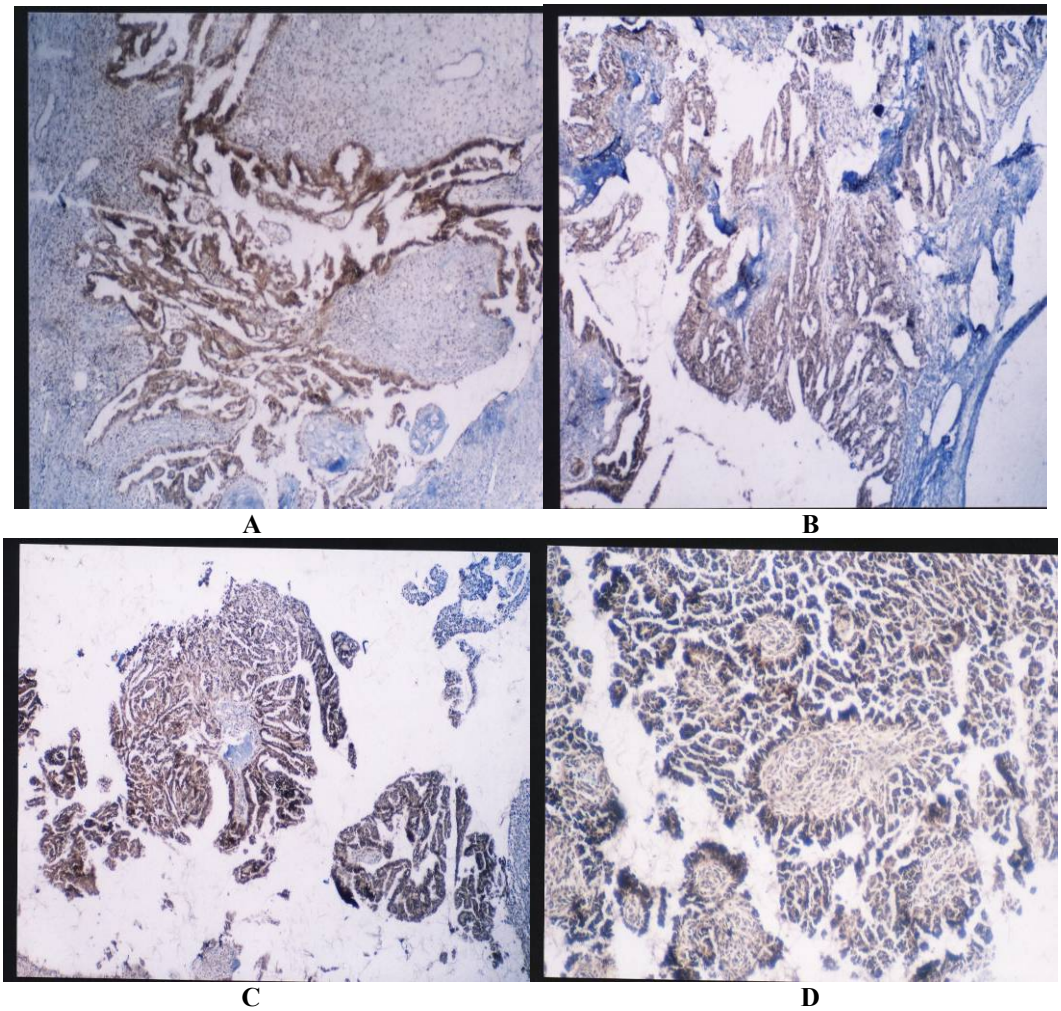
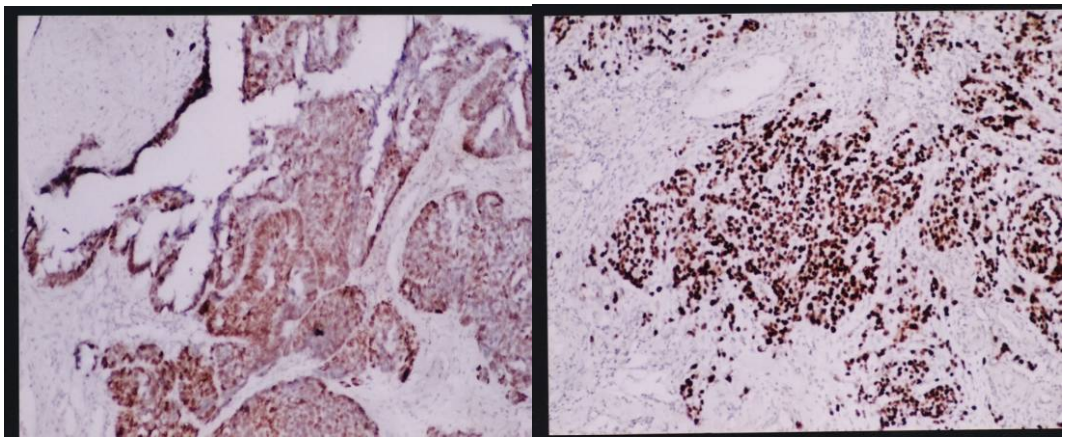


Figure (1) The age incidence of ovarian tumor in the study group.

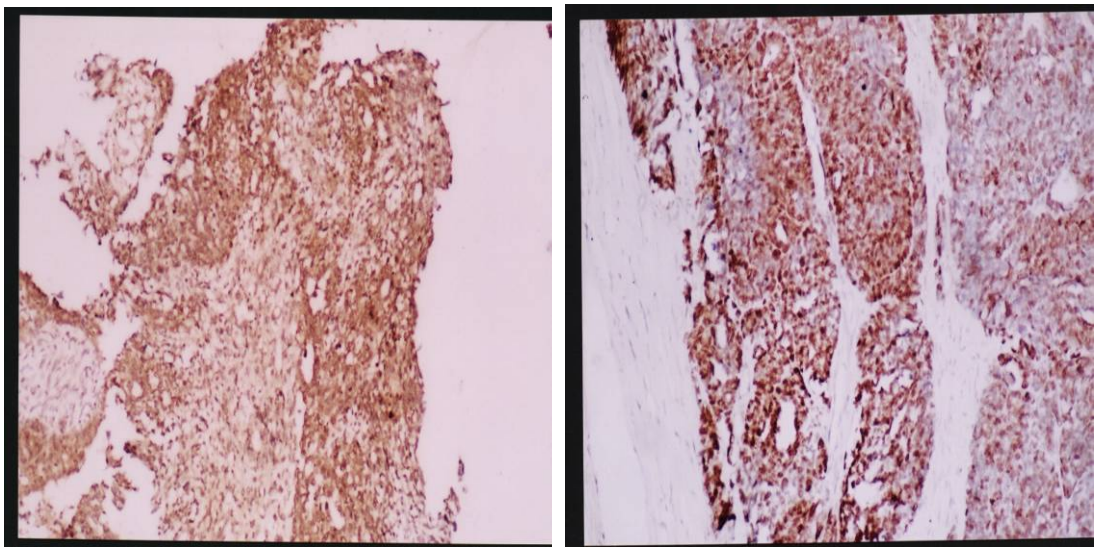


Figure(2)CD44v6 expression in ovarian tumors of LMP (A, B) and serous carcinoma (C, D).



A B

Figure(3) P53 expression in moderately(A) and poorly differentiated (B) carcinoma



A B

Figure (4) Bcl-2 expression in well differentiated ovarian carcinoma.

4. Discussion

Tumors of the ovarian epithelial surface account for about 80% of all ovarian neoplasms and exhibit a heterogeneous histological classification affecting survival. Tumors of low malignant potential, defined as borderline ovarian tumors (BOTs), have a markedly better survival and low recurrence, even if surgery still represents the common management for this type of cancer. It is still debated in the literature if BOTs can be considered as intermediate precursors in the progression to high grade ovarian tumors. Evidences now propose that high-grade serous carcinomas are not associated with a defined precursor lesion. Despite the large body of data summarized, a limited number of molecules proved to be useful in elucidating BOTs pathogenesis and only a few of these showed possible application in the therapy.

In this respect, great interest has been raised in studying LMP tumors of the ovary.

In this study, the immunohistochemical expression of CD44v6 was detected in (9/100 cases) of ovarian cancer. In univariate analysis, there was a statistically significant correlation between CD44v6 expression and the patient clinical stage (FIGO Stage I) (p<0.001) and low grade of the tumor (p<0.005). These results were in agreement with Saegusa *et al.,* 1999 who reported that CD44 loss of expression is associated with poor prognosis useing both immunohistochemistry and RT-PCR techniques in 115 cases of ovarian tumors. More recent studies (Ross *et al.,* 2001 and Sillanpaa *et al.,*2003) reported that high CD44 expression was significantly associated with relatively good prognosis in ovarian carcinoma. Schroder *et al.,* 1999 concluded that CD44v6 was associated with intra-peritoneal implantation in 50

cases of primary ovarian cancer with lymph node metastasis. However, the difference in the findings could be related to the fact that they analyzed the standard form (CD44s) while our study used the variant CD44v6. Zagorianakou *et al.,* 2004 found no statistically significant correlation between CD44s expression and the clinicopathological parameters or survival.

In the present study, CD44v6 expression was detected in (50%) of ovarian tumors of low malignant potential with statistically significant correlation between CD44v6 expression and tumors of LMP (p<0.0001). This could demonstrate that CD44v6 may be functionally involved in the pathogenesis of epithelial tumors of LMP and with progression to carcinoma, its expression decreased markedly. Zagorianakou *et al.,* 2004 found that the expression of CD44s in the LMP group was higher when compared to malignant lesions. Another possibility for the explanation of the CD44v6 findings were that LMP ovarian tumors could be a distinct category of ovarian tumors and not a precurse or premalignant step into carcinoma.

Together with histopathological studies, mutations of p53 gene, microsatellite instability (MSI) and under- or over-expression of many genes and proteins have been used to predict prognosis in borderline ovarian tumors and epithelial ovarian carcinoma. In the present study, we found that p53 over-expressed in (41% of ovarian tumors of LMP cases) and it was not correlated with the clinico-pathological factors of the patients but it could be related to the process of tumorgenesis (progressive strong expression of p53 from LMP to carcinoma). Gershenson *et al.,* 1999, analyzed p53 expression in advanced stage serous borderline tumors and found that over-expression of p53 was significantly associated with an increased probability of progression, recurrence and a decreased overall survival.

A statistically significant correlation was observed between P53 expression and advanced disease carcinoma in the present study (61% of positive cases at advanced clinical FIGO stages III and IV p<0.005) and serous histological type (p<0.003). This was in agreement with Ayadi *et al.,* 2010, Vergara *et al.,* 2010 who found P53 status was associated with advanced FIGO stage. P53 status was correlated with high grade tumor in our patients as (14/20 cases of high grades III and IV) showed reaction with p53. This was in agreement with the study of Baekelandt *et al.,* 1999, who found p53 protein positivity correlated with loss of differentiation and represents an independent prognostic factor in advanced stage III ovarian cancer.

Bcl-2 immuno-expression was detected in (43/50 cases 86%) of ovarian tumors of LMP in the present study. This increased expression in borderline ovarian tumors (BOT) and low grades ovarian carcinomas with decreased expression as the tumors increased in grading could be related to the process of tumorgenesis of the ovarian surface epithelium into malignant tumors with gradual loss of expression of Bcl-2. In univariate analysis, Bcl-2 expression was significantly associated with early FIGO stage (66% of the positive cases were at stages I and II (p<0.001), and low grade (92% of the positive cases at grades I and II p<0.001).There was no correlation between Bcl-2 expression and tumor histological type. These results were similar to those of Chan *et al.,* 2000 and Skirnisdottir *et al.,* 2002 who concluded that Bcl-2 immunostaining was most frequently associated with low tumor grade.

There was a statistically significant correlation between the immunohistochemical expression of two markers (P53 and Bcl-2) together and the advanced stage ovarian tumors as they were expressed together in 18/36 cases (50%) of stages III &IV, while they both were positive in 6/64 (9%) of stages I &II (p<0.001). Sasaki *et al.,* 2009 attempted to calrify critical pathway in the carcinogenesis of type II tumors. They found that genetic alteration in c-myc, Bcl-2, P53 and Ras pathway resulted in carcinosarcoma and poorly differentiated adenocarcinoma.

The regulation of apoptosis is through a balance between the pro and antiapoptotic genes and a tilt of the balance towards antiapoptotic genes is an important factor in genesis of most cancers including ovarian cancer. Together with aberrant tumor suppressor gene, the normal ovarian epithelial cells can be transformed into tumor cells. In the present study, both P53 and Bcl-2 were expressed in 14/50 (28%) of LMP ovarian tumors and this finding was in agreement with Kar *et al.,* 2007 who documented that the loss or dysfunction of activated p53 allows the unchecked replication of cells with genetic damage, which result in tumor development.

Conclusion

We concluded that (1)-CD44v6 expression is associated with localized, early stage, ovarian tumors and LMP and its loss of expression indicates progressive attitude into malignancy. (2)-P53 is correlated with increased aggressiveness of the ovarian carcinoma.(3)- Bcl-2 expression was related to the progression from LMP to neoplastic tumors and it could play a role in the process of oncogenesis of the ovarian tissue.(4) Detection of both P53 and Bcl-2 together indicated aggressive ovarian carcinoma.

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5. References

1-Parkin DM, Whelan SL, Ferlay J, Teppo L, editors. Cancer incidence in five countries, volume VIII. IARC Scientific publication No.155.Lyon (France): International Agency for Research on Cancer; 2002.

2-Visca P, DelNomo F, Botti C, Marandino F, Sebastiani V, DiTondo U, Donnorso RP, Trombetta G, Filippi S, Alo PL. Role and prognostic significance of CD44s expression in colorectal cancer. Anticancer Res., 2002;22:2671-15

3-Indinnimeo M, Cicchini C, Giarnieri E, *et al.* Evaluation of CD44 varient 6 expression and clinical pathological factors in pulmonary metastasis from colon carcinoma Oncol Rep., 2003; 10:1875-7.

4-Kokenyesi R. Ovarian carcinoma cells synthesize both condroitin sulfate and heparin sulfate cell surface proteoglycans that mediate cell adhesion to interstitial matrix. J Cellular Biochem ., 2001; 83:259-270

5-Aunoble SB, Sanches R, Didier E *et al.,* Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer (review). Int J Oncol., 2000; 16: 567-576.

6-Nielsen JS, Jakobsen E, Holund B *et al.* Prognostic significance of p53,Her-2 and EGFR overexpression in borderline and epithelial ovarian cancer, 2004(Nov-Dec);14(6):1086-96

7-Sagarra RA, Andrade LA, Martinez EZ *et al.* P53 and Bcl-2 as prognostic predictors in epithelial ovarian cancer. Int J Gynecol Cancer 2002; 12(6): 720-727.

8-Kupryjanzk J, Szymanska , Madry R *et al.* Evaluation of clinical significance of P53, Bcl-2, Bax and MEK1 expression in 229 ovarian carcinomas treated with platinum-based regimen. Br J Cancer 2003; 88: 848-854.

9-Shih IM and Kurman RJ. Ovarian tumorgenesis: a proposed odel based on morphological and molecular genetic analysis. Am J Patholo., 2004; 164(5): 1511-1518.

10-Okamura H and Katabuchi H. Pathophysiological dynamics of human ovarian surface epithelial cells in epithelial ovarian carcinogenesis. Int Rev. 2005; 242: 1-54.

11- Scully RE. International Histological Classification Of tumors . Histological typing Of Ovarian tumors 2nd edition, Heidelberg: Springer-erlag, 1998.

12- Tavassoli FA, Devilee P. World Health Organization Classification of Tumors: Patholoy and Genetics of tumors of the breast and female genital organds. Lyon IARC Press; 2003.

13- Berek JS, and Natarajian S (2007). Ovarian and fallopian tube cancers. In JS Berek and Novak's Gynaecology, 14th ed, pp.1457-1547, Pihiladelphia: Lippincott Williams and Wilkin.

14-Anwar F and Wood BL. CD44v6 expression in different subtypes of Hodgkin lymphoma. Mod Pathol 2000; 13(10): 1121-1127.

15- Diebold HJ, Baretton G, Feichner *et al.* Bcl-2 expression, P53 accumulation and apoptosis in ovarian carcinoma. Am J lin Pathol., 1996; 105(3): 341-9.

16- Henriksen R, Wilander E, Oberg K. Expression and prognostic significance of Bcl-2 in ovarian tumors. Br J Cancer, 1995; 72: 1324-1329.

17-Saegusa M , Machida D, Hshimura M *et al.* CD44 expression in benign, premalignant and malignant ovarian neoplasma: relation to tumor development and progression. J. Pathol., 1999; 189; 326-337.

18-Ross JS, Sheehan CE, Willam SS, Malfatano JH, Szyfelbein WM, Kallakury BV. Decreased CD44 standard form expression correlated with prognostic variables in ovarian carcinomas. Am J Clin Pathol., 2001; 116:122-128

19-Sillanpaa S, Anttila MA, Voutilainen K, Tammi RH, Tammi MI, Saarikoski SV, Kosma VM. CD44 expression indicates favorable prognosis in epithelial ovarian cancer. Clinical Cancer Research, 2003; 9: 5318-5324.

20-Schroder W, Rudlowski C, Biesterfeld S *et al.* Expression of CD44 (v5-v10) splicing variants in primary ovarian cancer and lymph node metastasis. Anti cancer Res., 1999; 19(5B): 3901-6.

21-Zagorianakou N, Stefanou D, Makrydimas G *et al.* CD44s expression in benign, borderline and malignant tumors of the ovarian surface epithelium : correlation with P53, steroid receptors status, proliferation indices (PCNA, MIBI) and survival. Anticancer Research, 2004; 24: 1665-1670.

22-Gershenson DM, Deavers M, Diaz S, Tortolero-Luna G, Miller BE, Bast RC, Mills GB, Silva EG. Prognostic significance of p53 expression in advanced stage ovarian serous borderline tumors. Clinical Cancer Research 1999;5:4053-4058.

23-Ayadi L, Chaabouni S, Khabir A *et al.* Correlation between immunohistochemical biomarkers expression and prognosis of ovarian carcinomas in Tunisian patients. World J Oncol., 2010; 1(3); 118-128.

24- Vergara D, inelli A, Martignago R *et al.* Biomolecular pathogenesis of borderline ovarian tumors: focusing target discovery through proteogenomics. Curr Cancer Drug Targets, 2010 Feb; 10(1): 107-16.

25-Baekelandt M, Kritenen GB, Nesland JM, Trope CG, Holm R. Clinical significance of apoptosis-related factors, p53, Mdm2 and Bcl-2 in advanced ovarian cancer. Journal Of Clinical Oncology, 1999; 17(7):2061-2068.

26-Chan WY, Cheung KK, Schorge JO *et al.* Bcl-2 and P53 proteins expression, apoptosis and P53 mutation in human epithelial ovarian cancers. Am J Pathol 2000; 150(2): 409-417.

27- Skirnisdottir I, Seidal T, Gerdin E *et al.* The prognostic importance of P53, Bcl-2 and Bax in early stage epithelial ovarian carcinoma treated with adjuvant chemotherapy. Int J gynaecol Cancer, 2002; 12(3): 265-276.

28-Sasaki R, Marisawa-Saito M, Yugawa T *et al.* Oncogenic transformation of human ovarian surface epithelial cells with defined cellular oncogenes. Carcinogenesis, 2009; 30(3): 423-431.

29-Kar R, Sen S, Singh A *et al.* Role of apoptotic regulators in human epithelial ovarian cancer. Cancer Biol Ther., 2007; 6: 1101-1105.

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