Role of GLUT-1 immunostaining in Diagnosis and prognosis of ovarian carcinoma.

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Abstract

Background: close relationship between GLUT-1 expression and carcinogenesis, tumor development, and the unfavorable prognosis of several malignant tumors.

Objective: To investigate the correlation of GLUT-1 immunostaining and clinic-pathologic features as well as prognosis of ovarian cancer patients.

Materials and methods: Paraffin blocks of 76 cases were retrieved from archives of pathology department, Mansoura University. These included 57 cases with malignant ovarian tumors, 11 cases with benign ovarian tumors, and 8 cases with borderline ovarian tumors. The sectioned samples were stained with polyclonal antibody for GLUT-1. The degree of immunostaining were correlated with clinic-pathologic features as well as prognosis of ovarian cancer patients.

Results: All benign tumors were negative for GLUT-1. Strong staining reaction for GLUT-1was significantly associated with the malignant phenotype (p=0.0001). Moreover, there was a statistically significant difference in staining intensity for GLUT-1 between borderline and malignant tumors (p=0.0028). There was a statistically significant correlation between high grade tumors and strong staining intensity (p=0.001). Correlation between staining reaction for GLUT-1 and developing metastases and/or recurrence was statistically significant (p=0.001).

Conclusion: GLUT-1 is related to carcinogenesis of ovarian carcinomas. The statistically significant correlation between staining intensity for GLUT-1 and malignant phenotype can make it a marker for target therapy for ovarian cancer patients. In addition, GLUT-1 can be considered as predictor for metastases and recurrence in ovarian carcinoma that needs to be validated in future trials.

Key words: Ovarian carcinoma, prognosis, GLUT-1, immunohistostaining

Introduction

Worldwide, ovarian cancer is reported to be sixth commonest cancer and the seventh commonest cause of cancer death in women (1,2). Similar to other malignant cells, ovarian cancer cells need glucose for their growth and survival as cancer cell growth is an energy-related process. GLUT-1 is a representative of a family of 14 closely related proteins known as glucose transporters that mediates glucose transport across cell membrane. The published studies have shown a close relationship between GLUT-1 expression and carcinogenesis, tumor biology, and the poor prognosis of many cancers (3,4,5,6,7).

Specifically, in ovarian tissue, Canturia et al. (2000) showed that GLUT1 staining was absent in benign ovarian epithelial tumors and a progressive increase in GLUT-1 expression from borderline tumors to frankly invasive carcinomas (8). It was found that focal or patchy distribution with weak

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to moderate intensity in borderline tumors. On the contrary, it shows diffuse expression with strong intensity in malignant tumors (8). Anti-GLUT-1 antibody may be useful in distinguishing invasive from noninvasive serous borderline implants (3). GLUT-1 expression was reported to be affected by the tumor grade and histologic type. Increasing histologic grade was associated with increasing GLUT-1 expression and serous carcinomas had greater GLUT-1 expression than the other tumor types (9,10,11,12,13).

In their study to investigate the association of GLUT-1 with response to chemotherapy and outcome in patients with ovarian carcinoma, Canturia et al. (14) reported that study of GLUT-1 status may reflect the outcome of patients with ovarian carcinoma. They found that GLUT-1 status is an independent prognostic factor of response to chemotherapy and disease free survival (DFS) in advanced stage ovarian carcinoma.

Despite being addressed in previous studies in benign, borderline, and malignant epithelial tumors of the ovary, the role of GLUT-1 in ovarian carcinogenesis and progression is still unclear. The aim of this study is to investigate the expression of GLUT-1 in benign, borderline, and malignant ovarian epithelial tumors, as well as its relation to metastases and recurrence of the tumor.

Materials and methods

Paraffin blocks of 76 cases were retrieved from archives of pathology department, Faculty of Medicine, Mansoura University. These included 57 cases with malignant ovarian tumors, 11 cases with benign ovarian tumors, and 8 cases with borderline ovarian tumors. These malignant cases represented patients diagnosed with epithelial ovarian carcinoma between January, 2011 and December, 2014. Paraffin blocks were cut into 4um thick sections on coated glass slides and stained with polyclonal antibody for GLUT-1(Cell Marque corporation product, California, USA) purified from rabbit antisera Cat. 355A-18 (7 ml) provided as pre-diluted antibody which is ready to use for IHC staining of formalin fixed paraffin embedded tissues. Positive control was colorectal carcinoma.

Patients' clinic-pathological data including age, histologic type, histologic grade, surgical stage (including metastases), as well as presence of recurrence in the follow up period from time of operation till their last follow up visits were retrospectively reviewed from patient files from department of Obstetrics and Gynecology, Mansoura University. Antigen retrieval was done through pressure cooker with tissue sections placed in EDTA solution (PH 9) for 10 min then slides were left to cool at room temperature.

Evaluation of immunohistochemical staining.

Results of IHC staining were assessed using a light microscope in blinded fashion. Staining was scored blinded to clinic-pathologic data. Only membranous and/or cytoplasmic reaction was considered positive. The extent of the expression was semi-quantitatively evaluated according to the following scoring system: 0, negative staining (0%); 1, weak positive (<10%); 2, moderate positive (10-50%) and 3, strong positive (>50%). Erythrocytes in each section were used as positive internal controls for GLUT-1. While stromal cells were negative internal control (6).

Data analysis

Statistical analysis was performed using Excel and SPSS 10.0 software. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. When 25% of the cells have expected count less than 5, Fisher exact test was used. Continuous variables were presented as mean ± SD (standard deviation) for parametric data and Median for non-parametric data. The two groups were compared with Student t test (parametric data) and Mann–Whitney test (non parametric data). The value of $P < 0.05$ was considered statistically significant.

Results

Seventy-six formalin fixed paraffin embedded specimens were immunostained with polyclonal antibody for GLUT-1. These included 57 cases malignant, 11 cases benign and 8 cases borderline tumors. Age range of studied cases was between 25 and 77 with the average age 55. Weak staining was mainly cytoplasmic and membranous staining was associated with higher intensities. Staining reaction was more evident in the areas away from the blood supply. Characters of studied cases were illustrated in table (1).

Correlation between staining intensity of GLUT-1and biological type of ovarian tumor

All benign tumors were negative for GLUT-1 (score 0). All borderline tumors revealed focal moderate staining intensity in 30-40% of the tumor examined (score 2). On the other hand cases diagnosed with malignant tumor revealed heterogeneous staining reaction. 4 cases were negative, 5 cases were weak positive (score 1), 19 cases were moderately positive (score 2), and 33 cases were strong positive (score 3)

Strong staining reaction for GLUT-1was significantly associated with the malignant phenotype (X2=71.140, p=0.0001). Moreover, there was a statistically significant difference in staining intensity for GLUT-1 between borderline and malignant tumors (p=0.0028)

Correlation between staining intensity of GLUT-1and grade of Malignancy

Our results showed that 71.9% of cases with strong reaction for GLUT1 were grade III. There was a statistically significant correlation between high grade tumors and strong staining intensity (p=0.001) as shown in table 2.

Correlation between staining intensity of GLUT-1and histotype of ovarian tumor

Expression of GLUT-1 was significantly correlated with serous histotypes. Strong staining reaction for GLUT-1 was more commonly encountered in serous, endometroid and clear cell tumors compared to mucinous and undifferentiated carcinomas with statistically significant difference (p=0.014) , table (3).

Correlation between staining intensity of GLUT-1and stage of ovarian carcinoma

All cases with strong staining reaction for GLUT1 were at advanced stage (IIIC &IV) with a statistically significant correlation (p=0.012). On the other hand, early stage patients under study were mostly associated with moderate reaction while only one of these cases was negative for GLUT1.

Correlation between staining intensity of GLUT-1 and prognosis of ovarian carcinoma

Cases positive for recurrence &/or metastases were 34, all of which, scored 3 regarding the intensity except for 7 cases (4 scored 2, and 3 scored 1).On the other hand 23 cases were negative for recurrence &/or metastases, 15 of which were score (2), 5 score (3), 2 score (1) and 1 case was negative (table 4).

79.4% of cases positive for recurrence &/or metastases were associated with strong staining intensity for GLUT-1. Correlation between staining reaction for GLUT-1 and developing metastases and/or recurrence was statistically significant (p=0.001).

Discussion

In contrast to normal differentiated cells, which depends mainly on mitochondrial oxidative phosphorylation to generate energy, most malignant cells instead rely on aerobic glycolysis, a phenomenon termed "Warburg effect." This gives the malignant cells the chance to survive under hypoxic conditions. Consequently, glucose is essentially needed by malignant cells for survival and proliferation. This implies a need for a concomitant increase in glucose transport across the cell membrane (6,7).

We have investigated the diagnostic role of GLUT-1 IHC staining in ovarian tumors. We found a gradual increase in staining intensity of GLUT-1 from borderline to malignant tumors with a statistically significant difference supporting that the expression of this transporter may be closely related to the malignant transformation of epithelial ovarian tumors. Our findings agreed with results of other studies (3,7,8,9,10,12).

Apart from the study done by Iida et al. (12) who reported 68% positive ratio for GLUT-1 in adenomas, our study as well as all the previously related studies demonstrated negative reaction for GLUT-1 in benign tumors. Regarding borderline tumors, all the studied cases revealed moderate staining reaction so they were assigned a score 2. On the other hand, in the study done by Canturia et al. (2000) 60% of borderline tumors revealed weak staining reaction (scored 1) and 40% (scored 2) (8).

The correlation GLUT-1 with the malignant tumor grade was statistically significant. This was in concordance with other authors (8,14). Ozcan et al. (10) also reported the increased staining reaction for GLUT-1 with increasing the tumor grade however; the correlation in his study wasn't statistically significant.

When the staining reaction for GLUT-1 was assessed in relation to the histologic type of the tumor we have found higher intensity in serous tumors, endometroid and clear cell carcinomas compared to undifferentiated and mucinous carcinomas with statistically significant differences. This was in agreement with Canturia et al. (8) who reported the stronger intensity of stain in serous carcinomas compared to other subtypes with a statistically significant difference. Similarly were the results of Iida et al. (12) who reported a significant higher staining reaction in serous carcinoma than mucinous carcinomas. Moreover, other studies reported that GLUT-1 overexpression was observed more frequently in serous and clear cell types (4,6). Tsukioka et al. (11) declared that the expression of GLUT1differed among the histological types, and the difference between serous and clear cell adenocarcinomas was significant. On the contrary, Canturia et al. (14) found no statistical association between GLUT-1 expression levels and histologic type.

The comparatively strong expression in serous tumors, especially in adenocarcinomas, is considered to be

attributed to the papillary proliferation of the tumor cells accompanied by scanty vasculature that may lead to a more hypoxic environment. It is speculated that mucinous adenocarcinoma may become less hypoxic compared with serous adenocarcinoma because the back-to-back arrangement of mucinous carcinoma nests and glands is accompanied by fine vascular stroma. Furthermore, papillary organization is one of the essential factors for stimulating GLUT-1 expression (12).

As regard the FIGO staging, there has been significant correlation with staining intensity for GLUT-1 ($P = 0.01$) which agreed with other authors (4,7,11). On the other hand, Canturia et al. (14) found no statistical association between GLUT-1 expression levels and FIGO staging.

Despite being not widely studied in relation to prognosis, GLUT-1 can be considered as a poor prognostic factor in ovarian cancer. This can be supported by results from our study together with that of Cai et al. (7) who reported stastically significant correlation between GLUT1 overexpression and ovarian tumor metastases. Similarly, Semaan et al. (4) reported a negative impact on patient survival associated with high GLUT-1 expression. In concordance, Iida et al. (12) declared that the prognosis of ovarian adenocarcinomas with both HIF-1α and GLUT-1 overexpression seemed to be unfavorable regardless of the stage. Similarly, Canturia et al. (14) observed the shorter disease free survival (DFS) associated with GLUT-1 overexpression in advanced stage ovarian cancer patients with complete response to chemotherapy. In addition, Kim et al. (6) found that the overall survival rate tended to decrease when each of GLUT-1, and VEGF was highly expressed but he considered whether each to be considered as independent prognostic factor is questionable. Moreover, it was reported that GLUT-1 expression is remarkably upregulated in EOC and predicts a poor overall survival (15). In contrast to these data, Ozcan et al. (10) reported that when GLUT-1 expression was analyzed against prognosis, no statistically significant difference was identified.

Conclusion

GLUT-1 is related to carcinogenesis of ovarian carcinomas. The statistically significant correlation between staining intensity for GLUT-1 and malignant phenotype can make it a marker for target therapy for ovarian cancer patients. In addition, GLUT-1 can be considered as predictor for metastases and recurrence in ovarian carcinoma that needs to be validated in future trials.

Conflict of interest disclosure:

The authors confirm no conflict of interest with contents of this manuscript.

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Table 1: Characters of studied cases.

Age	Range: 25-77	
Histologic type	Benign : 11(7 mucinous, 4 serous)	
	Borderline: 8(7 serous, 1 mucinous)	
	Malignant: 57	HGS:34
		LGS:6
		Mucinous:7
		Endometroid:4
		Clear cell:3
		Undifferentiated:3
Grade	I: 13	
	II:4	
	III:40	
Stage	IC:7	
	IIIC:17	
	IV:41	
Recurrence&/or metastases	Positive:34	
	Negative :23	

Table 2: correlation between GLUT1 and grade of malignancy.

			GRADE			TOTAL
			1	2	3	
INTENSITY	NEGATIVE	COUNT	0	0	1	1
		% within intensity	.0%	.0%	100%	100.0%
		% within grade	.0%	.0%	.2.5%	1.8%
		% of total	.0%	.0%	.1.8%	1.8%
	Weak	COUNT	2	0	3	5
		% within intensity	40.0%	.0%	60.0%	100.0%
		% within grade	15.4%	.0%	7.5%	8.8%
		% of total	3.5%	.0%	5.3%	8.8%
	Moderate	COUNT	6	0	13	19
		% within intensity	31.6%	.0%	68.4%	100.0%
		% within grade	46.2%	.0%	32.5%	33.3%
		% of total	10.5%	.0%	22.8%	33,3%
	Strong	COUNT	5	4	23	32
		% within intensity	15.6%	12.5%	71.9%	100.0%
		% within grade	46.2%	100.0%	62.2%	56.1%
		% of total	8.8%	7.0%	40.4%	56.1%
Total		COUNT	13	4	40	57
		% within intensity	22.8%	7.0%	70.2%	100.0%
		% within grade	100.0%	100.0%	100.0%	100.0%
		% of total	22.8%	7.0%	70.2%	100.0%

X2=27.541 p=0.001

Table 3: GLUT 1 in relation to tumor type.

			histotype					TOTAL
			serous	mucnous	endometroid	Clear cell	undfferentiateded	
INTENSITY	NEGA-TIVE	COUNT	4	7	0	0	1	12
		% within intensity	33.3%	58.3%	.0%	.0%	8.3%	100.0%
		% within grade	7.8%	36.7%	.0%	.0%	33.3%	15.8%
		% of total	5.3%	9.2%	.0%	.0%	1.3%	15.8%
	Weak	COUNT	4	0	0	0	1	5
		% within intensity	80.0%	.0%	.0%	.0%	20.0%	100.0%
		% within grade	7.8%	.0%	.0%	.0%	33.3%	6.6%
		% of total	5.3%	.0%	.0%	.0%	1.3%	6.6%
	Moderate	COUNT	21	4	0	1	1	27
		% within intensity	77.8%	14.8%	.0%	3.7%	3.7%	100.0%
		% within grade	41.2%	26.7%	.0%	50.0%	33.3%	35.5%
		% of total	27.6%	5.3%	.0%	1.3%	1,3%	35.5%
	Strong	COUNT	22	4	4	2	0	32
		% within intensity	68.6%	12.5%	12.5%	6.3%	.0%	100.0%
		% within grade	43.2%	26.7%	100.0%	66,7%	.0%	42.1%
		% of total	30.3%	5.3%	5.3%	1.3%	.0%	42.1%
Total		COUNT	51	15	4	3	3	67
		% within intensity	68.4%	19.7%	5.3%	2.6%	3.9%	100.0%
		% within grade	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
		% of total	68.4%	19.7%	5.3%	2.6%	3.9%	100.0%

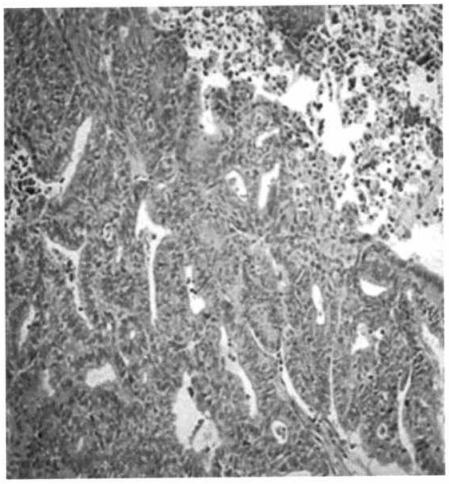
X=25.094 p = 0.014.

Table 4: Correlation between GLUT 1 and prognosis.

			prognosis		TOTAL
			positive	negative	
INTENSITY	NEGATIVE	COUNT	0	1	1
		% within intensity	.0%	100%	100.0%
		% within grade	.0%	4.3 %	1.8%
		% of total	.0%	1.8%	1.8%
	Weak	COUNT	3	2	5
		% within intensity	60.0%	40.0%	100.0%
		% within grade	8.8%	8.7%	8.8%
		% of total	5.3%	3.5%	8.8%
	Moderate	COUNT	4	15	19
		% within intensity	21.1%	78.9%	100.0%
		% within grade	11.8%	65.2%	33.3%
		% of total	7.0%	26.3%	33,3%
	Strong	COUNT	27	5	32
		% within intensity	84.4%	15.6%	100.0%
		% within grade	79.4%	21.7%	56.1%
		% of total	47.4%	8.8%	56.1%
Total		COUNT	34	23	57
		% within intensity	59.6%	40.4%	100.0%
		% within grade	100.0%	100.0%	100.0%
		% of total	59.6%	40.4%	100.0%

X2=21.366 p=0.001.

Figures.



Figure(1a): Endometroid adenocarcinoma (H&Ex100)

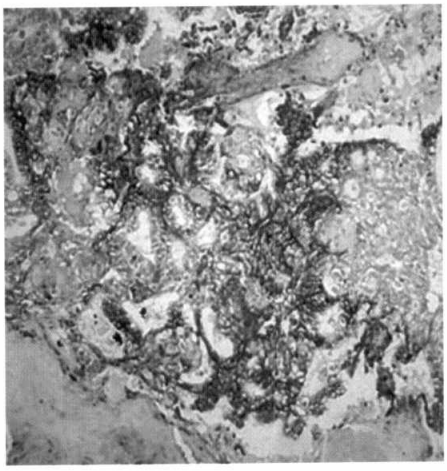


Figure (1b): Strong diffuse reaction for GLUT-1 in the same case (IHC x100)

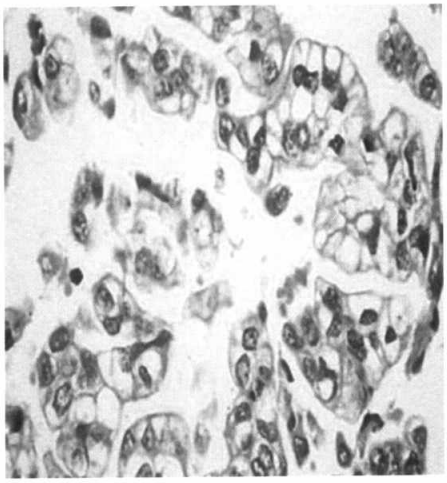


Figure (2a): A case of clear cell carcinoma (H&E x400)

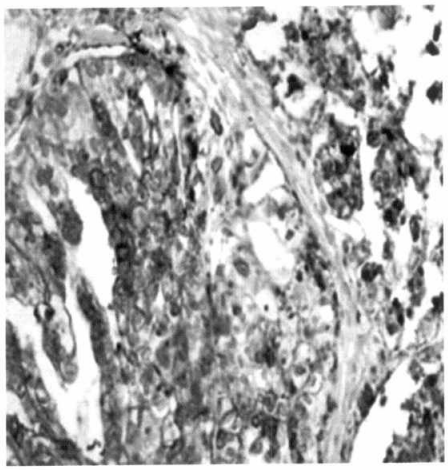


Figure (2b): Strong diffuse reaction for GLUT-1(IHCx200).

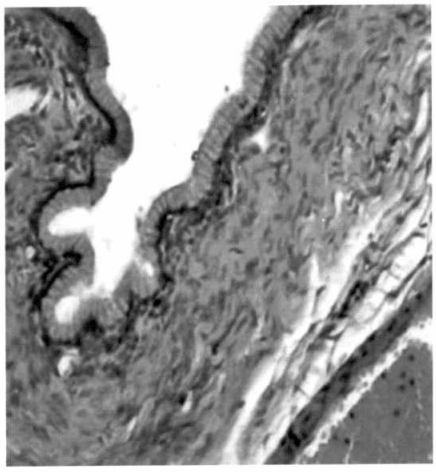
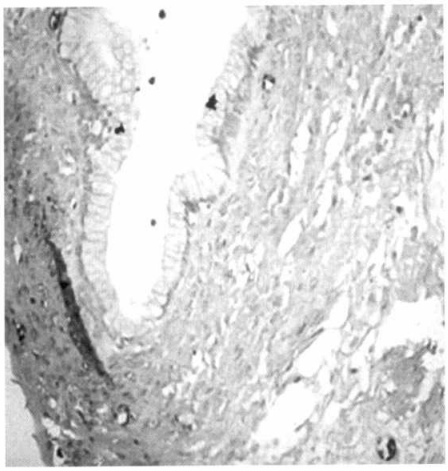


Figure (3a): Mucinous cystadenoma, single layer of cells with bland nuclei (H&E x200)



Figure(3b):Negative reaction for GLUT-1 in the same case(IHC x200)

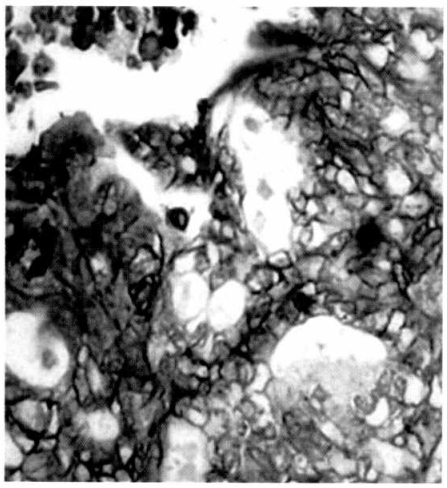


Figure (4a): Strong reaction for GLUT 1 in serous carcinoma (IHC x400)

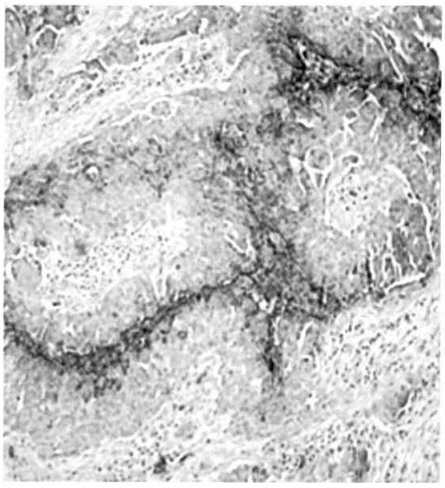
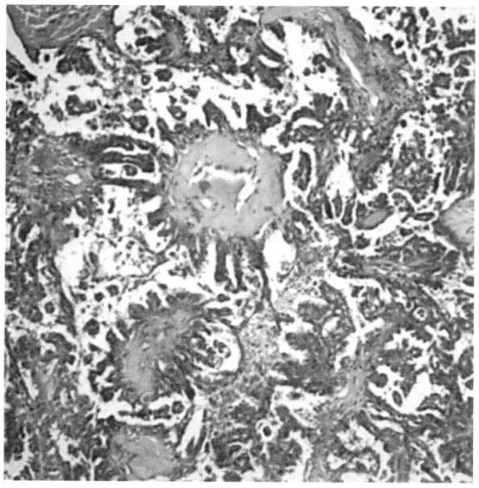


Figure (4b): High grade serous carcinoma, reaction for GLUT-1was intense at the periphery of the papillae where cells are more hypoxic (H&E x100).



Figure(5a): Noninvasive micropapillary serous carcinoma with medusa head like pattern (H&E x200,)

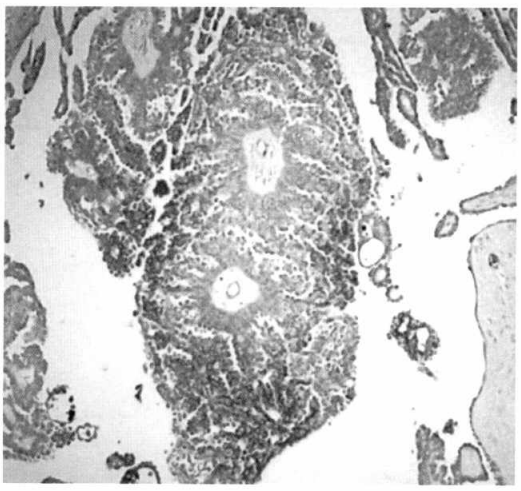


Figure (5b): Staining reaction for GLUT-1 in micropapillary serous borderline tumor (non invasive micropapillary serous carcinoma). Staining is moderate in the center and strong at the periphery which is more hypoxic. Confluence of micropapillae indicates diagnosis of non invasive micropapillary carcinoma (IHCx 100).