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Original Article

Quantification of Mean Vessel Density in Retinoblastoma and Its Correlation with Local Tumor Invasion and Patients Survival

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ABSTRACT

Background and Objectives: **Retinoblastoma is the most common intraocular pediatric malignancy. Angiogenic factor expression such as VEGF (vascular endothelial growth factor) in retinoblastoma can be confirmatory angiogenic potential of this tumor. This study was performed to determine the role of angiogenesis in local invasion of retinoblastoma and its correlation with patients' survival.**

Materials and methods: **This clinicopathological analysis was performed on 60 paraffin-embedded eyes with adequate tumoral tissue, which were stained using a CD34 antibody. Microvessel count was carried out in three tumor areas with the richest vascularity (hot spots) at a high magnification (× 400). The obtained data were correlated with histopathological characteristics and 5-years survival. Statistical analysis of the data was performed using student t-test and ANOVA test ($P<0.05$).**

Results: **Tumor with local invasion to choroids, optic nerve, sclera and cilliary body showed statistically significant higher mean vessel density ($P=0.00$, $P=0.041$, $P= 0.008$ and $P= 0.002$, respectively). In addition, a statistically significant correlation was detected between mean vascular density and 5-years survival ($P=0.031$).**

Conclusion: **The results suggest that in retinoblastoma, mean vessel density has a significant role in local invasion of tumor growth and a significant correlation with patient survival. Therefore, in these patients an anti-angiogenic therapy and minute diagnostic and follow up programs should be considered to identify metastasis.**

Key words: Angiogenesis, Retinoblastoma, Survival

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Introduction

Retinoblastoma is the most common intraocular pediatric malignancy, after uveal malignant melanoma and metastatic carcinoma is most common intraocular malignancy of any age (1). The most common clinical manifestation of retinoblastoma are leukokoria and strabismus, the tumors also could asymptomatic and small fundus lesion found on routine eye inspection (1). Macroscopically tumors usually are creamy white with chalky areas of calcification and necrotic yellowish regions (2). In microscopy, retinoblastoma composed of small round cells with large basophilic nuclei (3). Retinoblastoma therapy under going transition to globe conserving therapy with interest in combined systemic chemotherapy and focal laser tumor ablation, cause of this trend to chemotherapy is potential risk of primary radiotherapy for increasing of late secondary tumors (4). Most common rout for scope tumor from eye is optic nerve (4). Effective histopathological factors on prognosis are consist of invasion of the optic nerve, massive choroid invasion (5) and neovascularization of iris (1).

The role of vessel density in neoplastic disease particularly solid tumors has studied (6). Imbalance between proangiogenic factor opposite antiangiogenic events (resulting of hypoxia and genetic disorders) causes angiogenic switch (7, 8). Several studies show that tumor neovascularization is important in development of lesion, patient survival, or response to chemotherapy (6, 9). Angiogenic factor expression such as VEGF (vascular endothelial growth factor) in retinoblastoma can be confirmatory angiogenic potential of this tumor (10, 11).

In this study quantity of vascular density in patient with different clinical and histopathological characteristic was determined to define the role of angiogenesis in local tumor invasion and its correlation with patients' survival.

Materials and Methods

Patients

Among patient with retinoblastoma that treated by enucleation from 1992 to 2003 at Farabi Hospital, Tehran, Iran, 60 paraffin embedded eyes with adequate tumoral tissue to perform immunohistochemical staining were selected for the study. After histological review, suitable area for immunohistochemical analysis was determined.

Immunohistochemistery

Four-micron section slides were prepared of paraffin blocks and stained using a CD34 antibody (Dako, Denmark) with avidin- biotin peroxidase method. In parallel, slides were stained with hematoxylin and eosin. For evaluation of vessel density, an Olympus CH 30 light microscope was used. Primary, three tumor areas with the richest vascularity, so called "hot spots", selected on low magnification (× 40). In each hot spot, CD 34 positive vessels were quantified with a square field of 0.2 mm^2 at a high magnification (× 400). The means of three areas was recorded. Establishment of brown stain in each cell or cell cluster that clearly isolated from around micro vessels, tumor cells or connective component were accepted as a countable micro vessel. Vessel in necrotic area not scored.

Statistical Analysis

Statistical analysis was performed using SPSS ver. 11.5 soft ware. Student *t*- test was used for two group comparison and ANOVA test for more than two groups. Level of significance of 0.05 applied in all calculation.

Results

Patients consisted of 37 (61.7 %) males and 23 (38.3 %) females. The average age was 32.5 month (between 6 month to 8 year old), mean vessel stained with CD 34 was 8.7/ 0.2 mm^2 (rang; 4-19) and mean diameter of tumors was 17.98 mm (rang; 4-30mm). In retinoblastoma, distribution of vessels showed no particular pattern, tumors with scattered and other with numerous vessels could be identified (Fig.1).

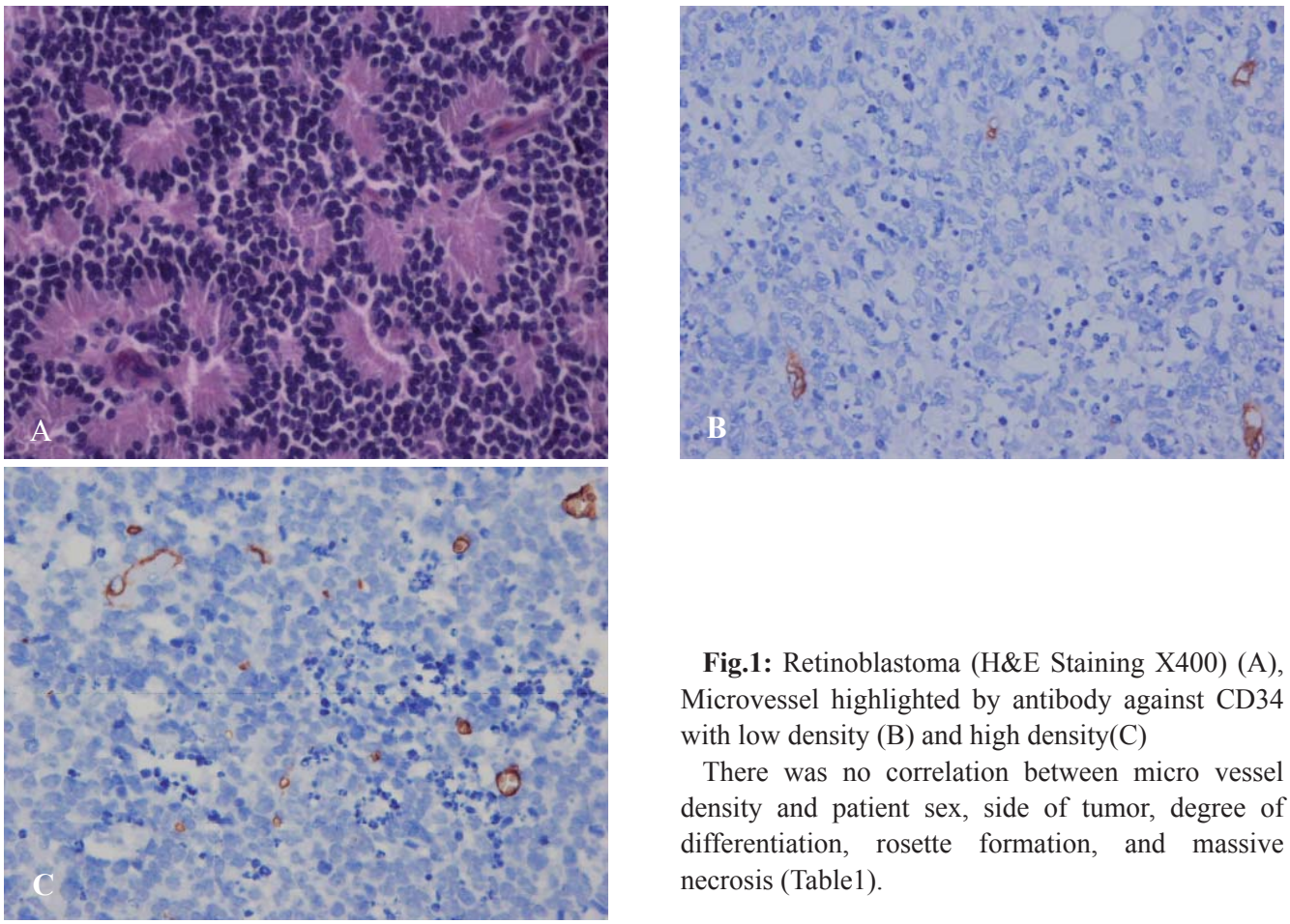

Fig.1: Retinoblastoma (H&E Staining X400) (A), Microvessel highlighted by antibody against CD34 with low density (B) and high density(C)

There was no correlation between micro vessel density and patient sex, side of tumor, degree of differentiation, rosette formation, and massive necrosis (Table1).

Table 1: CD 34 expression according to clinical and pathological variables

Variable		MVD/0.2 mm²	SD	SE	*P*
Sex	Male	8.86	3.15	0.52	
	Female	8.76	3.56	0.74	0.935[t]
Side	Right	9.13	3.52	0.73	
	Left	8.20	3.20	0.58	0.323 [t]
Differentiation	Well	7.40	1.98	0.60	
	Moderate	8.29	2.853	0.56	
	Poorly	9.81	3.95	0.82	0.094 [a]
Optic nerve end	Free	8.36	3.19	0.44	
	Tumor	11.26	3.26	1.23	0.041 [t]
Optic nerve	Free	7.60	2.27	0.38	
	Tumor	10.56	3.86	0.80	0.002 [t]
Sclera	Free	7.92	2.70	0.39	
	Tumor	11.63	3.67	1.11	0.008 [t]
Choroid	Free	6.83	1.67	0.43	
	Tumor	9.34	3.46	0.52	0.000 [t]
Cilliary body	Free	7.54	2.16	0.34	
	Tumor	11.05	3.90	0.92	0.002 [t]
Necrosis	Focal	8.46	3.23	0.55	
	Massive	9.06	3.39	0.68	0.495 [t]
Rosette	+	7.65	2.41	0.54	
	-	9.18	3.57	0.57	0.075 [t]
5 year survival	+	7.52	3.05	0.53	
	-	11.4	4.77	1.50	0.031[t]

MVD: mean vascular density, SD: standard deviation, SE: standard error

t: student *t*-test, a: ANONA test

Retinoblastoma that invade to choroid, cilliary body, head of optic nerve and optic nerve cut end with higher vessel density that this increase was statistically significant (P=0.00, P=0.002, P= 0.041 and P= 0.002 respectively). Also patient with 5 year survival comparison to dead patient in this period , have lower mean vessel density(MVD) that it difference was statistically significant (P=0.031) (Table 1).

Discussion

A statistically significant correlation was detected between vascular density and local invasion to choroids, optic nerve, sclera and cilliary body. In addition, analysis revealed angiogenesis parameters is a significant predictor for 5- year survival.

Three ways of vascular development are vasculogenesis, angiogenesis, and arteriogenesis. The vasculogenesis that mainly in embryonic period occur with differentiation of angioblasts to endothelial cells to form new vessels (12). Angiogenesis, the formation of new vessel from available ones, later in embryogenesis and in normal process such as female cycle reproduction and wound healing (12). Arteriogenesis is enlargement of the arterioles to sustain increase metabolic demand such as vascular stenosis or obstructive events (12). Tumors vessel formation mainly occurs by angiogenesis (13). In the vascular phase, shift to angiogenic factor, causes exponential tumor growth (14). New vessel formation is an important factor in metastasis because tumor cells via microvessels easily enter the circulation (15).

CD 34 marker was stained normal and neoplastic endothelial cells as well as a variety of soft tissue neoplasm (5). We used CD 34 marker, because this is highly sensitive for detection of microvessels, simplicity, and lowest background staining.

Recently, retinoblastoma has changed from almost fatal disease to one in which 95% of patients are cured (3). Metastatic risk factors for retinoblastoma increasingly studied, histological evidence of invasion of choroid, sclera, and optic nerve are predictive for metastasis and risk of death (16). After enucleation in retinoblastoma, clinician ask pathologist to determine metastatic risk. In the recent years, use of prophylactic therapy was based on histopathological evidence of tumor invasion to optic nerve and/or choroids. Commonly patient with post- laminar optic nerve invasion is recommended to adjuvant or prophylactic therapy. Reports of local invasion or metastatic retinoblastoma without any risk factor remind that these factors are insensitive. In evaluation of histopathological risk factors seems to inadequate tissue sampling for histological examination is most important. For dissolving this problem, sampling the entire specimen can be done but not usable in routine practice. Therefore, a test that can perform in a single slide with prognostic information is suitable for usual use.

In this study, we did not fined statistical difference in microvessel density with sex, side, or differentiation in retinoblastoma. Antiangiogenic therapy is a new treatment choice has tested in experimental model (17) and several tumors including retinoblastoma (12, 18,19). Concerning our findings vascular count is crucial for the tumor expansion, therefore tumors with higher vessel count benefit from addition of antiangiogenic therapy to chemotherapy.

Conclussion

Result of our study show tumors with infiltration of optic nerve, choroid, sclera and cilliary body have higher vessel density, the second important issue is that intra tumoral vessels play a significant role for patient survival. Therefore, in such patient, minute diagnostic and follow up programs to identified metastasis should be considered.

Acknowledgements

The authors declare that they have no conflicts of interest.

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