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Microvessel density and Ki-67 labeling index in esthesioneuroblastoma: is there a prognostic role?☆

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ABSTRACT

Esthesioneuroblastoma (ENB) is a malignant neuroectodermal tumor. Hyams grading has an established role in its prognostication. The importance of microvessel density (MVD) and Ki-67 labeling index (Ki-67 LI) is well studied in various tumors, but the same remains understated in ENB. The aims of the study were to estimate proliferation index and MVD in ENB and to correlate them with Hyams grade. Twenty-six ENB cases diagnosed over a period of 5 years were included. Hyams grade, MVD, and Ki-67 LI were evaluated for each of them. The cases were categorized as low (Hyams grades 1 and 2) and high (Hyams grades 3 and 4) grades. Microvessel density and Ki-67 LI were correlated with grade. The treatment response was analyzed in different grades. The commonest histologic grade was 4 (42%). The mean Ki-67 LI was 2%, 8.2%, 30.8%, and 40.5% and mean MVD was 81.67/mm^2, 37/mm^2, 24/mm^2, and 25.2/mm^2 in grades 1, 2, 3, and 4, respectively. A statistically significant correlation of grade with Ki-67 LI ($P < .001$) and MVD ($P < .007$) was noted. Hyams grade in ENB correlates well with treatment response. Ki-67 LI is an important prognostic factor in ENB. We propose a cutoff of 25% for Ki-67 LI to differentiate low- vs high-grade ENB, but larger studies are needed for validation. Contrary to epithelial tumors, there is a decrease in MVD with increasing grade in ENB.

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1. Introduction

Esthesioneuroblastoma (ENB) is a malignant neuroectodermal tumor that comprises approximately 6% of all sinonasal malignancies [1]. It is a malignant small round cell tumor with fibrillary stroma and variable presence of rosettes (Homer Wright and Flexner Wintersteiner). The tumor is immunopositive for synaptophysin (Syn), neuron-specific enolase (NSE) and chromogranin (CG) along with s100 positivity in sustentacular cells around the tumor islands [2]. It shows remarkable variation in biological activity ranging from indolent course to widely metastatic disease [3]. The paucity of large case series or randomized treatment trials makes the determination of prognostic factors perplexing in this rare tumor.

Neoangiogenesis is believed to play a pivotal role in occurrence and progression of solid epithelial tumors, but its prognostic value has not been studied and validated in many endocrine tumors [4]. Similarly, the proliferation indices have been studied extensively in various primary central nervous system and epithelial tumors, but the data on ENB are scant [5]. The aims of the present study are to estimate proliferation index and microvessel density (MVD) in ENB and their correlation with Hyams grade and to study response to treatment among various Hyams grades.

2. Materials and methods

Twenty-six consecutive cases of ENB diagnosed over a period of 5 years (2009-2013) in a tertiary care center were included in the study. The diagnosis in each case was established on the basis of standard histopathologic and immunohistochemical criteria [1]. A battery of immunostains including Syn (clone SP11; Spring Biosciences, Pleasanton, California; 1:200), CG (clone SP12; Spring biosciences; 1:200), NSE (clone NSE-P1; Sigma-Aldrich, St Louis, Missouri; 1:100), CD56 (clone 123C3; Spring Biosciences; prediluted), s100 (clone SP127; Spring Biosciences; 1:100), pancytokeratin (pan-CK; clone AE1/AE3; Thermo Scientific, Carlsbad, California; 1:50), epithelial membrane antigen (EMA; clone E 29; Thermo Scientific; 1:800), EBV LMP-1 (clone CS.1-4; Dako, Glostrup, Denmark; 1:200), and p40 (clone BC28; Biocare, Concord, California; prediluted) was used. Only those cases with suggestive morphology and immunohistochemical positivity for 3 or more neuroendocrine markers (CG, Syn, CD56, or NSE) along with immunonegativity for EMA and absence of dotlike positivity of pan-CK typical of small cell neuroendocrine carcinoma were diagnosed as ENB.

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The cases that showed positivity for pan-CK and EMA, and negativity for CK5/6, Syn, CG, CD56, S-100, P-40, and EBV LMP-1 were considered sinonasal undifferentiated carcinoma (SNUC) and were excluded. The cases with diffuse dotlike positivity of pan-CK characteristic of small cell neuroendocrine carcinoma were also excluded. Demographical parameters, clinical presentation, modified Kadish staging, and treatment modalities adopted were studied and analyzed. Hyams grading was done independently by 3 pathologists (L.S., R.R., M.K.S.). For the convenience of statistical analysis, the cases were subsequently categorized as low grade (Hyams grades 1 and 2) and high grade (Hyams grades 3 and 4) [6,7]. CD31 (clone SP164; Spring Biosciences; 1:100) staining was done to highlight the blood vessels and MVD estimation was done using image proplus software on digitally acquired images. Microvessels were counted on a ×20 objective (0.739 mm^2) in the area of highest MVD and were then expressed per millimeter square area [8]. For uniformity of assessment, any dark staining, endothelial cell or cell cluster clearly separating from adjacent structures was considered as a distinct vessel. Microvessel density was calculated in the areas of highest MVD (hot spots) as neoangiogenesis is not uniform within a given tumor [9]. Ki-67 (clone SP-6; Spring Biosciences; 1:200) staining was deemed as positive when either the entire nucleus or a portion of it was stained. Ki-67 labeling index (Ki-67 LI) was determined by estimating Ki-67 immunostaining in the highest proliferation areas. The LI or growth fraction was defined as the percentage of positive cells after evaluating a total of 500 cells per area (total of 6 areas) or 3000 cells per section (excluding labeled endothelial cells and lymphocytes) [10]. The anatomical location, Hyams grade, MIB LI, and MVD in each case is shown in Table 1.

With regard to the clinical outcome, all patients were followed up monthly for a minimum period of 6 months and 3 monthly thereafter (mean follow-up, 15 months). The follow-up evaluation was done by clinical examination, nasal endoscopy, and radiology, if and when required. Complete resolution of disease was considered as absence of any evidence of tumor during sequential monthly follow-up. Any evidence of disease before the period of 6 months was considered as residual, whereas after 6 months, it was designated as recurrence. Wherever available, the posttreatment biopsy was reviewed in recurrent and residual tumors. We analyzed and compared MVD and Ki-67 LI values in high-grade and low-grade ENBs. Because the sample size was limited, the exact Wilcoxon rank sum nonparametric test was used for comparing the distribution of MVD and MIB LI between the low- and high-grade ENBs. Statistical significance was assessed using an α level of .05. Statistical analyses were performed using STATA 11.2 software (StataCorp, College Station, Texas).

3. Results

The study included a total of 26 cases of ENB with a male-to-female ratio of 3.6:1. The age ranged from 7 to 63 years, with mean age being 32 years. The commonest clinical presentation was epistaxis (87.7%), followed by nasal obstruction (82%), anosmia (42%), and visual complaints (40%). Two patients had atypical presentations, in the form of syndrome of inappropriate antidiuretic hormone secretion (SIADH), consistent with the paraneoplastic presentation of ENB.

The commonest histologic grade was grade 4, seen in 11 patients (42%), followed by grade 3 and grade 2, each comprising 6 patients, (23%), with only 3 patients in grade 1 (12%). All 26 cases exhibited the classical morphology. The lower-grade tumors showed lesser degree of atypia, fewer mitosis (<4/10 high-power field) and no tumor necrosis (Fig. 1A, B), whereas the higher-grade tumors exhibited a much higher mitosis (>4/10 high-power field), nuclear atypia, and necrosis (Fig. 1C, D).

The immunohistochemical results were as expected in the case of ENB (CG, Syn, and S100 positive), but in our study, 8 cases showed pan-CK reactivity. The MIB LI was 2%, 8.2%, 30.8%, and 40.5% respectively, in grades 1, 2, 3, and 4. As compared with high-grade ENB (Fig. 2A), low-grade ENBs (Fig. 2B) had significantly higher Ki-67 LI ($P < .001$). The mean MVD was 81.67/mm^2, 37/mm^2, 24/mm^2, and 25.2/mm^2 in grades 1, 2, 3, and 4, respectively (Table 2). Significantly lower MVD was noted in high-grade ENBs (Fig. 2C) as compared with low-grade ENBs (Fig. 2D). The mean MVD was 59.2/mm^2 in low-grade ENBs as compared with 24.6/mm^2 in high-grade ENBs ($P = .007$; Fig. 3).

Table 1
Anatomical location, Hyams grade, MIB labeling index, and micro vessel density in each of 26 cases

S.No	Anatomical location	Hyams grade	Ki-67 LI	MVD/mm^2
1.	Rt sinonasal mass with I/C and I/O extension	4	30	5
2	Mass in Lt nasal cavity, sphenoid, ethmoid sinuses with I/O extension	2	2	26
3.	Nasal mass, extending to cribriform plate	3	20	4
4.	Rt sinonasal mass with I/C and I/O extension	4	55	38
5.	Sinonasal mass extending to basifrontal area with destruction of frontal skull vault	4	40	18
6.	Rt sinonasal mass	2	10	51
7.	Mass in Lt nasal cavity, sphenoid, ethmoid, I/O extension	3	30	6
8.	Mass in Lt nasal cavity, sphenoid, ethmoid, I/O extension	2	4	51
9.	Sinonasal mass with intracranial and intra orbital extension	4	40	35
10.	Rt nasal cavity mass, extending to cribriform plate, destroying Rt lamina papyrecea, posterior table of frontal sinus with I/C and I/O extension	3	30	41
11.	Mass in b/l nasal cavity, b/l ethmoids, Rt frontal sinus	4	40	21
12.	Mass in Rt nasal cavity, extending to cribriform plate	1	2	64
13.	Mass in b/l nasal cavity, b/l ethmoids, Rt frontal sinus	1	2	101
14.	Rt sinonasal mass with I/C and I/O extension	4	35	26
15.	Mass in Rt nasal cavity extending to sinuses with intraorbital extension	3	40	51
16.	Sinonasal mass with I/C and I/O extension	4	30	28
17.	Mass in Rt nasal cavity, paranasal sinuses, destroying cribriform plate	2	8	38
18.	Mass in Lt nasal cavity extending to sinuses with I/O extension	4	60	22
19.	Sinonasal mass with I/C and I/O extension	2	20	20
20.	Rt sinonasal mass, destroying the cribriform plate	1	3	80
21.	Lt sinonasal mass	2	5	36
22.	Rt nasal cavity mass, extending to cribriform plate, destroying Rt lamina papyrecea, post table of frontal sinus with I/C and I/O extension	3	35	20
23.	Lt sinonasal mass	3	30	22
24.	Sinonasal mass with I/C and I/O extension	4	38	21
25.	Rt nasal cavity mass with I/C and I/O extension	4	42	18
26.	Rt sinonasal mass with I/C and I/O extension	4	45	45

Abbreviations: b/l, bilateral; Lt, left; I/C, intracranial; I/O, intraocular; post, posterior; Rt, right.

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