HISTOPATHOLOGICAL CONSIDERATIONS IN THE DIAGNOSIS OF TUMOURS OF ADRENAL MEDULLA

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ABSTRACT

BACKGROUND

Tumours of the adrenal medulla can be heterogeneous in morphology and unpredictable in biological behaviour. Diagnosis of these tumours requires histopathological evaluation along with clinical and radiological correlation. These tumours are categorized into neuroblastoma and pheochromocytoma groups based on specific features. However, in some cases distinguishing malignancy becomes extremely difficult, especially in case of pheochromocytoma. Moreover, differential diagnosis of neuroblastoma and pheochromocytoma from adrenal cortical tumours and metastatic lesions poses many challenges to the pathologist. Careful gross and microscopic examination of these lesions is essential for an accurate diagnosis and immunohistochemistry is crucial for confirmation.

AIM

This work is taken up to study the incidence of adrenal medullary tumours and analyse the histopathological criteria for diagnosis and the utility of immunohistochemistry in differentiating them from cortical tumours and the metastatic lesions.

MATERIAL AND METHODS

This is a retrospective study of tumours of adrenal medulla that were reported in the Department of Pathology, Guntur Medical College. Gross examination of postoperative specimens and microscopic features on histological sections are analysed and supplemented by immunohistochemistry.

RESULTS

The clinical and morphological findings of tumours of adrenal medulla are tabulated and basing on the distinct features histological diagnosis of neuroblastoma and pheochromocytoma is made and confirmed by immunohistochemistry.

CONCLUSION

This study highlights the importance of thorough histopathological examination in diagnosis of tumours of adrenal medulla and specific criteria in grading neuroblastoma and identifying malignancy in pheochromocytoma and the role of immunohistochemistry in confirmation.

KEYWORDS

Adrenal Medullary Tumours, Neuroblastoma, Pheochromocytoma, Histopathology, Immunohistochemistry.

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INTRODUCTION

Tumours and tumour-like conditions of the adrenal medulla can be categorized into two groups, embryonal neural tumours and adult neuroendocrine tumours. The former is represented by neuroblastoma and ganglioneuroblastoma that arise from primitive sympathetic ganglion cells and the latter is represented by pheochromocytoma that arise from chromaffin cells. These groups are characterized by distinct clinical and morphological features. However, accurate diagnosis depends upon careful interpretation of histopathological criteria in correlation with clinical setting and imaging investigations. Immunohistochemistry (IHC) plays a crucial supplemental role in differentiation of medullary tumours from cortical tumours and extra-adrenal tumours, i.e. metastatic carcinomas or primary carcinomas of kidney or liver or malignant melanoma.

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MATERIAL AND METHODS

This is a retrospective study of adrenal medullary tumours that were reported during the last five years in the Department of Pathology, Guntur Medical College. The size and weight of postoperative specimens and gross features of external and serial cut surfaces and tumour capsule were recorded. Paraffin embedded tissue blocks and Haematoxylin and Eosin (H and E) sections were retrieved from the archives in the Department of Pathology and reviewed.

Special stains like periodic-Schiff reaction and reticulin stain were done to assess intracytoplasmic hyaline and distribution of reticulin fibres in the tumour tissue. A panel of immunohistochemical markers including inhibin, vimentin, chromogranin and neuron specific enolase (NSE) were used for confirmation of diagnoses and differentiation from cortical tumours and secondary tumours. Sections of 4-micron thickness were taken for H and E staining and Immunohistochemical study.

RESULTS

The clinical presentation and morphological findings of 5 cases of adrenal medullary tumours were tabulated (Table I). The cases were classified according to the WHO classification.^[1] and categorized into neuroblastoma and pheochromocytoma groups after histopathological assessment (Table II).

Three cases (Cases No. 1, 2 and 3) were reported as neuroblastoma. The age of these patients ranged from 5 months to 3 years with male-to-female ratio of 2:1. The weights of neuroblastomas varied between 144 g to 288 g with an average weight of 209.3 g. The largest diameter ranged between 8 and 9 cm with an average of 8.6 cm (Fig. 1a).

Two cases of pheochromocytoma (Cases No. 4 and 5) were diagnosed both in women in fourth and fifth decades of

life. The clinical presentation in both cases was hypertension with episodic headache. Postoperative adrenal masses measured 6 to 8 cm in the largest dimension, while weight ranged from 65 gm to 85 gm. On gross examination, replacement of the adrenal gland by the tumours was evident in both the lesions. Cut surface showed heterogeneous appearance with areas of haemorrhage and foci of necrosis in one case.

Features	Case 1 F 5 months	Case 2 M 3y	Case 3 M 2y	Case 4 F 35y	Case 5 F 41y
Clinical presentation	Abdominal mass	Abdominal mass, para-aortic lymphadenopathy	Abdominal mass	Retro-peritoneal mass	Hypertension
Tumour size	9x7x4 cm	8x5x5 cm,	9x7x6 cm	6x4.5x4 cm	8.5x4.5x4 cm
Tumour weight	196 g	144 g	288 g	65 g	86 g
Margins	Infiltrative	Infiltrative	Infiltrative	Capsulated	Capsulated
Cut surface	Solid, grey white/brown/yellow	Grey white/brown	Lobulated, grey white	Nodular, grey white/brown/yellow	Grey brown/yellow, soft
Haemorrhage	Present	Present	Present	Present	Present
Necrosis	Present	Present	Present	-	-

Table 1: Clinical and Gross Features of Tumours of Adrenal Medulla

Features	Case 1 F 5/12y	Case 2 M 3y	Case 3 M 2y	Case 4 F 35y	Case 5 F 41y
Cells	Small round cells	Round to oval cells	Round to oval cells, tumour giant cells	Cells clear/amphophilic cytoplasm	Cells clear/amphophilic cytoplasm
Nuclei	Round, deeply staining	Round, deeply staining	Round, deeply staining	Hyperchromatic prominent nucleoli	Hyperchromatic prominent nucleoli
Arrangement	Nodules with intervening fibrous septa	Clusters, Homer Wright rosettes	Alveolar pattern, rosettes	Zellballen pattern	Zellballen pattern
Background	Neurofibrillary	Stroma Poor	Fibrillary	Fibrovascular Stroma	Fibrovascular Stroma
Mitotic Activity	10/10 HPF	>100 karyorrhectic index			
Haemorrhage	Present	Present	Present	Present	Present
Necrosis	Present	Present	Present	-	-
Calcification	Present	Present	Present	-	-
Vascular Invasion	-	-	-	-	-
Capsular Invasion	-	-	-	-	-
Histological Diagnosis	Neuroblastoma, poorly differentiated	Neuroblastoma with deposits in lymph nodes	Neuroblastoma anaplastic	Pheochromocytoma	Pheochromocytoma

Table 2: Microscopy of Tumours of Adrenal Medulla

Fig. 1: Neuroblastoma

a. Gross Cut Surface

b. Cells Arranged in Nodules H&E 10x

Fig. 3: Neuroblastoma

a. Deposit in Lymph Node H&E 10x

Fig. 2: Neuroblastoma Cellular Features

c. Organoid Pattern H&E 10x

a. High Mitotic Activity H&E 40x

b. IHC Positive for NSE

Fig. 4: Pheochromocytoma

b. Homer Wright Pseudorosettes H&E 40x

a. CT Images

b. Arrangement of Cells H&E 10x

b. NSE Positivity

c. Cellular Features H&E 40x

Fig. 5: Pheochromocytoma IHC

a. Chromogranin Positivity

DISCUSSION

Neuroblastoma

Majority of embryonal tumours are neuroblastomas, which are heterogeneous with respect to location, morphology and biological behaviour. They can regress spontaneously, mature to a benign ganglioneuroma or behave aggressively with metastatic spread and fatal outcome.^[2] Neuroblastoma is almost exclusively seen in children and it is the third most common childhood cancer.^[3] Young et al. observed that 85% of all neuroblastomas occur in the first 4 years of life and that there is no sex predilection.^[4] In the present study also, all 3 cases of neuroblastoma (Cases No. 1, 2, 3) occurred in children with ages ranging from 5 months to 3 years.

Samia et al. reported that tumour size ranged from 9.5 to 20cm with an average size of 14.56 cm.^[5] The tumour size of our cases in the largest dimension varied from 8 cm to 9 cm and weight varied from 144 g to 288 g (Table I). The histological diagnosis of neuroblastoma was made in these cases (Cases No. 1, 2, 3) basing on the presence of monotonous small blue round to oval cells in lobules with prominent intervening fibrovascular stroma (Fig. 1b, 1c). Prominent mitotic activity (Fig. 2a) and typical Homer-Wright pseudorosettes were present in 2 cases (Fig. 2b). Extensive areas of necrosis and calcification were present in all 3 cases. These observations are in accordance with Shimada et al.^[6] and Lack et al.^[7] who reported that neuroblastoma is characterized by lobular pattern with delicate and incomplete fibrovascular septa.

Histological classification system of neuroblastoma was first proposed by Shimada.^[8] This was later modified by International Neuroblastoma Pathology Classification System (INPC).^[9] with prognostic implications. According to these systems, neuroblastoma is classified into 3 subtypes: undifferentiated, poorly differentiated and differentiated. One of our cases (Case No. 1) was graded III basing on the presence of differentiated elements and graded II basing on mitotic activity. In the second case of neuroblastoma (Case No. 2), microscopy of lymph nodes showed metastatic deposits (Fig. 3a). Third case (Case No. 3) showed mononucleated and multinucleated giant cells and was categorized as anaplastic

neuroblastoma. Capsular invasion and angioinvasion were observed in 2 cases.

The differential diagnosis of neuroblastoma includes rhabdomyosarcoma, Ewing's Sarcoma (ES)/Primitive Neuroectodermal Tumour (PNET) and blastematos Wilms' tumour. Immunohistochemistry helps in confirming the diagnosis of neuroblastoma. Negativity for CD99 differentiates neuroblastoma from ES/PNET. NSE is positive in 85 to 100% of cases of neuroblastoma. In this respect, Liu et al. reported that NSE was expressed in small cells of neuroblastoma.^[10] In our cases, expression of NSE was observed in majority of the cells of neuroblastoma.

Pheochromocytoma

Pheochromocytoma can affect any age group. The two cases of pheochromocytoma in our study were seen in the fourth decade. In this regard, Lack recorded that the peak age at diagnosis is in the fifth decade.^[7] Both cases in the study occurred in females, though roughly equal sex incidence has been observed in other studies.^[1] Ozgur Mete suggested careful examination of adjacent adrenal gland for any small nodules.^[11] On gross examination, both cases in the study showed encapsulation and there was no suggestion of infiltration into the kidney or adjacent tissues. A compressed rim of adrenal cortex was found at the periphery of the lesion with intervening peritumoral collagen.

The presence of typical Zellballen pattern of arrangement of tumour cells surrounded by sustentacular cells and fibrovascular stroma favour a diagnosis of pheochromocytoma (Fig. 4b, 4c). Reticulin stain clearly demonstrates the Zellballen pattern. Majority of the tumour cells were polygonal, whereas some cells were round to oval to spindle shaped. Literature shows that oncocyctic change, spindle cells and lipid cell change with clear cell morphology in pheochromocytoma can mimic cortical lesions.^[1,12] Cytoplasm of tumour cells was granular and eosinophilic/basophilic.

Immunohistochemical staining for neuroendocrine markers chromogranin A, synaptophysin and NSE was done for confirmation. Pace et al. and Feng et al. reported that pheochromocytomas are strongly reactive for chromogranin A and neuron specific enolase.^[13,14] Positive reaction for chromogranin A is the most reliable marker to differentiate pheochromocytoma from adrenal cortical and non-neuroendocrine metastatic deposits.^[13] Distinctive granular chromogranin positivity was evident in our cases, thereby confirming the diagnosis of pheochromocytoma (Fig. 5a). Diffuse NSE positivity also was apparent in the cytoplasm (Fig. 5b). Samia et al. observed that positivity for NSE also helps in differentiating from adrenocortical tumours.^[5] Achilles et al. found that malignant tumours were devoid of S-100 positive cells and felt that the presence of S-100 positive cells may help in excluding malignancy in pheochromocytoma.^[15] Negativity for EMA ruled out the possibility of renal cell carcinoma in our cases.

Malignancy in Pheochromocytoma

The definition of malignancy in medullary tumours is stringent. It is defined by the presence of metastasis to sites, where paraganglionic tissue is not normally found.^[1,16] It is very difficult to determine malignancy in pheochromocytomas before the occurrence of metastasis. Macroscopic features like

larger and nodular tumours with variegated appearance favour malignant pheochromocytoma, but pleomorphism of tumour cells alone is insufficient in establishing malignancy in these tumours. Histological findings like capsular invasion/vascular invasion, extension to periadrenal fat, increased cellularity, hyperchromasia, smaller cells with high N/C ratio, macronucleoli, increased mitotic activity and atypical mitotic figures may suggest aggressive course, but they are not diagnostic of malignancy.

In the absence of metastasis accurate prediction of biological behaviour is difficult, but it is recognized that extensive local invasion could be lethal. Thomson LD proposed a system for categorizing these tumours, 'Pheochromocytoma of the Adrenal gland Scoring Scale' (PASS) basing on weighted analysis of a set of features.^[17] All tumours that metastasized were reported to have score of 4 or more. Nuclear hyperchromasia, prominent nucleoli and abundant clear cytoplasm were evident in one of our cases, but no secondaries were detected in our cases of pheochromocytoma. Immunohistochemical study with Ki-67 labelling also has been tried to assess malignant potential by some workers.^[18,19] but their usefulness is still limited to research. Kimura et al. proposed a scoring system combining histologic, immunohistochemical and biochemical characteristics to predict metastatic potential and prognosis of tumours with metastasis.^[18] However, currently there is no universally agreed staging system.

CONCLUSION

In spite of many advances taking place in the evaluation of tumours of adrenal medulla, careful gross examination and histological interpretation of H and E sections still remain the fulcrum basing on which an accurate diagnosis is made. Neuroblastomas can be heterogeneous in morphology as well as behaviour and should be graded basing on distinct histologic criteria. It is important to distinguish malignant tumours from benign counterparts to plan appropriate management, but determination of malignancy in pheochromocytomas is difficult. They tend to metastasize late and behave unpredictably and hence they should never be diagnosed as benign. At the same time, they should not be designated malignant in the absence of demonstrable secondary deposits in lymph nodes or distant sites. Immunohistochemistry plays an important supplemental role in differentiating tumours of medulla from cortical and metastatic tumours.

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