Adrenocortical Neoplasms in Childhood and Adolescence: Analysis of Prognostic Factors Including DNA Content

Claudia Zerbini, M.D., Harry P.W. Kozakewich, M.D.,
David S. Weinberg, M.D., Ph.D., Diane J. Mundt, Ph.D.,
James A. Edwards III, B.S., H.T. (A.S.C.P.), and
Ernest E. Lack, M.D.

Abstract

Thirty-two adrenocortical neoplasms in children and adolescents were evaluated for prognostic factors including clinical and morphological parameters and DNA ploidy. The patients were segregated into two groups according to clinical outcome: group A, represented by patients with clinically benign neoplasms (n = 15), and group B, patients with clinically malignant tumors as evidenced by local recurrence, metastases, or fatal outcome (n = 17). Clinical and morphological parameters in these two groups were evaluated using appropriate statistical methods. Parameters with a significant predictive value in terms of prognosis were age (p = .04), tumor size (p = .0003), median tumor weight (p = .0001), mitotic count (p = .04), and 25% tumor necrosis or more (p = .03). Twenty-three cases were studied for DNA ploidy: 10 cases by image analysis and 13 by both image analysis and flow cytometry. By ploidy analysis, 17 of 23 cases—12 of 14 in group A and 5 of 9 in group B—were found to be aneuploid. Multiple aneuploid peaks were found in 5 of 23 cases—4 of 14 cases in group A and 1 of 9 cases in group B. In tumors studied by both image analysis and flow cytometry, there was no discrepancy between results of ploidy analysis. There was no statistically significant association demonstrated between clinical outcome and DNA ploidy pattern. DNA ploidy heterogeneity, characterized by multiple aneuploid populations of cells, was also detected in both benign and malignant neoplasms. Based on our results, aneuploidy is relatively frequent in pediatric adrenocortical tumors and does not appear to have predictive value for biological behavior. Endocr Pathol 3:116–128, 1992.
in the first two decades of life were statistically analyzed for prognostic significance. Epidemiological, clinical, and pathological features of 30 tumors described herein were published recently [20]. A pertinent review of the literature related to DNA ploidy for adrenocortical neoplasms is provided.

**Materials and Methods**

**Cases**

The 32 cases of adrenocortical tumors were obtained from the National Cancer Institute, Bethesda, Maryland \((n = 16)\), Children's Hospital, Boston, Massachusetts \((n = 15)\), and Georgetown University Medical Center, Georgetown, Washington, DC \((n = 1)\). The age and sex of the patients, clinical data, size and weight of each tumor, and the original pathological diagnosis were obtained in all cases from the clinical records and pathology reports. A more detailed study of the clinico-pathological features, treatment, and follow-up of 30 of these patients is published elsewhere [20].

The patients were segregated into two groups based on clinical follow-up: Group A included patients who were alive (or who had died of other causes) without evidence of recurrent or metastatic adrenocortical tumor \((n = 15)\). Included in group A were tumors that had originally been diagnosed as adrenocortical adenomas \((ACA, n = 5)\), adrenocortical carcinomas \((ACC, n = 9)\), and adrenocortical tumor with indeterminate malignant potential \((ACI, n = 1)\). Group B consisted of patients with recurrence or documented metastases or fatal outcome due to tumor \((n = 17)\).

The length of follow-up varied from 11 months to 17 years. Sex, age, laterality, the presence of an endocrine syndrome, weight and size of the tumor, and original pathological diagnosis \((ACA \text{ or } ACC)\) were evaluated in groups A and B using statistical methods.

**Pathological Features**

Using statistical analysis, the following parameters were compared in groups A and B: growth pattern \((alveolar \text{ or } nesting, \text{ diffuse } \text{ or solid}, \text{ trabecular}, \text{ or mixed})\), the presence of capsular invasion and vascular invasion, broad fibrous bands, nuclear pseudo-inclusions, mitotic activity \((including \text{ atypical } \text{ mitoses})\), and microcalcifications, and the proportion of cells with compact, eosinophilic cytoplasm versus pale lipid-rich cells. Nuclear pleomorphism was graded as 0 \((absent)\), 1+ \((mild)\), 2+ \((moderate)\), or 3+ \((marked)\). The mitotic rate was evaluated in 50 high-power fields \((hpf)\) after selecting the areas with greatest mitotic activity. The amount of tumor necrosis was visually estimated from examination of the histological slides and was expressed as a percentage of total area of tumor.

**DNA Ploidy Analysis**

DNA ploidy was measured in 23 of the 32 tumors, 10 cases by image analysis only \((Cases 7, 11, 12, 14, 16, 19, 20, 22, 24, 30)\) and 13 cases by both image analysis and flow cytometry \((Cases 1-6, 8, 9, 13, 15, 21, 25, 29)\). Paraffin blocks of 13 different tumors were available. For image analysis, 5-µm sections were cut from selected paraffin blocks and were stained for DNA by the Feulgen method (Cell Analysis System, Elmhurst, IL). In 10 cases, hematoxylin and eosin-stained slides were destained and restained by the same Feulgen method \((Cases 7, 11, 12, 14, 16, 19, 20, 22, 24, 30)\). DNA content of nuclei in Feulgen-stained sections was measured by image analysis using the CAS-200 System \((Cell \text{ Analysis System})\) with the Quantitative Ploidy Analysis software \((version 2.5)\). This software provides a filter function that automatically classifies nuclei into six classes according to nuclear area, shape, DNA content \((in \text{ picograms})\), and optical density. The classes were previously defined in such a way that classes 1 and 2 corresponded to diploid and class 5 to tetraploid cells; classes 3 and 4 had DNA content between diploid and tetraploid, and class 6 represented hypertetraploid cells. In every case, at least 100 nuclei were measured. Only 1 of the 23 cases stained by the Feulgen method \((Case 13)\) was not accepted by this filter function because all cells were in the hypodiploid range \((suboptimal staining)\).

From the 13 cases in which paraffin blocks were available, nuclear suspensions were prepared from thick sections \((50 \mu m)\) by the method of Hedley and co-workers [12] as modified by Stephenson and associates [27]. Two additional 5-µm hematoxylin and eosin-stained sections \((before \text{ and after the...)}\).