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ARTICLE

Apoptosis and P53, Bcl-2 and Bax Gene Expression in Parathyroid Glands of Patients with Hyperparathyroidism

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Altogether 107 patients were operated on at the Department of Transplantation and Surgery of Semmelweis University in the past four years, for clinical symptoms of hyperparathyroidism. Clinical and laboratory data of the patients supported the diagnosis of primary or secondary hyperparathyroidism. Chronically impaired renal function was found in 52 cases. The removed parathyroid glands showed hyperplasia in 54, adenoma in 50 and carcinoma in 3 cases. The majority of parathyroid lesions in primary hyperparathyroidism were adenomas (41 cases) and in secondary hyperparathyroidism were hyperplasias (43 cases). The ratio of oxyphil to chief cells as well as occasional mitotic and apoptotic figures were determined. The oxyphil component was present in both hyperplastic and tumorous lesions. Apoptosis and mitosis were rarely seen in hyperplasias and adenomas (under 2%), whereas in carcinomas 3% of the tumor cells were apoptotic and 4% showed mitosis. Cytoplasmic p53 positivity could be observed in 3 of the adenomas and in 2 of the hyperplasias. The carcinomas, four adenomas and 3 hyperplasias showed nuclear p53 positivity. Bcl-2 and Bax were detected in the cytoplasm of the tumor cells in the majority of adenomas and in the cells of hyperplasias. Oxyphil cells were more frequently positive than chief cells or clear cells. Colocalization of Bcl-2 and Bax was found randomly in all types of lesions. The very low incidence of carcinoma, the low mitotic and apoptotic ratio in adenomas and hyperplasias suggest a potent antiproliferative defense mechanism in the parathyroid cell population. This may also be reflected in the cytoplasmic colocalization of various gene products which regulate cell death and cell proliferation. No significant differences in the p53, Bcl-2 and Bax spectrum were found between the primary and secondary (i.e. renal failure) parathyroid alterations. (Pathology Oncology Research Vol 10, No 2, 98–103)

Keywords: Parathyroid, adenoma, hyperplasia, carcinoma, mitosis, apoptosis, Bcl-2, p53, Bax

Introduction

Hyperplasia and adenomas of the parathyroid glands are not uncommon, but malignant tumors of this gland are extremely rare.[1] Hyperplasias may be primary but in most cases parathyroid hyperplasia is secondary to chronic renal failure. Adenomas may appear in both primary and secondary hyperparathyroidism (HP), but the majority of adenomas are not related to renal disease.[2] Of course, renal damage develops regularly as a result of an elevated serum Ca level in HP.

The difficulties in differentiating between hyperplasia, adenoma and carcinoma of the parathyroid gland are well known. Most studies refer to morphological criteria and biological behaviour of the tumours.[3] The significance of DNA diploidy indicating the benign or malignant character of parathyroid tumours was studied by Bocsi et al.[4] They found that the DNA index had no value in deciding the benign or malignant character of a given sample.

Few but important data are available on p53 and Bcl-2 expression in parathyroid hyperplasia, adenoma and carcinoma. Bcl-2 and P53 expression was found both in hyperplasias and adenomas, but carcinomas failed to express

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Table 1. **Characteristic laboratory findings in HP patients**

	PH		*SH*	
	Pre-operative	*Post-operative*	*Pre-operative*	*Post-operative*
Se Ca (mmol/l)	3(2,73-3,5)	2,23 (1,4-2,62)	2,74 (2,2-3,21)	2,14 (1,44-2,7)
Se P (mmol/l)	0,83 (0,59-1,24)		1,71 (0,63-3,0)	
Se PTH (pg/ml)	247,9 (68,6-1301)	20,3 (1,86-49,2)	821,9 (77,3-2510)	50,6 (1,2-251)
Se creatinine (dialysis) (μ mol/l)			769 (180-1194)	
Se creatinine (renal graft) (μ mol/l)			183 (95-338)	

Bcl-2.[5,6] The role of other gene products in the differential diagnosis of parathyroid neoplasia, like p27, cyclin D1, and Ki67, was also studied.[7,8,9]

We investigated the mitotic and apoptotic activity and also the expression of p53, Bcl-2 and Bax in hyperplasia and benign as well as malignant tumours of the parathyroid glands. The aim of this study was to detect differences in this respect between the above-mentioned proliferative conditions and, further, to demonstrate enhancing or inhibitory factors of apoptotic activity which may explain the low incidence of parathyroid carcinomas.

Materials and Methods

Clinical and laboratory parameters

The parathyroid lesions presented here were clinically diagnosed and surgically removed at the Clinical Department of Transplantation and Surgery of Semmelweis University, Budapest, Hungary. Altogether 107 patients were operated on because of hyperparathyroidism (HP), of which 55 proved to have primary (PH) and 52 secondary hyperparathyroidism (SH). No MEN I or MEN II/a cases were found in our material. Clinical symptoms and laboratory parameters (serum Ca and P, creatinine and parathormone levels as well as Tc99 labeling) leading to the diagnosis of HP were registered together with the time between the onset of clinical symptoms (or the start of hemodialysis, or the date of renal transplantation) and the diagnosis of HP. Localization and number of enlarged and removed parathyroid glands; postoperative serum Ca and parathormone levels were also registered.

Histological studies

The removed parathyroid glands were fixed in buffered neutral formalin and embedded in paraffin. Eight m thin sections were cut and stained with hematoxylin and eosin. Immunoperoxidase reactions to detect p53, Bcl-2 and Bax as well as TUNEL reaction to demonstrate apoptosis were performed. Sections were treated with proteinase K, incubated in methanol and H_2O_2. Anti-p53, anti-Bcl-2 and anti-Bax antibodies were the products of DAKO (Glostrup, Denmark). The antibodies were diluted 1:100 and applied overnight at 37°C. The secondary anti-mouse IgG (DAKO, Glostrup, Denmark) was used in a dilution of 1:100 the next day. Diaminobenzidine (DAB) served as chromogen and methyl green as counterstain. The TUNEL reaction was performed using

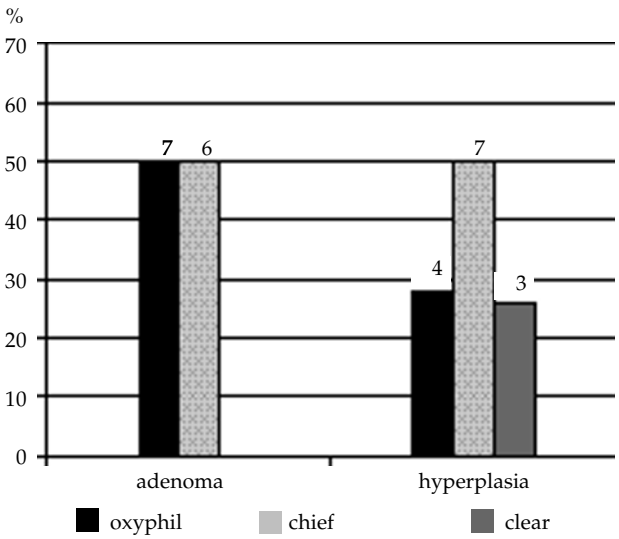

Figure 1. *Distribution of cell types in hyperplasia and adenoma*

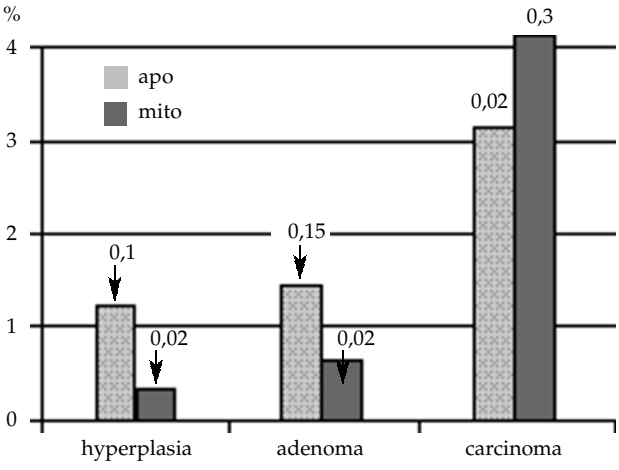

Figure 2. *Apoptotic and mitotic index*

Apop Detec (Simply sensitive In Situ Detection System Kit, DAKO) which detects DNA fragmentation along nucleosomes and stains the nuclei of apoptotic cells. Morphological criteria of apoptosis, observed in H the E stained sections were also considered.

Cellular constitution of the parathyroid lesions and mitotic index (counting 1000 cells) were determined using H&E stained sections. The criteria described by Roth[1] were applied to differentiate between hyperplasia, adenoma and carcinoma. The apoptotic index was established after TUNEL reaction considering 1000 cells. Both mitotic and apoptotic indices were expressed as per cent. Bcl-2 and Bax, as well as p53 expression were studied in chief cells, oxyphil cells, and clear cells. Positivity was considered if 50% or more of cellis showed cytoplasmic or nuclear reaction with DAB.

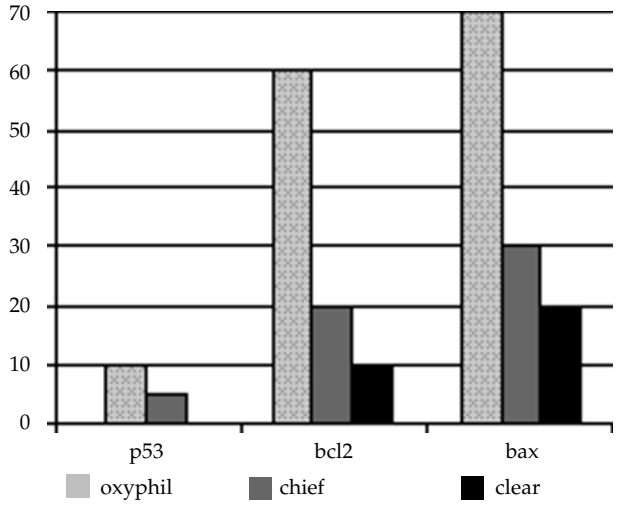

Figure 3a. *P53, Bcl2 and Bax expression in parathyroid hyperplasia*

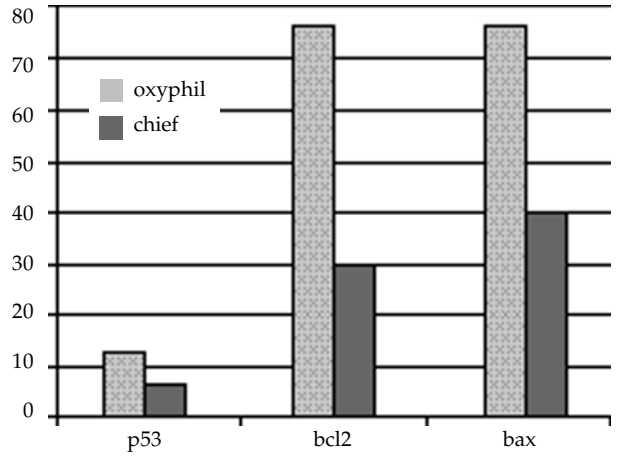

Figure 3b. *P53, Bcl2 and Bax expression in parathyroid adenoma*

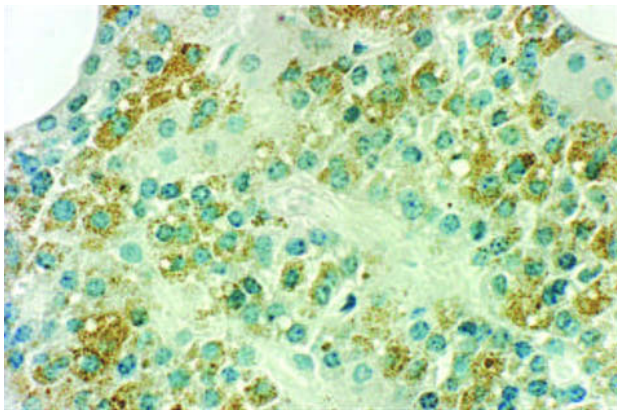

Figure 4a. *Parathyroid hyperplasia. Notice the cytoplasmic positivity for Bcl_2 in oxyphil cells and chief cells (Anti-Bcl_2 immunoperoxidase, x 300)*

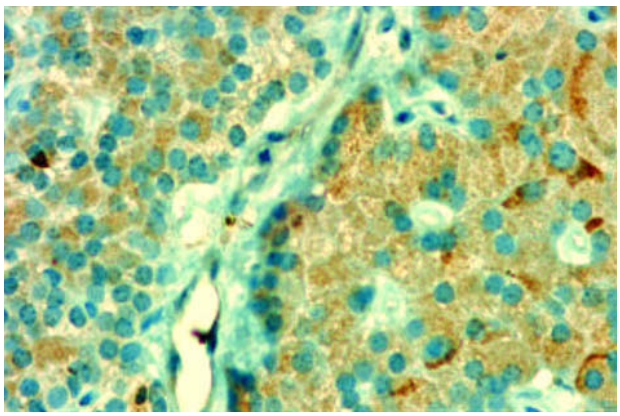

Figure 4b. *Parathyroid hyperplasia. Notice the cytoplasmic positivity for Bax in oxyphil cells and chief cells. (Anti-Bax immunoperoxidase, x 300)*

Results

The age and sex distribution. The overwhelming majority of patients with primary lesions was female (49 females and 6 males). The frequency of SH was nearly equal in men and women where there were 25 male and 27 female patients. The average age of patients suffering from PH (60 years in females and 59.2 in males) was higher than in patients with SH (43.3 in females and 49.5 in males). In PH-s there were 41 adenomas, 11 hyperplasias and only three carcinomas were found. Carcinomas were verified clinically, one carcinoma recurred three times and the patient died because of local invasion, while two carcinomas gave lymph node metastases. Among the secondary lesions hyperplasia was the most common. In SH 9 adenomas and 43 hyperplasias were found.

The clinical symptoms and events. PH resulted in nephrolithiasis in 23 caes and osteoporosis in 25 cases as

well as other bone and joint alterations in 13 cases. There were bone fractures in 3 cases and serum Ca and P were elevated in 9 cases. SH was predictable by the fact of pre-existing chronic renal failure treated by dialysis in 19 cases or renal grafting in 26 cases. 16 patient had suffered from bone and joint pain. The most characteristic *laboratory findings* were high serum Ca and P levels as well as parathormone (PTH) level which were found in both PH and SH , higher values being characteristic for SH. Preoperative serum Ca and PTH levels reflected elevated parathyroid activity the details of which can be viewed in *Table 1*. Definitive preoperative diagnosis was made by ultrasound in 35 PH caes and 34 SH-s and/or CT imaging in 39 PH-s and 41 SH-s as well as by isotope scanning (Tc 99) in 52 PH-s and 48 SH-s. In 34 cases of PH postoperative Ca administration became necessary. Subcutaneous autotransplation of parathyroid tissue was performed postoperatively in 13 cases of SH. Serum creatinine level was high in nearly all SH patients with chronic renal disease.

The time gap between the onset of symptoms in PH was relatively long (5.6 years in average) calling the attention to the difficulties of the clinical diagnosis of HP. The period between the start of dialysis or the renal grafting was in average 4,2 and 9 years, respectively.

In 48 PH cases only one parathyroid gland and in 23 cases of SH all four glands and in 16 SH three glands were removed.The rest had two glands removed. Primary lesions were solitary in the majority of cases, and were localized in the inferior right and left parathyroid glands.

Postoperative complications such as bleeding, infection, or damage of the recurrent laryngeal nerve occurred in 6 cases. All operations for PH and all but two operations for SH proved to be successful as judged by laboratory findings.

Histological examination of the removed parathyroid glands showed the structural and cellular features of hyperplasia, adenoma or carcinoma. The cellular composition of adenomas and carcinomas is shown in *Figure 1*. Chief and oxyphil cells were both present in the lesions, and a clear cell component was also found in hyperplasia. No difference could be detected between primary and secondary HP.

Apoptotic index was equally low in adenomas and hyperplasias, and so was the mitotic ratio. The carcinomas showed slight elevation in mitotic and apoptotic activity *(Figure 2.)*.

The ratio of p53, Bcl-2 and Bax expression was studied in chief cells, oxyphil cells and clear cells. Hyperplasias showed p53 positivity in the cytoplasm of the oxyphil cells in two cases, chief cells were positive in one case. Nuclear positivity was observed in oxyphil cells in three cases and in chief cells in two cases. Bcl-2 positivity of the cytoplasm of oxyphil cells was found in 33 cases, in chief cells in 11 cases and in clear cells in 5 cases. The oxyphil cells were bax positive in 36 cases, chief cells showed positivity in 12 cases and clear cells in 10 cases *(Figure 3a)*. *Figure 4a* shows Bcl-2 positive cells in hyperplasia. Bax positive cells in hyperplasia are shown in *Figure 4b*. Co-expression of Bcl2 and Bax, as well as p53 was registered in most of the positive cases. No significant differences were registered regarding Bcl-2, Bax or p53 expression between hyperplasias found in case of primary or secondary hyperparathyroidism. Adenomas expressed p53 in the cytoplasm of the oxyphil cells in three cases, and in chief cells in two cases. Nuclear positivity for p53 was found in oxyphil cells in four cases and in chief cells in three cases *(Figure 5a, 5b)*. Bcl-2 positivity was found in the oxyphil cells in 39 samples and in the chief cells in 15 samples. Bax was positive in oxyphil cells in 38 and in the chief cells in 20 cases *(Figure 3b)*. *Figure 6a.* and *6b.* shows Bcl-2 and Bax positive cells of adenomas, respectively. Co-expression of the three genes was characteristic to adenomas as well.

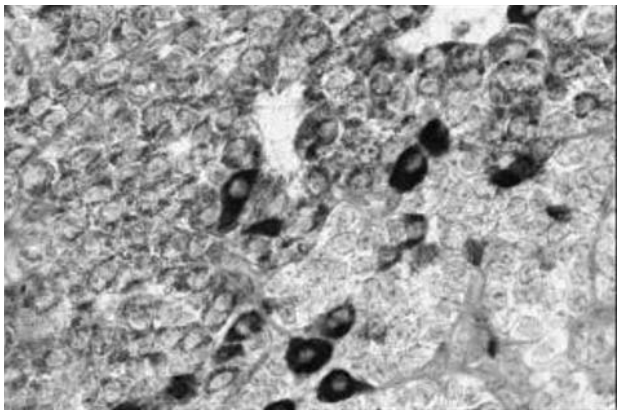

Figure 5a. *Parathyroid adenoma. Notice the cytoplasmic positivity for p53 in oxyphil cells (middle, strong positivity) and chief cells. (Anti-p53 immunoperoxidase, x 300)*

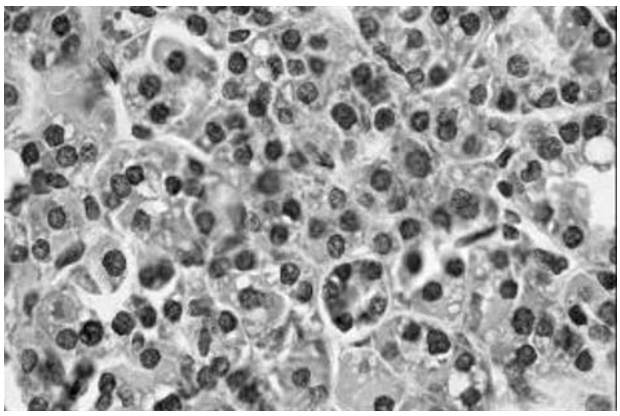

Figure 5b. *Parathyroid adenoma. Notice nuclear positivity for p53 in oxyphil cells. (Anti-p53 immunoperoxidase, x 300).*

The carcinomas showed nuclear P53 positivity *(Figure 7a.)* and the cytoplasm was negative for Bcl-2 and Bax *(Figure 7b.)*.

Discussion

The results of our study on a relatively large number of parathyroid hyperplasias and adenomas clearly indicate no differences in their mitotic or apoptotic activity, both mitotic and apoptotic indices were equally low. In agreement with the study of Ricci et al,[5] p53 and Bcl-2 expression was found in hyperplasias as well as in adenomas. The percent of p53 or Bcl-2 positive cases was slightly higher in adenomas, but differentiating between these two entities could not be based on p53 or Bcl-2 immunostaining. The same was found with Bax expression. To our knowledge, expression of Bax has not yet been studied in these parathyroid lesions. The most interesting finding in our study was the co-expression of Bcl-2 and Bax in most hyperplasias and adenomas. The co-localization of Bcl-2 and Bax may neutralize the effect of both genes on apoptosis. Oxyphil cells and chief cells were studied separately for p53, Bcl-2 and Bax expression. Oxyphil cells were positive for these gene products in a higher proportion of cases than chief cells or modified (clear) chief cells. This fact may be due to the abundance of mitochondria in oxyphil cells. The appearance of p53 positivity in the cytoplasm in some hyperplastic and adenomatous lesions may point to the increased activity of inhibitor of p53 amino acid terminal nuclear signal[12] which means an increased defense against transport of mutant p53 protein into the nucleus. The antibody used by us labels predominantly mutant p53 by immunostaining. Nuclear positivity of some of the adenomas and hyperplasias may be the sign of a tendency towards malignant transformation.

The very low number of carcinomas does not allow definite conclusions in comparing these tumours with adenomas. In accordance with the literature[10,11] mitotic as well as apoptotic activity were slightly elevated in carcinomas. Nuclear p53 positivity and the abscence of Bcl-2 as well as

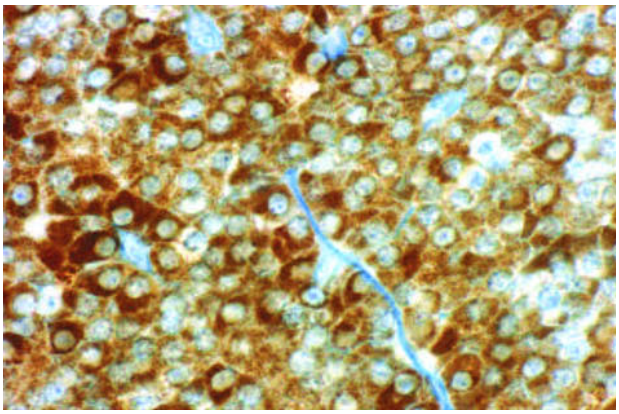

Figure 6a. *Paratohyroid adenoma. Notice the cytoplasmic positivity for Bcl_2 in oxyphil cells (Anti-Bcl_2 immunoperoxidase, x 300)*

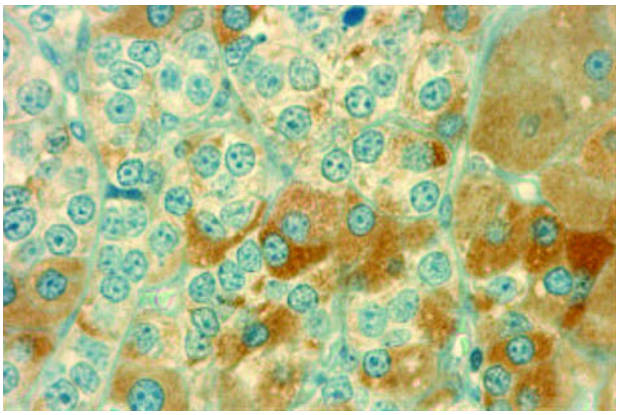

Figure 6b. *Parathyroid adenoma. Notice the cytoplasmic positivity for Bax in oxyphil cells (strong positivity) and chief cells (Anti-Bax immunoperoxidase, x 600)*

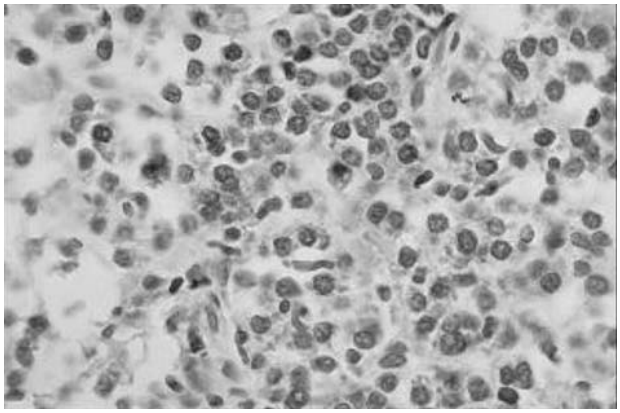

Figure 7a. *Parathyroid carcinoma. Notice the nuclear positivity for p53. (Anti-p53 immunoperoxidase, x 300)*

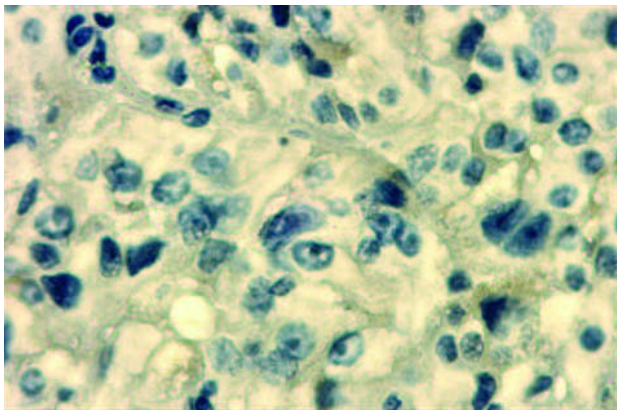

Figure 7b. *Parathyroid carcinoma. negative immunostaining for Bcl_2. (Anti-Bcl_2 immunoperoxidase, x 300)*

Bax expression in carcinomas are in accordance with the findings of Stojadinovic et al.[6] Co-expression of P53, Bcl-2 and Bax may be a factor responsible for the low mitotic and apoptotic activities. The very low rate of malignant tumours in the parathyroid glands may also have some relation to the low mitotic and apoptotic ratio in benign proliferative lesions, but this issue should be investigated further.

Our clinical findings may contribute to the differential diagnosis of hyperplasia and adenoma of the parathyroid glands. Single lesions are more likely adenomas than multiple ones. Adenomas occurred overwhelmingly in females. The gender distribution in the case of hyperplasia was practically even. The average age of patients with PH was close to 60 years, while SH occurred in younger patients.

Serum Ca and PTH levels were significantly higher in hyperplasias compared to adenomas. Chronic renal failure, chronic dialysis or renal grafting point to SH and the likelihood of hyperplasia. The presence of bone and joint lesions in both PH and SH and the very long time between the onset of these symptoms and the surgical removal of the parathyroid lesions call the attention to the importance of considering HP in patients with osteoporosis.

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