Correlation Of Histomorphologic, Histochemical And Immunophenotypic Findings In Pituitary Adenomas Showing Prolactin Activity

Prolaktin Aktivitesi Gösteren Pituiter Adenomalarda Histomorfolojik, Histokimyasal Ve İmmünofenotipik Bulguların Korelasyonu

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Abstract: *Objective:* In order to study clinical, histomorphological, histochemical and immunohistochemical characteristics in hyperproloactinemic patients, we undertook a comparative study of 30 pituitary adenomas.

Methods: We used a group of nuclear, cytoplasmic and staining parameters for the evaluation of morphologic features of the tumor types. Tumors showed a large spectrum from the point of histomorphological features. By using Gomori reticulin stain, we distinguished hyperplasia from neoplasia. Additionally, Periodic Acid Schiff staining was used for the detection of glycoprotein content in tumor cell cytoplasm.

Twenty-six cases have been analysed using anti-prolactin monoclonal antibody, and an overall of 23 cases for anti-growth hormone monoclonal antibody immunohistochemically.

Results: We found diffuse positivity in 15 cases, sparse positivity in 8 cases for anti-prolactin antibody. Three cases showed no immunoreactivity for prolactin. Six cases showed diffuse positivity and 2 cases showed sparse positivity for anti-growth hormone monoclonal antibody. Fifteen cases were immunonegative for anti-growth hormone monoclonal antibody. Five cases were immunonegative for PRL but positive for GH. Four of those cases had increased serum GH levels and were presented with acromegaly.

Conclusion: We concluded that there is no direct correlation between serum hormone levels and immunophenotypic expressivity of pituitary adenomas. The reason of

Özet: *Amaç:* Hiperprolaktinemisi olan 30 pitüiter adenomalı hastanın klinik, histomorfolojik, histokimyasal ve immümhistokimyasal bulguları karşılaştırmalı olarak çalışıldı.

Yöntem: Olguların ışık mikroskopundaki morfolojik özellikleri, nükleusa ve sitoplazmaya ait şekil, büyüklük ve boyanma özellikleri baz alınarak parametrize edildi. Tümörler histomorfolojileri bakımından geniş bir sipektrumda dağıldılar. Hiperplaziler neoplazilerden Gomori retikülün boyası kullanılarak ayrıldı. Tümör hücreleri stoplazmasında glikoprotein varlığını tespit etmek için PAS boyası kullanıldı.

26 olguya prolaktin (PRL), 23 olguya ise büyüme hormonu (GH) immunohistokimyası uygulandı.

Bulgular: PRL ile 15 olguda diffüz pozitivite ve 8 olguda hafif pozitivite gözlenirken 3 olguda immünreaktivite saptanmadı. GH ile 6 olgu diffüz pozitivite ve 2 olgu hafif pozitivite gösterdi. 15 olgu ise immünnegatifti. Serum prolaktin seviyesi yüksek olan 5 olguda PRL immunohistokimyasının negatif olduğu saptanırken, bu olgularda GH immunopozitivitesi izlendi. Bu olgulardan biyokimyasal değerleri bilinen 4 hastada serum GH seviyelerinin de yüksek olduğu saptandı ve klinikte akromegali ile prezente oldukları dikkati çekti.

Sonuç: Sonuç olarak, endokrin aktivite ile pitüiter adenoma tipi arasında bir ilişki olsa da, serum hormon düzeyleri ile adenomun immünfenotipik görüntüsü arasında direkt korelatif bir ilişki olmadığı saptandı. GH immunopozitivitesi gösteren olgularda serum PRL

prolactinemia in cases showing GH immunopositivity was thought to be a stalk effect. Using electron microscopy has to be taken into consideration to identify the type of the pituitary adenoma while having insufficient results for immunohistochemical studies.

Key Words: Hyperprolactinemia, immunohistochemistry, pituitary adenoma.

seviyelerindeki artışın sap basısı nedeniyle olabileceği gözönüne alındı. İmmunohistokimyanın tümör tipini belirlemede yetersiz kaldığı durumlarda elektron mikroskopisinin kullanılması gerektiği düşünüldü.

Anahtar Kelimeler: Hiperprolaktinoma, immünhistokimya, pituiter adenoma

INTRODUCTION

The mostly encountered pathologies in lesions that effect sellar region are pituitary adenomas. The 10-20% of the intracranial neoplasms consists of pituitary adenoma (14).

With the development of clinical and biochemical researches Radioimmunoassay (RIA), computerized tomography (CT), magnetic resonance imaging (MRI), surgery techniques (trans-sphenoidal adenomectomy) electron microscopy and immunohistochemical techniques, our knowledge about pituitary adenomas has increased to a large extent (5,6,7).

In the light of the knowledge that began to accumulate with the definitions Nakane made for the immunohistochemistry of the pituitary cell types and improved with the development of immunohistochemistry, "tinctorial classification" based on the eosinophilic, basophilic and chromophobic staining of the cell on pituitary adenomas has lost its significance (12). When the "tinctorial classification" is compared to the "functional classification" that gained significance with the improvements in immunohistochemistry, matchings can be observed on various types of tumors in tinctorial classification. For example, with the introduction of functional classification, it has been understood that the eosinophlic adenomas which were formerly thought to secrete growth hormone (GH) can secrete GH and / or prolactin (PRL) and that some of them can secrete no hormone at all (5,9,13).

It has been observed that some histomorphologic and ultrastructural findings contribute in defining the adenoma type in pituitary adenomas (8). For example, the parameters such as cellularity, pleomorphism or monotony, nuclear irregularities, the granule type they include and heterogeneous immunoreactivity are evaluated as the findings observed in various adenoma types. Besides, it has been observed in various studies that there is a close relationship between the endocrine activity of the tumor and immunophenotype (4,17). But, in case the functional classification is not satisfactory, it is necessary to show the origin of the cell using electron microscope.

In this study, the relation of the pituitary adenomas that showed prolactin endocrine activity with histomorphological parameters and the conventional chemistry together were compared with the immunohistochemical results.

MATERIALS AND THE METHOD

Within the study, 55 pituitary adenoma cases that were admitted to the Laboratory of Neuropathology of Marmara University Institute of Neurological Sciences a period of 3 years. Among these cases, 30 pituitary adenomas (54.4%) that showed prolactin hyperactivity in terms of blood biochemistry were selected. 22 of the cases were female and 8 of them were male. These cases that showed prolactin hyperactivity were found to be 35.9 years old on average.

The tumor cell nuclei which could be observed histomorphologically diversely were parameterised in terms of their sizes and their localisation within the cell. The nuclei more than two times bigger than an erythrocyte was considered as "big". The tumor cases most of the nuclei of which were observed as pushed into oneside of the cell were included in the eccentrically localised group and those observed in the center were included in the central group.

The parameters which could be used to determine the endocrine tumor type were separated into two groups as those vesicular nucleus cases that had slack chromatin patterns and those that had more intense chromatin patterns.

The tumor cell cytoplasms were parameterised in terms of their acidophilic, basophilic and chromophobic dying characteristics which are used

in tinctorial classification. And, they were separated in two groups, as those rich in granule and those sparsely containing granule.

Gomori Reticuline dye was applied to the selected cases for tumor-hyperplasia differentiation. The tumor cases were dyed with Periodic Acid Schiff (PAS) for glycoprotein inquiry into the tumor cell cytoplasm.

IMMUNOHISTOCHEMISTRY

a. Histochemistry of PRL and GH

To the 26 cases of 30 pituitary adenomas that were studied PRL and to the 23 cases of them GH immunohistochemistry (PRL, monoclonal mouse IgG, BioGenex; Growth Hormone, monoclonal mouse IgG, BioGenex) was applied. In the both hormone immunohistochemistry applications, normal pituitary sections were used as control groups.

b. Evaluation of the PRL and GH immunohistochemistry

The cases were evaluated in terms of the prolactin and growth hormone immunohistochemistry dying without knowing their clinical findings and the blood hormone expressivity values. The tumor sections were separated into three groups according to their dying characteristic (11). They were grouped as the cases with 80% or more positive tumor cells the cases with 20% or less positive tumor cells, and the immunonegative cases (11). The cells that were rarely positive in tumor section and that were not in conformity with the general tumor cell morphology were considered as normal cells in between; and thus, this kind of cases were included in the immunonegative group.

RESULTS

Clinical Findings

In the clinical stories of the cases studied the most frequently observed findings that made them consult a doctor were headache and sight failings. Besides, the endocrine related symptoms galactorrhea, acromegaly and amenorrhea were observed in the patients. Some of the patients were showing multiple symptoms.

Conventional Histochemistry Findings

The reticuline dying which was applied to all of the cases for the query of the tissue frame was used for tumor-hyperplasia separation. All of the cases showed intra-tumoral reticulolysis. In the cases studied, no hyperplasia was observed. Applying the PAS dye, which is used for querying into the basal membrane and the glycoprotein content, to all of the cases, it was detected that the tumor cell cytoplasms did not show PAS positivity (Figure 1).

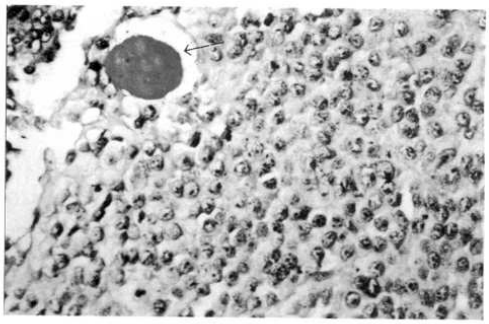

Figure 1. X200, Periodic Acid Schiff stain, intravascular PAS (+) glycoprotein material is shown by arrow. Tumor cells do not exhibit PAS positivity.

Histomorphological Findings

When the 30 pituitary adenoma cases dyed with hematoxyline and eosin were examined, the findings in table 1 were obtained (Table 1).

Immunohistochemistry Findings

a. PRL Immunohistochemistry

In the 57.6% of the cases the dying percentage of which are shown in table 5, more than 80% of the tumor cells showed immunopositivity in conformity with endocrine activity (Figure 2) (Table 2).

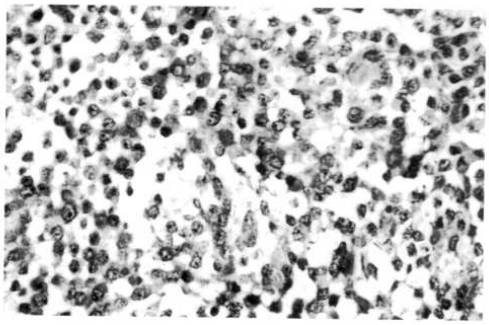

Figure 2. X200, Prolactin immunohistochemistry (BioGenex), more than 80% of tumor cells show immunopositivity.

Table II: Evaluation of prolactin immunoexpressivity in 26 cases

	Results of PRL Immunohistochemistry: 26 cases		
	Positivity in more than % 80 cell	Positivity in less than % 20 cell	Immunonegative cases
Number of cases	15	3	8

Table III: Evaluation of GH immunoexpressivity in 23 cases

	Results of GH Immunohistochemistry: 23 cases		
	Positivity in more than % 80 cell	Positivity in less than % 20 cell	Immunonegative cases
Number of cases	6	2	15

Table IV: The clinical, biochemical and PRL (immunohistochemistry) evaluation of the 6 cases immunopositive with GH

	6 cases showing positivity with GH		
	GH Biochemistry	Symptoms	PRL immunohistochemistry
1	High	Ac, G, A	(-)
2	?	A, G	(-)
3	High	Ac	(-)
4	Normal	(-)	(+)
5	High	Ac	(-)
6	High	Ac	(-)

Ac : Acromegaly G : Galactorrhea A : Amenorrhea

After the GH immunohistochemistry findings were evaluated; the clinical, biochemical and the PRL immunohistochemical findings related to the 6 cases that showed positivity at more than 80% cells with GH immunohistochemistry were re-examined (Table 4).

DISCUSSION

The high prolactin serum hormone levels were female in 73.3% of the 30 pituitary adenoma cases. The most frequently observed clinical symptom among these cases was galactorrhea. In a comparative study, lower preoperative prolactin level in young female patients in comparison with older female and particularly male patients was found by authors (3).

It was observed that the cases with similar biochemical profile (hyperprolactinemia) had diverse histomorphological findings. Tumor cell nuclei, being big and small both specifically and on the case base showed heterogeneity in size. Besides the nucleus size, diversity was observed in the placement of the nuclei within the cells, as well. While the nucleus was eccentrically positioned in same cases, it was centrally positioned in a few cases; and in most of the cases, there were cells with the nucleus positioned both way. In the literature, the tumor group with the small, nucleus showing monotony and centrally positioned within the cell rather matches with prolactinomas (1,9,13,16). Considering the cytoplasm, most of the cases featured acidophil or acidophil-chromophobe in dying. Thus, within the functional classification where tinctorial classification was inadequate for the distinctive diagnosis of these tumors with the biochemical profiles showing prolactin activity, it was thought that distinctive diagnosis could be made by using different antibodies (13). When the granule content was considered, it was observed that nearly half of the cases were poor in granule. In a study from the literature where 1043 pituitary adenomas were examined, while the percentages of the GH secreting adenoma types observed as rich or poor in granule were close to each other, the type poor in granule was more frequently observed in the adenomas secreting PRL (5,14).

In most of the cases, the chromatin pattern was slack and they were showing vesicular nuclei. In the literature, it has been reported that the prolactin secreting pituitary adenomas mostly had intense patterns (1,9,13,16).

With Gomori reticuline, intratumoral reticulolysis was observed in all of the cases. In one of the cases, microadenoma, with pituitary cells showing hyperplasia around, was observed. In this case, the intratumoral reticulolysis in the microadenoma and the reticulogenesis surrounding the cell groups that showed hyperlasia proved that it was necessary to apply routine reticuline to distinguish between neoplasia and hyperplasia. In all of the cases that were applied PAS dying, it was detected that the tumor cell cytoplasms were PAS negative. Thus, the corticotroph cell adenomas with tumor cell cytoplasms giving positive signal and the gonadotroph and the thyrotroph cell adenomas giving weak signal were excluded from the distinguishing diagnosis.

With the PRL immunohistochemistry applied to 26 pituitary adenomas showing PRL hyperactivity, 15 cases (57,6 %) showed diffuse positivity and in the 3 cases no staining was observed. In some of the 8 cases, some cell groups were positively stained; and in some cases, singularly stained cells were rarely observed. Biochemically, it was detected that the immunohistochemical profiles of the endocrine active prolactinomas were in hormone specific confirmity by 60 percent. The fact that the immunohistochemical profiles of the hyperprolactinemic cases were immunonegative by nearly 12 percent made us think that the reason for prolactinemia was non-neoplastic ("stalk effect"). Besides, it was concluded that there was quantitative direct relationship between the immunohistochemical fenotype and the biochemical hormone expressivity, and that the immunohistochemical profile may not be a "real-time" reflection of every biological process, as well. In the literature, while many cases have been stained with the suspected hormone positively, some cases without clinical findings have been stained with various hormones positively (10).

When the GH immunohistochemistry applied to the 23 pituitary adenomas showing prolactin hyperactivity, 6 cases (26%) showed diffuse positivity. There was no staining in 15 cases and 2 cases showed positive staining, partially in groups and as single cells. When the clinical, biochemical, histomorphological and immunohistochemical data related to the 6 cases that were positively stained as diffuse were re-examined, the findings obtained were in confirmity with the GH secreting pituitary adenomas. Clinically, it was detected that 4 of the 6 cases were acromegaly, their blood biochemistry values were high in terms of GH, and that 5 cases were stained negatively for PRL. Histomorphologically, pleomorphism, binucleation, vesicular nuclei and eccentrically positioned nuclei which have been mentioned in literature as related to GH adenomas were observed in these cases (2,9,13).

These findings made us think that the adenomas secreting prolactin had diverse morphologies within a heterogenous spectrum. Besides, it may be thought that the GH secreting adenomas are a more homogenous group, but they may show increase in prolactin because of some reasons such as "stalk effect", inhibiting the Prolactin Inhibiting Factor (PIF) oscillation or mixed GH-PRL adenomas.

For the cases with acromegaly and hyperprolactinemia in the literature, it has been reported that increased PRL secretion may stem from adenoma cells, and that, as the transportation or the secretion of the hyphothalamic PIF are destroyed by the GH producing tumor, PRL may possibly be produced from the PRL cells around the adenoma (15).

As a conclusion the relation of the pituitary adenomas that showed prolactin endocrine activity with histomorphological parameters and immunohistochemical results are not always completely correlated. All of histomorphology, histochemistry and immunohistology have their shortcomings; therefore more than one method must be used in diagnosis of pituitary adenomas. Although there is a close relationship between the endocrine activity of the tumor and immunophenotype it is necessary to show the origin of the cell using electron microscope.

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REFERENCES

1. Asa SL: Tumors of the pituitary gland, in Burger PC, Scheithauer BW (ed) Atlas of the Tumor Pathology, Third series, fascicle 22, Washington,DC: Armed Forces Institute of Pathology, 1997:1-131
2. Bassetti M, Spada A, Birina M, Giannattasio G: GH and PRL: Hormone studies and immunocytochemical correlates, in Landolt AM, Heitz PU, Zapf J, Girard J, Del Pozo E (ed) Advances in Pituitary Adenoma Research. Oxford, New York: Pergamon Press,1988:23-26
3. Calle-Rodrigue RD, Giannini C, Scheithauer BW, Lloyd RV, Wollan PC, Kovacs KT, Stefaneanu L, Ebright AB, Abboud CF, Davis DH: Prolactinomas in male and female patients: a comparative clinicopathologic study. Mayo Clin Proc 73;1046-1052, 1998
4. Furuhata S, Kameya T, Tsurata T, Naritaka H, Toya S: Colocalization of growth hormone (GH) and glycoprotein subunit alpha in GH-producing pituitary adenomas in acromegalic patients. Acta Neuropathologica 87: 568-571, 1994
5. Horvath E, Kovacs K: Pituitary gland. Path Res Pract 183:129-142, 1988
6. Horvath E, Kovacs K, Smyth HS, Killinger DW, Scheithauer BW, Randall R, Laws ER, Singer W: A novel type of pituitary adenoma: Morphological features and clinical correlations. Journal of Clinical Endocrinology and Metabolism 66:1111-1118, 1988
7. Horvarth E, Kovacs K: The Adenohypophysis, in Kovacs K, Asa SL (ed), Functional Endocrine Pathology, Boston: Blackwell Scientific Publications 1991:245-281
8. Irsy G, Goth M, Balint K: Comparison of the serum hormone levels and histological findings in pituitary adenomas. Exp Clin Endocrinol 88: 224-236, 1986
9. Kontogeorgos G, BassioukaI, Giannou P, Vamvassakis E, Rologis D, Orphanidis G: Diagnosis of pituitary adenomas on touch preparations assisted by immunocytochemistry. Acta Cytologica 39:141-152,1995
10. Kovalic JJ, Mazoujian G, McKeel DW, Fineberg BB, Grigsby PW: Immunohistochemistry as a predictor of clinical outcome in patients given postoperative radiation for subtotally resected pituitary adenomas. J Neuro Oncol 16: 227-232, 1993
11. Kujas M, Pleau-Varet J, Peillon F, Derome P: Correlations between immunocytochemical and clinical data in 675 cases of pituitary adenomas, in Landolt AM, Heitz PU, Zapf J, Girard J, Del Pozo E (ed), Advances in Pituitary Adenoma Research. Oxford, New York: Pergamon Press, 1988;21-22
12. Nakane PK: Classification of anterior pituitary cell types with immunoenzyme histochemistry. J Histochem Cytochem 18:9-20, 1970
13. Parisi J, Scheithauer BW: Pituitary tumors, in Nelson JS (ed), Principles and Practice of Neuropathology, USA: Mosby 1993:267-295
14. Rock JP, Babu VR, Drumheller T, Chason J: Cytogenetic findings in pituitary adenoma: Results of a pilot study. Surg Neurol 40: 224-229, 1993
15. Saegar W, Kant P, Caselitz J, Lüdecke DK: Electron microscopical morphometry of pituitary adenomas: Comparison of tumours in acromegaly and hyperprolactinemia. Path Res Pract 183: 17-24, 1988
16. Sheithauer BW: The pituitary and sellar region, in Sternberg SS (ed), Surgical Pathology, New York: Raven Press 1989: 371-393
17. Trouillas J, Girod C, Sassolas G, Claustrat B: Immunocytochemistry, what does it add to clinical management, in Landolt AM, Heitz PU, Zapf J, Girard J, Del Pozo E (ed), Advances in Pituitary Adenoma Research. Oxford, New York: Pergamon Press, 1988:11-20.

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Lack of prolactin receptor signaling in mice results in lactotroph proliferation and prolactinomas by dopamine-dependent and -independent mechanisms.

Schuff KG, Hentges ST, Kelly MA, Binart N, Kelly PA, Iuvone PM, Asa SL, Low MJ.

Hypothalamic dopamine inhibits pituitary prolactin secretion and proliferation of prolactin-producing lactotroph cells by activating lactotroph dopamine D2 receptors (D2Rs). Conversely, prolactin (PRL) stimulates hypothalamic dopamine neurons via PRL receptors (PRLRs) in a short-loop feedback circuit. This study showed that PRL inhibits lactotrophs by two distinct mechanisms: (a) indirectly by activation of hypothalamic dopamine neurons and (b) directly within the pituitary in a dopamine-independent fashion.