RESEARCH ARTICLE

Lack of Correlations among Histopathological Parameters, Ki-67 Proliferation Index and Prognosis in Pheochromocytoma Patients

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Abstract

Background: In this study prognostic correlations of histopathologic parameters and the Ki-67 proliferation index and as well as the diagnostic value of immunohistochemical markers in pheochromocytomas were evaluated. Materials and Methods: A total of 22 patients diagnosed with a pheochromocytoma between 2000-2010 in Izmir Katip Celebi University Ataturk Training and Research Hospital were included. Diagnostic value of the PASS scoring system, and prognostic correlations of histopathologic parameters and Ki-67 proliferation index were investigated. SPSS for Windows 17.0 software was used for statistical analysis. Results: There was no statistically significant correlation between recurrence and clinicopathologic parameters or the PASS score (PASS>4). In addition, there were no statistically significant correlations between PASS score and clinicopathologic parameters, such as diameter (5 cm), weight (>100g), gender (female/male ratio) and age (25-45/45-55/>55). Besides, there were no significant correlation between diameter and clinicopathological parameters and also recurrence. However, there was a statistically significant correlation between Ki-67 proliferation index and capsule invasion (p=0.047). Conclusions: Some but not most of the findings in our study were concordant with the literature. To clarify relationships, investigations with standard scoring systems which are not affected by subjective factors and feature appropriate histopathological criteria should be made on larger study groups.

Keywords: Pheochromocytoma - Ki 67 - grading - prognosis

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Introduction

Pheochromocytomas are rare tumors and more than 2/3 of the cases are functional and symptomatic, often manifest as hypertensive crises, and 80-90% of pheochromocytomas are benign, mostly full recovery is ensured with a successful total surgery (Ernest, 2007; Rosai, 2011).

In general, studies on pheochromocytomas focused on the mutations, proliferation markers, and recently histological parameters that may be useful for malignancy as well as different combinations of these parameters, and the correlation of these combinations with malignancy. Therefore, it was aimed to identify mutations associated with pheochromacytoma and to determine the significance of these mutations for the diagnosis, and to establish a standard combination of histological parameters (investigated to date). In this context, some histological parameters were determined under the name of Pheochromocytoma In Adrenal Gland Scalled Score (PASS) (Thompson, 2002). The PASS system will help to differentiate tumors for having a potential to behave biologically aggressive (PASS >or=4) or benign (PASS <4) (Strong et al., 2008; Agarwal et al., 2010; Szalat et al., 2011; de Wailly et al., 2012).

Studies indicating a correlation between proliferation markers such as Ki-67 (MIB-1) and prognosis have supported the benefits of MIB-1 assessment in determining malign progression. In different assessment studies, 3 threshold values have been specified by using the areas with maximum cell density: >2% (Nagura et al., 1999), >10% (3) and >20 cells/200× (Kimura et al., 1994). In two other studies, the threshold values of 2% and 3% have confirmed malignancies with only 50% sensitivity (Clarke et al., 1998; van der Harst et al., 2000).

In the light of all this information, we intended to investigate the prognostic values of the histopathological parameters with recognized diagnostic and prognostic significance, PASS scoring system, as well as Ki-67 proliferation index in a sample constituted of 22 pheochromocytoma cases who have been operated in our hospital between 2000 and 2010.

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Materials and Methods

Twenty two patients who have been pathologically diagnosed as pheochromocytoma by immunohistochemistry and histomorphological sign in the Medical Pathology department of Izmir Ataturk Education and Research Hospital between 2000-2010 were included in the study. All patients were re-evaluated retrospectively. Demographic data of the patients were obtained from records of the hospital information system.

Patient files were reviewed in terms of the parameters that may affect the diagnosis and prognosis of the cases (i.e. age, gender, recurrence, accompanied medullary thyroid cancer, tumor size, tumor location, tumor weight, increased cellularity, increased mitotic figures (>3/10 high-power field-HPF), atypical mitotic figures, large nests or diffuse growth, hyperchromasia, becoming fusiform of tumor cells, capsule invasion, nuclear pleomorphism, makronucleol, focal or confluent necrosis, vascular invasion, invasion to periadrenal fat tissue, cellular monotony, Ki-67 proliferation index, PASS score (PASS> or=4 or PASS <4). Histological parameters such as vascular invasion, capsule invasion, nuclear pleomorphism and hyperchromasia were given 1 point, and for the remaining parameters, namely peridrenal fat tissue invasion, large nests or diffuse growth, focal or confluent necrosis, increased cellularity, becoming fusiform of tumor cells, cellular monotony, increased mitotic figures, atypical mitotic figures 2 points were given in the PASS scoring system (Thompson, 2002). Most of the patients were reach by phone or mail and questioned about survival and recurrence, and eventually 11 of the 22 patients were reached.

In the microscopic evaluation, representative hematoxylin and eosin stained sections representing tumor were selected and histopathological parameters, such for capsular invasion, vascular invasion, extension into periadrenal fat tissue, large and combined nests, diffuse growth pattern, necrosis, cellularity, becoming fusiform of tumor cells, nuclear pleomorphism, cellular monotony, nuclear hyperchromasia, makronucleol, increased mitotic figures, atypical mitotic figures, presence/absence of hyaline globule were re-evaluated with light microscopy. Hyalin globuline re-eveluated with PAS stain.

In the pathology archive records of the cases, without any necrosis and artefacts paraffin embedded blocks were selected and 3-4 micron thick sections of each were prepared for immunohistochemical analysis.

For the proliferation marker Ki-67 nuclear staining was considered positive with lymphoma tissue. Under High Power Field(HPF) of the light microscope, the ratio of the positive cells to all neoplastic cells within the area with the most intense staining of tumor cells was semiquantitatively expressed as a percentage. A thousand tumor cells were counted in the most cellular area of the tumor, especially at HPF (×400, Olympus light microscope CXP) and the number of tumor cells among these which show nuclear immunoexpression with Ki-67 (Figure 1) were counted, and averaged, and finally a proportional value was calculated. Ki-67 proliferation index and parameters such as tumor size, tumor weight, accompanied medullary thyroid carcinoma, and recurrence, as well as PASS score and its components, namely histopathological parameters, were compared.

SPSS for Windows 17.0 program was used for statistical analysis of data. Compliance with the normal distribution and homogeneity of the data was evaluated again. A parametric test, the Independent t-test was used for paired comparison of homogeneous variables with a normal distribution, while Spearman's Rho test was used for comparison of the relationship between the variables. Chi-square test was used to compare categorical variables, and Fisher's exact test for correction continiuty. The results were analysed in 95% confidence interval. A p value <0.05 was considered significant.

Results

A total of 22 pheochromocytoma patients were included in the study. The mean age was 48.1±3.30 years (min: 25, max: 78) and 14 patients (63.6%) were female and 8 (36.4%) were male. According to the age groups, 10 (45.4%) patients were between 25-45 years, 6 (27.3%) were between 46-55 years, and 6 (27.3%) were over 55 years of age, respectively.

Out of the patients, 11 (50%) had right adrenal tumor, 7 (31%) had left adrenal tumor, and 2 (9.5%) had bilateral tumor, while 2 (9.5%) had extraadrenal tumor. Tumor size was <5 cm in 6 (27.3%), >5cm in 16 (72.7%) patients, respectively. Tumor weight was <100g in 17 (77.3%), while >100 g in 5 (22.7%) patients, respectively.

According to the hospital records, 5 (22.7%) patients were found to be associated with thyroid medullary carcinoma. Out of the patients that we could reach, 3 (13.6%) patients experienced recurrence whereas 8 (36.4%) did not. According to the PASS Scoring System, PASS<4 was found in 10 (45.5%) patients, while PASS>4 in 12 (54.5%) patients, respectively.

Histopathological parameters of the cases were analyzed, capsule invasion (Figure 2) was determined in 7 (31.8%), vascular invasion in 5 (22.7%), extension into periadrenal fat tissue in one (4.5%), large combined layers in 11 (50%), diffuse growth in 9 (40.9%), necrosis in 3 (13.6%), moderately increased cellularity in 12 (54.5%) and marked in 10 (45.5%), becoming slightly fusiform in 11 (50%) and marked in 11 (50%), cellular monotony in 5 (22.7%), nuclear hyperchromasia in 11 (50%), with macronucleol in (13.6%), increased mitotic figure in 2 (9.1%), atypical mitotic figure in 3 (13.6%), with hyaline globule in 16 (72.8%) cases were detected. No statistically significant relationship was not detected between PASS scores and diameter and weight of the tumor, and gender and age of the patients. (p=0.221; p=0.457; p=0.746; p=0.457).

Diameter of the tumor and capsule invasion, vascular invasion, extension into periadrenal fat tissue, large combined nests, diffuse growth, necrosis, increased cellularity, becoming fusiform, marked pleomorphism, cellular monotony, nuclear hyperchromasia, macronucleol, increased mitotic figures, atypical mitotic figures, hyaline globules, and recurrence did not display any statistically significant correlation (p=0.350; p=0.119; p=0.531 ;

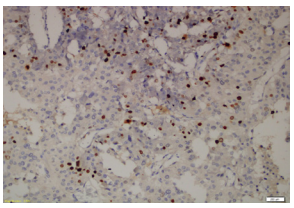

Figure 1. Nuclear Positive Ki-67 Staining in Pheochromocytoma, ×400

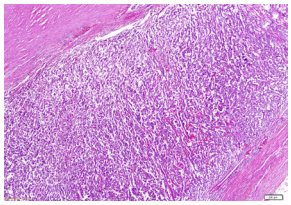

Figure 2. Capsule Invasion in Pheochromocytoma, H&E, ×100

p=0.560; p=0.157; p=0.254; p=0.557; p=1.000; p=0.494; p=0.467; p=1.000; p=0.800; p=0.364; p=0.800; p=0.517; p=0.425).

In the comparison of recurrence with capsule invasion, vascular invasion, expanded and combined nests, diffuse growth, necrosis, increased cellularity, becoming fusiform of the tumor cells, cellular monotony, increased mitotic figures (>3/10 HMF), any atypical mitotic figures, pleomorphism, nuclear hyperchromasia, and the absence of hyaline globule and PASS scores (>4) in patients with pheochromocytoma, any statistically significant correlation could not be found (p=0.621; p=0.214; p=0.782; p=0.621; p=0.521; p=0.632; p=0.064, p=0.214; p=0.870; p=0.870; p=0.782; P=0.621; p=0.490; p=0.125).

Ki-67 <2% was found in 13 (59.1%) patients while Ki-67> 2% in 9 (40.9%) cases, respectively. The chi-square test showed a significant relationship between Ki-67 proliferation index and capsule invasion (P=0.047), while no significant relationship was detected between Ki-67 proliferation index and diameter (>5cm), weight (>100 g), accompanied medullary thyroid carcinoma, recurrence, PASS (<4 and >4), vascular invasion, extension into periadrenal fat tissue, large combined nests, diffuse growth, necrosis, increased cellularity, becoming fusiform, marked pleomorphism, cellular monotony, nuclear hyperchromasia, atypical mitotic figures, increased mitotic figures, and hyaline globule (p=0.595; p=0.962; p=0.962; p=0.387; p=0.690; p=0.660; p=0.323; p=0.394; p=0.665; p=0.779; p=0.329; p=0.674; p=0.193; p=0.595; p=0.962; p=0.193; p=0.075; p=0.329; p=0.882).

Patients were re-evaluated in terms of histo/immuno-chemical markers which had been used at the time of diagnosis. PAS-positive hyaline globule was present in 12 patients, while not in 2 cases. Out of these 12 patients, hyaline globules were stained positively with d- PAS in 9 cases.

Discussion

Pheochromocytoma is called as sympathetic paraganglioma of adrenal medulla (Ronald et al., 2004; Rosai, 2011). Some researchers prefer to use pheochromocytoma for tumors manifested in adrenal medulla, while paraganglioma for tumors arised from ganglia outside of the adrenal medulla (Rosai, 2011). Clinical features of these tumors (depending on the secreted hormones) and pathological characteristics are similar. More than 2/3 of the pheochromocytomas are functional and symptomatic (Ernest, 2007).

The age range of the patients in our study was 25-78. In the study of Agarwal et al., the age distribution of the study group was between 13-65 years of age and showed similar characteristics to our study (Agarwal et al., 2010).

In our study, there was no significant relationship between tumor weight and recurrence. In this respect, our results were consistent with the results of Agarwal et al. (Agarwal et al., 2010). Again, in their study, Linnoila et al. (Linnoila et al., 1990) reported higher values for tumor weight but noted that tumor weight cannot be sufficient to predicate the malignancy of the tumor.

Tumor size has been recognized significant to indicate malignancies in some publications, hence general surgeons refrain from removing/operating tumors larger than 6 cm laparoscopically (Harrison et al., 1999). However, in larger series like the study of Amit Agarwal et al. (Agarwal et al., 2010), a statistically significant relationship was not found between tumor diameter and malignancy. In our study, tumor diameter was compared with each histopathological parameter separately, and no significant relationship was found.

In general, studies on pheochromocytomas focused on the mutations, proliferation markers, and recently histological parameters that may be useful for malignancy as well as different combinations of these parameters, and the correlation of these combinations with malignancy. Therefore, it was aimed to identify mutations associated with pheochromacytoma and to determine the significance of these mutations for the diagnosis, and to establish a standard combination of histological parameters. In this context, Thompson determined some histological parameters under the name of a scoring system (PASS) that can be used in pheochromacytoma (Thompson, 2002). Yet again, this scoring system was evaluated also by

Amit Agarwal et al. (Agarwal et al., 2010). Numerous studies reported that the PASS system would be beneficial in differentiating tumors for having a potential to behave biologically aggressive (PASS >or=4) or benign (PASS <4) (Strong et al., 2008; Agarwal et al., 2010; Szalat et al., 2011; de Wailly et al., 2012).

In his study, Thompson stated that PASS scoring systems (PASS> 4) can show the metastatic potential of the tumor (Thompson, 2002). The study data of Szalat et al.(Szalat et al., 2011) supported Thompson's data. Similarly, Strong et al. concluded that PASS scoring system was definitely significant in pointing out malignancy, and confirmed with PASS> 4 of their malignant cases, while PASS <4 of benign cases (Strong et al., 2008).

In the study of Agarwal et al., 41% of patients with tumors >6cm had PASS >4, and metastases was observed in none of these patients. On the other hand, among patients with tumors <6 cm, 81% had PASS <4, while 19% had PASS> 4, again metastases was not observed in any of these cases. Therefore, a comparison could not be made between benign and malignant cases (Agarwal et

al., 2010). In our study, out of the patients that we could reach, 3 (13.6%) experienced recurrence, and all these had PASS> 4. Out of the 8 (36.4%) patients without recurrence, 4 (50%) had PASS> 4. In our study, there was no significant relationship between recurrence and PASS. In this context, even though all patients with recurrence had PASS> 4 and this may suggest that PASS is beneficial in determining malignancy, the fact that 50% of patients without recurrence had PASS> 4 dis not coincide with the result concluding that PASS supports prediction of malignancy or recurrence.

In our study, we could not determine any significant relationship between the size of the tumor and PASS, in parallel to the data of Wu et al. (Wu et al., 2009), which have pointed out cases with PASS> 4 and PASS <4 showed no differences in terms of the tumor sizes. Given that pheochromacytomas are clinically effective through hormones rather than mass effect, hormone active tumors manifest clinical findings at smaller sizes and hence are operated, and this can justify above mentioned statistical insignificance (Wu et al., 2009).

PASS scoring system and recurrence did not exhibit any statistically significant relationship, nevertheless each of other histopathologic parameters were separately evaluated statistically. As a result, similar to that between the PASS system and recurrence, no statistically significant relationship could be found between recurrence and capsule invasion, vascular invasion, large and combined nests, diffuse growth, necrosis, increased cellularity, becoming fusiform of tumor cells, cellular monotony, increased mitotic figures (>3/10 HMF), any atypical mitotic figures, pleomorphism, nuclear hyperchromasia, lack of hyaline globule were found.

In the studies conducted by Nagura S, et al. and Kimura N, et al. the relationship of proliferation markers, such as Ki-67 proliferation index, with prognosis was investigated (Kimura et al., 1994; Nagura et al., 1999). These studies have demonstrated the benefit of determining the malignant course by means of immunohistochemical expression of Ki-67. In the evaluation studies, 3 cut-off values were determined :>2% (Nagura et al., 1999), >10% (Thompson, 2002), and >20 cells / 200× magnification, at the most intense cell field (Kimura et al., 1994). In our study, Ki-67 proliferation index and capsule invasion showed a significant relationship (p=0.047). However, no significant result was found between Ki-67 proliferation index and other parameters, i.e. Diameter> 5 cm, weight> 100 g, accompanied medullary thyroid carcinoma, recurrence, PASS<4, PASS> 4, vascular invasion, periadrenal fat tissue extension, large and combined nests, diffuse growth, necrosis, increased cellularity, becoming fusiform of tumor cells, marked pleomorphism, cellular monotony, nuclear hyperchromasia, atypical mitotic figures, increased mitotic figures, and hyaline globule.

In our study, particularly Ki-67 proliferation index and capsular invasion was proportionally significant. Insignificant results between Ki-67 proliferation index and recurrence might be that we could not reach all cases and thus enrolled limited number of cases. In one of their studies, Nagura et al. have determined threshold of Ki-67 proliferation index as 2% and obtained 1.4% Ki-67 proliferation index in benign cases while 3.3% in malignant tumors, which was considered statistically significant (Nagura et al., 1999). In the same study, parameters such as age, gender, concomitant endocrine neoplasia was found to be statistically insignificant. Van der Harst et al. and Clarke et al. determined higher values for Ki-67 proliferation index like 2% and 3%, respectively, for tumor cases in which malignancies were proven only with 50% sensitivity (Clarke et al., 1998; van der Harst et al., 2000).

Similar to that specified in the literature, with immunohistochemistry, 95% immunoexpression was observed with ChromograninA in our cases (Ronald et al., 2004). Immunoexpression was observed in all of the cases with Synaptophysin. With S-100, 95% immunoexpression was determined in sustentacular cells. These findings have supported our diagnosis. Focal immunoexpression was determined in 20% of our patients with Melan A. This low level of immunoexpression was consistent with neuromelanin pigment in pheochromacytoma. Immunoexpression obtained with EMA in all cases have supported the differential diagnosis with renal cell carcinoma. Hyaline globule showed positive staining with PAS in 12 cases, whereas resistance to diastase in 9 cases, which was consistent with the literature (Ronald et al., 2004).

The diagnostic characteristics achieved with immunohistochemical markers Chromogranin A, synaptophysin, and S-100 in this study were in accordance with the results in the literature. The presence of PAS (+) and diastase resistant hyaline globules in tumor cells was considered to be diagnostic value. However, our data analysis based on 4, which has been reported as the threshold of the PASS scoring system in most studies, was incompatible with the literature, and so was not considered as a significant prognostic value. Again, the relationship between Ki-67 proliferation index and malignancy and recurrence was evaluated separately and insignificant results were found, which was also incompatible with the literature. However, many different results are available in the literature (Agarwal et al., 2010; Szalat et al., 2011; de Wailly et al., 2012).

We were limited in terms of obtaining statistically significant results in three spots. First, our study group was not very large. Second, some of the parameters were relatively subjective. And third, we could not reach all of the cases.

To demonstrate the relationship between any of the histopathological parameters and tumor size, we could not come to the conclusion that the statistical data of the important parameters such as increased mitotic activity can be used as a diagnostic value. We speculate that a standard scoring system of objective histological criteria should be established in larger series in order to more fully and clearly manifest the relationship between various findings in pheochromacytoma.

As a result, we conclude that evaluating the parameters in the scoring system in large series, with new approaches, new parameters, and new criteria would lead to obtain more accurate results in the diagnosis and prognosis of pheochromacytoma tumors.

References

Agarwal A, Mehrotra PK, Jain M, et al (2010). Size of the tumor and Pheochromocytoma of the Adrenal Gland Scaled Score (PASS): Can They Predict Malignancy? *World J Surg*, **34**, 3022-8.

Clarke MR, Weyant RJ, Watson CG, et al (1998). Prognostic markers in pheochromocytoma. *Hum Pathol*, **29**, 522-6.

DeLellis RA, Lloyd RV, Heitz PU, et al (2004). World Health Organization Classification of Tumours Pathology & Genetics. Tumours of Endocrine Organs, 151-5.

de Wailly P, Oragano L, Radé F, et al (2012). Malignant pheochromocytoma: new malignancy criteria. *Langenbecks Arch Surg*, **397**, 239-46.

Ernest E. Lack (2007). Tumors of the adrenal gland. In 'Diagnostic Histopathology of Tumors 3rd Edition', Eds Christopher D.M. Fletcher. Elsevier Ltd, Philadelphia pp 1109-15.

Harrison LE, Gaudin PB, Brennan MF (1999). Pathologic features os prognostic significance for adrenocortical carcinoma after curative resection. *Arch Surg*, **134**, 181-5.

Kimura N, Miura W, Noshiro T, et al (1994). Ki-67 is an indicator of progression in neuroendocrine tumours. *Endocr Pathol*, **5**, 223-8.

Linnoila RI, Keiser HR, Steinberg SM, et al (1990). Histopathology of benign versus malignant sympathoadrenal paragangliomas: clinicopathologic study of 120 cases including unusual histologic features. *Hum Patol*, **21**, 1168-80.

Nagura S, Katoh R, Kawaoi A, et al (1999). Immunohistochemical estimations of growth activity to predict biological behavior of pheochromocytomas. *Mod Pathol*, **12**, 1107-11.

Rosai J (2011). Pheochromocytoma. In 'Surgical Pathology 10th Edition', Eds Rosai and Ackermans. MOSBY Elsevier Inc. pp 1076-8.

Strong VE, Kennedy T, Al-Ahmadie H, et al (2008). Prognostic indicators of malignancy in adrenal pheochromocytomas: clinical, histopathologic, and cell cycle/apoptosis gene expression analysis. *Surgery*, **143**, 759-68.

Szalat A, Fraenkel M, Doviner V, et al (2011). Malignant pheochromocytoma: Predictive factors of malignancy and clinical course in 16 patients at a single tertiary medical center. *Endocrine*, **39**, 160-6.

Thompson LD (2002). Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases. *Am J Surg Pathol*, **26**, 551-66.

van der Harst E, Bruining HA, Jaap Bonjer H, et al (2000). Proliferative index in phaeochromocytomas: does it predict the occurrence of metastases? *J Pathol*, **191**, 175-80.

Wu D, Tischler AS, Lloyd RV, et al (2009). Observer variation in the application of the pheochromocytoma of adrenal Gland Scaled Score. *Am J Surg Pathol*, **33**, 599-608.