Comparison of Ki-67 Index Values Between Patients With Operated Giant Prolactinomas and Macroprolactinomas

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A B S T R A C T

Background Data on whether there is a histopathological difference in cellular features as determined with Ki-67 between giant prolactinomas and smaller macroprolactinomas are not fully clear. In this study, we aimed to compare Ki-67 value between patients followed-up with diagnosis of macroprolactinoma and giant prolactinoma and operated for different reasons.

Material and Methods Files of 15 patients with giant prolactinomas and 16 patients with macroprolactinomas who had been operated with various indications were retrospectively evaluated. Similar number of patients were included to carry out a reasonable analysis. Patients' demographics (age and gender), age at the time of diagnosis, tumor diameter during the diagnosis and at the last follow-up visit, initial and last PRL and Ki-67 values were compared between the groups. Ki-67 value was studied with MIB-1 monoclonal antibody method.

Results The mean age and gender were similar between the patients with macroprolactinomas and giant prolactinomas. The mean longest tumor diameter at diagnosis was measured as 18.13 ± 9.42 mm in the macroprolactinoma and 47.07 ± 9.70 mm in the giant prolactinoma group ($p<0.001$). The mean PRL level at diagnosis was found as 4534.93 ± 12923.56 in the macroprolactinoma and 5513.08 ± 7077.87 in the giant prolactinoma group ($p=0.008$). The mean Ki-67 value was found as 31.06 ± 28.82 in the macroprolactinoma and 31.60 ± 30.78 in the giant prolactinoma group. There was no significant difference between the groups in the Ki-67 values ($p=0.922$).

Conclusions Ki-67 value was similar between macroprolactinomas and giant prolactinomas, suggesting that mitotic activity as determined by Ki-67 value is not practical in indicating growth and proliferation characteristics of prolactinomas.

Introduction

Prolactinomas are the most common hormone secreting adenomas of the pituitary, accounting for approximately 60% of all pituitary adenomas that cause clinical symptoms.[1] The prevalence of prolactinomas has been reported as 500 cases per million and the annual incidence as 27 cases per million.[2] Prolactinomas are classified based on their longest diameter measured on magnetic resonance imaging (MRI) as microprolactinomas (<10 mm), macroprolactinomas (>10 mm) and giant prolactinomas (> 40 mm).[3] Macroprolactinomas are more commonly encountered in women, leading to menorrhea, infertility and galactorrhea, are usually confined to sella turcica and do not cause compressive symptoms. Macroprolactinomas are more common in men and exhibit hyperprolactinemia, often leading to signs and symptoms of mass effect, including hypopituitarism, headaches and visual impairment due to compression of the optic chiasm and extracellular diffusion.[1,4,5]

Giant prolactinomas on the other hand are extremely rare, accounting for only 1-5% of all prolactinomas.[6] Giant prolactinomas are usually seen in men aged 20-50 years with a male-to-female ratio of ~9:1.[7] Giant prolactinomas are in general of benign nature, although these tumors are invasive and aggressive, extending into the suprasellar area and invading cavernous sinuses. The optic chiasm is often involved, causing visual defects and sometimes ophthalmoplegia. Patients with giant prolactinomas usually present with elevated prolactin (PRL) levels up to 100,000 ng/mL.[8] Male patients may complain of erectile dysfunction, hypogonadism, visual problems, headaches and weakness.

Treatment goals are similar between giant prolactinomas and macroprolactinomas, and include normalization of prolactin levels, tumor shrinkage with decompression of adjacent structures, especially the optic chiasm and cranial nerves, and normalization of testosterone levels.[8] Medical treatment of prolactinomas is mostly successful using dopamine agonists (DAs) as the first line therapy. However, several indications may require pituitary surgery as the second line treatment in a selected group of patients, including those who cannot tolerate or are resistant to medical treatment with DAs, patients that desire fertility, those with prolactinomas that impinge on the optic chiasm, psychiatric patients with contraindication to treatment with DAs and those and patients presenting with CSF leak or pituitary apoplexy.[1,9,10]

Ki-67 is one of the immunohistochemical markers of growth and proliferation used in the histological evaluation of different tumors. The utility of Ki-67 expression in the active cell cyle of prolactinomas is controversial.[11,12] In addition, data on whether there is a histopathological difference in cellular features as determined with Ki-67 between giant prolactinomas and smaller macroprolactinomas are not fully clear. In this study, we aimed to compare Ki-67 index between patients followed-up with the diagnosis of macroprolactinoma and giant prolactinoma and operated for different reasons.

Material and Methods

The files of 31 patients with 15 having giant prolactinomas and 16 macroprolactinomas, who had been operated with various indications and who had Ki-67 index studied through histochemical examinations in the Endocrinology and Metabolism clinic of our hospital between 2015 and 2019 were evaluated and included in this retrospective study. Similar number of patients were included to carry out a reasonable analysis. Tumor diameters were measured with gadolinium-enhanced pituitary MRIs performed at diagnosis and upon follow-up. The pituitary adenomas were evaluated in two groups, including those with a tumor diameter between 10-40 mm defined as macroprolactinomas and those >40 mm as giant prolactinomas.

Patients' demographics (age and gender), age at the time of diagnosis, tumor diameter during the diagnosis and at the last follow-up visit, initial and last PRL and Ki-67 values were recorded and compared between the groups. Data used in this study were obtained from the electronic information system and hospital archives.

Table 1. Demographic characteristics of the patients.

	Macroprolactinoma (n=16)	Giant prolactinoma (n=15)	Total (n=31)	p value
Age at diagnosis (year) (mean±SD)	34.06±7.92	39.13±10.84	36.52±9.63	0.188
Gender				0.01*
Male n (%)	5 (31.25)	12 (80)	17 (54.84)	
Female n (%)	11 (68.75)	3 (20)	14 (45.14)	

*Chi-square, p<0.05; SD: standard deviation.

Ki-67 Immunostaining

Ki-67 antigen was determined using MIB-1 monoclonal antibody. Surgical specimens were fixed in 10% buffered formalin and then embedded in paraffin blocks. Avidin-biotin-peroxidase method was used for Ki-67 Immunostaining.[13] Sections of 5 μm were cut and put onto glass slides, dried and were then incubated with MIB-1 antibody at 4 °C for 24 hours. The areas with highest concentrations of MIB-1 positive nuclei were analyzed at 400x magnification. The Ki-67 value was calculated in each slide as the rate of immunopositive nuclei based on 1,000. Hematogenous cells were excluded and only dark brown stained nuclei were considered positive.

Statistical Analysis

Data obtained in this study were statistically analyzed with SPSS v. 23 (SPSS, Statistical Package for Social Sciences, IBM Inc., Chicago, IL, USA) statistical software. Normality of the variables was tested with the Kolmogorov-Smirnov method. Since the variables were non-normally distributed, Mann-Whitney U method among the non-parametric tests was used in comparison of the continuous variables between the groups. Categorical parameters were compared using the Chi-square tests. Continuous variables are expressed as mean±standard deviation descriptive statistics and categorical variables as frequency (number, percentage). The statistical significance level was set at $p<0.05$.

Ethical Considerations

The study protocol was approved by the local ethics committee of the hospital. Patient consents were not needed as the study was retrospective in design, but the necessary permission was obtained from the hospital management for using archive files. The study was performed in accordance with the 1964 Declaration of Helsinki (DoH) and its later amendments.

Results

A total of 31 patients with prolactinomas were included in the study with 17 (54.84%) being male and 14 (45.14%) female. Sixteen (51.61%) of the patients had macroprolactinomas and 15 (48.39%) had giant prolactinomas. Five (31.25%) patients were male and 11 (68.75%) patients were female in the macroprolactinoma group, while 12 (80.00%) patients were male and 3 (20.00%) patients were female in the giant prolactinoma group. The male:female ratio was significantly higher in the giant prolactinoma group compared to the macroprolactinoma group (p=0.01).

The mean age at diagnosis was 36.52±9.63 year overall, 34.06±7.92 years in the macroprolactinoma group and 39.13±10.84 years in the giant prolactinoma group. No statistically significant difference was found between both groups in terms of the mean age (p=0.188). Demographic data of the patients are given in Table 1.

The mean tumor diameter was measured as 32.13±17.45 mm overall, 18.13±9.42 mm in

the macroprolactinoma group and 47.07±9.70 mm in the giant prolactinoma group (p<0.001). The mean tumor diameter at the end of follow-up was measured as 13.82±16.31 mm overall, 1.00±3.61 mm in the macroprolactinoma group and 24.93±14.72 mm in the giant prolactinoma group (p<0.001).

The mean PRL level at diagnosis was found as 5024.01±10249.95 ng/mL overall, 4534.93±12923.56 ng/mL in the macroprolactinoma group and 5513.08±7077.87 ng/mL in the giant prolactinoma group (p=0.008). The mean PRL level at the end of follow-up was found as 996.52±2463.35 ng/mL overall, 55.00±103.75 ng/mL in the macroprolactinoma group and 2155.32±3392.46 ng/mL in the giant prolactinoma group (p<0.001).

In the histopathological examinations; the mean Ki-67 value was found as 31.32±29.28 overall, 31.06±28.82 in the macroprolactinomas group and 31.60±30.78 in the giant prolactinoma group. There was no statistically significant difference between the groups in terms of the Ki-67 values (p=0.922) (*Figure 1*).

Discussion

Giant prolactinomas are distinguished from smaller macroprolactinomas with some clinical and biological characteristics. In the current literature, patients with a pituitary adenoma > 40 mm in diameter and a PLR level exceeding 1,000 ng/mL are considered to have giant prolactinomas, while macroprolactinomas are defined as adenomas with a diameter between 10-40 mm.[5,12] In the present study, we compared giant prolactinomas and macroprolactinomas in terms of clinical and histochemical characteristics.

Both macroprolactinomas and giant prolactinomas have a male predominance with a reported male to female ratio of 9:1 in giant prolactinomas.[6,14] Espinosa et al.[5] reported the rate of males as 47% in macroprolactinomas and 89% in giant prolactinomas. Artz et al.[15] reported the rate of male patients with macroprolactinomas as 56.7%. In our study, the rate of the males was 31.3% in the macroprolactinoma and 80% in the giant prolactinoma group. The difference between the studies in the rate of male patients might be resulted from the number of patients and inclusion criteria.
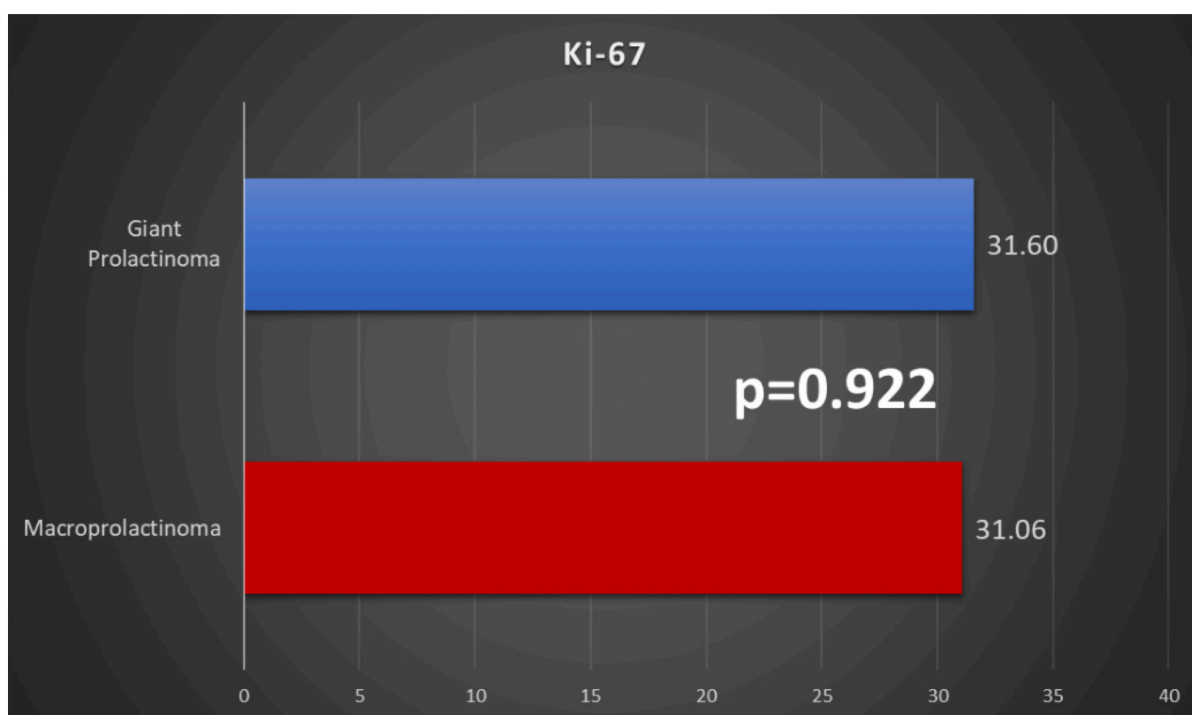
Figure 1. Ki-67 values of the patient groups with prolactinomas.

Approximately 60% of the adult males with macroprolactinomas are diagnosed before 40 years of age.[16] Although macroprolactinomas are most commonly seen in young men, they have also been reported in elderly males.[17] Whereas, giant prolactinomas are usually diagnosed in the 20-50 years age group.[8] Almalki et al.[18] reported the mean age at diagnosis as 38.1 years and Iglesias et al.[16] as 40 years. In our previous study, we found the mean age as 34.4 years.[12] In the present study, the mean age of all patients was found as 36.52 years and our finding was in the age range reported in the literature. There was no significant difference between the macroprolactinoma and giant prolactinoma groups in terms of the mean age (34.06 vs 39.13, p=0.188).

Normalization of PLR levels has been reported as 70-80% for macroprolactinomas and 60-68% for giant prolactinomas.[6,14] These rates are not surprising, because mitotic rate and proliferation of the giant tumors are only mildly increased in these adenomas compared to macroprolactinomas.[8] In our study, normalization of PLR levels was found as 100% in the macroprolactinoma and 80% in the giant prolactinoma group at the end of the follow-up.

Although most of the prolactinomas show a slow growth, some exhibit aggressive or invasive biological behavior.[19] However, there is no generally accepted marker available to identify invasiveness of pituitary adenomas. Recently, several cell cycle specific nuclear antigens have been used with various immunohistochemical methods to evaluate biological tumor characteristics. Among these antigens, Ki-67 is typically expressed at G1, S, G2 and M phases of the cell cycle during proliferation.[20] Ki-67 value can be readily obtained with monoclonal antibody MIB-1, which enables detection of the Ki-67 antigen in formalin-fixed, paraffin-embedded tissues.[19] Ki-67 has been reported to be useful in evaluating various brain tumors, providing information about cell proliferation and thus, prognosis.[21] However, it is still controversial whether Ki-67 is related to tumor aggressiveness or invasiveness in prolactinomas. Peak et al.[19] found no significant differences in the Ki-67 index in relation to age, gender and type of pituitary adenomas. In our previous study, we also did not observe a significant difference in

Ki-67 values between invasive and non-invasive prolactinomas.[12] On the contrary, Pizarro et al.[22] argued that mitotic activity evaluated by the detection of Ki-67 antigen was significantly higher in invasive than in non-invasive pituitary adenomas and that Ki-67 could be used in therapeutic postoperative management since cut-off values associated with aggressive behavior of the tumor can be established. Bălinișteanu et al.[23] reported higher Ki-67 values in prolactinomas secreting PRL. However, in our study we compared Ki-values between macroprolactinomas and giant prolactinomas for the first time in the literature and could not find a statistically significant difference between the two types of pituitary adenomas (31.06 vs 31.60, p=0.922).

Study Limitations

This study has several limitations. The study was designed as retrospective and included a relatively small number of patients. In addition, correlations of Ki-67 with different parameters could not be analyzed due to sample size. Finally, we could not exactly compare our findings, because there is only one study comparing macroprolactinomas and giant prolactinomas, but it did not evaluate Ki-67 (Espinosa). Therefore, our study is the first to investigate the utility of Ki-67 value in indicating biological behavior of pituitary adenomas. We believe that our findings will raise a new debate in the utility of Ki-67 index in distinguishing pituitary adenomas in terms of invasiveness.

Conclusions

Ki-67 value, which is used as a marker of invasiveness and biological behavior of different tumors, was similar between macroprolactinomas and giant prolactinomas. This finding suggests that mitotic activity as determined by Ki-67 value is not effective on growth and proliferation characteristics of prolactinomas. However, our results should be supported with further comprehensive prospective studies with a larger series of patients.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contribution

Study Conception: SC, OOG; Study Design: SC; Supervision: SC, OOG; Data Collection and/or Processing: SC, OOG; Statistical Analysis and/or Data Interpretation: SC; Literature Review: SC; Manuscript Preparation: SC; and Critical Review: OOG.

References

1. Cander S, Oz Gul O, Eylemer E, Gullulu Boz E, Gunes E, Hakyemez B, Yilmazlar S, Ersoy C. Comparison of the effects of medical and surgical treatments in giant prolactinoma: a single-center experience. Hormones (Athens). 2021;20(3):491-8. doi: 10.1007/s42000-021-00307-0.

2. Glezer A, Bronstein MD. Prolactinomas. Endocrinol Metab Clin North Am. 2015 Mar;44(1):71-8. doi: 10.1016/j.ecl.2014.11.003.

3. Pekic S, Soldatovic I, Miljic D, Stojanovic M, Doknic M, Petakov M, Popovic V. Familial cancer clustering in patients with prolactinoma. Horm Cancer. 2019 Feb;10(1):45-50. doi: 10.1007/s12672-018-0348-3.

4. Cooper O, Greenman Y. Dopamine agonists for pituitary adenomas. Front Endocrinol (Lausanne). 2018 Aug 21;9:469. doi: 10.3389/fendo.2018.00469.

5. Espinosa E, Sosa E, Mendoza V, Ramírez C, Melgar V, Mercado M. Giant prolactinomas: are they really different from ordinary macroprolactinomas? Endocrine. 2016 Jun;52(3):652-9. doi: 10.1007/s12020-015-0791-7.

6. Maiter D, Delgrange E. Therapy of endocrine disease: the challenges in managing giant prolactinomas. Eur J Endocrinol. 2014 Jun;170(6):R213-27. doi: 10.1530/EJE-14-0013.

7. Moraes AB, Silva CM, Vieira Neto L, Gadelha MR. Giant prolactinomas: the therapeutic approach. Clin Endocrinol (Oxf). 2013 Oct;79(4):447-56. doi: 10.1111/cen.12242.

8. Shimon I. Giant prolactinomas. Neuroendocrinology. 2019;109(1):51-6. doi: 10.1159/000495184.

9. Wildemberg LE, Fialho C, Gadelha MR. Prolactinomas. Presse Med. 2021 Dec;50(4):104080. doi: 10.1016/j.lpm.2021.104080.

10. Molitch ME. Management of medically refractory prolactinoma. J Neurooncol. 2014;117(3):421-8. doi: 10.1007/s11060-013-1270-8.

11. Yuhan L, Zhiqun W, Jihui T, Renlong P. Ki-67 labeling index and Knosp classification of pituitary adenomas. Br J Neurosurg. 2021 Apr 27:1-5. doi: 10.1080/02688697.2021.1884186.

12. Cander S, Gül ÖÖ, Ertürk E, Tuncel E, Ersoy C. Prolactin levels and gender are associated with tumour behavior in prolactinomas but Ki-67 index is not. Endokrynol Pol. 2014;65(3):210-6. doi: 10.5603/EP.2014.0029.

13. Bratthauer GL. The avidin-biotin complex (ABC) method and other avidin-biotin binding methods. Methods Mol Biol. 2010;588:257-70. doi: 10.1007/978-1-59745-324-0_26.

14. Gillam MP, Molitch ME, Lombardi G, Colao A. Advances in the treatment of prolactinomas. Endocr Rev. 2006 Aug;27(5):485-534. doi: 10.1210/er.2005-9998.

15. Arzt E, Bronstein M, Guitelman M (eds): Pituitary Today II: New molecular, physiological and clinical aspects. Front Horm Res. Basel, Karger 2010; 38:50-8. doi: 10.1159/isbn.978-3-8055-9445-5.

16. Iglesias P, Díez JJ. Macroprolactinoma: a diagnostic and therapeutic update. QJM. 2013 Jun;106(6):495-504. doi: 10.1093/qjmed/hcs240.

17. Kitchen T, Coles R, Smith A, Baglioni P, Okosieme OE. Giant prolactinoma in an elderly man. J Am Geriatr Soc. 2010 Jul;58(7):1402-4. doi: 10.1111/j.1532-5415.2010.02922.x.

18. Almalki MH, Aljohani N, Alzahrani S, et al. Clinical features, therapeutic trends, and outcome of giant prolactinomas: A single-center experience over a 12-year period. Clin Med Insights Endocrinol Diabetes. 2020;13:1179551420926181. doi: 10.1177/1179551420926181.

19. Paek KI, Kim SH, Song SH, Choi SW, Koh HS, Youm JY, Kim Y. Clinical significance of Ki-67 labeling index in pituitary macroadenoma. J Korean Med Sci. 2005 Jun;20(3):489-94. doi: 10.3346/jkms.2005.20.3.489.

20. Parkins CS, Darling JL, Gill SS, Revesz T, Thomas DG. Cell proliferation in serial biopsies through human malignant brain tumours: measurement using Ki67 antibody labelling. Br J Neurosurg. 1991;5(3):289-98. doi: 10.3109/02688699109005189.

21. Thotakura M, Tirumalasetti N, Krishna R. Role of Ki-67 labeling index as an adjunct to the histopathological diagnosis and grading of astrocytomas. J Cancer Res Ther. 2014 Jul-Sep;10(3):641-5. doi: 10.4103/0973-1482.139154.

22. Pizarro CB, Oliveira MC, Coutinho LB, Ferreira NP. Measurement of Ki-67 antigen in 159 pituitary adenomas using the MIB-1 monoclonal antibody. Braz J Med Biol Res. 2004 Feb;37(2):235-43. doi: 10.1590/s0100-879x2004000200011.

23. Bălinişteanu B, Cîmpean AM, Ceauşu AR, Corlan AS, Melnic E, Raica M. High Ki-67 expression is associated with prolactin secreting pituitary adenomas. Bosn J Basic Med Sci. 2017 May 20;17(2):104-8. doi: 10.17305/bjbms.2017.1750.