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Original Article

Role of topoisomerase II α and KI -67 biomarkers in pituitary tumors

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Keywords:

Immunohistochemistry; Ki67; Pituitary adenoma; Prognosis; Topoisomerase IIα.

ABSTRACT

Background: Pituitary Adenoma, a neoplastic proliferation of anterior pituitary hormone producing cells, is mainly considered benign but some are clinically aggressive and recurrent and very rarely malignant. From recent advances in pathological and molecular study, there is a continuous search for numerous prognostic biomarkers, to analyze better tumor behavior and prediction of response to treatment and recurrences. This study was conducted to see the epidemiological spectrum of pituitary tumors and to evaluate the correlation of TOPOISOMERASE IIα (Topo 2A), and Ki-67 biomarker with recurrence, aggressiveness, hormone subtype, radiological invasiveness, and prognostic grade of pituitary tumors.

Materials and methods: This was a prospective study with a total of 54 cases. We studied the clinical behavior, radiology, histopathological findings, and immunohistochemistry with Ki-67, Topoisomerase IIα, over 3 years at a tertiary care center.

Results: When comparing Ki-67 expression with aggressiveness, a high degree of statistical significance was found (Mann -Whitney U Test, p-value <0.001). All of our aggressive tumors (8/54) had a Ki-67 level of≥3% while most of the nonaggressive tumors (46/54) had a Ki-67 level of <3%. When comparing Topo 2A expression with recurrence, a high degree of statistical significance was found (Mann Whitney U test, P value <0.001). Most of the recurrent tumor (11/15) had Topo 2A index of 1 % or more.

Conclusions: The benign, aggressive, recurrence, or malignant nature of PA can be effectively predicted with the help of immunohistochemistry such as TOPO 2A and Ki67, thereby guiding better patient management.

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INTRODUCTION

Pituitary adenoma (PA) is neoplastic proliferation of anterior pituitary hormone producing cells. Pituitary adenomas are the third most frequent histological type, constituting approximately 15%–20%, of all primary intracranial neoplasms.[1] According to the presence of endocrine symptoms or not, PA is classified clinically into functioning & non-functioning adenomas.[2] The signs and symptoms of a functioning PA are the results of the excess secretion of specific hormones. Nonfunctioning tumors are large, and most present with mass effects whereas endocrinologically

functional tumors are often small in size. Mostly, these are noninvasive and benign in nature, and exhibit slow expansive growth displacing surrounding tissues but invasive adenoma are also frequent. Invasiveness is defined radiologically as an extension into the sellar floor bone, the cavernous sinus, and/or the diaphragm sella. Despite the presence of marked dural invasion, so-called 'invasive' pituitary adenomas display benign behavior. Truly malignant pituitary tumors (pituitary carcinomas) are very uncommon with an incidence of 0.2% of symptomatic pituitary tumors and are defined by the presence of cerebrospinal or systemic metastases.[3] Based on imaging dimensions, adenomas are designated as microadenoma < 1cm, macroadenoma >1 -4 cm, and giant adenoma >4 cm. Macroadenoma and giant adenoma are likely to invade into the cavernous sinus.[4]

As per the 2017 World Health Organization (WHO) classification, the subtyping of pituitary adenoma is done according to hormone secretion because each subtype has its own clinical presentation. Immunohistochemistry is necessary for the diagnosis of pituitary tumors and for classification into 7 main subtypes, Somatotroph, Lactotroph, Gonadotroph, Corticotroph, Thyrotroph, Null cell (immunonegative adenoma, and transcription factor negative) and Pleurihormonal adenoma.[1] WHO in 2017 recognized the relevance of lineage-restricted pituitary transcription factors (TFs) to classify tumors of adenohypophyseal cells and divided them into the three following lineages: PIT1 (pituitary specific transcription factor 1), TPIT (pituitary cell restricted factor), SF1 (splicing transcription factor 1). The detection of TFs using IHC has not yet been fully validated in our study period. The routine use of immunohistochemistry for pituitary transcription factors (PIT1, TPIT, SF1, GATA3, and ERα) is endorsed in the 2021 WHO classification. The major PIT1, TPIT, and SF1 lineage-defined PitNET types and subtypes feature distinct morphologic, molecular, and clinical differences.[5] But our study was before that classification period.

French five-tiered prognostic clinicopathological classification was proposed in 2013.[6] This classification takes into consideration the tumor diameter, tumor type, and grading. The grading is based on invasion and proliferation. Recurrence is not associated with suprasellar expansion, the invasion of dura mater forming the diaphragm sellae.[7] Mainly histological evidence of invasion of bone and the respiratory mucosae breaching into the sphenoid sinus was taken into consideration.[7]

The proliferation was evaluated based on the mitotic count, Ki-67 labeling index, and p53. The classification was based on the following three characteristics: [6]

1: Tumour diameter division into micro (<10 mm), macro (>10 mm), and giant (>40 mm), measured by MRI.

2: Tumour type division into GH, PRL, ACTH, FSH/LH, and TSH by immunocytochemistry.

3: Tumou**r** grad**e** division based on the following criteria:

   a. Invasion is defined as histological and/or radiological (MRI) signs of cavernous or sphenoid sinus invasion.

   b. Proliferation considered on the presence of at least two of the three criteria:

      Ki-67: ≥3 % (formalin fixative)

      Mitoses: n >2/10 HPF

      TP-53: positive (10 strongly positive nuclei/10 HPF)

Based on these criteria, the Pituitary adenomas were divided into the following five grades:

- Grade 1a: non-invasive tumour
- Grade 1b: non-invasive and proliferative tumor
- Grade 2a: invasive tumour
- Grade 2b: invasive and proliferative tumor
- Grade 3: metastatic tumor (cerebrospinal or systemic metastases)

A tumor is regarded as aggressive if there is an unusually rapid growth rate, more frequent recurrences requiring repeated surgeries, and clinically relevant growth in spite of standard therapies.[8] Thus, the terms invasive and non-invasive should refer only to imaging or morphological findings, and aggressive and non-aggressive pituitary adenomas to their clinical behavior.[9]

Ki-67 is a nuclear antigen expressed in the G1, G2, and synthesis phases of the cell cycle but not in the resting G0 phase.[10] A labeling index ≥3% has been suggested to have prognostic value. This cut-off value is recommended by WHO and five-tiered classification .[6]

In rapidly proliferating tumor cells, DNA topoisomerase content is higher than in normal cells.[11] DNA topoisomerases enzymes wind and unwind DNA during replication and have been divided into two types: Types I and II. Type II plays an important role in DNA replication and mitosis.[12] Topo IIα expression has been found to be significantly higher in invasive than noninvasive tumors. This knowledge about the property of Type II topoisomerase has been utilized to develop several effective anticancer drugs.[13,14]

The objective of our study was to evaluate the correlation of TOPOISOMERASE IIα (TOPO 2A), and Ki-67 biomarker with tumor recurrence, aggressiveness, hormone subtype, radiological invasiveness, and prognostic grade of pituitary adenomas.

MATERIALS AND METHODS

This was a prospective cross-sectional study conducted from January 2017 to January 2020. The study was conducted in collaboration with the Department of Pathology,

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Endocrinology, and Neurosurgery at a tertiary care hospital. The patients with clinical and radiological evidence of pituitary tumor, selected for operation were included in this study. The patients who were debilitated and who refused surgery were excluded from the study. The invasion was assessed by MRI. Only Grade III and IV tumors of Knosp's classification were taken into account for invasiveness. After surgery, histopathological studies were done in the department of pathology for confirmation of a diagnosis of PA by hematoxylin and eosin staining (H&E). Specific hormone immunohistochemistry (IHC) were done for the subtyping of different type of adenoma. Cytoplasmic positivity of ≥ 10 % cell population was taken as positive for hormone IHC. IHC studies were done to know the Ki-67 labeling index and TOPO 2A indexing. It was performed on 4-5 µm thick, polyl-lysine precoated slides on formalin-fixed, paraffin-embedded tissue sections.

Ki-67 proliferation index was calculated by calculating the percentage of labeled nuclei per 1000 nucleated cells in an area with a maximum population of cells at high power view. A labeling index ≥3% was used as a cut-off value, as per WHO and French Five Tiered Prognostic classification.[6]

The TOPO 2A index was determined by counting the number of positive nuclear staining divided by 1000 tumor cell nuclei. Areas of necrosis and haemorrhage were excluded.[15] TOPO 2A exceeding > 1% is taken into account.[16]

All data were thoroughly maintained in a Microsoft Excel worksheet. Median, mean values with standard deviation were calculated for quantitative variables, whereas proportions represented qualitative variables. Mann Whitney U test, Kruskal Wallis, Fischer's exact tests were employed for statistical analyses, as appropriate, using SPSS software version 20.0 (IBM, Armonk, New York, USA) and MedCalc version 15.8 [Mariakerke, Belgium: MedCalc Software bvba;2015]. Two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

In our study, we evaluated a total of 54 cases of PA over 3 years at a tertiary super specialty hospital that caters largely to the Eastern part of India. Our patients ranged from 1-80 yrs, mainly in the 41-60 yrs age group (46%), with the mean age being 41.19 ± SD 12.918. There was no significant sex predilection in our study M: F (1:1.6). 18 patients (33.33%) presented with mass related symptoms like headache & visual defects in our study. 36 (66.6%) patients presented with hormone related symptoms. Radiology was useful in assessing the tumor size and tumor invasion. In our study, 46 cases (85%) were macroadenomas, according to Knosp classification radiologically. We found 34/54 (63.%) cases with cavernous sinus invasion in our study and 20/54(37%) cases did not show invasion. Among them, 8/54 cases were noninvasive microadenoma. On H&E staining 40 cases were eosinophilic (74%), 10 cases (18.51%) were basophilic, and 4 cases (7.4%) of chromophobic adenoma. Immunohistochemical staining of pituitary adenomas was performed (fig.1).

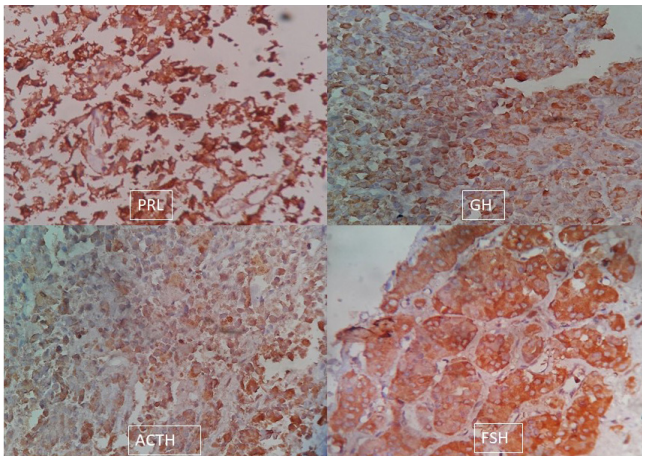

Figure 1: *Immunohistochemical staining of pituitary adenomas for Prolactin (PRL), Growth hormone (GH), Adrenocorticotropic hormone (ACTH) and Follicle-stimulating hormone (FSH). (IHC, 40x)*

We received 13 cases (24%) of lactotroph adenoma, 14 (25.92%) of somatotroph adenomas, 6 cases (11.1%) corticotroph adenomas, 8 cases (14 .81%) mixed somatotroph and lactotroph adenoma, 7 cases (12 .96%) of hormone immunonegative (TF not tested) adenoma, 3 (5.55%) cases of gonadotroph adenoma and 3 (5.55%) of pleurihormonal adenoma. We didn't come across any thyrotroph adenoma. A total of 15 (27 .77%) cases showed recurrence after surgery (diagnosed by MRI or by hormone tests) between 6 months to 24 months during 36 months of follow-up study.

Table 1: Clinico-radiological parameters of patients in this study.

	Clinical Parameters	n (%)
Age group	1-20	4 (7.4)
	21-40	23 (42.59)
	41-60	25 (46.29)
	61-80	2 (3.7)
Tumor size	Macroadenoma	46 (85.19)
	Microadenoma	8 (14.81)
Invasiveness	Invasive	34 (63)
	Non-invasive	20 (37)
Hormone subtype	Lactotroph	13 (24)
	Gonadotroph	3 (5.55)
	Somatotroph	14 (25.92)
	Corticotroph	6 (11.1)
	Mixed somatotroph-Lactotroph	8 (14.81)
	Pleurihoemonal	3 (5.55)
	Immunonegative	7 (12.96)
Recurrence	Recurrent	15 (27.77)
	Nonrecurrent	39 (72.22)

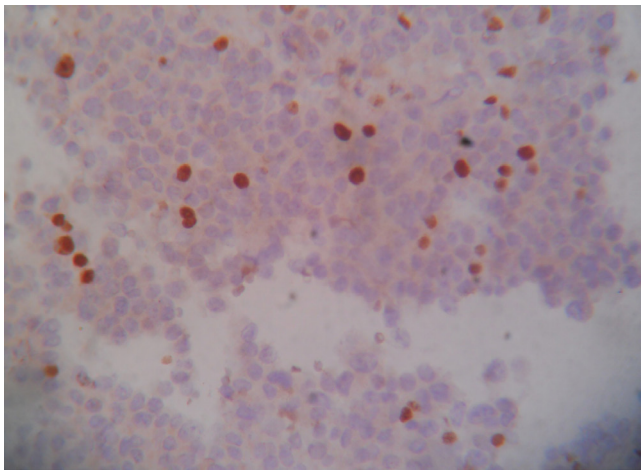

Figure 2: *Nuclear staining of KI 67. (IHC 40x)*

Values of Ki 67 ranged between 0.1 % to 10% with a median value of 0.55% in our study. (fig. 2) When comparing Ki -67 (fig. 2) expression with recurrence a high degree of significance was found (Mann-Whitney U test, P value <0.001). (Table 2)

Table 2: Comparison of Ki67 expression between recurrent and Non-recurrent subgroups of pituitary adenoma [Mann-Whitney test (independent samples)]

	Recurrent	Non-Recurrent	
Sample size	15	39	P < 0.001
Lowest value	0.5	0.1	
Highest value	8.0	10.0	
Median	3.0	0.5	

When comparing Ki-67 expression with aggressiveness, a high degree of significance was found (Mann -Whitney U Test, p-value < 0.001). All of our aggressive tumors (8/54) had a Ki-67 level of≥3% while most of the nonaggressive tumors (46/54) had a Ki-67 level of <3%.

Table 3: Comparison of Ki67 expression between aggressive and non-aggressive subgroups of pituitary adenomas. [Mann-Whitney test (independent samples)]

	Aggressive	Non-aggressive	
Sample size	8	46	P < 0.001
Lowest value	3.0	0.1	
Highest value	10.0	4.0	
Median	4.0	0.5	

The highest median value of Ki 67 (4%) was observed in Gonadotroph adenoma. On comparison with Ki-67 across different tumor subgroups no significant correlation was found (Kruskal Wallis test, p=0.060). The highest median value of Ki-67 (3.5%) was observed in Prognostic grade group 2B. On comparison with Ki-67 across different prognostic grade groups significant correlation was found (Kruskal Wallis test, p<0.001).

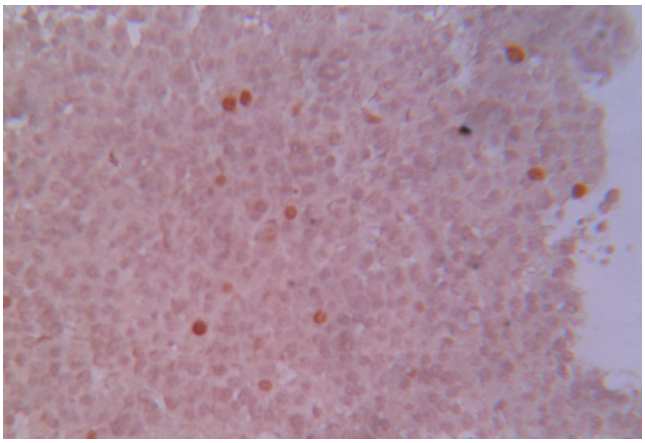

Figure 3: *Nuclear staining of Topoisomerse IHC. (IHC, 40x)*

Values of Topo 2A ranged between 0.1 % to 7% with a median value of 0.6%. (fig.3) Highest median value of the Topo 2A (fig. 3) index was found in gonadotroph adenoma (2.4%). When comparing Topo 2A expression with recurrence, a high degree of significance was found (Mann Whitney U test, *P*-value <0.001) Most of the recurrent tumors (11/15) had a Topo index of 1% or more**.** (Table 4)

Table 4: Comparison of Topoisomerase expression between recurrent and non-recurrent subgroups of pituitary adenoma

	Non-recurrent	Recurrent	
Sample size	39	15	P < 0.001
Lowest value	0.10	0.50	
Highest value (topoisomerase)	3.00	7.00	
Median	0.60	2.00	

In our study, we observed a significant correlation between Topo 2A expression status and Knosp invasiveness (Mann -Whitney u test, P value =0.029). When comparing Topo 2A expression with aggressiveness, a high degree of significance was found (Mann -Whitney U test, *P* value <0.001). All of our aggressive tumors (8/54) had high Topo 2A index values (range 2% to 7% with a median value of 2.2**%**). (Table 5)

Table 5: Comparison of Topoisomerase expression between aggressive and non-aggressive subgroups of pituitary adenomas [Mann-Whitney test (independent samples)].

	Aggressive	Non-aggressive	
Sample size	8	46	P < 0.001
Lowest value	2.0	0.1	
Highest value	7.0	2.0	
Median	2.2	0.6	

Upon comparison of Topo 2A across different tumor subgroups and prognostic grade groups, a significant correlation was found. (Kruskal Wallis test, p=0.032 and p<0.001 respectively). The highest median value of the Topo 2A index 2% was observed in Prognostic grade group 2B**.** **(Table 6)**

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Table 6: Comparison of Topoisomerase expression between prognostic grade subgroups. (n=54)

Prognostic grade subgroup	n	Minimum	Median	Maximum	p-value
1A	19	0.3000	0.600	0.900	P < 0.001
1B	1	1.0000	1.000	1.000	
2A	22	0.1000	0.600	1.500	
2B	12	1.0000	2.000	7.000	
	Total sample size: 54				

DISCUSSION

We received 54 cases of pituitary tumors in our study over 3 years. Our study chiefly focused on the findings of one tertiary care center in the eastern part of India. Mahta et al have done a study with 85 cases.[18] In India Rishi et al have conducted a study with 151 PA.[19]

In our study, maximum patients were found in the age distribution of 41-60 yrs age group (n=25: 46.29%). 23 patients (42.59%) were in the 21-40 yrs age group and 04 patients were in the 01-20 yrs age group & 02 patients were in the 61-80 yrs age group. According to WHO pituitary tumors are rarely seen in the pediatric population.[20] In a study by Kleinschmidt-DeMasters, the most common age group of pituitary adenomas was 30-70 yrs which corroborates with our findings.[21]

As per WHO, there is no sex predilection of pituitary adenomas, which corroborates our results (M: F=1:1.6).[20] In our study, we did not encounter any pediatric patient but a study by Kanter SL et al pituitary adenomas show a predilection for females in the pediatric age group.[22] Though panhypopituitarism with features of hypogonadism, hypoadrenalism, etc. are frequent, people rarely come with these symptoms.[20] They seek medical advice with visual difficulties e.g. hemianopia, and other mass effect related symptoms, eg. headache, vomiting, etc commonly. We found 18 patients (33.33% presented with visual difficulties & mass effects. 36 (66.6%) patients presented with hormone related symptoms. Few patients had an overlap of symptoms having both mass effects and symptoms of hormone excess (4/54), as patients usually reported sooner if symptoms of hormone excess such as gigantism, obesity, etc were present. In a study by Rishi et al, the most common presenting symptoms were visual symptoms & headache.[19]

According to Knosp classification, radiologically, the most common PAs were macroadenomas 46/54 (85%) with cavernous sinus invasion (Grade III & IV). 20/ 54 (37%) cases were without invasion, and among them, 08/54 cases were noninvasive microadenoma.

Our study with Knosp classification corroborates with the study by Ortiz-Plata et al who found 18% of cases belonging to Grade I & II, with the majority being Grade IV PA.[24]

Values of Ki 67 IHC ranged between 0.1 % to 10% with a median value of 0.55% in our study. Saeger et al showed a range of 0.16 - 15.48%.[17]

When comparing Ki-67 expression with aggressiveness, a high degree of significance was found (Mann -Whitney U test, p<0.001). All aggressive tumors (8/54) in our study had a Ki-67 level of>3% while most of the non-aggressive tumors (46/54) had a Ki-67 level of <3%. Thapar et al.[25] reported that a 3% Ki67 cut-off value is associated with 72.7% sensitivity and 97.3% specificity and a positive and negative predictive value of 96% and 80% respectively, in distinguishing non-invasive from invasive pituitary adenomas. Righi et al. showed in a series of 166 patients with pituitary tumors that invasion is significantly associated with recurrence/ progression and that a Ki-67 index of 3 % has high specificity and low sensitivity.[26]

When comparing the Ki-67 index with Knosp radiological invasiveness no statistical significance was found (Mann-Whitney U test, p=0.3213) in our study. Salehi F et al showed that the ability of the Ki67 index to predict tumor invasiveness remains controversial as discrepant results have been reported.[27] This corroborated our study. In the study by Saeger et al, invasive pituitary adenomas had a higher Ki-67 value of 2.01+/-3.15%, and non-invasive ones had a value of 1.12+/-1.87 %.[17] This did not corroborate our study.

Values of Topo 2A ranged between 0.1 % to 7% with a median value of 0.6% in our study. The highest median value of Topo 2A index was found in gonadotroph adenoma (2.4%). In studies by Vidal et al or Wolfsberger et al. the highest topo IIα expression levels were observed in pituitary carcinomas, silent -ACTH adenomas, prolactinomas, somatotropinomas, and silent subtype 3 adenomas.[15,28] The results of our study were slightly different, which may be due to a small group of the study population or different ethnic origins.

When comparing Topo 2A expression with recurrence, a high degree of statistical significance was found (Mann Whitney U test, *P* value <0.001) Most of the recurrent tumours (11/15) had a Topo index of 1% or more. The hazard ratio for recurrence when topoisomerase expression >1 % is 9.892 (95% CI 2.557 to 38.274). Małgorzata TrofimiukMuldner et al also showed that at the cut-off level of a Topo IIα index of 1%, topo 2A expression showed 63.6% sensitivity and 86.8% specificity in predicting tumor recurrence or progression.[16] In our study we got a significant correlation between Topo 2A expression status with Knosp invasiveness (Mann -Whitney u test, P value =0.029) corroborating with the findings of Trofimiuk et al. [16]

In our study, we did not observe a significant correlation between Topo 2A index with age and sex, and histology. This is in agreement with Trofimiuk et al.[16] Vidal et al also found a negative correlation between Topo 2A and age. [15] Wolfsberger et al. found that the female sex is significantly associated with a higher Topo 2A index in their study which

is different from our study.[28]

CONCLUSIONS

Despite considerable progress in understanding the pathogenesis of pituitary adenomas, no single marker has been found yet that predicts the aggressive behavior of pituitary adenomas independently. The Topo IIα level has been identified to be superior over Ki 67 as a prognostic/predictive marker for Pituitary adenomas.

Limitations:

The authors acknowledge the relatively low sample size in this study and that no thyrotroph adenomas were encountered. Some patients were lost to follow-up, and their data related to tumor behavior, and recurrences were lost too. Also, due to the limited period of the study, follow-up of the patients treated with TOPO 2A inhibitors and the clinical outcome of the therapy could not be evaluated.

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