Alexandria Journal of Medicine xxx (2016) xxx–xxx

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Contents lists available at ScienceDirect

Alexandria Journal of Medicine

journal homepage: http://www.elsevier.com/locate/ajme

Original Article

Proliferaton index in pituitary adenomas from a black African population

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ARTICLE INFO

Article history:
Received 21 August 2016
Revised 4 November 2016
Accepted 19 November 2016
Available online xxxx

Keywords:
Pituitary adenoma
Immunohistochemistry
Atypical
Ki67

ABSTRACT

Background: The WHO has recognized a variant of pituitary adenomas with potential aggressive behaviour which have been termed atypical pituitary adenomas. This group of tumours are recognized by their mitotic rate of more than >3%, p53 expression and invasion of surrounding structures. There has however been no study of the occurrence of these tumours in a black African population. This study is a preliminary attempt to examine this group of tumours in blacks.
Methods: This study retrospectively reviewed fifty-seven histologically diagnosed and immunohistochemically characterized pituitary adenomas received in our department over a twenty-one year period. Specimens were stained with ki67, a nuclear marker of cell proliferation which has been identified as the single best predictor of atypical pituitary adenoma.
Results: Twelve of the tumours showed atypical features with eight (67%) of these tumours being prolactinomas. Two of the tumours were gonadotrophs and two were null cell adenomas. There was no correlation with age or gender. Two of the tumours required neurosurgical re-exploration with one of these showing a higher mitotic index in the second biopsy.
Conclusion: The study suggests similarity in the rate of occurrence of pituitary adenomas with atypical features in a black African population with what is seen in Caucasians. Prolactinomas constitute a significant percentage of the tumours with this feature.

1. Introduction

The most common tumour occurring in the sellar area is the pituitary adenoma and it constitutes about 10–15% of all intracranial tumours.[1–3] Pituitary adenomas are benign epithelial tumours composed of adenohypophyseal cells, some of which secrete hormonal substances.[3,4] Most of the tumours are slow growing with the cells appearing monomorphic. They often grow in an expansive pattern and do not invade surrounding structures. Occasional tumours show invasion of surrounding structures with increase in proliferation.[4] The WHO classification of 2004 has identified this group of tumours as atypical adenomas and they are characterized by local invasion and increased mitosis.[5,6] The atypical adenomas usually show an increased MIB-1 labelling index of more than 3% and often show p53 reactivity which is not seen in benign tumours.[2,5] This group of tumours are known to confer a poor prognosis due to their high recurrence rate.[7] There is no proof at present to describe these tumours as premalignant lesions despite their clinical behaviour. However, the clinical relevance of these tumours has been questioned based on studies which have not been able to show any correlation with invasiveness and rate of proliferation.[7–9] Several studies have shown that the ki67 index is the single best predictor of atypia in pituitary adenoma compared with the other parameters.[10,11]

Many studies have shown prolactinomas, and corticotroph adenomas as the most common tumours showing atypical features, while null cell and gonadotroph adenomas as the least common; others have shown lack of uniformity in the secretory status of cells showing invasion.[2,12,5,13] No study has been carried out in an African population to determine the occurrence of this variant of pituitary adenomas in the black African population. This is a pre-

Peer review under responsibility of Alexandria University Faculty of Medicine.
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http://dx.doi.org/10.1016/j.ajme.2016.11.003
2090-5068/

liminary study examining the occurrence of pituitary adenomas with atypical features in a predominantly black African population.

2. Materials and method

This study was conducted on immunohistochemically characterized pituitary adenomas from surgical specimens obtained at the University College Hospital, Ibadan over a twenty-one year period. The tumours were removed via Pterional craniotomy with microsurgical total tumour removal for the giant tumours and endoscopic trans-septal trans-sphenoidal pituitary resection for the intrasellar tumours. The pituitary adenomas had been identified according to the earlier described method.[14] Ethical approval for the study was obtained from the joint University of Ibadan/University College Hospital ethical committee. The age, sex and clinical history of the patients were obtained from their records. There was no record of accompanying dura in the gross description of any of the biopsies. The archived slides and paraffin wax blocks were retrieved and the slides were reviewed to ascertain the accuracy of the initial diagnoses.

Each of the tissue blocks was stained with antibodies for ki67. The streptavidin-biotin-peroxidase method was used and the protocol followed that of previous researchers.[12,13,15]

Three serial sections, cut at 5 μm each, were obtained from the archived paraffin blocks for each case and deparaffinised. Antigen unmasking, using the heat induced epitope retrieval method, was done by first pre heating the retrieval buffer to a temp of 90–95 °C in an incubator (microwave) and subsequently immersing the slides in preheated citrate buffer diluted to 1:10 with distilled water for 10–20 min. They were then allowed to cool for 20 min in cold water and thereafter rinsed in TBST (Tris buffered saline with Tween). Blocking of enzyme activities/peroxidase was done with 3% hydrogen peroxide for 10–15 min. Each specimen was incubated for 20–30 min with 40–130 μl of an appropriately characterized and diluted (1:200) KI67 rabbit primary antibody (Thermofisher scientific Clone SP6). This was followed by incubation with an undiluted labelled polymer Horse Radish Peroxidase (HRP) conjugated anti-mouse secondary antibody for 30 min. Diaminobenzidene [DAB] solution was added to cover the specimen. Counterstaining was done with Haematoxylin. A tonsil biopsy with a histological diagnosis of reactive follicular hyperplasia and a normal pituitary gland were used as positive and negative controls respectively.

The proliferation index was assessed using a modification of the earlier described methods.[8,9] A total of 1000 nuclei from the neoplastic cells were counted from ten randomly selected fields with the aid of a graticle. This was done on each slide at 40× magnification. The total number of stained nuclei was expressed as a percentage of the absolute number of counted nuclei. Using modified 2004 World Health Organisation criteria, adenomas with a Ki67 index of $\geqslant 3\%$ were classified as atypical adenomas.[6] Statistical analysis was performed using Student *t*-test for comparison of continuous variables and chi-squared test for comparison of discontinuous variables, to determine whether there was any association between the clinical and immunohistochemical data. The level of statistical significance was set at $p \leqslant 0.05$.

3. Result

A total of 134 patients were diagnosed with pituitary adenoma from the institutional cancer registry record during the study period. However only seventy-two of the biopsies were received in the surgical department during the study period (many of the patients received drug therapy while some defaulted and were lost to follow up). Fifty-seven of the surgical biopsies of pituitary adenomas were included in the study. Fifteen of the biopsies were excluded either due to unavailability of their tissue blocks while some had been exhausted during earlier diagnostic procedures. The specimens were obtained from patients with ages ranging from 9 to 73 yrs. The tumours occurred more frequently in patients in their fifth decade with 17 (30%) pituitary adenomas seen in this age group (Table 1).

The ki67 index in the tumours ranged from 0.2 to 10.2 with a mean index of 2.05. The benign tumours were 45 (79%) while the pituitary adenomas with atypia constituted 12 (21%). Eight of the pituitary adenomas with atypia (67%) occurred in patients in the fourth and fifth decades. There was however no statistical significance between the age and the level of atypia in these tumours ($p = 0.456$). Thirty (52.6%) of the tumours were from female patients while 27 (47.4%) were obtained from male patients. Seven of the pituitary adenomas with atypical features occurred in females while five occurred in males (Fig. 1). There was no statistical significance between the sex and tumour atypia ($p = 0.751$).

Eight (67%) of the pituitary adenomas with atypia showed positive staining for prolactin. This was significant ($p = 0.001$). Only two gonadotroph and null cell adenomas had atypical features, even though they are the most frequent adenomas seen (Table 2). Twenty-two (38.6%) of the pituitary adenomas showed microscopic dural invasion while only three (13.6%) of these invasive tumours showed high ki67 index.

Four out of the fifty-seven pituitary adenomas had surgical re-exploration with two of these tumours having features of atypia. Only one of the recurrent adenoma with atypical features was a prolactinoma while the other was a null cell adenoma. The lone case of prolactinoma required re-exploration after the pathologist identified the tumour as atypical and the second biopsy from the re-exploration showed a markedly higher index score (Fig. 2).

4. Discussion

Invasive pituitary adenomas do not show typical histological features of malignancy that identify tumours in other organs.[18] The exact pathway that determines progression in pituitary adenomas is yet to be fully understood although the *Ras* gene mutation has been identified in some cases of pituitary carcinoma.[4,19] Ikeda and Yoshimoto have also demonstrated that adenomas with increased ki67 index tend to show increased expression of both c-myc and bcl-2 genes.[20] Protein kinase C (PKC) and Pituitary Tumour Transforming Gene (PTTG-1) are among the few other identified gene mutations associated with increased aggressiveness.[1,4,21] Several growth factors have however been associated with increased ki67 index including epithelial growth factor receptor (EGFR) and isoforms of basic fibroblast growth factor receptor (FGFR4).[4,19,21]

The 2004 WHO definition of atypical adenomas identified several characteristics which include high mitotic rate, ki67 index greater than 3%, increased expression of p53 gene or gross invasion of neighbouring structures.[6,21] The proliferation index can be mea-

Table 1
Distribution of benign and atypical adenoma according to age in decades.

Age in decades	Atypical	Benign
1–10	0	1
11–20	2	2
21–30	1	2
31–40	3	11
41–50	5	12
51–60	0	12
61–70	1	4
71–80	0	1