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Immunohistochemical Expression of β-catenin and Epidermal Growth Factor Receptor in Adamantinomatous Craniopharyngioma

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***Abstract*—**Introduction: Craniopharyngiomas (CPs) are rare epithelial tumors located mainly in the sellar/parasellar region. CPs have been classified histopathologically, genetically, clinically and prognostically into two distinctive subtypes: adamantinomatous and papillary variants. Aim: To examine the pattern of expression of both the β-catenin and epidermal growth factor receptor (EGFR) in surgically resected samples of adamantinomatous CP, and to asses for the possibility of using anti-EGFR in the management of ACP patients. Materials and methods: β-catenin and EGFR immunostaining was performed on paraffin-embedded tissue sections of 18 ACP cases. Result: 17 out of 18 cases (94%) of ACP exhibited strong nuclear/cytoplasmic expression of β-catenin, 15 (83%) of APC cases were positive for EGFR. Conclusion: Nuclear accumulation of β-catenin is a diagnostic hallmark of ACP. EGFR positivity in most cases of ACP could qualify the use of anti-EGFR therapy.

***Keywords*—**Craniopharyngioma, adamantinomatous, papillary, epidermal growth factor receptor, B-catenin.

I. Introduction

CPs are benign epithelial tumors originate from sellar, parasellar, and suprasellar region. CPs account for 2–4.6% of all intracranial tumors and it is the commonest intracranial neoplasm of non-glial origin in children representing 9% of brain tumors in pediatric age group [1]-[3]. Although CPs are considered histologically as benign tumors (grade I according to WHO classification), they infiltrate adjacent structures such as the pituitary, hypothalamus, optic nerves, blood vessels and 3rd ventricle, thereby causing significant morbidity and mortality [4]. There are two main subtypes of CP: The admantinomatous type, which is more common in children, and the papillary type occurring predominantly in adults. Some mixed forms also have been reported [5]. Two theories have been reported to explain the origin of CP, namely, embryogenetic and metaplastic theories. The embryogenetic theory suggests that ACP arises from epithelial remnants of the craniopharyngeal duct or Rathke's pouch while the metaplastic theory relates to metaplasia of squamous epithelial cells that make up part of anterior pituitary [6], [7].

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The clinical presentation of CP is not specific, and depends on the patient's age, location of the tumor, its size, and how pressure affects the nearby vital structures of the brain. The presenting features include increased intracranial pressure, visual field deficits, and hormonal disturbances of hypothalamus and pituitary glands [8], [9].

Histopathologically, ACP is charactarized by wet keratin nodules and a mixture of cystic and solid areas with a stratified squamous epithelium, as well as the presences of calcification, fibrosis and cholesterol crystals. The presence of these features histologically will support the diagnosis of ACP. In contrast, papillary CP often consists of encapsulated solid parts similar to the oropharyngeal mucosa with a papillary growth pattern that has no calcifications. These classical pathological features are not present in all cases so there is a critical need for adjuvant immunohistochemical markers to confirm the diagnosis [10].

The most important pathway in promoting cellular proliferation and determining cellular fate during tissue development and homeostasis is the Wnt pathway. β-catenin is the key player of this pathway. Recently, it has been found that mutations of the Glycogen Synthase Kinase 3 binding domain (GSK3b) of β-catenin is present in ACP. Therefore, it has been used as an immunohistochemical marker to confirm the diagnosis [10].

EGFR belongs to a family of receptor tyrosine kinase. These receptors share a similar structure, which consists of intracytoplasmic tyrosine kinase domain and extracellular ligand binding domain. Activation of this receptor will lead to a recruitment of enzymes and proteins that are responsible for different intracellular complexes, leading to cellular differentiation, proliferation, and motility [11], [12].

Recently, the EGFR cascade was found to play a role in Wnt-signaling activation, most likely due to a direct interaction between EGFR and β-catenin [13]. Aberrant EGFR-signaling was identified in several types of cancers such as non–small-cell lung cancer (NSCLC), colorectal carcinoma, breast cancer, head and neck squamous cell carcinoma, bladder cancer, and pancreatic cancer [14]-[17].

The aim of the study is to examine the immunohistochemical expression of β-catenin in ACP and assess the status of EGFR expression.

II. Materials and Methods

Eighteen Formalin-fixed, paraffin-embedded tissue blocks of ACP were obtained retrospectively from the archives of the

Departments of Pathology at Alnoor General Hospital in Makkah during the period between 2005 and 2013.

Immunohistochemical analysis was performed with the following commercially available antibodies: β-catenin, EGFR.

Four μm thick sections were cut from routine paraffin embedded blocks then deparaffinized in xylene and hydrated by graded alcohols. Immunostaining was performed with the Dako Autostainer. The slides were incubated with peroxidase-blocking reagent followed by the primary antibody, and then the visualization reagent (secondary goat-antimouse immunoglobulin and horseradish peroxidase linked to a dextran polymer backbone) was used. After rinsing with distilled water, the slides were incubated with DAB (3, 3-diaminobenzidine), a substrate–Chromagen solution and a Mayer Hematoxylin counter stain was applied before cover slipping.

A. Scoring Methods

For β-catenin expression, the staining pattern similar to that of the normal maxillary sinus mucosal tissue. Homogeneously positive staining at the cell membrane was evaluated as negative (normal maxillary sinus mucosal tissue from patients with CP treated by trans-sphenoidal surgery served as controls according to the supplier's protocols). The cytoplasm/nuclear immunoreactivity was evaluated separately from membranous immunoreactivity, and the nuclear staining was scored as strong (2), weak (1), or negative (0).

Regarding EGFR-staining, only staining of the tumor cell membranes was considered to be specific. The staining pattern of tumor cell membranes was further classified as incomplete staining and complete staining. The following scoring approach was used in the assessment of EGFR immunostaining: score 0 = no staining or unspecific staining of the tumor cells; score 1 = weak (intensity) and incomplete staining (quality) of more than 10% of the tumor cells (quantity); score 2 = moderate and complete staining of more than 10% of the tumor cells; score 3 = strong and complete staining of more than 10% of the tumor cells [18].

B. Statistical Analysis

The data were analyzed using SPSS version 12.0.0 for Windows (SPSS Inc., Chicago, IL). Scale variables were presented as the mean ± standard deviation (mean ±SD) or percentages, as appropriate.

III. Results

A. Haematoxylin and Eosin

Histologically, all the eighteen cases of ACP showed the presence of a complex epithelial pattern with epithelial cells disposed in sheets, cords, nests, and anastomosing trabeculae with peripherally palisading columnar epithelial cells with darkly stained nuclei and little cytoplasm. Those areas merge with loosely cohesive aggregates of squamous cells. Wet keratin was identified in all cases (Fig. 1 [A]). Calcification, foreign body giant cells, and cholesterol clefts were detected in eleven cases either singly or in combination. In nine cases, cystic cavities containing squamous debris were present.

B. Immunohistochemical Findings

The detailed immunohistochemical characteristics found in ACP using β-catenin and EGFR are summarized in Table I.

1. β-catenin

Examination revealed a shift from membrane-bound to nuclear/cytoplasmic accumulation of β-catenin in seventeen out of eighteen cases (94%) of the ACP, predominantly in the compactly cohesive epithelial cells within the whorl-like arrays and occasionally in palisaded cells (Fig. 1 [C]). One case showed only β-catenin membranous immunoreactivity, without cytoplasm/nuclear accumulation, therefore it was considered as negative.

Sheets of epithelial cells showed weak to moderate cytoplasmic and membranous staining. Peripherally palisading cells showed somewhat stronger expression in both the cytoplasm and membrane. Clusters of cells with strong nuclear immune-reactivity were seen among the whorl-like arrays of the epithelial cells.

2. EGFR Family

Regarding EGFR, fifteen out of eighteen cases (83%) of APC were positive.

TABLE I
B-Catenin and EGFR Expression in ACP

Specimen no	β-catenin	EGFR
1	Positive	Positive
2	Positive	Negative
3	Positive	Positive
4	Positive	Positive
5	Positive	Positive
6	Negative	Negative
7	Positive	Positive
8	Positive	Positive
9	Positive	Positive
10	Positive	Positive
11	Positive	Positive
12	Positive	Positive
13	Positive	Negative
14	Positive	Positive
15	Positive	Positive
16	Positive	Positive
17	Positive	Positive
18	Positive	Positive

IV. Discussion

CPs are classified into adamantinomatous and papillary subtypes [5]; however, histopathologic differentiation between the two can be challenging sometimes because the typical features are not always present, which necessitates the use of adjuvant immunohistochemical markers to confirm the diagnosis and therefore choose the appropriate management of the patient. Recently, disturbances in the Wnt pathway as demonstrated by β-catenin mutations and/or nuclear accumulation have been found in ACP exclusively. Nuclear

shift from the originally membrane-bound β-catenin as detected by immunohistochemical testing was the whole mark in the diagnosis of ACP in specimens lacking the classical histological features [21].

Management of CP forms a big challenge to most neurosurgeons because of its big size and its pressure effect on nearby vital brain structures. Surgical removal of these tumors is not always possible, especially if calcification is present. Radiation therapy can be used in patients with remnant tumor tissue in whom surgical removal was not completely done or in patients with recurrent disease after complete resection [3], [9].

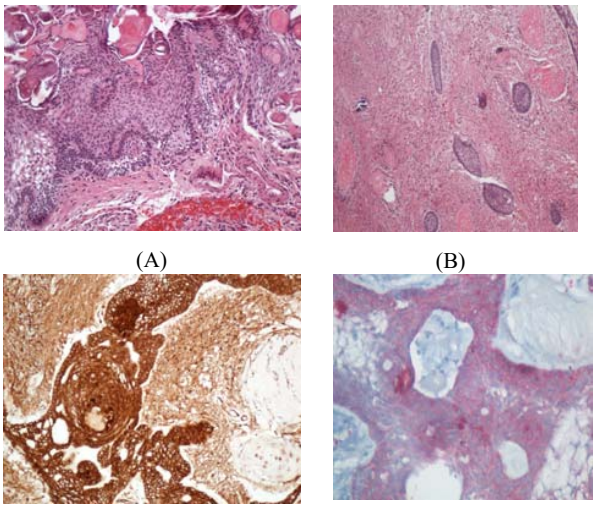

Fig. 1 Adamantinomatous CP exhibitingwhorl-like cell cluster of epithelial cells at different grades of differentiation, peripherally palisading cells and nodules of ‘‘wet keratin (A) and ghostcells (B) (H&E original magnification 200×). (C) Immunohistochemical staining of β-catenin demonstrating strong nuclear and cytoplasmic expression which was predominantly in compactly cohesive epithelial cells within the whorl-like arrays (200 x). (D) EGFR immunostaining showing positive expression in the neoplastic cells (400 x)

Due to the limitations in the treatment options and the need for development of some adjuvant chemotherapeutic options in patients with CP, we explored weather or not ACP harbors EGFR activation to see if it can be a treated with anti EGFR therapy.

This study included eighteen samples of ACP, and our results showed that 94% of our samples were positive for β-catenin and showed cytoplasmic/nuclear expression. On the other hand, 83% of the cases exhibited positivity for EGFR.

The distinctive β-catenin expression patterns observed in our study, i.e. nuclear accumulation, was observed more in the cohesive cell clusters within the whorl-like area. This result suggests that aberrant β-catenin is expressed in ACP, and it plays an important role in its tumorigenicity. This finding was in keeping with that of previously published data [20]. This characteristic pattern is a result of activating mutations of the gene-encoding β-catenin and it has been detected exclusively in the adamantinomatous subtype, but not in the papillary subtype. The β-catenin protein is encoded by the CTNNB1 gene and is a key mediator of the canonical WNT signaling pathway, which is essential in the control of stem cell pluripotency, cellular differentiation, proliferation, and migration [19]. These CTNNB1 mutations affect serine phosphorylation of CTNNB1 at residue 33 by glycogen synthase kinase 3 beta (GSK-3 beta), which is critical for CTNNB1 degradation, resulting in translocation of CTNNB1 to the nucleus. This, in turn, forms a complex with the T-cell factor/lymphoid enhancer binding factor family, acting as a transcription factor to reactivate the Wnt pathway. Mutation in the β-catenin gene has been demonstrated as playing an important role in tumor morphogenesis and epithelial differentiation of ACP [19], [20].

Our result of the activated EGFR in ACP was compatible with Holsken et al.’s study, which concluded with the same result. Moreover, they found that the activated EGFR is located in the cohesive cell clusters within the whorl-like area, and that this activation is associated with nuclear co-localization of activated β-catenin suggesting an interplay of both pathways in ACP [21]. They also concluded that the EGFR activation plays an important role in tumor cell migration through identification of EGFR-P positive cells aggregated in whorls at the tumor’s brain invasion border [21].

In summary, our preliminary study proved that β-catenin is an important diagnostic marker for ACP and is expressed in a unique pattern in the areas of tumor cell clusters. We also concluded that EGFR is activated in ACP, a finding which qualifies the patient as a candidate for anti-EGFR therapy.

We suggest further investigations and subsequent studies with a larger sample size, and more investigations on the EGFR mutation at the molecular basis, which should be correlated with immunohistochemical expression.

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